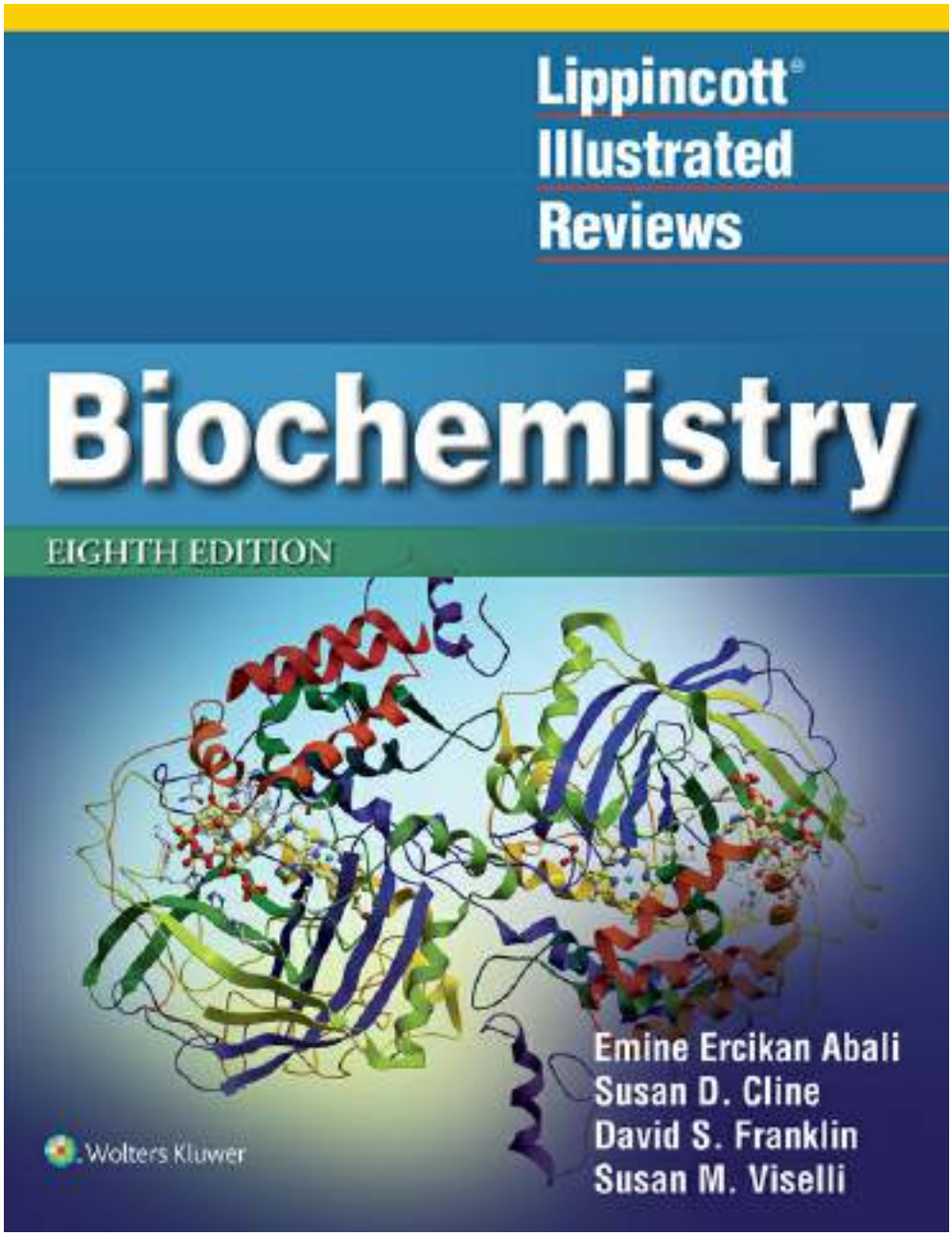

Lippincott® Illustrated Reviews: Biochemistry

Eighth Edition

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Dedication

This edition is dedicated to those we teach and to those who taught us.

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Acknowledgments

We extend gratitude to the founding authors of this title, the late Dr. Pamela Champe and the late Dr. Richard Harvey, who created the first four editions, and to Dr. Denise Ferrier, who coauthored or authored the next three editions. We have strived to carry on their tradition of excellence with the current edition.

We value the many members of the Association of Biochemistry Educators who provided critical peer review of the new materials produced for this edition.

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Preface

Biochemistry is the study of how our bodies utilize the nutritional substances in our diet to make building blocks, fuels, and communication molecules for our cells. It also includes the processes by which we convert chemicals within our bodies and eliminate chemicals from our bodies. This book provides a succinct and illustrative review of these complex mechanisms. In doing so, the book also offers examples of a useful organizational tool called a concept map. Here is an explanation of concept maps so that you may use them as you study biochemistry, and perhaps create your own concept maps in your studies.

Concept Maps

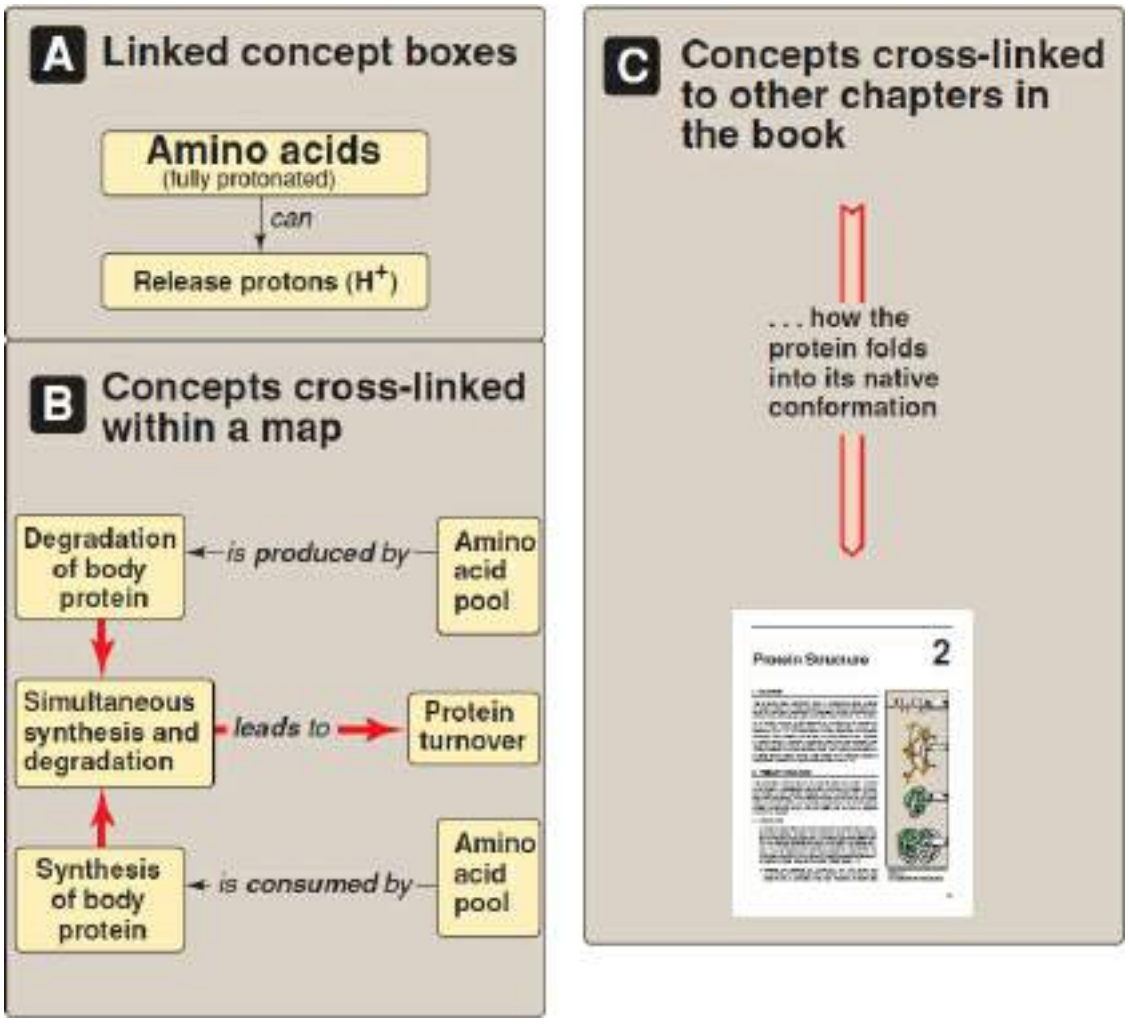
Students sometimes view biochemistry as a list of facts or equations to be memorized, rather than a body of concepts to be understood in context of the whole person. Details provided to enrich understanding of these concepts inadvertently turn into distractions. What seems to be missing is a guide, or type of road map—one that provides the student with an understanding of the context of how various topics fit together to tell a story. In this text, a series of biochemical concept maps have been created to graphically illustrate relationships between ideas and connections between concepts. These are presented near the end of each chapter to show how the information can be grouped or organized. A concept map is, thus, a tool for visualizing the connections between concepts. Material is represented in a hierarchical fashion, with the most inclusive, most general concepts at the top of the map, and the more specific, less general concepts arranged beneath. The concept maps ideally function as templates or guides for organizing information, so the student can readily find the best ways to help with the integration of new information with knowledge they already possessed. Concept map construction is described below.

A: Concept boxes and links

Educators define concepts as “perceived regularities in events or objects.” In the biochemical maps, concepts include abstractions (e.g., free energy), processes (e.g., oxidative phosphorylation), and compounds (e.g., glucose 6-phosphate). These broadly defined concepts are prioritized with the central idea positioned at the top of the page. The concepts that follow from this central idea are then drawn in boxes (see figure, part A). The size of the type indicates the relative importance of each idea. Lines are drawn between concept boxes to show which are related. The label on the line defines the relationship between two concepts, so that it reads as a valid statement (i.e., the connection creates meaning). The lines with arrowheads indicate in which direction the connection should be read.

B: Links to other parts of a map

Unlike linear flow charts or outlines, concept maps may contain cross-links that allow the reader to visualize complex relationships between ideas represented in different parts of the map (see figure, part B) or between the map and other chapters in this book (see figure, part C) or to other books in the Lippincott® Illustrated Reviews series (e.g., *Lippincott® Illustrated Reviews: Cell and Molecular Biology*). These links can help identify concepts that are central to more than one topic in biochemistry, empowering students to be effective in clinical situations and on professional licensure examinations that require integration of material. These maps with links provide a visual aid to represent nonlinear relationships between facts, in contrast to cross-referencing within linear text and concepts. The first example of a complete concept map can be found at the end of Chapter 1 (Fig. 1.13).



Recommended use of this textbook and other resources

This book is a comprehensive review of biochemistry. In addition to concept maps and illustrative figures, clinical boxes are included to offer students biologic or medical application of concepts. Students are also encouraged to challenge their understanding of the information that they have read through the completion of study questions at the

end of each chapter and in the larger question bank available online.

Contents

UNIT VI: Medical Nutrition

UNIT VII: Storage and Expression of Genetic Information

UNIT I:
Protein Structure and Function

Amino Acids and the Role of pH 1

I. OVERVIEW

Proteins are the most abundant and functionally diverse molecules in living systems. Virtually every life process depends on this class of macromolecules. For example, enzymes and polypeptide hormones direct and regulate metabolism in the body, whereas contractile proteins in muscle permit movement. In bone, the protein collagen forms a framework for the deposition of calcium phosphate crystals, acting like the steel cables in reinforced concrete. In the bloodstream, proteins, such as hemoglobin and albumin, transport molecules essential to life, whereas immunoglobulins fight infectious bacteria and viruses. In short, proteins display an incredible diversity of functions, yet all share the common structural feature of being linear polymers of amino acids. This chapter describes the properties of amino acids and the importance of pH to normal protein and body function. Chapter 2 explores how these simple building blocks are joined to form proteins that have unique three-dimensional structures, making them capable of performing specific biologic functions.

II. STRUCTURE

Although more than 300 different amino acids have been described in nature, only 20 are commonly found as constituents of mammalian proteins. These 20 standard amino acids are the only amino acids that are encoded by DNA, the genetic material in the cell. Nonstandard amino acids are produced by chemical modification of standard amino acids. Each amino acid has a carboxyl group, a primary amino group (except for proline, which has a secondary amino group), and a distinctive side chain or R group bonded to the α-carbon atom.

At physiologic pH (~7.4), the carboxyl group of an amino acid is dissociated, forming the negatively charged carboxylate ion ($-COO^-$), and the amino group is protonated ($-NH_3^+$) (Fig. 1.1A). In proteins, almost all of these carboxyl and amino groups are combined through peptide linkage and, in general, are not available for chemical reaction except for hydrogen bond or ionic bond formation (Fig. 1.1B). Amino acids within proteins are referred to as *residues* in reference to the residual structure remaining after peptide bond formation between consecutive amino acids within a peptide chain. It is the nature of the side chains that ultimately dictates the role an amino acid plays in a protein. Therefore, it is useful to classify the amino acids according to the properties of their side chains, that is, whether they are nonpolar, with an even distribution of electrons, or polar with an uneven distribution of electrons, such as acids and bases (Figs. 1.2 and 1.3).

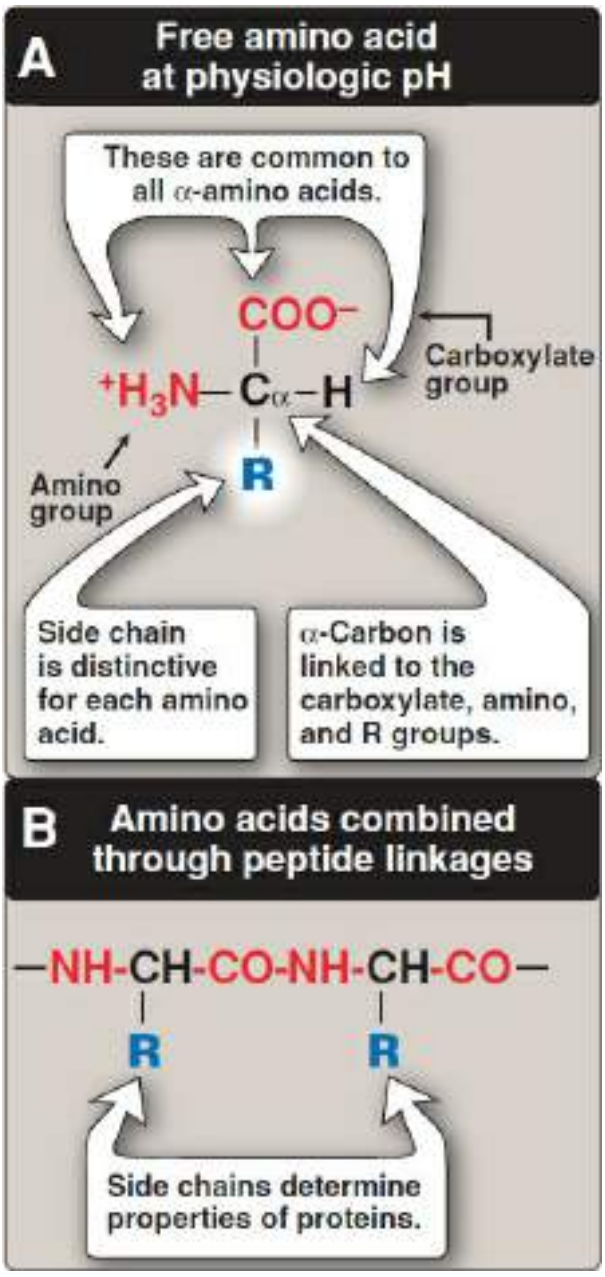


Figure 1.1
A, B: Structural features of amino acids.

A. Amino acids with nonpolar side chains

Each of the amino acids in this category has a side chain that does not gain or lose protons or participate in hydrogen or ionic bonds (see Fig. 1.2). The side chains of these amino acids can be thought of as “oily” or lipid like, a property that promotes hydrophobic interactions (see Fig. 2.10).

1. Location in proteins: In proteins found in polar environments such as aqueous solutions, the side chains of nonpolar amino acids tend to cluster together in the

interior of the protein (Fig. 1.4). This phenomenon is known as the hydrophobic effect and is the result of the hydrophobicity of the nonpolar R groups, which act much like droplets of oil that coalesce in an aqueous environment. By occupying the interior of the folded protein, these nonpolar R groups help give proteins their three-dimensional shape.

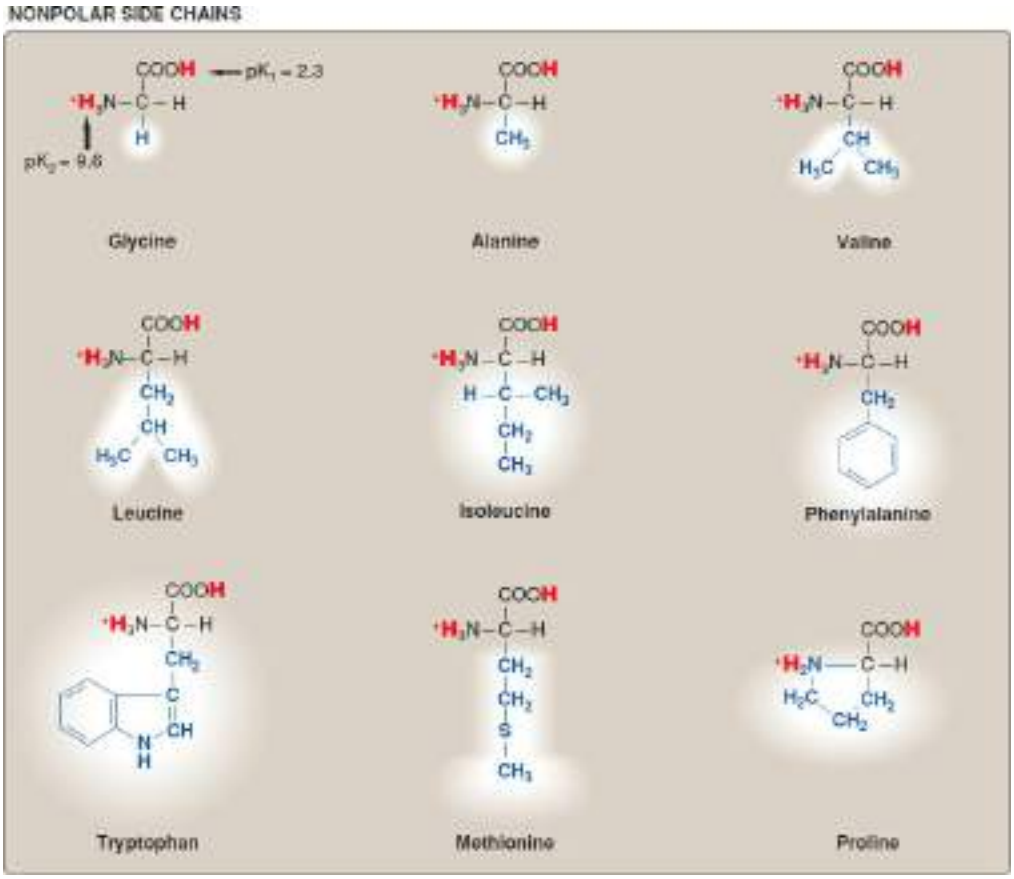


Figure 1.2
Classification of the 20 standard amino acids, according to the charge and polarity of their side chains at acidic pH, is shown here and continues in Figure 1.3. Each amino acid is shown in its fully protonated form, with dissociable hydrogen ions represented in *red*. The pK values for the α-carboxyl and α-amino groups of the nonpolar amino acids are similar to those shown for glycine.

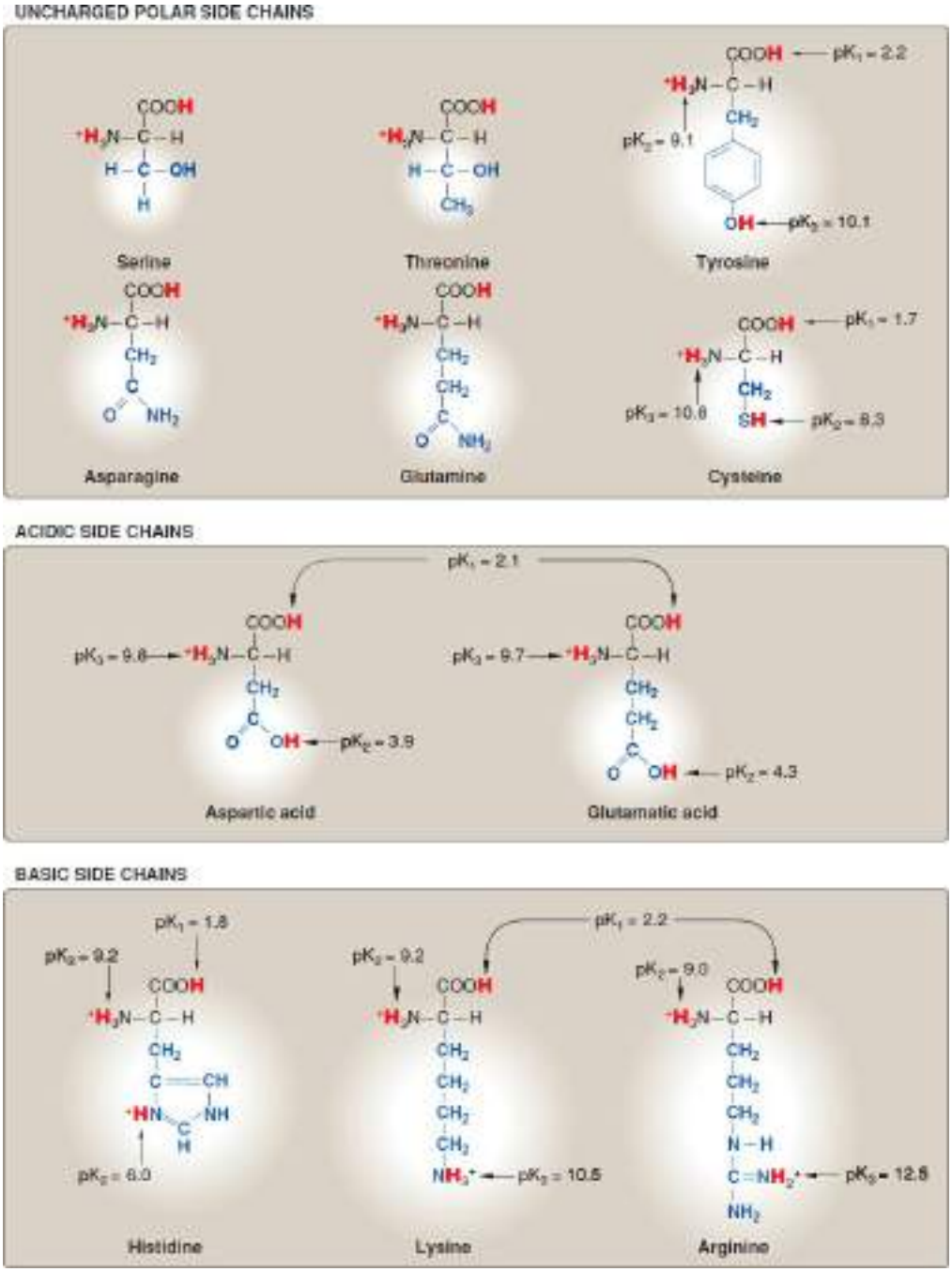


Figure 1.3
Classification of the 20 standard amino acids, according to the charge and polarity of their side chains at acidic pH (continued from Fig. 1.2). (Note: At physiologic pH (7.35 to 7.45), the α-carboxyl groups, the acidic side chains, and the side chain of free histidine are deprotonated.)

For proteins located in a hydrophobic environment, such as within the hydrophobic core of a phospholipid membrane, nonpolar R groups are found on the outside surface of the protein, interacting with the lipid environment (see Fig. 1.4). The importance of these hydrophobic interactions in stabilizing protein structure is discussed in Chapter 2.

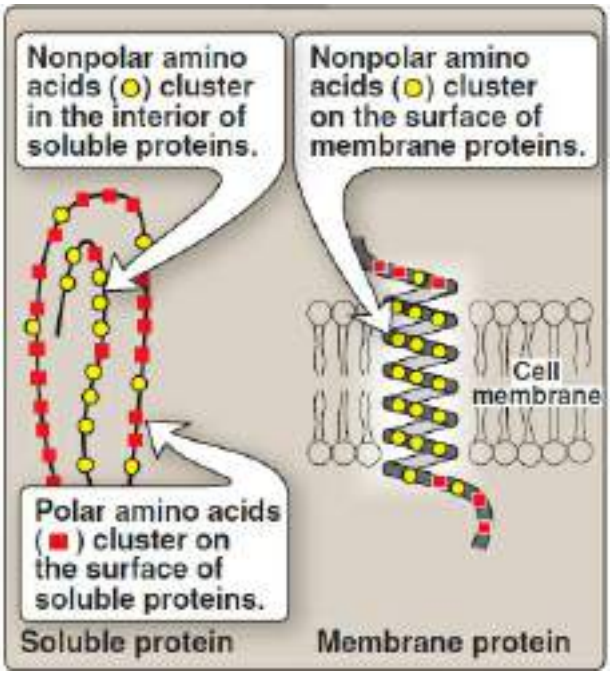


Figure 1.4
Location of nonpolar amino acids in soluble and membrane proteins.

> Sickle cell anemia, a disease that causes red blood cells to become sickle shaped rather than disc shaped, results from the replacement of polar glutamate with nonpolar valine at the sixth position in the β subunit of hemoglobin A (see Chapter 4).

2. Unique features of proline: Proline differs from other amino acids in that its side chain and α-amino nitrogen form a rigid, five-membered ring structure (Fig. 1.5). Proline, then, has a secondary (rather than a primary) amino group and is frequently referred to as an "imino acid." The unique geometry of proline contributes to the formation of the extended fibrous structure of collagen (see Chapter 4, II Collagen B. Structure), but it interrupts the α-helices found in more compact globular proteins (see Chapter 2, III Secondary structure).

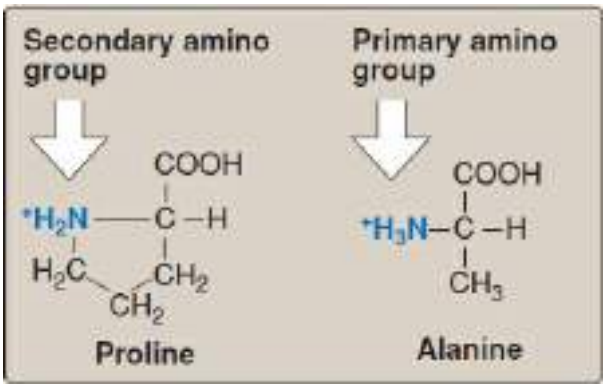


Figure 1.5

Comparison of the secondary amino group found in proline with the primary amino group found in other amino acids such as alanine.

B. Amino acids with uncharged polar side chains

These amino acids have zero net charge at physiologic pH of approximately 7.4, although the side chains of cysteine and tyrosine can lose a proton at an alkaline pH (see Fig. 1.3). Serine, threonine, and tyrosine each contain a polar hydroxyl group that can participate in hydrogen bond formation (Fig. 1.6). The side chains of asparagine and glutamine each contain a carbonyl group and an amide group, both of which can also participate in hydrogen bonds.

1. Disulfide bond formation: The side chain of cysteine contains a sulfhydryl (thiol) group (−SH), which is an important component within the active site of many enzymes. In proteins, the –SH groups of two cysteines can be oxidized to form a covalent cross-link called a disulfide bond (−S–S–). Two cysteine residues that form a disulfide bond are referred to as cystine. (See Chapter 2 Section IV. B. for a further discussion of disulfide bond formation.)

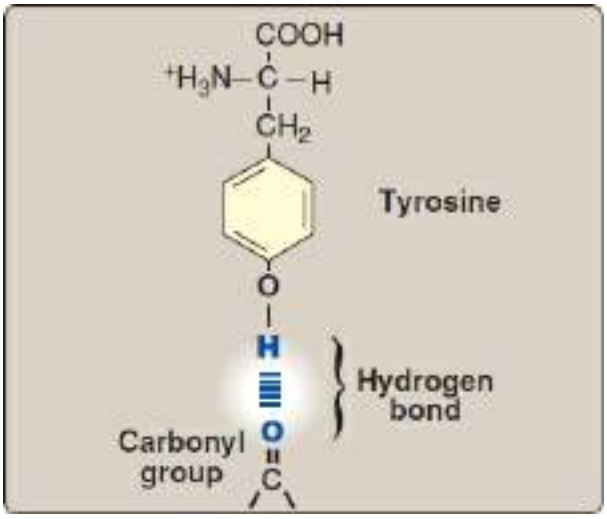


Figure 1.6
Hydrogen bond between the phenolic hydroxyl group of tyrosine and another molecule containing a carbonyl group.

Many extracellular proteins are stabilized by disulfide bonds. Albumin, a protein that functions in the transport of a variety of molecules in the blood, is one example. Fibrinogen, a blood protein converted to fibrin to stabilize blood clots, is another example.

2. Side chains as attachment sites for other compounds: The polar hydroxyl group of serine, threonine, and tyrosine can serve as a site of attachment for phosphate groups. Kinases are enzymes that catalyze phosphorylation reactions. Phosphatases are enzymes that remove the phosphate group. The changes in phosphorylation status of proteins (whether phosphorylated or not),

especially of enzymes, alters their activation status; some enzymes are more active when phosphorylated while others are less active. In addition, the amide group of asparagine, as well as the hydroxyl group of serine or threonine, can serve as a site of attachment for oligosaccharide chains in glycoproteins (see also Chapter 14 Section VII.).

C. Amino acids with acidic side chains

The amino acids aspartic acid and glutamic acid are proton donors. At physiologic pH, the side chains of these amino acids are fully ionized, containing a negatively charged carboxylate group ($-COO^-$). The fully ionized forms are called aspartate and glutamate.

D. Amino acids with basic side chains

The side chains of the basic amino acids accept protons (see Fig. 1.3). At physiologic pH, the R groups of lysine and arginine are fully ionized and positively charged. In contrast, the free amino acid histidine is weakly basic and largely uncharged at physiologic pH. However, when histidine is incorporated into a protein, its R group can be either positively charged (protonated) or neutral, depending on the ionic environment provided by the protein. This important property of histidine contributes to the buffering role it plays in the functioning of proteins including hemoglobin (see Chapter 3). Histidine is the only amino acid with a side chain that can ionize within the physiologic pH range (7.35 to 7.45).

Clinical Application 1.1: Slower, Longer-Acting Insulin Created by Substituting Amino Acids

Insulin glargine was first approved for use in the United States in the year 2000. It is a slower-acting form of insulin created in the laboratory by replacing the asparagine normally at position 21 on the A chain of insulin with glycine, and extending the carboxy terminus by two additional arginine residues. The result of these changes is a less water-soluble form of insulin with a net charge of +0.2, which is closer to 0, causing a slower absorption of insulin glargine from the site of injection. The glycine substitution prevents deamidation of the asparagine at acidic pH in the neutral, subcutaneous space. The additional arginine residues shift the isoelectric point from pH 5.4 to pH 6.7, making the molecule more soluble at acidic pH and less soluble at neutral pH. Insulin glargine is therefore a form of insulin that acts slowly, has longer activity, and requires less frequent injection. This form of insulin can be useful in the treatment of diabetes mellitus and help patients achieve better glycemic control. (See Chapter 23 for the structure of insulin.)

E. Abbreviations and symbols for commonly occurring amino acids

Each amino acid has an associated three-letter abbreviation and a one-letter symbol (Fig. 1.7). The one-letter codes are determined by the following rules:

1. Unique first letter: If only one amino acid begins with a given letter, then that letter is used as its symbol. For example, V = valine.

2. Most commonly occurring amino acids have priority: If more than one amino acid begins with a particular letter, the most common of these amino acids receives this letter as its symbol. For example, glycine is more common than glutamate, so G = glycine.
3. Similar sounding names: Some one-letter symbols sound like the amino acid they represent. For example, F = phenylalanine.
4. Letter close to initial letter: For the remaining amino acids, a one-letter symbol is assigned that is close in the alphabet as possible to the initial letter of the amino acid, for example, K = lysine. Furthermore, B is assigned to Asx, signifying either aspartic acid or asparagine; Z is assigned to Glx, signifying either glutamic acid or glutamine; W is used for tryptophan and X is used to represent an unidentified amino acid.

F. Amino acid isomers

Because the α-carbon of an amino acid is attached to four different chemical groups, it is an asymmetric or chiral atom. Glycine is the exception because its α-carbon has two hydrogen substituents. Amino acids with a chiral α-carbon exist in two different isomeric forms, designated D and L, which are enantiomers, or mirror images (Fig. 1.8). (Note: Enantiomers are optically active. If an isomer, either D or L, causes the plane of polarized light to rotate clockwise, it is designated the [+] form.) All amino acids found in mammalian proteins are of the L configuration. However, D-amino acids are found in some antibiotics and in bacterial cell walls. (Note: Racemases enzymatically interconvert the D- and L-isomers of free amino acids.)

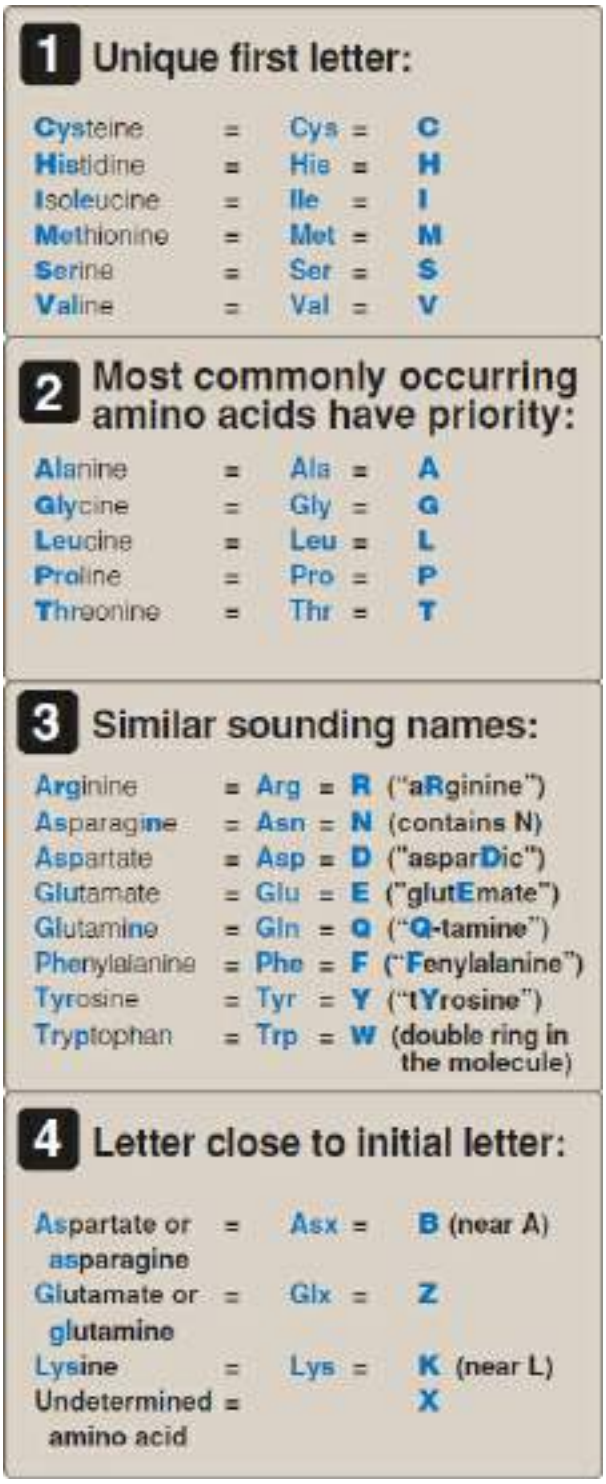


Figure 1.7
Abbreviations and symbols for the standard amino acids.

III. ACIDIC AND BASIC PROPERTIES

Amino acids in an aqueous solution contain weakly acidic α-carboxyl groups and weakly

basic α-amino groups. In addition, each of the acidic and basic amino acids contains an ionizable group in its side chain. Thus, both free amino acids and some amino acids combined in peptide linkages can act as buffers. Acids may be defined as proton donors and bases as proton acceptors. Acids (or bases) described as weak ionize to only a limited extent.

A. pH

The concentration of protons ($[H^+]$) in aqueous solution is expressed as pH.

$$pH = \log 1/ [H^+] \text{ or } -\log [H^+]$$

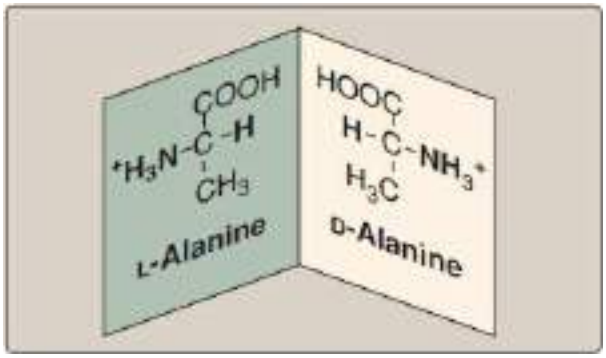


Figure 1.8
D and L forms of alanine are mirror images (enantiomers).

1. Dissociation constants: The salt or conjugate base, A^-, is the ionized form of a weak acid. By definition, the dissociation constant of the acid, K_a, is:

$$K_a = \frac{[H^+][A^-]}{[HA]}$$

The larger the K_a, the stronger the acid, because most of the HA has dissociated into H^+ and A^-. Conversely, the smaller the K_a, the less acid has dissociated and, therefore, the weaker the acid.

2. Henderson–Hasselbalch equation: By solving for the $[H^+]$ in the above equation, taking the logarithm of both sides of the equation, multiplying both sides of the equation by −1, and then substituting pH = −log $[H^+]$ and pK_a = −log K_a, we obtain the Henderson–Hasselbalch equation:

$$pH = pKa + \log [A-] / [HA]$$

This equation demonstrates the quantitative relationship between the pH of the solution and concentration of a weak acid (HA) and its conjugate base (A^-).

B. Buffers

A buffer is a solution that resists a change in pH following the addition of an acid or base and can be created by mixing a weak acid (HA) with its conjugate base (A^-). If an acid is added to a buffer, A^- can neutralize it, being converted to HA in the process. If a base is added, HA can likewise neutralize it, being converted to A^- in the process.

Maximum buffering capacity occurs at a pH equal to the pK_a, but a conjugate acid–base pair can still serve as an effective buffer when the pH of a solution is within approximately ±1 pH unit of the pK_a. If the amounts of HA and A^- are equal, the pH is equal to the pK_a. As shown in Figure 1.9, a solution containing acetic acid (HA = $CH_3 – COOH$) and acetate ($A^- = CH_3 – COO^-$) with a pKa of 4.8 resists a change in pH from 3.8 to 5.8, with maximum buffering at pH 4.8. At pH values less than the pKa, the protonated acid form ($CH_3 – COOH$) is the predominant species in solution. At pH greater than the pK_a, the deprotonated base form ($CH_3 – COO^-$) is the predominant species.

1. Dissociation of the carboxyl group: The dissociation constant of the carboxyl group of an amino acid is called K_1, rather than K_a, because the molecule contains a second titratable group. The Henderson–Hasselbalch equation can be used to analyze the dissociation of the carboxyl group of alanine:

$$K_1 = [H^+]\ [II] / [I]$$

where I is the fully protonated form of alanine and II is the isoelectric form of alanine (Fig. 1.10). This equation can be rearranged and converted to its logarithmic form to yield:

$$pH = pK_1 + \log [II] / [I]$$

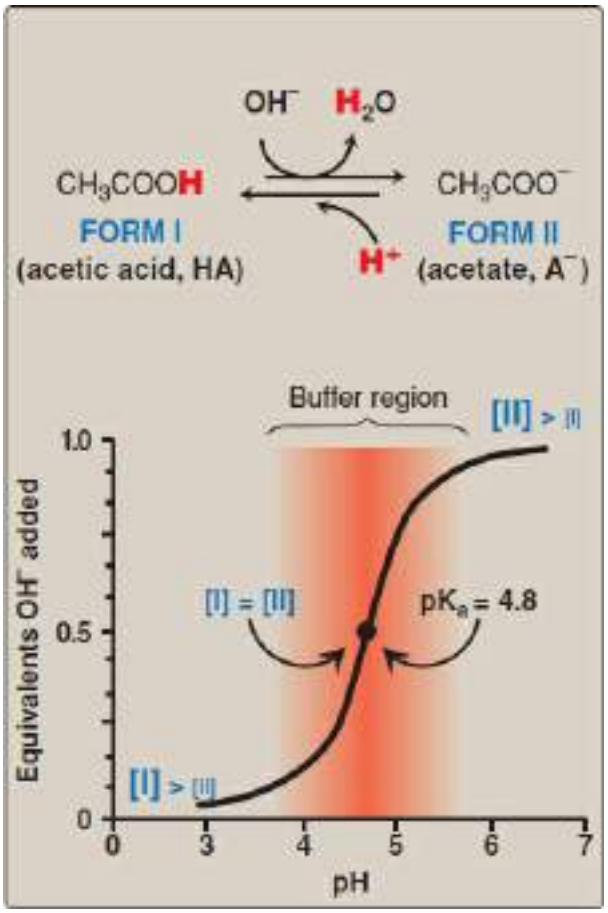


Figure 1.9
Titration curve of acetic acid.

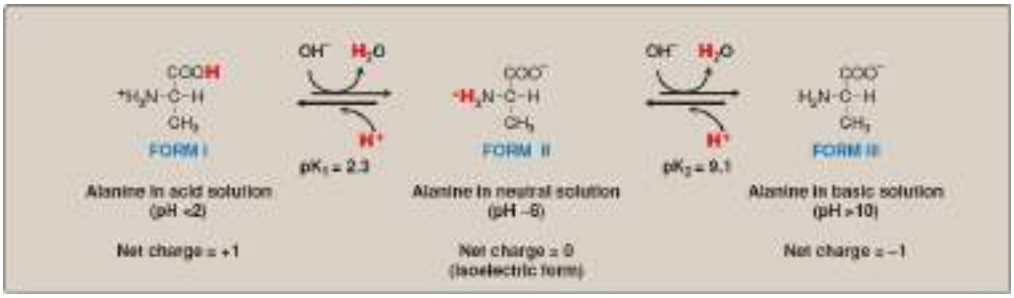


Figure 1.10
Ionic forms of alanine in acidic, neutral, and basic solutions.

2. Amino group dissociation: The second titratable group of alanine is the amino ($-NH_3^+$) group. Because this is a much weaker acid than the –COOH group, it has a much smaller dissociation constant, K_2. (Note: Its pK_a is, therefore, larger.) Release of a H^+ from the protonated amino group of form II results in the fully deprotonated form of alanine, form III.

3. pKs and sequential dissociation: The sequential dissociation of H^+ from the

carboxyl and amino groups is summarized in Figure 1.10 using alanine as an example. Each titratable group has a pK_a that is numerically equal to the pH at which exactly one half of the H^+ have been removed from that group. The pK_a for the most acidic group (−COOH) is pK_1, whereas the pK_a for the next most acidic group ($-NH_3^+$) is pK_2. (Note: The pK_a of the α-carboxyl group of amino acids is ~2, whereas the pK_a of the α-amino group is ~9.)

By applying the Henderson–Hasselbalch equation to each dissociable acid group, it is possible to calculate the complete titration curve of a weak acid. Figure 1.11 shows the change in pH that occurs during the addition of base to the fully protonated form of alanine (I) to produce the fully deprotonated form (III).

a. Buffer pairs: The –COOH/$–COO^-$ pair can serve as a buffer in the pH region around pK_1, and the $–NH_3^+$/$–NH_2$ pair can buffer in the region around pK_2.

b. When pH = pK: When the pH is equal to pK_1 (2.3), equal amounts of forms I and II of alanine exist in solution. When the pH is equal to pK_2 (9.1), equal amounts of forms II and III are present in solution.

c. Isoelectric point pI: At neutral pH, alanine exists predominantly as the dipolar form II in which the amino and carboxyl groups are ionized, but the net charge is zero. The isoelectric point (pI) is the pH at which an amino acid is electrically neutral, that is, when the sum of the positive charges equals the sum of the negative charges. For alanine, with only two dissociable hydrogens (one from the α-carboxyl and one from the α-amino group), the pI is the average of pK_1 and pK_2 (pI = [2.3 + 9.1]/2 = 5.7) as shown in Figure 1.11. The pI is, thus, midway between pK_1 (2.3) and pK_2 (9.1). pI corresponds to the pH at which the form II (with a net charge of zero) predominates and at which there are also equal amounts of forms I (net charge of +1) and III (net charge of −1).

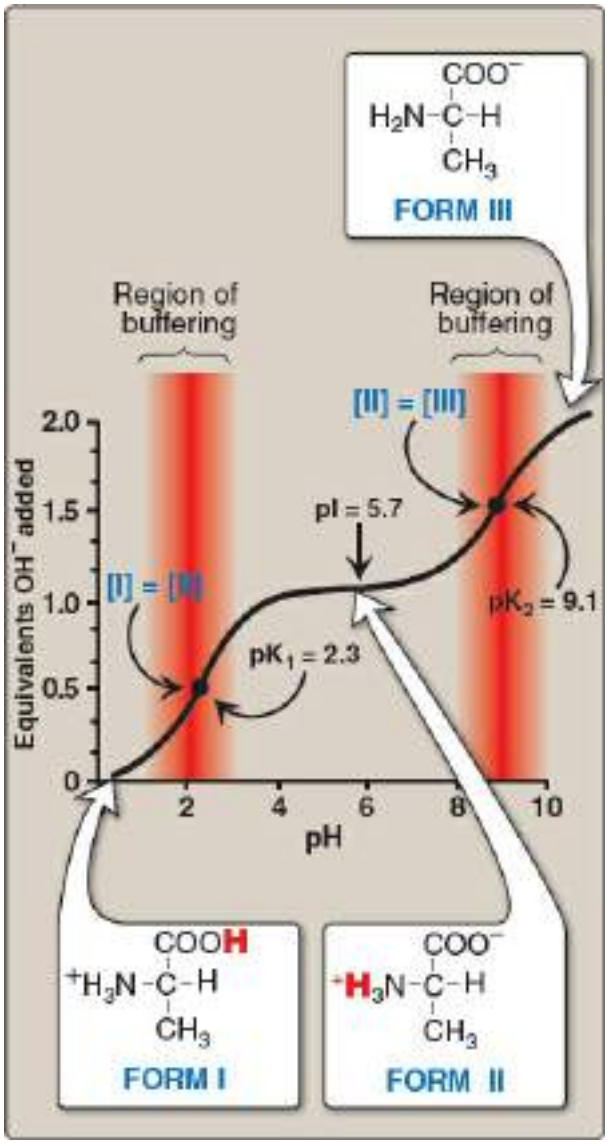


Figure 1.11
The titration curve of alanine.

In the laboratory, separation of plasma proteins by charge is typically done at a pH above the pI of the major proteins. Therefore, at a high pH (alkaline) the charge on the proteins is negative. In an electric field, the proteins will move toward the positive electrode at a rate determined by their net negative charge. Variations in the mobility pattern are suggestive of certain diseases.

4. Net charge at neutral pH: At physiologic pH, amino acids have a negatively charged group ($-COO^-$) and a positively charged group ($-NH_3^+$), both attached to the α-carbon. Glutamate, aspartate, histidine, arginine, and lysine have additional potentially charged groups in their side chains. Substances such as amino acids that can act either as an acid or a base are described as

amphoteric.

C. Buffering the blood, the bicarbonate buffer system

The pH within our blood is maintained in the slightly alkaline range of 7.35 to 7.45 by the bicarbonate buffer system. Most proteins function optimally at this physiologic pH and their amino acid constituents exist in the chemical form; exceptions include some digestive enzymes that function at acidic pH of the stomach between pH 1.5 and 3.5. Lysosomal enzymes also function at an acidic pH range between pH 4.5 and 5.0. Maintaining arterial pH at 7.40 ± 0.5 is important for health; normally the bicarbonate buffer system is able to keep pH within the acceptable range.

The bicarbonate ion concentration, [HCO_3^-], and the carbon dioxide concentration [CO_2] influence the pH of the blood, as depicted in Figure 1.12A. The need for a buffering system can be appreciated by considering that organic acids (e.g., lactic acid) are generated during metabolism and that glucose and fatty acid oxidation generate CO_2, the anhydrous form of H_2CO_3 (carbonic acid). The relatively water-insoluble CO_2 is converted by the enzyme carbonic anhydrase to the water-soluble HCO_3^- (bicarbonate), which is carried through the blood to the lungs where dissolved CO_2 is exhaled. Therefore, lungs regulate the loss and retention of CO_2 by altering the breathing rate. The kidneys are also important in regulating acid–base balance. Kidneys retain or excrete bicarbonate, H^+, ammonia, and other acids/bases that may appear in the blood.

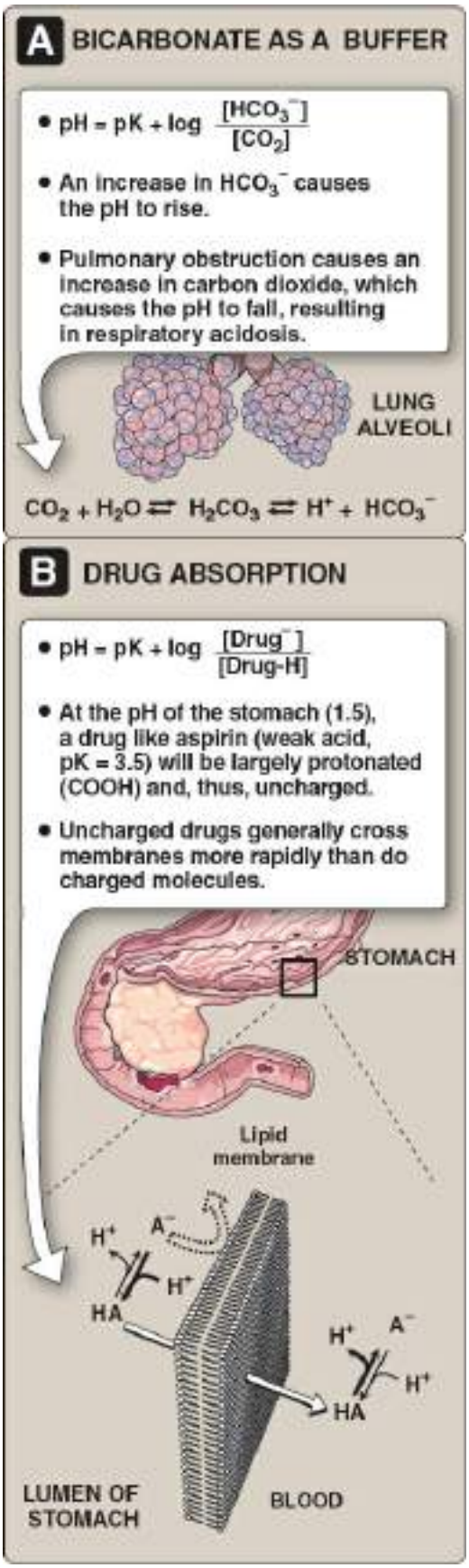


Figure 1.12
The Henderson–Hasselbalch equation is used to predict: **(A)** changes in pH as the concentrations of bicarbonate (HCO_3^-) or carbon dioxide (CO_2) are altered and **(B)** the ionic forms of drugs.

D. pH and drug absorption

Many drugs are administered orally and must be transported across intestinal epithelial cells in order to be absorbed into the blood. Most drugs are either weak acids or weak bases. Acid drugs (HA) release a H^+, causing a charged anion (A^-) to form. Weak bases (BH^+) can also release a H^+; however, the protonated form of basic drugs is usually charged and the loss of a proton produces the uncharged base (B).

$$HA \leftarrow \rightarrow H^+ + A^-$$
$$BH^+ \leftarrow \rightarrow B + H^+$$

Drugs are best absorbed at a pH where dissociation of their side chains results in the most neutral molecule. The effective concentration of the permeable form of each drug at its absorption site is determined by the relative concentrations of the charged and uncharged forms. (Fig. 1.12B). It is believed that the transport of drugs occurs via transport proteins and often occurs through active transport, although the systems are not well characterized.[1]

E. Blood gases and pH

As a consequence of certain disease processes or poisons, blood pH can become abnormal. Acidemia is defined as an arterial pH <7.35 and alkalemia is defined as an arterial pH >7.45. In the bicarbonate buffer system, CO_2 is an acid and bicarbonate is a base. Because the bicarbonate buffer is an open system and CO_2 is released in the breath, changes in breathing can impact the acid–base balance in the body. Hyperventilation can cause release of too much acid, causing alkalosis; on the other hand, generation of excess metabolic acids (e.g., lactic acidosis or ketoacidosis that can accompany type 1 diabetes mellitus) can cause acidosis. Loss of excess acid through vomiting can cause an acid–base disturbance as well. Neither renal compensation nor compensation by breathing rate changes (respiratory compensation) will bring pH back toward normal physiologic range if excess metabolic acids have been generated. It should be noted that neither the lungs nor the kidneys can fully compensate or overcompensate for pH imbalances. Measuring CO_2 and bicarbonate along with pH can help to determine the acid–base imbalance that may be present in a patient (Table 1.1).

Table 1.1 Disturbances in Acid–Base Balance

pH	[H^+]	Initial Issue	Response	Disorder
Decreased	Increased	Hypoventilation; increased retention of CO_2 (more acid)	Increased renal retention of HCO_3^- (more base)	Respiratory acidosis; lungs not excreting enough acid as CO_2, as in COPD
Increased	Decreased	Hyperventilation; increased release of CO_2 (less acid)	Decreased renal retention of HCO_3^- (less base)	Respiratory alkalosis; lungs excreting too much acid as CO_2, as in hyperventilation and asthma
Decreased	Increased	More acid generated	Less CO_2 released in breath (hypoventilation); HCO_3^- will be low to attempt to buffer the acid	Metabolic acidosis; body generates acid that cannot be excreted by lungs, as in lactic acidosis, diabetic ketoacidosis, ingestion of acid
Increased	Decreased	HCO_3^- increases	More CO_2 released in breath (hyperventilation); renal excretion of HCO_3^-	Metabolic alkalosis; when blood is alkaline and not caused by respiratory imbalance, as in excess loss of acid in vomiting or ingestion of a base

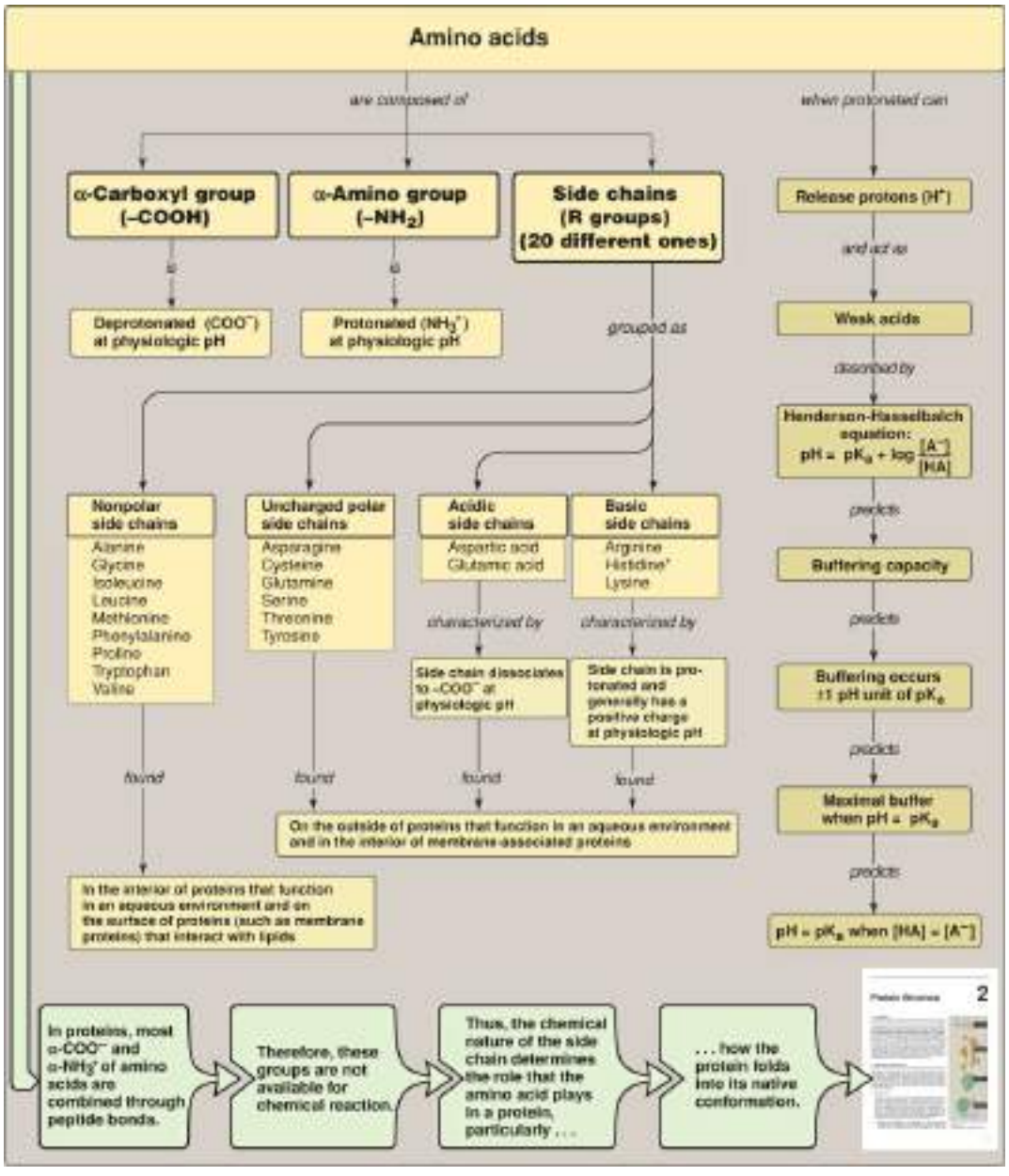


Figure 1.13
Key concept map for amino acids. (Note: *Free histidine is largely deprotonated at physiologic pH,

but when incorporated into a protein, it can be protonated or deprotonated depending on the local environment.)

IV. Chapter Summary

- Each amino acid has an **α-carboxyl group** and a primary **α-amino group** (except for **proline**, which has a **secondary amino group**) (Fig. 1.13).
- Because the α-carbon of each amino acid (except glycine) is attached to four different chemical groups, it is asymmetric (**chiral**), and amino acids exist in D- and L-isomeric forms that are optically active mirror images (**enantiomers**). The L-form of amino acids is found in proteins synthesized by the human body.
- At physiologic pH, the α-carboxyl group is dissociated, forming the negatively charged carboxylate ion ($-COO^-$), and the α-amino group is protonated ($-NH_3^+$).
- Each amino acid also contains one of 20 distinctive **side chains** attached to the α-carbon atom.
- The chemical nature of this **R group** determines the function of an amino acid in a protein and provides the basis for classification of the amino acids as **nonpolar**, **uncharged polar**, **acidic** (**polar negative**), or **basic** (**polar positive**).
- All free amino acids, plus charged amino acids in peptide chains, can serve as **buffers**.
- The quantitative relationship between the pH of a solution and the concentration of a weak acid (HA) and its conjugate base (A^-) is described by the **Henderson–Hasselbalch equation**. Buffering occurs within ±1 pH unit of the pK_a and is maximal when pH = pK_a, at which $[A^-] = [HA]$.
- The pH within blood is maintained in the slightly alkaline range of 7.4 ± 0.5 by the bicarbonate buffer system; the lungs regulate the acid CO_2 by altering breathing rate and the kidneys retain or release acids and base.

Study Questions

Choose the ONE best answer.

1.1 The peptide Val-Cys-Glu-Ser-Asp-Arg-Cys:

A. Contains asparagine.
B. Contains a side chain with a secondary amino group.
C. Contains a side chain that can be phosphorylated.
D. Cannot form an internal disulfide bond.
E. Cannot move toward the cathode during electrophoresis at pH 5.

Correct answer = C. The hydroxyl group of serine can accept a phosphate group. Asp is aspartate, not asparagine. Proline contains a secondary amino group and is not within this peptide. The two cysteine residues can, under oxidizing conditions, form a disulfide bond. The net charge on the peptide at pH 5 is negative, and it would move to the anode.

1.2 An amino acid has a secondary amino group that is geometrically incompatible with a right-handed spiral of an alpha helix. It is observed to insert a kink in the amino acid chain and to interfere with the normally smooth, helical structure of the alpha helix, and is found in high concentration in collagen. The amino acid described is:

A. Ala
B. Cys
C. Gly
D. Pro
E. Ser

Correct answer = D. Proline differs from other amino acids in that its side chain and a-amino nitrogen form a rigid, 5-membered ring structure and therefore contains a secondary amino group. It interrupts α helices in globular proteins, contributes to the structure of collagen, and is found in high concentration in collagen. None of the other

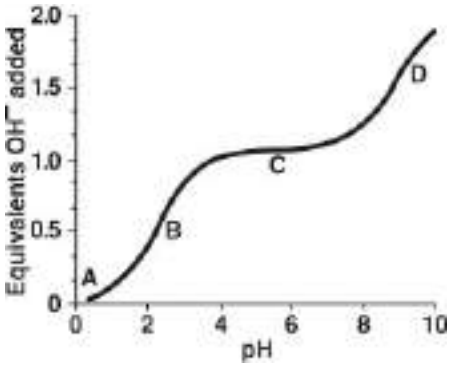
amino acids have these properties.

1.3 An amino acid that may have its side chain phosphorylated by the action of a kinase is:

A. Arg
B. Cys
C. Gly
D. Thr
E. Val

Correct answer = D. The polar hydroxyl group found within Ser, Thr, and Tyr can serve as a site of attachment for phosphate groups. Kinases are enzymes that catalyze phosphorylation reactions. None of the other amino acids contain a hydroxyl group susceptible to phosphorylation by a kinase.

1.4 Concerning the titration curve for a nonpolar amino acid where the letters A through D designate certain regions on the curve below,



A. Point A represents the region where the amino acid is deprotonated.
B. Point B represents a region of minimal buffering.
C. Point C represents the region where the net charge on the amino acid is zero.
D. Point D represents the pK of the amino acid's carboxyl group.
E. The amino acid could be lysine.

Correct answer = C. Point C represents the isoelectric point, or pI, and as such is midway between pK_1 and pK_2 for a nonpolar amino acid. The amino acid is fully protonated at Point A. Point B represents a region of maximum buffering, as does Point D. Lysine is a basic amino acid, and free lysine has an ionizable side chain in addition to the ionizable α-amino and α-carboxyl groups.

1.5 An 18-year-old female with a 15-year history of type 1 diabetes mellitus is brought to the Emergency Department for evaluation of nausea, vomiting, and altered consciousness. Her blood glucose is 560 mg/dl (reference range for random glucose, <200 mg/dl). Her arterial blood pH is 7.15 (reference range is 7.35 to 7.45) and bicarbonate is 12 mEq/l (reference range, 22 to 28 mEq/l). Which of the following is the expected type of compensation in her body in response to this acid–base imbalance?

A. Increased respiration
B. Increased renal release of acid
C. Increased renal retention of base
D. Decreased respiration
E. Decreased renal release of acid

Correct answer = A. In response to a metabolic acidosis, compensation is respiratory. Increased respiration removes acid in the form of CO_2 from the body. Since the acid is being generated metabolically (diabetic

ketoacidosis suspected) altered renal release of acid or retention of base would not be compensatory.

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[1]For further discussion of drug transport, see *LIR Cell and Molecular Biology*, 2nd Edition, Chapter 16.

Protein Structure 2

I. OVERVIEW

Proteins are composed of amino acids that are joined together by peptide bonds in a linear sequence, and then folded into a unique three-dimensional shape that determines function. The complexity of protein structure is best analyzed by considering the molecule in terms of four organizational levels: primary, secondary, tertiary, and quaternary (Fig. 2.1). An examination of these hierarchies of increasing complexity has revealed that certain structural elements are repeated in a wide variety of proteins, suggesting that there are general rules regarding the ways in which proteins achieve their native (functional) form. These repeated structural elements range from simple combinations of α-helices and β-sheets forming small motifs to the complex folding of polypeptide domains of multifunctional proteins (see Chapter 2 Section IV.).

II. PRIMARY STRUCTURE

The linear sequence of amino acids in a protein is the primary structure of the protein. Many genetic diseases result in proteins with abnormal amino acid sequences, causing improper folding and loss or impairment of normal function. If the primary structures of the normal and the mutated proteins are known, this information may be used to diagnose or study the disease.

A. Peptide bond

In proteins, adjacent amino acids are joined covalently by peptide bonds, which are amide linkages between the α-carboxyl group of one amino acid and the α-amino group of the next amino acid. For example, valine and alanine can form the dipeptide valylalanine through the formation of a peptide bond (Fig. 2.2). Peptide bonds are resistant to conditions that denature proteins, such as heat and high concentrations of urea. Prolonged exposure to a strong acid or base at elevated temperatures is required to nonenzymatically break these bonds.

1. Naming the peptide: By convention, the free amino end (N-terminal) of the peptide chain is written to the left and the free carboxyl end (C-terminal) to the right. Therefore, all amino acid sequences are read from the N- to the C-terminal end. For example, in Figure 2.2A, the order of the amino acids in the dipeptide is valine, alanine. Linkage of 50 or more amino acids through peptide bonds results in an unbranched chain called a polypeptide, or protein. Each component amino acid is called a residue, because it is the portion of the amino acid remaining after the water atoms are lost during the formation of the peptide

bond. When a peptide is named, all amino acid residues have their suffixes (-ine, -an, -ic, or -ate) changed to -yl, with the exception of the C-terminal amino acid. For example, a tripeptide composed of an N-terminal valine, a glycine, and a C-terminal leucine is called valylglycylleucine.

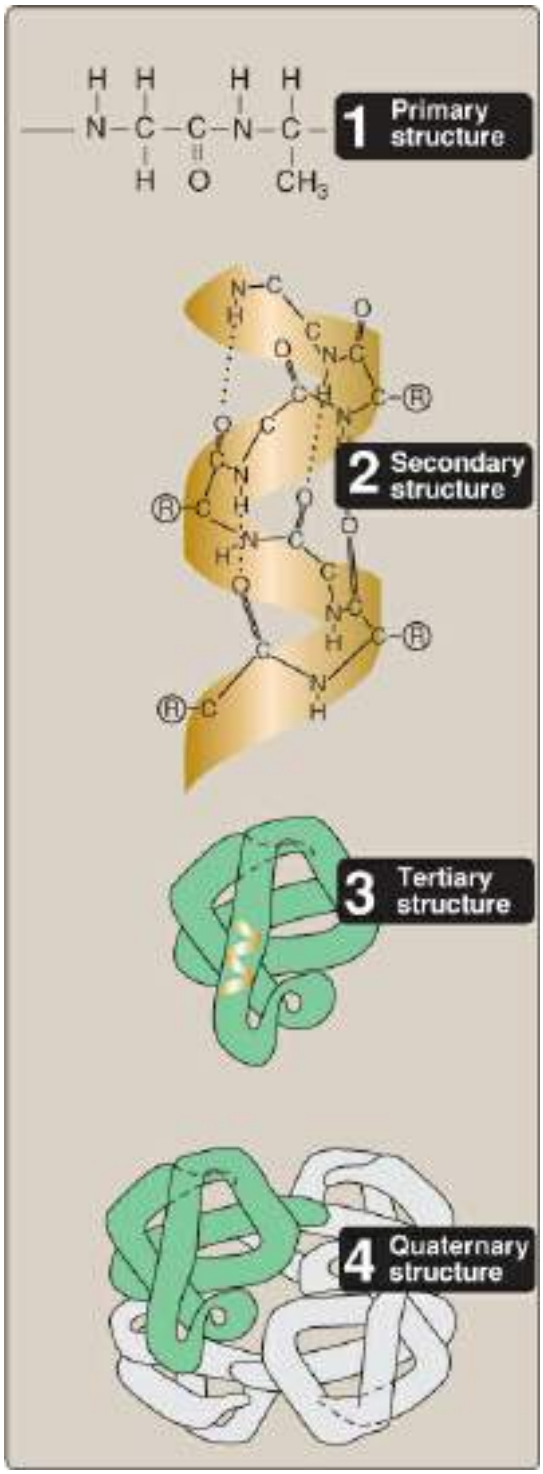


Figure 2.1

Four hierarchies of protein structure.

2. Peptide bond characteristics: The peptide bond has a partial double-bond character; that is, it is shorter than a single bond and is rigid and planar (Fig. 2.2B). This prevents free rotation around the bond between the carbonyl carbon and the nitrogen of the peptide bond. However, the bonds between the α-carbons and the α-amino or α-carboxyl groups can rotate freely (although they are limited by the size and character of the R groups). This allows the polypeptide chain to assume a variety of possible conformations. The peptide bond is almost always in the trans configuration (instead of the cis; see Fig. 2.2B), largely because of steric interference of the R groups (side chains) when in the cis position.

3. Peptide bond polarity: Like all amide linkages, the −C = O and −NH groups of the peptide bond are uncharged, and neither accept nor release protons over the pH range of 2 to 12. The charged groups present in polypeptides consist solely of the N-terminal (α-amino) group, the C-terminal (α-carboxyl) group, and any ionized groups present in the side chains of the constituent amino acids. However, the −C = O and −NH groups of the peptide bond are polar, and are involved in hydrogen bonds (e.g., in α-helices and β-sheets), as described on page 17.

B. Determining the amino acid composition of a polypeptide

The first step in determining the primary structure of a polypeptide is to identify and measure its constituent amino acids. A purified sample of the polypeptide to be analyzed is first hydrolyzed by strong acid to cleave the peptide bonds, and release the individual amino acids. These can then be separated by cation-exchange chromatography. Amino acids bind to the chromatography column with different affinities, depending on their charges, hydrophobicity, and other characteristics. Each amino acid is then sequentially released from the chromatography column by eluting with solutions of increasing ionic strength and pH (Fig. 2.3), and the separated amino acids are quantitated spectrophotometrically. The analysis described above is performed using an amino acid analyzer, an automated machine whose components are depicted in Figure 2.3.

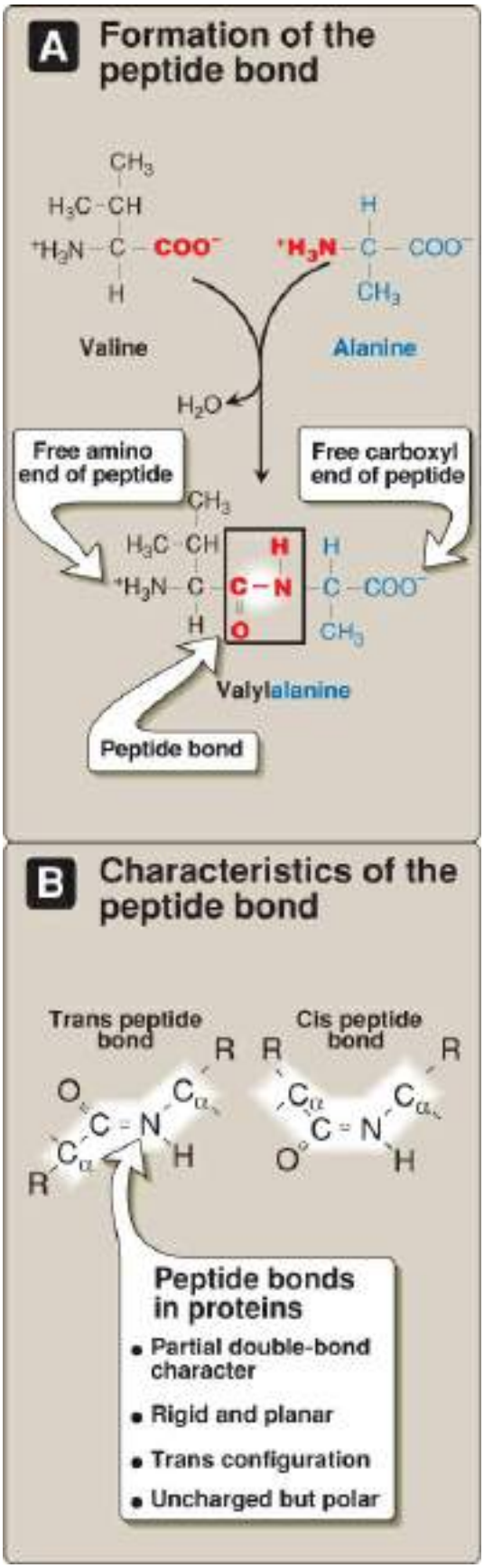


Figure 2.2
A: Formation of a peptide bond, showing the structure of the dipeptide valylalanine. **B:** Characteristics of the peptide bond. (Note: Peptide bonds involving proline may have a cis configuration.)

C. Sequencing the peptide from its N-terminal end

Sequencing is a stepwise process of identifying the specific amino acid at each position in the peptide chain, beginning at the N-terminal end. Automated sequencers are used now; the historical process to produce amino acid derivatives is shown in Figure 2.4.

D. Cleaving the polypeptide into smaller fragments

Many polypeptides have a primary structure composed of more than 100 amino acids. Such molecules cannot be sequenced directly from end to end. However, these large molecules can be cleaved at specific sites and the resulting fragments sequenced (Fig. 2.5). Enzymes that hydrolyze peptide bonds are termed peptidases or proteases. (Note: Exopeptidases cut at the ends of proteins and are classified into aminopeptidases and carboxypeptidases. Carboxypeptidases are used in determining the C-terminal amino acid. Endopeptidases cleave within a protein.)

E. Determining a protein's primary structure by DNA sequencing

The sequence of nucleotides in a protein-coding region of the DNA specifies the amino acid sequence of a polypeptide. Therefore, if the nucleotide sequence can be determined, knowledge of the genetic code allows the sequence of nucleotides to be translated into the corresponding amino acid sequence of that polypeptide. This indirect process, although routinely used to obtain the amino acid sequences of proteins, has the limitations of not being able to predict the positions of disulfide bonds in the folded chain and of not identifying any amino acids that are modified after their incorporation into the polypeptide (posttranslational modification). Therefore, direct protein sequencing is an extremely important tool for determining the true character of the primary sequence of many polypeptides. (See also Chapter 34 for discussion of related techniques.)

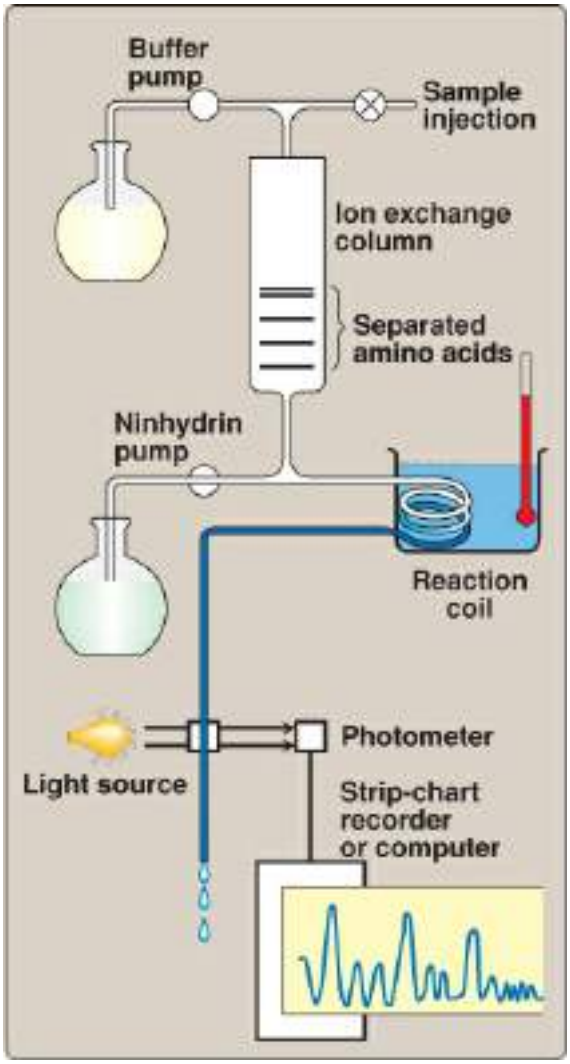


Figure 2.3
Determination of the amino acid composition of a polypeptide using an amino acid analyzer.

III. SECONDARY STRUCTURE

The polypeptide backbone does not assume a random three-dimensional structure but, instead, generally forms regular arrangements of amino acids that are located near each other in the linear sequence. These arrangements are termed the secondary structure of the polypeptide. The α-helix, β-sheet, and β-bend (or, β-turn) are examples of secondary structures commonly encountered in proteins. Each is stabilized by hydrogen bonds between atoms of the peptide backbone. (Note: The collagen α-chain helix, another example of secondary structure, is discussed in Chapter 4.)

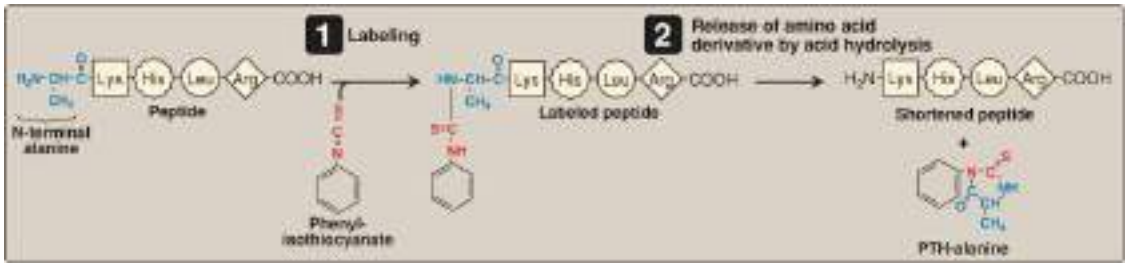


Figure 2.4
Determination of the amino (N)-terminal residue of a polypeptide by Edman degradation. PTH = phenylthiohydantoin.

A. α-Helix

Several different polypeptide helices are found in nature, but the α-helix is the most common. It is a rigid, right-handed spiral structure, consisting of a tightly packed, coiled polypeptide backbone core, with the side chains of the component L-amino acids extending outward from the central axis to avoid interfering sterically with each other (Fig. 2.6). A very diverse group of proteins contain α-helices. For example, the keratins are a family of closely related, rigid, fibrous proteins whose structure is nearly entirely α-helical. They are a major component of tissues such as hair and skin. In contrast to keratin, myoglobin, whose structure is also highly α-helical, is a globular, flexible molecule found in muscles (see Chapter 3 Section II. B.).

1. Hydrogen bonds: An α-helix is stabilized by extensive hydrogen bonding between the peptide bond carbonyl oxygens and amide hydrogens that are part of the polypeptide backbone (see Fig. 2.6). The hydrogen bonds extend up and are parallel to the spiral from the carbonyl oxygen of one peptide bond to the –NH group of a peptide linkage four residues ahead in the polypeptide. This ensures that all but the first and last peptide bond components are linked to each other through intrachain hydrogen bonds. Hydrogen bonds are individually weak, but they collectively serve to stabilize the helix.

2. Amino acids per turn: Each turn of an α-helix contains 3.6 amino acids. Thus, amino acids spaced three or four residues apart in the primary sequence are spatially close together when folded in the α-helix.

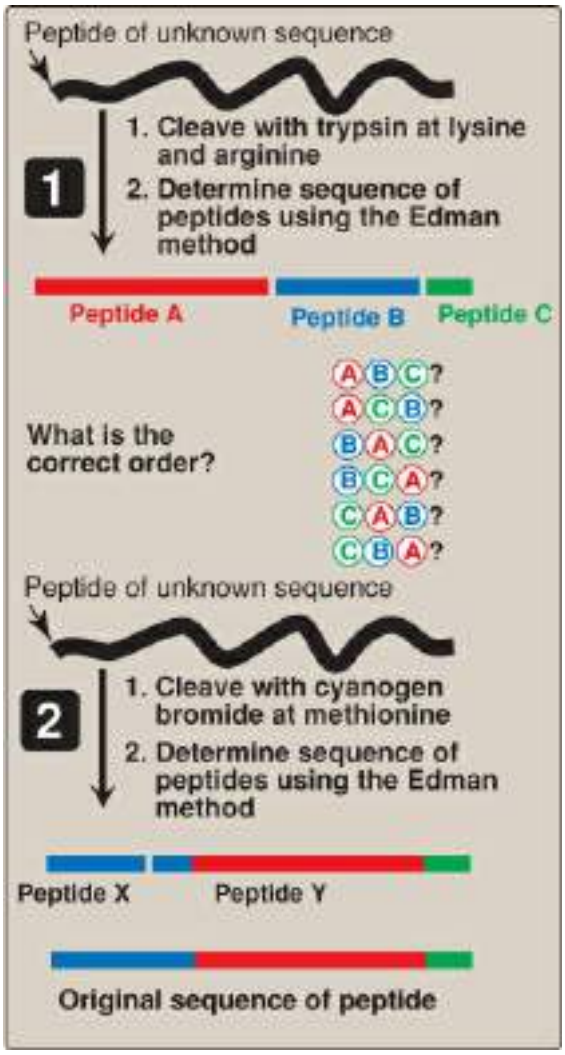


Figure 2.5
Overlapping of peptides produced by the cleavage action of trypsin and cyanogen bromide.

3. Amino acids that disrupt an α-helix: The R group of an amino acid determines its propensity to be in an α-helix. Proline disrupts an α-helix because its rigid secondary amino group is not geometrically compatible with the right-handed spiral of the α-helix. Instead, it inserts a kink in the chain, which interferes with the smooth, helical structure. Glycine, with hydrogen as its R group, confers high flexibility. Additionally, amino acids with charged or bulky R groups, such as glutamate and tryptophan, respectively, and those with a branch at the β-carbon, the first carbon in the R group (e.g., valine), are less likely to be found in an α-helix.

B. β-Sheet

The β-sheet is another form of secondary structure in which all of the peptide bond components are involved in hydrogen bonding (Fig. 2.7A). Because the surfaces of β-sheets appear to be folded or to form “pleats,” they are often called β-pleated

sheets. Pleating results from successive α-carbons being slightly above or below the plane of the sheet. Illustrations of protein structure often show β-strands as broad arrows (Fig. 2.7B).

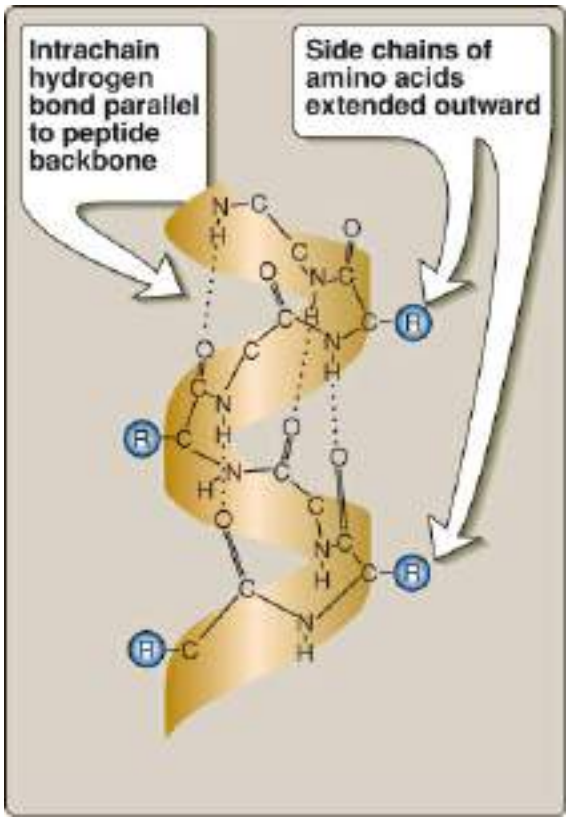


Figure 2.6
Structure of an α-helix.

1. Formation: A β-sheet is formed by two or more peptide chains (β-strands) aligned laterally and stabilized by hydrogen bonds between the carboxyl and amino groups of amino acids that either are far apart in a single polypeptide (intrachain bonds) or are in different polypeptide chains (interchain bonds). The adjacent β-strands are arranged either antiparallel to each other (with the N-termini alternating as shown in Fig. 2.7B) or parallel to each other (with the N-termini together as shown in Fig. 2.7C). On each β-strand, the R groups of adjacent amino acids extend in opposite directions, above and below the plane of the β-sheet. β-sheets are not flat and have a right-handed curl (twist) when viewed along the polypeptide backbone.
2. Comparing α-helices and β-sheets: In β-sheets, the β-strands are almost fully extended and the hydrogen bonds between the strands are perpendicular to the polypeptide backbone (see Fig. 2.7A). In contrast, in α-helices, the polypeptide is coiled and the hydrogen bonds are parallel to the backbone (see Fig. 2.6).

The orientation of the R groups of the amino acid residues in both the α-helix and the β-sheet can result in formation of polar and nonpolar sides in these secondary structures, thereby making them amphipathic.

C. β-Bends

β-bends, also called reverse turns and β-turns, reverse the direction of a polypeptide chain, helping it form a compact, globular shape. They are usually found on the surface of protein molecules and often include charged residues. β-bends were given this name because they often connect successive strands of antiparallel β-sheets. They are generally composed of four amino acids, one of which may be proline, the amino acid that causes a kink in the polypeptide chain. Glycine, the amino acid with the smallest R group, is also frequently found in β-bends. β-bends are stabilized by the formation of hydrogen bonds between the first and last residues in the bend.

D. Nonrepetitive secondary structure

Approximately one-half of an average globular protein is organized into repetitive structures, such as the α-helix and β-sheet. The remainder of the polypeptide chain is described as having a loop or coil conformation. These nonrepetitive secondary structures are not random but rather simply have a less regular structure than those described above. The term “random coil” refers to the disordered structure obtained when proteins are denatured (see Chapter 2 Section IV. D.).

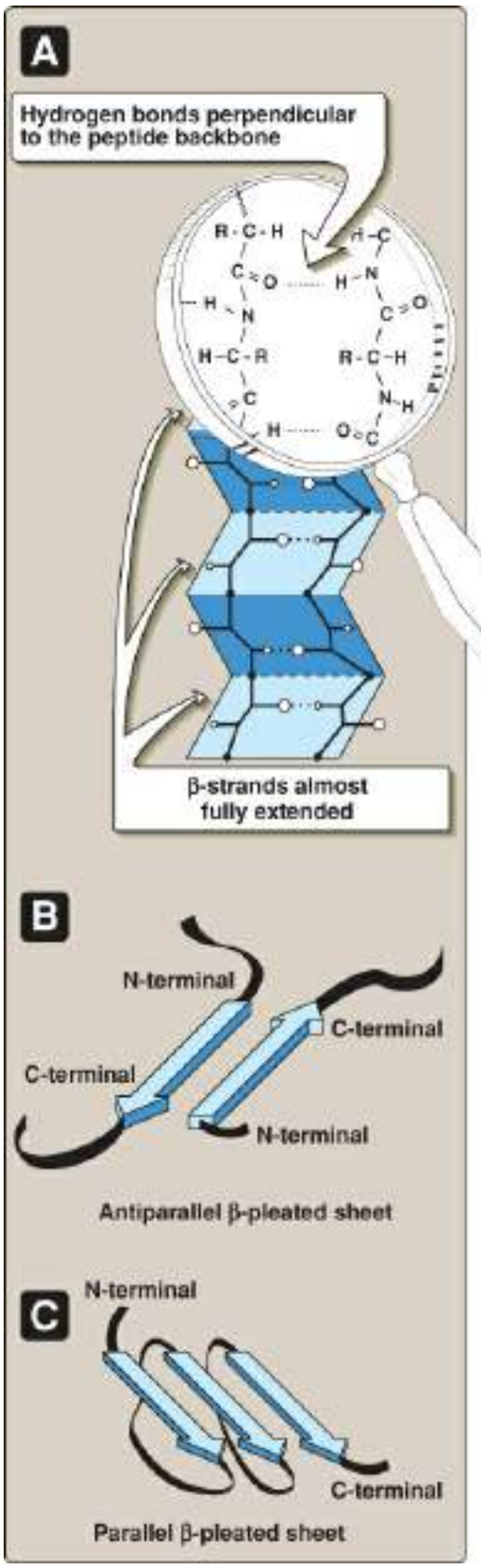


Figure 2.7
A: Structure of a β-sheet. **B:** An antiparallel β-sheet with the β-strands represented as broad arrows. **C:** A parallel β-sheet formed from a single polypeptide chain folding back on itself.

E. Supersecondary structures (motifs)

Globular proteins are constructed by combining secondary structural elements including α-helices, β-sheets, and coils, producing specific geometric patterns, or motifs. These form primarily the core (interior) region of the molecule. They are connected by loop regions (e.g., β-bends) at the surface of the protein. Supersecondary structures are usually produced by the close packing of side chains from adjacent secondary structural elements. For example, α-helices and β-sheets that are adjacent in the amino acid sequence are also usually (but not always) adjacent in the final, folded protein. Some of the more common motifs are illustrated in Figure 2.8.

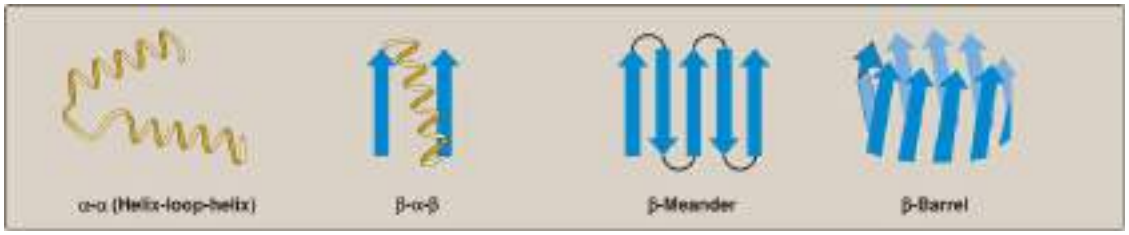


Figure 2.8
Common structural motifs involving α-helices and β-sheets. The names describe their schematic appearance.

Motifs may be associated with particular functions. Proteins that bind to DNA contain a limited number of motifs. The helix–loop–helix motif is an example found in a number of proteins that function as transcription factors (see Chapter 31).

IV. TERTIARY STRUCTURE

The primary structure of a polypeptide chain determines its tertiary structure. The term tertiary refers both to the folding of domains (the basic units of structure and function; see A. below) and to the final arrangement of domains in the polypeptide. The tertiary structure of globular proteins in aqueous solution is compact, with a high density (close packing) of the atoms in the core of the molecule. Hydrophobic side chains are buried in the interior, whereas hydrophilic groups are generally found on the surface of the molecule.

A. Domains

Domains are the fundamental functional and three-dimensional structural units of polypeptides. Polypeptide chains that are more than 200 amino acids in length generally consist of two or more domains. The core of a domain is built from

combinations of supersecondary structural elements (motifs). Folding of the peptide chain within a domain usually occurs independently of folding in other domains. Therefore, each domain has the characteristics of a small, compact globular protein that is structurally independent of the other domains in the polypeptide chain.

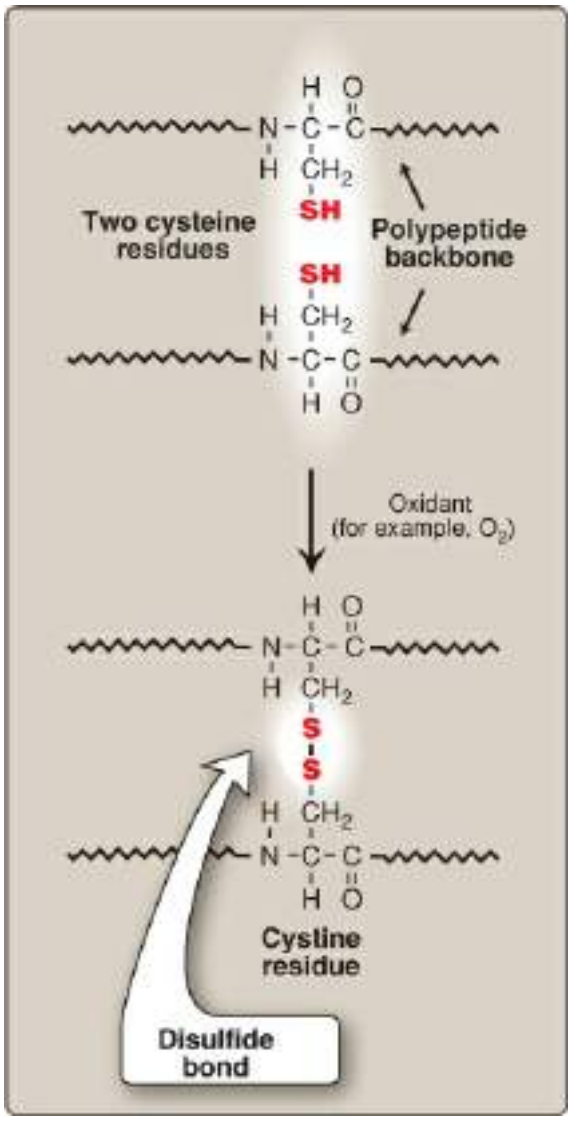


Figure 2.9
Formation of a disulfide bond by the oxidation of two cysteine residues, producing one cystine residue. O_2 = oxygen.

B. Stabilizing interactions

The unique three-dimensional structure of each polypeptide is determined by its amino acid sequence. Interactions between the amino acid side chains guide the folding of the polypeptide to form a compact structure. The following four types of interactions cooperate in stabilizing the tertiary structures of globular proteins.

1. Disulfide bonds: A disulfide bond (–S–S–) is a covalent linkage formed from the sulfhydryl group (−SH) of each of two cysteine residues to produce a cystine residue (Fig. 2.9). The two cysteines may be separated from each other by many amino acids in the primary sequence of a polypeptide or may even be located on two different polypeptides. The folding of the polypeptide(s) brings the cysteine residues into proximity and permits covalent bonding of their side chains. A disulfide bond contributes to the stability of the three-dimensional shape of the protein molecule and prevents it from becoming denatured in the extracellular environment. For example, many disulfide bonds are found in proteins such as immunoglobulins that are secreted by cells. It should be noted that protein disulfide isomerase breaks and reforms disulfide bonds during folding.

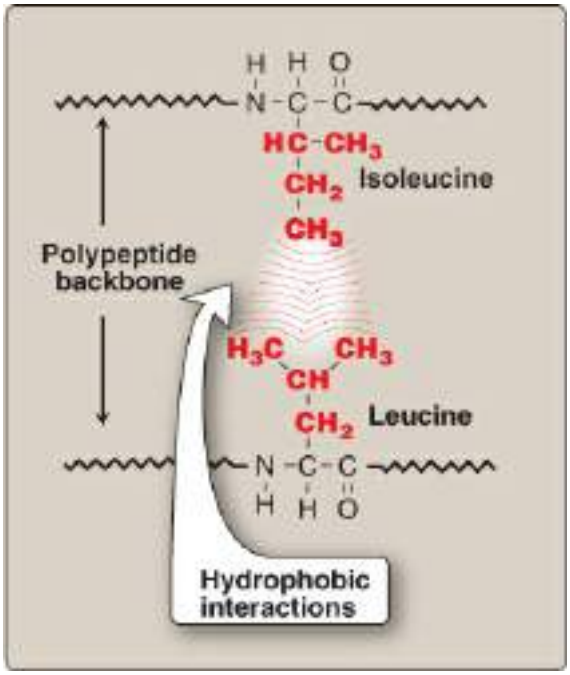


Figure 2.10
Hydrophobic interactions between amino acids with nonpolar side chains.

2. Hydrophobic interactions: Amino acids with nonpolar side chains tend to be located in the interior of the polypeptide molecule, where they associate with other hydrophobic amino acids (Fig. 2.10). In contrast, amino acids with polar or charged side chains tend to be located on the surface of the molecule in contact with the polar solvent. In each case, a segregation of R groups occurs that is energetically most favorable.

3. Hydrogen bonds: Amino acid side chains containing oxygen- or nitrogen-bound hydrogen, such as in the alcohol groups of serine and threonine, can form hydrogen bonds with electron-rich atoms, such as the oxygen of a carboxyl group or carbonyl group of a peptide bond (Fig. 2.11; see also Fig. 1.6).

Formation of hydrogen bonds between polar groups on the surface of proteins and the aqueous solvent enhances the solubility of the protein.

4. Ionic interactions: Negatively charged groups, such as the carboxylate group ($-COO^-$) in the side chain of aspartate or glutamate, can interact with positively charged groups such as the amino group ($-NH_3^+$) in the side chain of lysine (see Fig. 2.11).

C. Protein folding

Interactions between the side chains of amino acids determine how a linear polypeptide chain folds into the intricate three-dimensional shape of the functional protein. Protein folding, which occurs within the cell in seconds to minutes, involves nonrandom, ordered pathways. As a peptide folds, secondary structures form, driven by the hydrophobic effect; hydrophobic groups come together as water is released. These small structures combine to form larger structures. Additional events stabilize secondary structure and initiate formation of tertiary structure. In the last stage, the peptide achieves its fully folded, native (functional) form characterized by a low-energy state (Fig. 2.12). Some biologically active proteins or segments thereof lack a stable tertiary structure and are referred to as intrinsically disordered proteins.

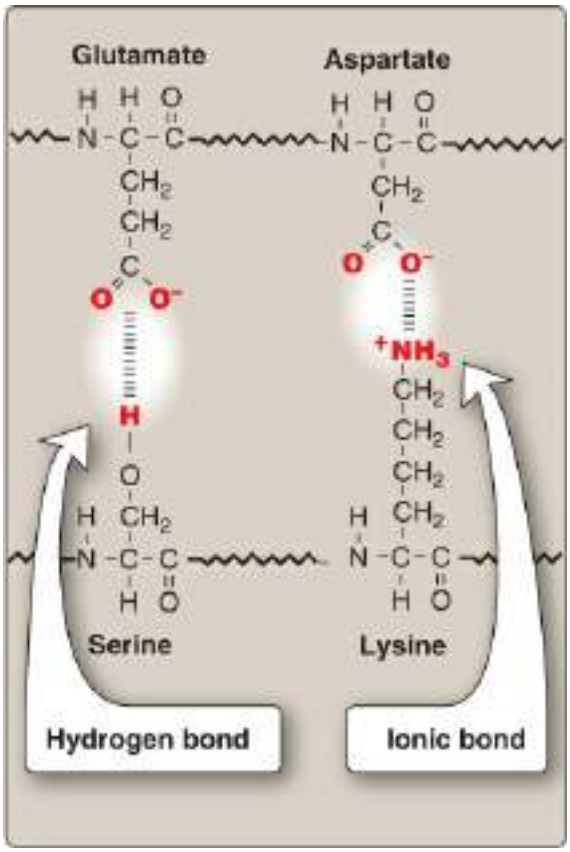


Figure 2.11

Interactions of side chains of amino acids through hydrogen bonds and ionic bonds (salt bridges).

D. Protein denaturation

Denaturation results in the unfolding and disorganization of a protein's secondary and tertiary structures without the hydrolysis of peptide bonds. Denaturing agents include heat, urea, organic solvents, strong acids or bases, detergents, and ions of heavy metals such as lead. Denaturation may, under ideal conditions, be reversible, such that the protein refolds into its original native structure when the denaturing agent is removed. However, most proteins remain permanently disordered once denatured. Denatured proteins are often insoluble and precipitate from solution.

E. Chaperones in protein folding

The information needed for correct protein folding is contained in the primary structure of the polypeptide. However, most denatured proteins do not resume their native conformations even under favorable environmental conditions. This is because, for many proteins, folding is a facilitated process that requires ATP hydrolysis and a specialized group of proteins, referred to as molecular chaperones. The chaperones, also known as heat-shock proteins (HSPs), interact with a polypeptide at various stages during the folding process. Some chaperones bind hydrophobic regions of an extended polypeptide and are important in keeping the protein unfolded until its synthesis is completed (e.g., Hsp70). Others form cage-like macromolecular structures composed of two stacked rings. The partially folded protein enters the cage, binds the central cavity through hydrophobic interactions, folds, and is released (e.g., mitochondrial Hsp60).

Chaperones, then, facilitate correct protein folding by binding to and stabilizing exposed, aggregation-prone hydrophobic regions in nascent and denatured polypeptides, preventing premature folding.

V. QUATERNARY STRUCTURE

While many proteins consist of a single polypeptide chain and are defined as monomeric proteins, other proteins consist of two or more polypeptide chains that may be structurally identical or totally unrelated. The arrangement of these polypeptide subunits is the quaternary structure of the protein. Subunits are held together primarily by noncovalent interactions including hydrogen bonds, ionic bonds, and hydrophobic interactions. Subunits may function independently of each other or may work cooperatively, as in hemoglobin, in which the binding of oxygen to one subunit of the tetramer increases the affinity of the other subunits for oxygen (see Chapter 3 Section II. E. 1.).

Isoforms of particular proteins all perform the same function but have different primary structures. They can arise from different genes or from tissue-specific processing of the product of a single gene. If the

proteins function as enzymes, they are referred to as isoenzymes (see page 70.).

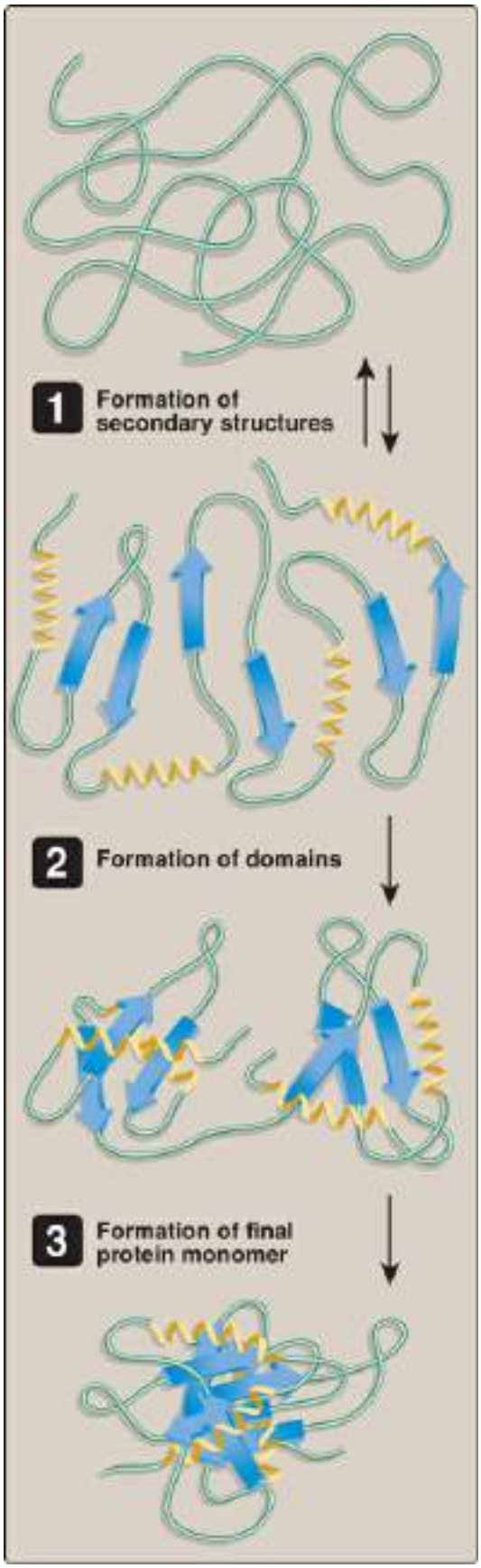


Figure 2.12
Steps in protein folding (simplified).

VI. PROTEIN MISFOLDING

Protein folding is a complex process that can sometimes result in improperly folded molecules. These misfolded proteins are usually tagged and degraded within the cell (see Chapter 19 Section II.). However, this quality control system is not perfect, and intracellular or extracellular aggregates of misfolded proteins can accumulate, particularly as individuals age. Deposits of misfolded proteins are associated with a number of diseases.

A. Amyloid diseases

Misfolding of proteins may occur spontaneously or be caused by a mutation in a particular gene, which then produces an altered protein. In addition, some apparently normal proteins can, after abnormal proteolytic cleavage, take on a unique conformation that leads to the spontaneous formation of long, fibrillar protein assemblies consisting of β-pleated sheets. Accumulation of these insoluble fibrous protein aggregates, called amyloids, has been implicated in neurodegenerative disorders such as Alzheimer disease (AD) and Parkinson disease.

The dominant component of the amyloid plaque that accumulates in AD is amyloid β (Aβ), an extracellular peptide containing 40 to 42 amino acid residues with a β-pleated sheet secondary structure in nonbranching fibrils. This peptide, when aggregated in a β-pleated sheet conformation, is neurotoxic and is the central pathogenic event leading to the cognitive impairment characteristic of the disease. The Aβ that is deposited in the brain in AD is derived by enzymatic cleavages (by secretases) from the larger amyloid precursor protein, a single transmembrane protein expressed on the cell surface in the brain and other tissues (Fig. 2.13).

The Aβ peptides aggregate, generating the amyloid that is found in the brain parenchyma and around blood vessels. Most cases of AD are not genetically based, although at least 5% of cases are familial. A second biologic factor involved in the development of AD is the accumulation of neurofibrillary tangles inside neurons. A key component of these tangled fibers is an abnormal form of the tau (τ) protein which is hyperphosphorylated and insoluble; in its healthy version, τ helps assemble and stabilize the microtubule structure. The defective τ protein appears to block the actions of its normal counterpart. In Parkinson disease, amyloid is formed from α-synuclein protein.

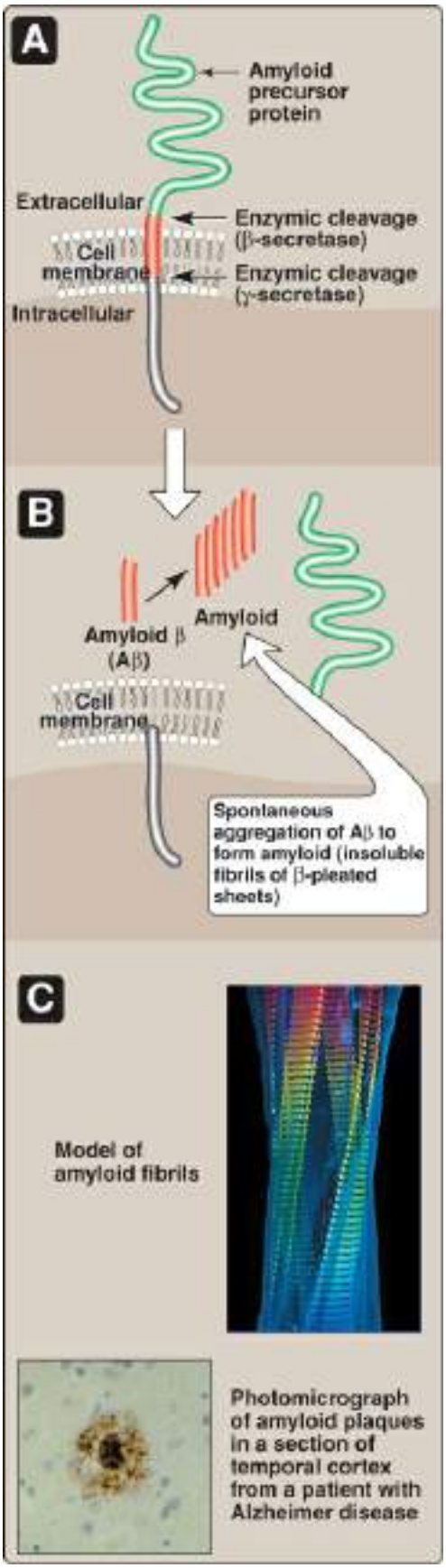


Figure 2.13
A–C: Formation of amyloid plaques found in Alzheimer disease (AD). (Note: Mutations to presenilin, the catalytic subunit of γ-secretase, are the most common cause of familial AD.)

B. Prion diseases

Prions or proteinaceous infectious particle are associated with certain diseases. The prion protein (PrP) is the causative agent of transmissible spongiform encephalopathies (TSEs), including Creutzfeldt–Jakob disease in humans, scrapie in sheep, and bovine spongiform encephalopathy, popularly called “mad cow disease,” in cattle. The infectivity of the agent causing scrapie in sheep is associated with a single-protein species that was not complexed with detectable nucleic acid. This infectious protein is designated PrP^{Sc} (Sc = scrapie). It is highly resistant to proteolytic degradation and tends to form insoluble aggregates of fibrils, similar to the amyloid found in some other diseases of the brain. A noninfectious form of PrP^{C} (C = cellular), encoded by the same gene as the infectious agent, is present in normal mammalian brains on the surface of neurons and glial cells. Thus, PrP^{C} is a host protein. No primary structure differences or alternate posttranslational modifications have been found between the normal and the infectious forms of the protein. The key to becoming infectious apparently lies in changes in the three-dimensional conformation of PrP^{C} Research has demonstrated that a number of α-helices present in noninfectious PrP^{C} are replaced by β-sheets in the infectious form (Fig. 2.14). This conformational difference is presumably what confers relative resistance to proteolytic degradation of infectious prions and permits them to be distinguished from the normal PrP^{C} in infected tissue. The infective agent is, thus, an altered version of a normal protein, which acts as a template for converting the normal protein to the pathogenic conformation. The TSEs are invariably fatal, and no treatment is currently available that can alter this outcome.

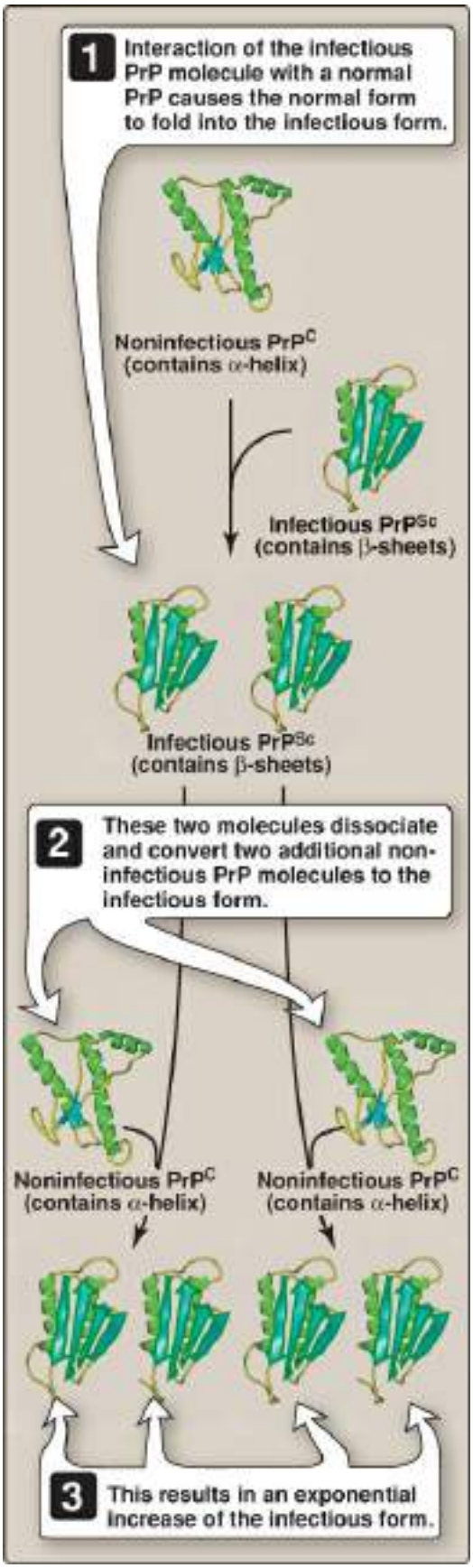


Figure 2.14
One proposed mechanism for multiplication of infectious prions. PrP = prion protein; PrP^{c} = prion protein cellular; PrP^{Sc} = prion protein scrapie.

VII. Chapter Summary

- A protein's native confirmation is the functional, fully folded protein structure (Fig. 2.15).
- The unique three-dimensional structure of a protein is determined by its **primary structure**, or amino acid sequence.
- Interactions between the amino acid side chains guide the folding of the polypeptide chain to form **secondary**, **tertiary**, and sometimes **quaternary** structures, which cooperate in stabilizing the native conformation of the protein.
- A specialized group of proteins named **chaperones** is required for the proper folding of many species of proteins.
- **Protein denaturation** results in the unfolding and disorganization of the protein's structure, which is not accompanied by hydrolysis of peptide bonds.
- Disease can occur when an apparently normal protein assumes a conformation that is cytotoxic, as in the case of **Alzheimer disease (AD)** and the **transmissible spongiform encephalopathies** (**TSE**), including **Creutzfeldt–Jakob disease**.
- In AD, normal proteins, after abnormal chemical processing, take on a unique conformational state that leads to the formation of neurotoxic **amyloid β peptide** (**Aβ**) assemblies consisting of β-pleated sheets. In TSE, the infective agent is an altered version of a normal **prion protein** that acts as a template for converting normal protein to the pathogenic conformation.

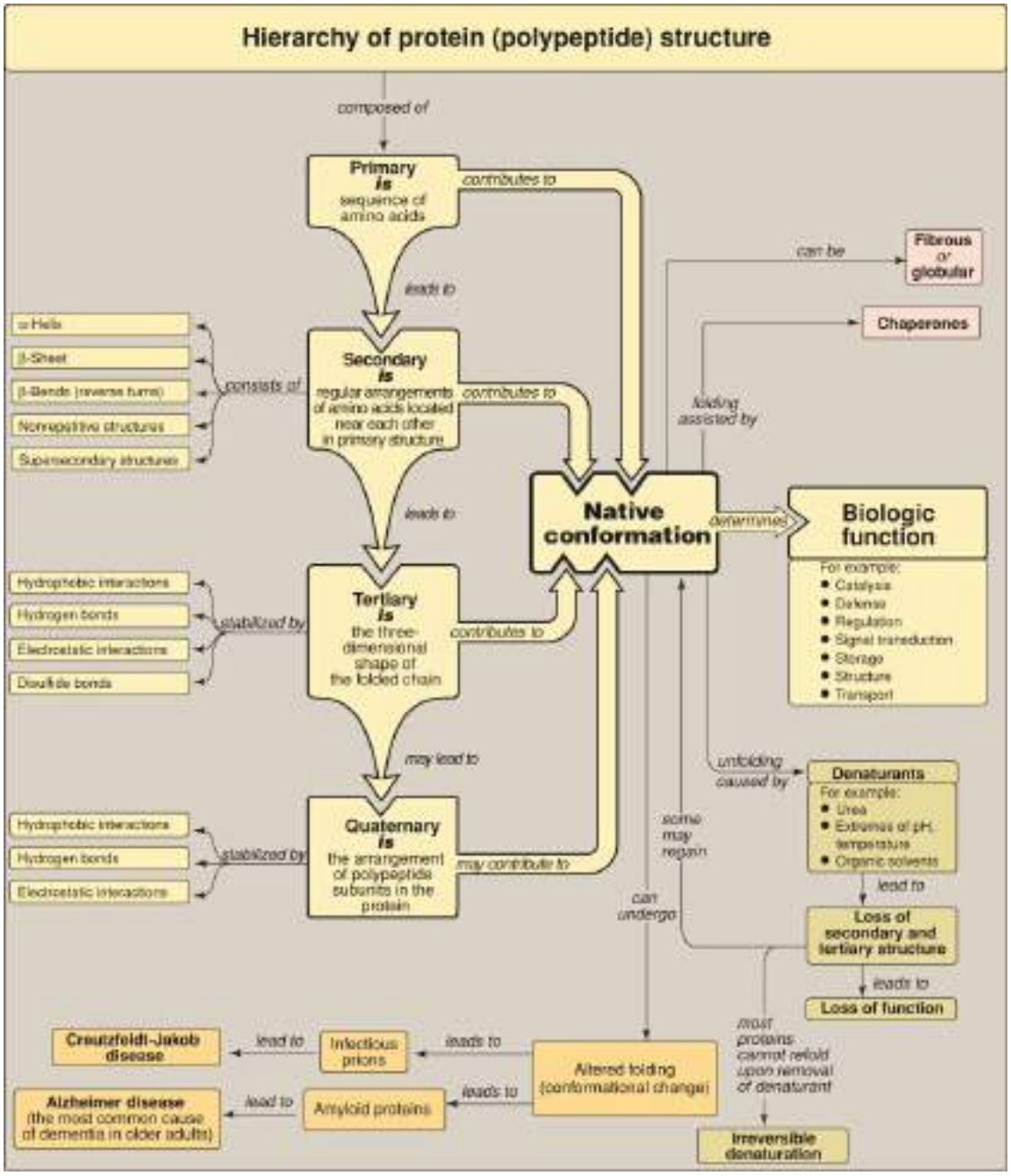


Figure 2.15
Key concept map for protein structure.

Study Questions

Choose the ONE best answer.

2.1 When considering protein structure:

A. Proteins with one polypeptide have quaternary structure stabilized by covalent bonds.
B. Peptide bonds that link amino acids most commonly occur in the cis configuration.
C. Disulfide bonds in proteins are between cysteine residues adjacent in the primary structure.
D. Denaturation of proteins leads to irreversible loss of secondary structural elements.

E. The primary driving force for protein folding is the hydrophobic effect.

Correct answer = E. The hydrophobic effect, or the tendency of nonpolar entities to associate in a polar environment, is the primary driving force of protein folding. Quaternary structure requires more than one polypeptide, and, when present, it is stabilized primarily by noncovalent bonds. The peptide bond is almost always trans. The two cysteine residues participating in disulfide bond formation may be a great distance apart in the amino acid sequence of a polypeptide (or on two separate polypeptides) but are brought into close proximity by the three-dimensional folding of the polypeptide. Denaturation may be reversible or irreversible.

2.2 A particular point mutation results in disruption of the α-helical structure in a segment of the mutant protein. The most likely change in the primary structure of the mutant protein is:

A. Glutamate to aspartate.
B. Lysine to arginine.
C. Methionine to proline.
D. Valine to alanine.

Correct answer = C. Proline, because of its secondary amino group, is incompatible with an α-helix. Glutamate, aspartate, lysine, and arginine are charged amino acids, and valine is a branched amino acid. Charged and branched (bulky) amino acids may disrupt an α-helix. The flexibility of glycine's R group (a H) can also disrupt an α-helix.

2.3 Which statement is true of β-sheets only, and not α-helices?

A. They may be found in typical globular proteins.
B. They are stabilized by interchain hydrogen bonds.
C. They are examples of secondary structure.
D. They may be found in supersecondary structures.

Correct answer = B. The β-sheet is stabilized by interchain hydrogen bonds formed between separate polypeptide chains, and by intrachain hydrogen bonds formed between regions of a single polypeptide. The α-helix, however, is stabilized only by intrachain hydrogen bonds. Statements A, C, and D are true for both of these secondary structural elements.

2.4 Stability of tertiary protein structure is provided in part by:

A. Alpha helices.
B. Aminopeptidases.
C. Beta meanders.
D. Disulfide bond formation.

Correct answer = D. Disulfide bonds along with hydrophobic interactions, hydrogen bonds, and ionic interactions are used to stabilize tertiary structure of proteins. Alpha helices and beta meanders are examples of secondary structures. Aminopeptidases are enzymes that cleave amino acids from the N-terminus of proteins and do not stabilize tertiary structure.

2.5 An 80-year-old male presented with impairment of intellectual function and alterations in behavior. His family reported progressive disorientation and memory loss over the last 6 months. There is no family history of dementia. The patient was tentatively diagnosed with Alzheimer disease (AD). If this diagnosis is correct then his condition:

A. Involves β-amyloid, an abnormal protein with an altered amino acid sequence.
B. Resulted from accumulation of denatured proteins with random conformations.
C. Occurred as the result of accumulation of amyloid precursor protein.
D. Is associated with the deposition of neurotoxic amyloid β peptide aggregates.
E. Was acquired from environmental damage unrelated to the genetics of the individual.

Correct answer = D. AD is associated with long, fibrillar protein assemblies consisting of β-pleated sheets found in the brain and elsewhere. The disease is associated with abnormal processing of a normal protein. The accumulated altered protein occurs in a β-pleated sheet conformation that is neurotoxic. The amyloid β that is deposited in the brain in AD is derived by proteolytic cleavages from the larger amyloid precursor protein, a single transmembrane protein expressed on the cell surface in the brain and other tissues. Most cases of AD are sporadic, although at least 5% of cases are familial.

Globular Proteins 3

I. OVERVIEW

The previous chapter described the types of secondary and tertiary structures that are the bricks and mortar of protein architecture. By arranging these fundamental structural elements in different combinations, widely diverse proteins capable of various specialized functions can be constructed. Two important protein structures are globular proteins and fibrous proteins (or scleroproteins). As the name implies, globular proteins are spherical (or “globelike”) in overall shape. They are usually somewhat water-soluble, possessing many hydrophilic amino acids on their outer surface, facing the aqueous environment. More nonpolar amino acids face the interior of the protein, providing hydrophobic interactions to further stabilize the globular structure. This is in contrast to fibrous proteins, which form long rodlike filaments, are relatively inert or water-insoluble, and provide structural support in the extracellular environment. This chapter examines the relationship between structure and function for clinically important globular hemeproteins, such as hemoglobin and myoglobin. Fibrous structural proteins, such as collagen and elastin, are discussed in Chapter 4.

II. GLOBULAR HEMEPROTEINS

Hemeproteins are a group of specialized globular proteins that contain heme as a tightly bound prosthetic group (see p. 59 for a discussion of prosthetic groups). The function of the heme group is dictated by the three-dimensional structure of the protein. In the mitochondrial electron transport chain, the cytochrome protein structure allows for rapid and reversible oxidation–reduction electron transfer of the heme-coordinated iron, reversibly transitioning between its ferrous (Fe^{2+}) and ferric (Fe^{3+}) states (see p. 83). In the enzyme catalase, the heme group is structurally part of the enzyme’s active site, which catalyzes the breakdown of hydrogen peroxide (see p. 163). The protein structure of hemoglobin can affect the alignment of the ferrous (Fe^{2+}) iron with respect to the plane of the heme prosthetic group. Changes in this alignment can affect the binding affinity and transport of oxygen by hemoglobin between the lungs and tissues.

A. Heme structure

Heme is a planar structure, comprised of a porphyrin ring with ferrous iron (Fe^{2+}) coordinated in the porphyrin ring center, as shown in Figure 3.1. The iron is held in the center of the heme molecule by bonds to four nitrogens of the porphyrin ring. The heme Fe^{2+} can form two additional bonds, one on each side of the planar porphyrin ring. In hemoglobin, one of these positions is coordinated to the side

chain of a histidine residue of the globin molecule, whereas the other position is available to bind O_2 (Fig. 3.2).

B. Myoglobin structure and function

Myoglobin, a hemeprotein present in heart and skeletal muscle, functions both as an oxygen reservoir and as an oxygen carrier that increases the rate of oxygen transport within the muscle cell. Myoglobin consists of a single polypeptide chain that is structurally similar to the individual polypeptide chains of the tetrameric hemoglobin molecule. This homology makes myoglobin a useful model for interpreting some of the more complex properties of hemoglobin.

1. α-Helical content: Myoglobin is a compact molecule, with ~80% of its polypeptide chain folded into eight stretches of α-helix. These α-helical regions, labeled A to H in Figure 3.2A, are terminated either by the presence of proline, whose five-membered ring cannot be accommodated in an α-helix (see p. 16) or by β-bends and loops stabilized by hydrogen bonds and ionic bonds (see p. 19). (Note: Ionic bonds are also termed electrostatic interactions or salt bridges.)

2. Location of polar and nonpolar amino acid residues: The interior of the globular myoglobin molecule is composed almost entirely of nonpolar amino acids. Nonpolar amino acids are packed closely together, forming a structure stabilized by hydrophobic interactions between these clustered residues (see p. 19). In contrast, polar amino acids are located almost exclusively on the surface, where they can form hydrogen bonds, both with each other and with water.

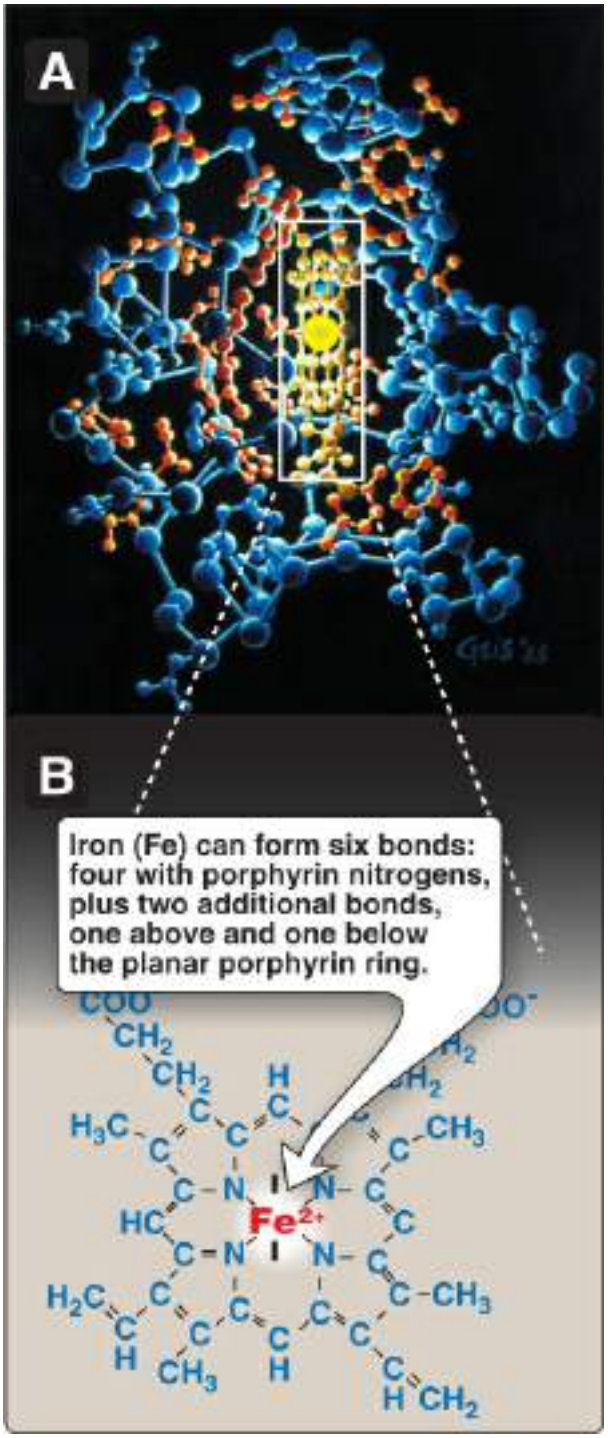


Figure 3.1
A: Hemeprotein (cytochrome c). B: Structure of heme.

3. Binding of the heme group: The heme prosthetic group of the myoglobin molecule sits in a crevice, which is lined with nonpolar amino acids. Notable exceptions are two histidine residues, which are basic amino acids (Fig. 3.2B). One of the two histidine residues, the proximal histidine (F8), binds directly to

the Fe^{2+} of heme. The second, or distal histidine (E7), does not directly interact with the heme group but helps stabilize the binding of O_2 to Fe^{2+}. Thus, the protein, or globin, portion of myoglobin creates a special microenvironment for the heme that permits oxygenation, the reversible binding of one oxygen molecule. The simultaneous loss of electrons by Fe^{2+} (oxidation to the ferric [Fe^{3+}] form) occurs only rarely.

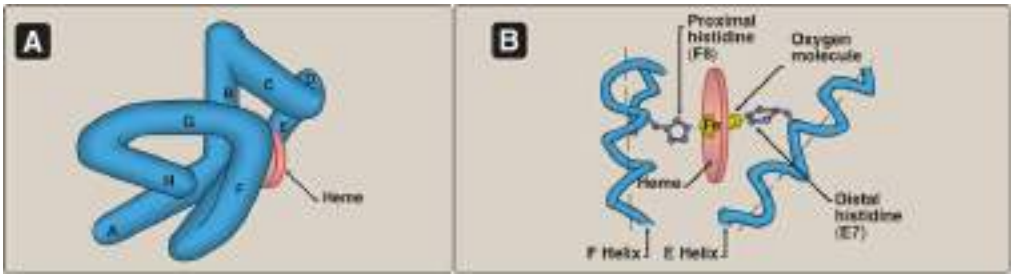


Figure 3.2
A: Model of myoglobin showing α-helices A to H. B: Schematic diagram of the oxygen-binding site of myoglobin.

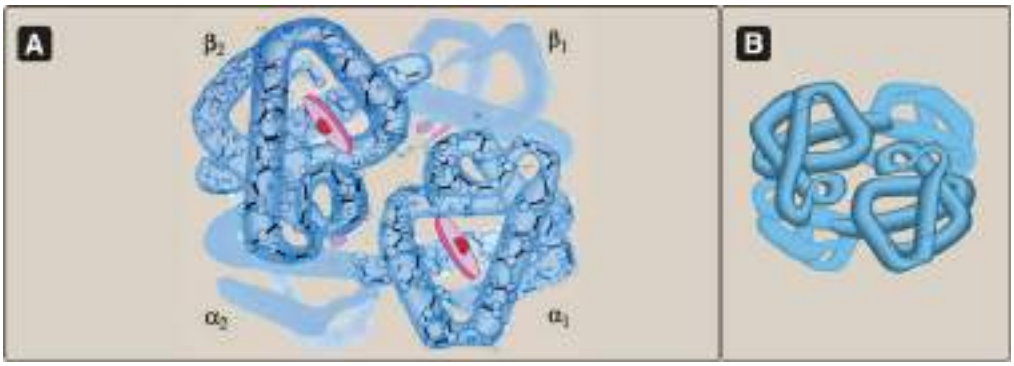


Figure 3.3
A: Structure of hemoglobin showing the polypeptide backbones. B: Simplified drawing showing the α-helices.

C. Hemoglobin structure and function

Hemoglobin is found exclusively in red blood cells (RBCs), where its main function is to transport O_2 from the lungs to the capillaries of the tissues. Hemoglobin A (HbA), the major hemoglobin in adults, is composed of four polypeptide chains (two α chains and two β chains) held together by noncovalent interactions (Fig. 3.3). Each chain (subunit) has stretches of α-helical structure and a hydrophobic heme-binding pocket similar to that described for myoglobin. However, the tetrameric hemoglobin molecule is structurally and functionally more complex than myoglobin. For example, hemoglobin can transport protons (H^+) and carbon dioxide (CO_2) from the tissues to the lungs and can carry four molecules of O_2 from the lungs to the cells of the body. Furthermore, the oxygen-binding properties of hemoglobin are regulated by interaction with allosteric effectors (see p. 30).

Obtaining O_2 from the atmosphere solely by diffusion greatly limits the size of organisms. Circulatory systems overcome this, but transport molecules such as hemoglobin are also required because O_2 is only slightly soluble in aqueous solutions such as blood.

1. Quaternary structure: The hemoglobin tetramer can be envisioned as composed of two identical dimers, $\alpha\beta_1$ and $\alpha\beta_2$. The two polypeptide chains within each dimer are held tightly together primarily by hydrophobic interactions (Fig. 3.4). (Note: In this instance, hydrophobic amino acid residues are localized not only in the interior of the molecule but also in a region on the surface of each subunit. Multiple interchain hydrophobic interactions form strong associations between the α-subunit and the β-subunit in each of the dimers.) In contrast, the two dimers are held together primarily by polar bonds. The weaker interactions between the dimers allow them to move with respect to one other. This movement results in the two dimers occupying different relative positions in deoxyhemoglobin as compared with oxyhemoglobin (see Fig. 3.4).

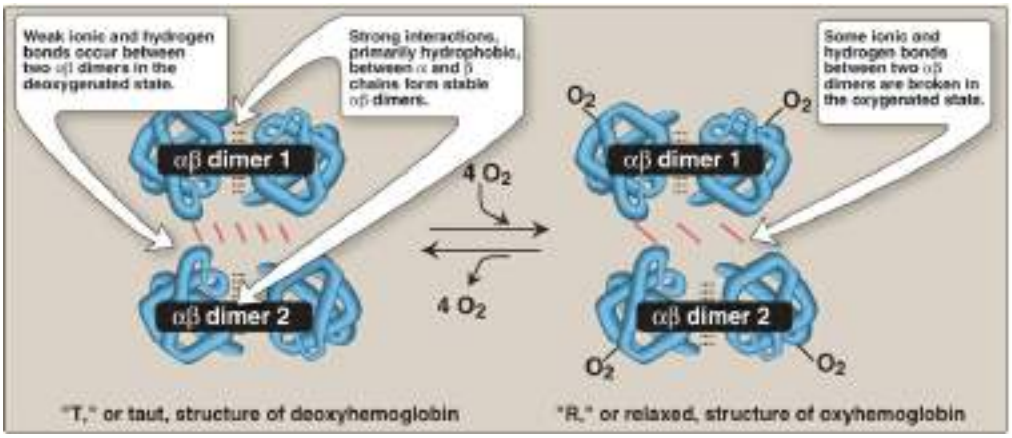


Figure 3.4
Schematic diagram showing structural changes resulting from oxygenation and deoxygenation of hemoglobin.

a. T form: The deoxy form of hemoglobin is called the "T," or taut (tense) form. In the T form, the two αβ dimers interact through a network of ionic bonds and hydrogen bonds that constrain the movement of the polypeptide chains. The iron (Fe^{2+}) is pulled out of the heme planar structure. The T conformation is the low–oxygen-affinity form of hemoglobin.

b. R form: The binding of O_2 to hemoglobin causes the rupture of some of the polar bonds between the two αβ dimers, allowing movement of the Fe^{2+} with respect to the planar heme structure. Specifically, the binding of O_2 to the heme Fe^{2+} pulls the iron more directly into the plane of the heme ring structure (Fig. 3.5B). Because the iron is also linked to the proximal histidine (F8), the resulting movement of the globin chains alters the interface

between the αβ dimers, leading to a structure called the “R,” or relaxed form (see Fig. 3.4). The R conformation is the high–oxygen-affinity form of hemoglobin.

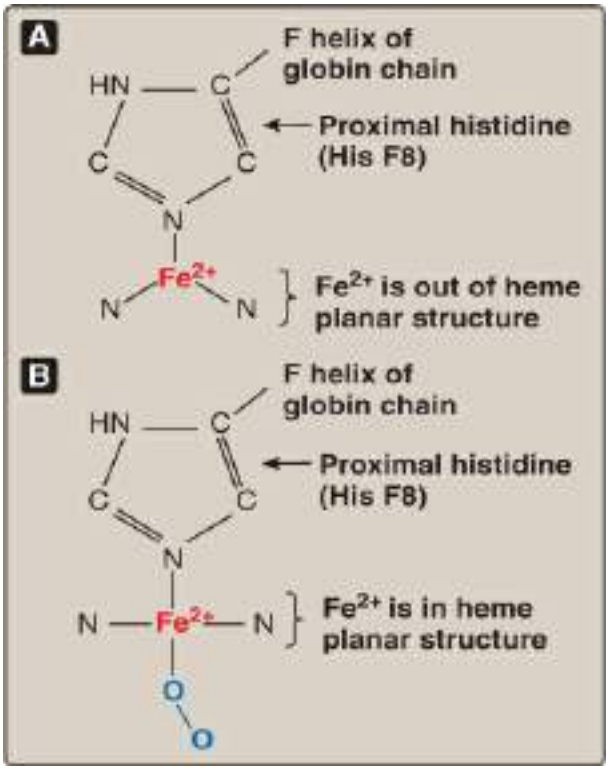


Figure 3.5
Movement of heme iron (Fe^{2+}). A: Out of the plane of the heme when oxygen (O_2) is not bound. B: Into the plane of the heme upon O_2 binding.

D. Oxygen binding to myoglobin and hemoglobin

Myoglobin can bind only one molecule of O_2, because it contains only one heme group. In contrast, hemoglobin can bind four molecules of O_2, one at each of its four heme groups. The degree of saturation (Y) of these oxygen-binding sites on all myoglobin or hemoglobin molecules can vary between zero (all sites are empty) and 100% (all sites are full), as shown in Figure 3.6. (Note: Pulse oximetry is a noninvasive, indirect method of measuring the oxygen saturation of arterial blood based on differences in light absorption by oxyhemoglobin and deoxyhemoglobin.)

1. Oxygen-dissociation curve: A plot of the degree of saturation (Y) measured at different partial pressures of oxygen (pO_2) is called the oxygen-dissociation curve. (Note: pO_2 may also be represented as PO_2.) The curves for myoglobin and hemoglobin show important differences (see Fig. 3.6). This graph illustrates that myoglobin has a higher oxygen affinity at all pO_2 values than does hemoglobin. The partial pressure of oxygen needed to achieve half saturation of

the binding sites (P_{50}) is ~1 mm Hg for myoglobin and 26 mm Hg for hemoglobin. The higher the oxygen affinity (i.e., the more tightly O_2 binds), the lower the P_{50}.

a. Myoglobin: The oxygen-dissociation curve for myoglobin has a hyperbolic shape (see Fig. 3.6). This reflects the fact that myoglobin reversibly binds a single molecule of O_2. Thus, oxygenated (MbO_2) and deoxygenated (Mb) myoglobin exists in a simple equilibrium:

$$Mb + O_2 \rightleftarrows MbO_2$$

The equilibrium is shifted to the right or to the left as O_2 is added to or removed from the system. (Note: Myoglobin is designed to bind O_2 released by hemoglobin at the low pO_2 found in muscle. Myoglobin, in turn, releases O_2 within the muscle cell in response to oxygen demand.)

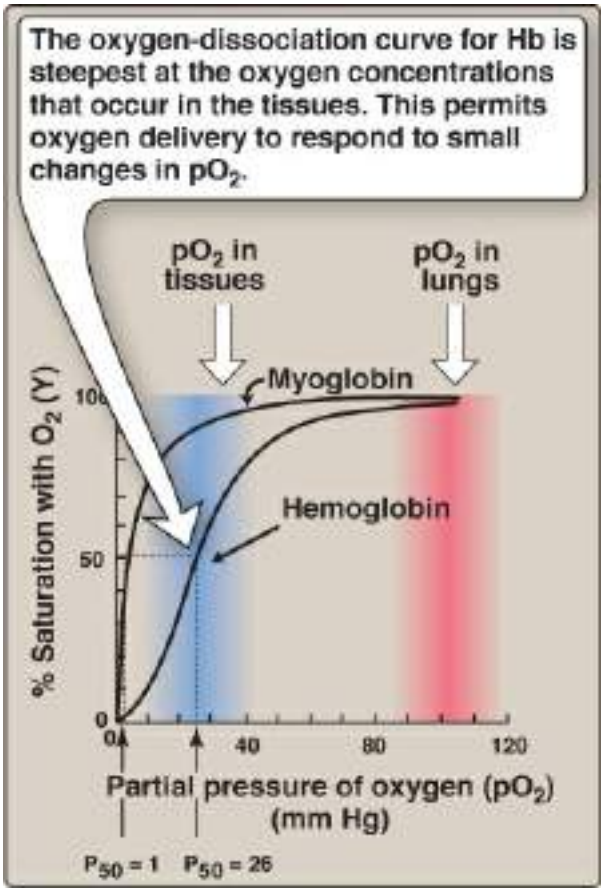


Figure 3.6
Oxygen-dissociation curves for myoglobin and hemoglobin (Hb).

b. Hemoglobin: The oxygen-dissociation curve for hemoglobin is sigmoidal in shape (see Fig. 3.6), indicating that the subunits cooperate in binding O_2.

Cooperative binding of O_2 by the four subunits of hemoglobin means that the binding of an oxygen molecule at one subunit increases the oxygen affinity of the remaining subunits in the same hemoglobin tetramer (Fig. 3.7). Although it is more difficult for the first oxygen molecule to bind to hemoglobin, the subsequent binding of oxygen molecules occurs with high affinity, as shown by the steep upward curve in the region near 20 to 30 mm Hg (see Fig. 3.6).

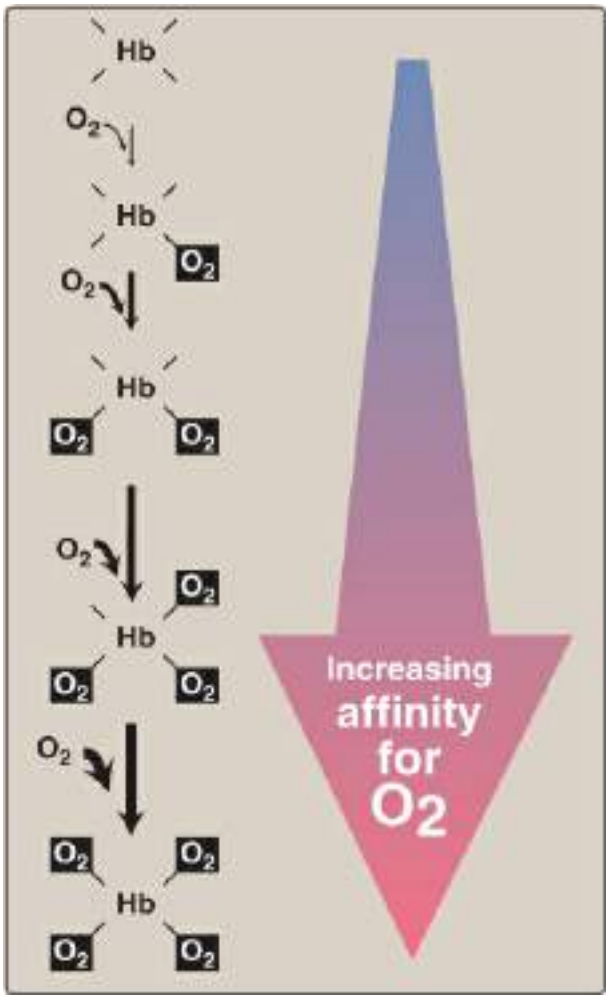


Figure 3.7
Hemoglobin (Hb) binds successive molecules of oxygen (O_2) with increasing affinity.

E. Allosteric effectors

The ability of hemoglobin to reversibly bind O_2 is affected by the pO_2, the pH of the environment, the partial pressure of carbon dioxide (pCO_2), and the concentration of 2,3-bisphosphoglycerate (2,3-BPG). These are collectively called allosteric ("other site") effectors, because their interaction at one site on the tetrameric hemoglobin molecule causes structural changes that affect the binding of O_2 to the heme iron at

other sites on the molecule. (Note: The binding of O_2 to monomeric myoglobin is not influenced by allosteric effectors.)

1. Oxygen: The sigmoidal oxygen-dissociation curve reflects specific structural changes that are initiated at one subunit and transmitted to other subunits in the hemoglobin tetramer. The net effect of this cooperativity is that the affinity of hemoglobin for the last oxygen molecule bound is ~300 times greater than its affinity for the first oxygen molecule bound. Oxygen, then, is an allosteric effector of hemoglobin. It stabilizes the R form.

   a. Loading and unloading oxygen: The cooperative binding of O_2 allows hemoglobin to deliver more O_2 to the tissues in response to relatively small changes in the pO_2. This can be seen in Figure 3.6, which indicates pO_2 in the alveoli of the lung and the capillaries of the tissues. For example, in the lung, oxygen concentration is high, and hemoglobin becomes virtually saturated (or “loaded”) with O_2. In contrast, in the peripheral tissues where the pO_2 is much lower than in the lungs, oxyhemoglobin releases (or “unloads”) much of its O_2 for use in the oxidative metabolism of the tissues (Fig. 3.8).

   b. Significance of the sigmoidal oxygen-dissociation curve: The steep slope of the oxygen-dissociation curve over the range of oxygen concentrations that occur between the lungs and the tissues permits hemoglobin to carry and deliver O_2 efficiently from sites of high to sites of low pO_2. A molecule with a hyperbolic oxygen-dissociation curve, such as myoglobin, could not achieve the same degree of O_2 release within this range of pO_2. Instead, it would have maximum affinity for O_2 throughout this oxygen pressure range and, therefore, would deliver no O_2 to the tissues.

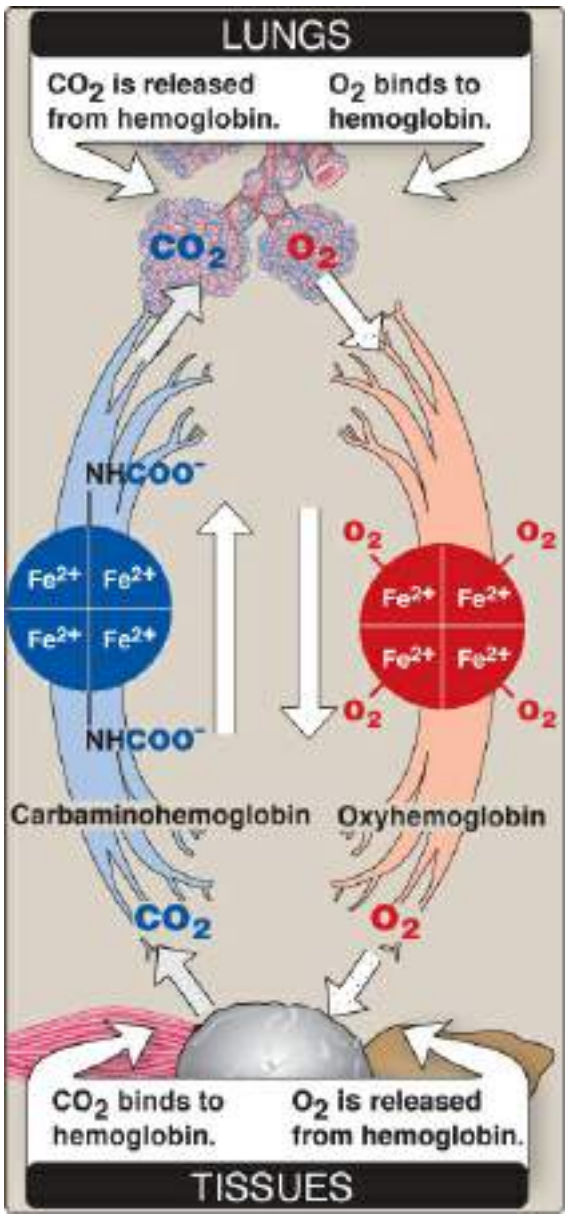


Figure 3.8
Transport of oxygen and carbon dioxide by hemoglobin. Fe = iron.

2. Bohr effect: The release of O_2 from hemoglobin is enhanced when the pH is lowered (proton concentration $[H^+]$ is increased) or when the hemoglobin is in the presence of an increased pCO_2. Both result in decreased oxygen affinity of hemoglobin and, therefore, a shift to the right in the oxygen-dissociation curve (Fig. 3.9). Both, then, stabilize the T (deoxy) form. This change in oxygen binding is called the Bohr effect. Conversely, raising the pH or lowering the concentration of CO_2 results in a greater oxygen affinity, a shift to the left in the oxygen-dissociation curve, and stabilization of the R (oxy) form.

a. Source of the protons that lower pH: The concentration of both H^+ and CO_2

in the capillaries of metabolically active tissues is higher than that observed in alveolar capillaries of the lungs, where CO_2 is released into the expired air. In the tissues, zinc-containing carbonic anhydrase converts CO_2 to carbonic acid:

$$CO_2 + H_2O \rightleftarrows H_2CO_3$$

which spontaneously ionizes to bicarbonate (the major blood buffer) and H^+:

$$H_2CO_3 \rightleftarrows HCO_3^- + H^+$$

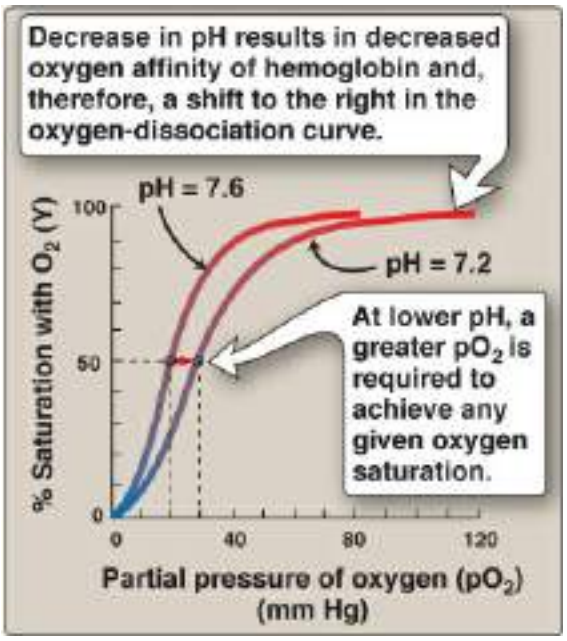


Figure 3.9
Effect of pH on the oxygen affinity of hemoglobin. Protons are allosteric effectors of hemoglobin.

The H^+ produced by this pair of reactions contributes to the lowering of pH. This differential pH gradient (i.e., lungs having a higher pH and tissues having a lower pH) favors the unloading of O_2 in the peripheral tissues and the loading of O_2 in the lung. Thus, the oxygen affinity of the hemoglobin molecule responds to small shifts in pH between the lungs and oxygen-consuming tissues, making hemoglobin a more efficient transporter of O_2.

b. Mechanism of the Bohr effect: The Bohr effect reflects the fact that deoxyhemoglobin has a greater affinity for H^+ than does oxyhemoglobin. This is caused by ionizable functional groups such as specific histidine side chains that have a higher pK_a (see p. 7) in deoxyhemoglobin than in oxyhemoglobin. Therefore, an increase in the concentration of H^+ (resulting in a decrease in pH) causes these groups to become protonated (charged)

and able to form ionic bonds (salt bridges). These bonds preferentially stabilize deoxyhemoglobin, producing a decrease in oxygen affinity. (Note: Hemoglobin, then, is an important blood buffer.)

The Bohr effect can be represented schematically as:

$$\underset{\text{Oxyhemoglobin}}{HbO_2} + H^+ \rightleftarrows \underset{\text{Doxyhemoglobin}}{HbH} + O_2$$

where an increase in H^+ concentration (or a lower pO_2) shifts the equilibrium to the right (favoring deoxyhemoglobin), whereas an increase in pO_2 (or a decrease in H^+ concentration) shifts the equilibrium to the left.

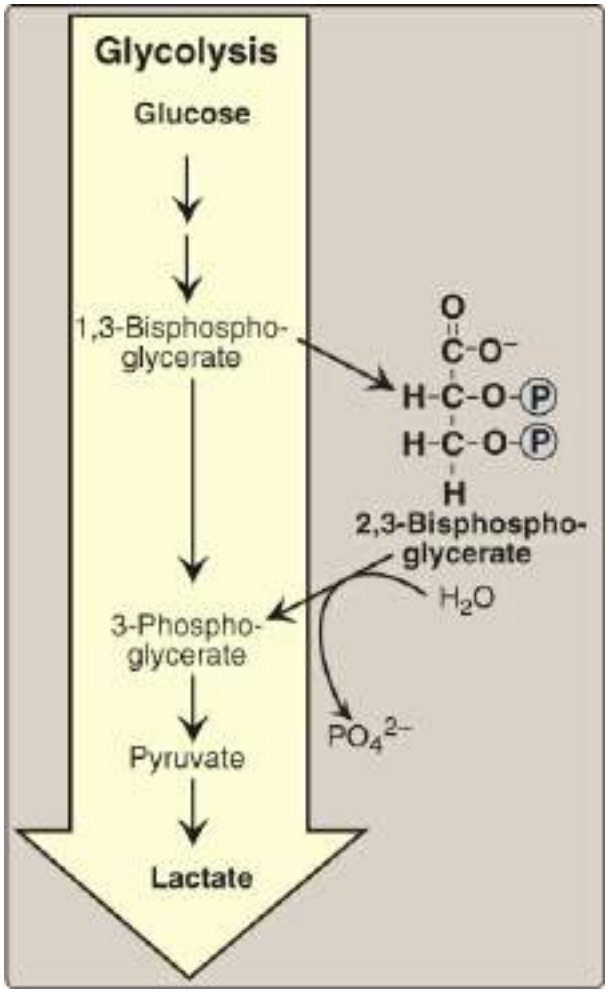


Figure 3.10
Synthesis of 2,3-bisphosphoglycerate. (Note: Ⓟ is a phosphoryl group, PO_3^{2-}.) In older literature, 2,3-bisphosphoglycerate (2,3-BPG) may be referred to as 2,3-diphosphoglycerate (2,3-DPG).

3. 2,3-BPG effect on oxygen affinity: 2,3-BPG is an important regulator of the

binding of O_2 to hemoglobin. It is the most abundant organic phosphate in the RBCs, where its concentration is approximately that of hemoglobin. 2,3-BPG is synthesized from an intermediate of the glycolytic pathway (Fig. 3.10; see p. 32 for a discussion of 2,3-BPG synthesis in glycolysis).

a. 2,3-BPG binding to deoxyhemoglobin: 2,3-BPG decreases the oxygen affinity of hemoglobin by binding to deoxyhemoglobin but not to oxyhemoglobin. This preferential binding stabilizes the T conformation of hemoglobin. The effect of binding 2,3-BPG can be represented schematically as:

$$HbO_2 + \text{2,3-BPG} \rightleftarrows Hb - \text{2,3-BPG} + O_2$$

Oxyhemoglobin Doxyhemoglobin

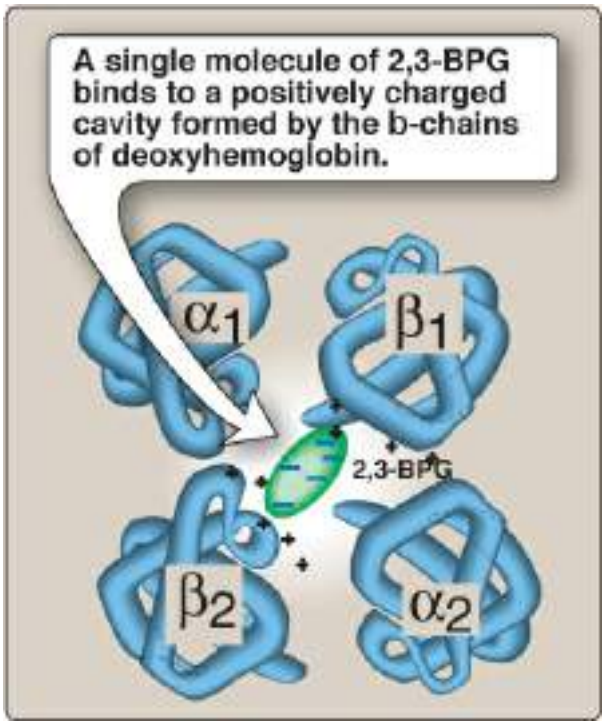


Figure 3.11
Binding of 2,3-bisphosphoglycerate (2,3-BPG) by deoxyhemoglobin.

b. 2,3-BPG–binding site: One molecule of 2,3-BPG binds to a pocket, formed by the two β-globin chains, in the center of the deoxyhemoglobin tetramer (Fig. 3.11). This pocket contains several positively charged amino acids that form ionic bonds with the negatively charged phosphate groups of 2,3-BPG. (Note: Replacement of one of these amino acids can result in hemoglobin variants with abnormally high oxygen affinity that may be compensated for by increased RBC production [erythrocytosis].) Oxygenation of hemoglobin narrows the pocket and causes 2,3-BPG to be released.

c. Oxygen-dissociation curve shift: Hemoglobin from which 2,3-BPG has been

removed has high oxygen affinity. However the presence of 2,3-BPG significantly reduces the oxygen affinity of hemoglobin, shifting the oxygen-dissociation curve to the right (Fig. 3.12). This reduced affinity enables hemoglobin to release O_2 efficiently at the partial pressures found in the tissues.

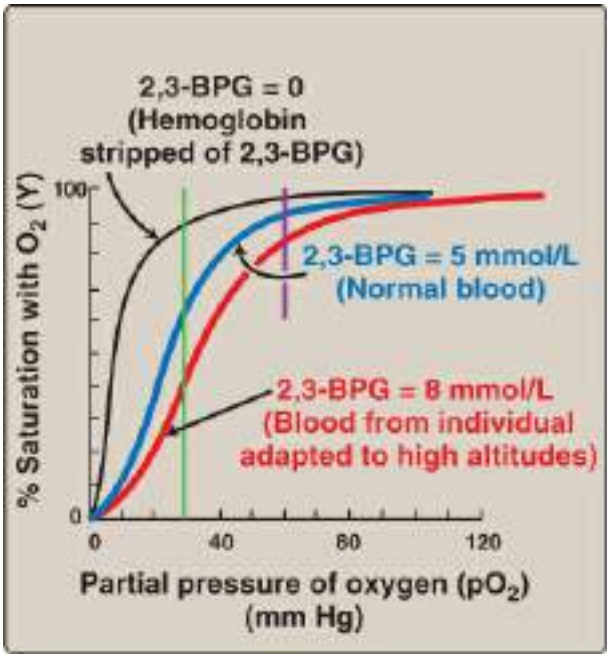


Figure 3.12
Allosteric effect of 2,3-bisphosphoglycerate (2,3-BPG) on the oxygen affinity of hemoglobin. Partial pressure of oxygen in the tissues is indicated by the *green line*. Partial pressure of oxygen in the lungs at high altitude is indicated by the *purple line*.

d. 2,3-BPG levels in chronic hypoxia or anemia: The concentration of 2,3-BPG in the RBCs increases in response to chronic hypoxia, such as that observed in chronic obstructive pulmonary disease (COPD) like emphysema, or at high altitudes, where pO_2 is lower and circulating hemoglobin may have difficulty receiving sufficient O_2. Intracellular levels of 2,3-BPG are also elevated in chronic anemia, in which fewer than normal RBCs are available to supply the body's oxygen needs. Elevated 2,3-BPG levels lower the oxygen affinity of hemoglobin, permitting greater unloading of O_2 in the capillaries of tissues (see Fig. 3.12).

e. 2,3-BPG in transfused blood: 2,3-BPG is essential for the normal oxygen transport function of hemoglobin. However, blood bank–stored blood gradually becomes depleted in 2,3-BPG. Consequently, stored blood displays an abnormally high oxygen affinity and fails to unload its bound O_2 properly in the tissues. Thus, hemoglobin deficient in 2,3-BPG would act as an oxygen "trap" rather than as an oxygen delivery system. Transfused RBCs are able to restore their depleted supplies of 2,3-BPG in 6 to 24 hours.

However, severely ill patients may be compromised if transfused with large quantities of such 2,3-BPG–depleted blood. Stored blood, therefore, is treated with a "rejuvenation" solution that rapidly restores 2,3-BPG. (Note: Rejuvenation also restores ATP lost during storage.)

Clinical Application 3.1: 2,3-BPG Offloads Oxygen to the Tissues

To illustrate the use of 2,3-BPG to offload oxygen to the tissues, consider two conditions: one individual living at sea level with 5 mmol/L 2,3-BPG, who travels to a high altitude where the pO_2 is lower, and another individual who lives at a high altitude and compensates by elevating their 2,3-BPG levels to 8 mmol/L. Hemoglobin in the lungs of the individual with 5 mmol/L 2,3-BPG will be fully saturated at sea level (Fig. 3.12). In the tissues, their hemoglobin is ~60% saturated (indicated by the *green line*), delivering ~40% of the bound oxygen to their tissues. At high altitudes with 5 mmol/L of 2,3-BPG, this same individual's hemoglobin will be only 90% saturated in the lungs (indicated by the *purple line*), so oxygen delivery to their tissues is only 30%. However, the individual living at a high altitude has adapted to have hemoglobin with 8 mmol/L of 2,3-BPG. The oxygen-binding curve shifts to the right. Oxygen saturation in the lungs is now only ~80% (indicated by the *purple line*) and oxygen saturation in the tissues is ~40% (indicated by the *green line*), providing a similar 40% delivery of the bound oxygen to the tissues by the increase in 2,3-BPG levels. The shift in O_2-binding affinity allowed a comparable 40%-oxygen delivery to the tissues.

4. CO_2 binding: Most of the CO_2 produced in metabolism is hydrated and transported as bicarbonate ion (see Fig. 1.12 on p. 9). However, some CO_2 is carried as carbamate bound to the terminal amino groups of hemoglobin (forming carbaminohemoglobin as shown in Fig. 3.8), which can be represented schematically as follows:

$$Hb - NH_2 + CO_2 \rightleftarrows Hb - NH - COO^- + H^+$$

The binding of CO_2 stabilizes the T, or deoxy, form of hemoglobin, resulting in a decrease in its oxygen affinity (see p. 30) and a right shift in the oxygen-dissociation curve. In the lungs, CO_2 dissociates from the hemoglobin and is released in the breath.

5. CO binding: Carbon monoxide (CO) binds tightly (but reversibly) to the hemoglobin iron, forming carboxyhemoglobin. When CO binds to one or more of the four heme sites, hemoglobin shifts to the R conformation, causing the remaining heme sites to bind O_2 with high affinity. This shifts the oxygen-dissociation curve to the left and changes the normal sigmoidal shape toward a hyperbola. As a result, the affected hemoglobin is unable to release O_2 to the tissues (Fig. 3.13). (Note: The affinity of hemoglobin for CO is 220 times greater than for O_2. Consequently, even minute concentrations of CO in the environment can produce toxic concentrations of carboxyhemoglobin in the blood. For example, increased levels of CO are found in the blood of tobacco

smokers. CO toxicity appears to result from a combination of tissue hypoxia and direct CO-mediated damage at the cellular level.) CO poisoning is treated with 100% O_2 at high pressure (hyperbaric oxygen therapy), which facilitates the dissociation of CO from the hemoglobin. (Note: CO also inhibits Complex IV of the electron transport chain (see p. 84).) Nitric oxide gas (NO) also is carried by hemoglobin. NO is a potent vasodilator (see p. 166). It can be taken up (salvaged) or released from RBCs, thereby modulating NO availability and influencing blood vessel diameter.

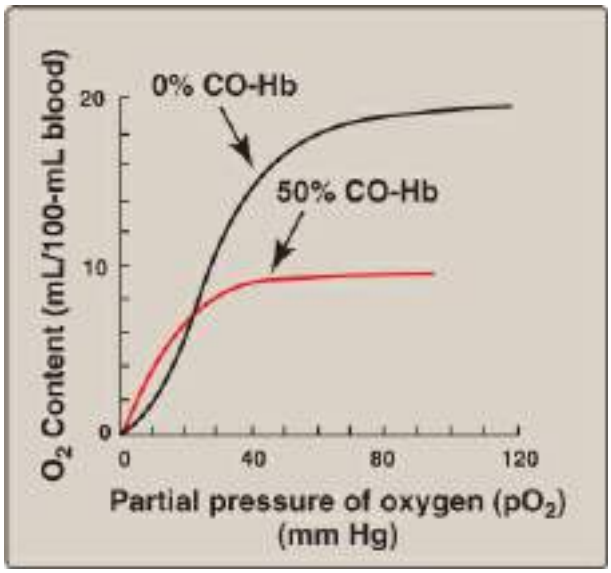


Figure 3.13
Effect of carbon monoxide (CO) on the oxygen affinity of hemoglobin. CO competes with O_2 for binding the heme iron. CO-Hb = carboxyhemoglobin (carbon monoxyhemoglobin).

F. Minor hemoglobins

It is important to remember that human HbA is just one member of a functionally and structurally related family of proteins, the hemoglobins (Fig. 3.14). Each of these oxygen-carrying proteins is a tetramer, composed of two α-globin (or α-like) polypeptides and two β-globin (or β-like) polypeptides. HbF is synthesized during fetal development, but is represented as <2% of the hemoglobin in adult blood. HbF is concentrated in RBCs known as F cells. HbA_2 is also synthesized in the adult, although at low levels compared with HbA. HbA can become modified by the covalent addition of a hexose (HbA_{1c}, see II.F.3. below).

Form	Chain composition	Fraction of total hemoglobin
HbA	$\alpha_2\beta_2$	90%
HbA_2	$\alpha_2\delta_2$	2%–3%
HbF	$\alpha_2\gamma_2$	<2%
HbA_{1c}	$\alpha_2\beta_2$-glucose	4%–6%

Figure 3.14
Human hemoglobins found in adult blood. HbA_{1c} is a subtype of HbA (or HbA_1). (Note: The α chains in these hemoglobins are identical.) Hb = hemoglobin.

1. Fetal hemoglobin: HbF is a tetramer consisting of two α chains identical to those found in HbA, plus two γ chains ($\alpha_2\gamma_2$; see Fig. 3.14). The γ chains are members of the β-globin gene family (see p. 36).

a. HbF synthesis during development: In the first month after conception, embryonic hemoglobins such as Hb Gower 1, composed of two α-like zeta (ζ) chains and two β-like epsilon (ε) chains ($\zeta_2\varepsilon_2$), are synthesized by the embryonic yolk sac. In the fifth week of gestation, the site of globin synthesis shifts, first to the liver and then to the marrow, and the primary product is HbF. HbF is the major hemoglobin found in the fetus and newborn, accounting for ~60% of the total hemoglobin in the RBCs during the last months of fetal life (Fig. 3.15). HbA synthesis starts in the bone marrow around the eighth month of pregnancy and gradually replaces HbF. Figure 3.15 shows the relative production of each type of hemoglobin chain during fetal and postnatal life.

b. 2,3-BPG binding to HbF: Under physiologic conditions, HbF has a higher oxygen affinity than does HbA as a result of HbF weakly binding 2,3-BPG. (Note: The γ-globin chains of HbF lack some of the positively charged amino acids that are responsible for binding 2,3-BPG in the β-globin chains.) Because 2,3-BPG serves to reduce the oxygen affinity of hemoglobin, the weaker interaction between 2,3-BPG and HbF results in a higher oxygen affinity for HbF relative to HbA. In contrast, if both HbA and HbF are stripped of their 2,3-BPG, they then have a similar oxygen affinity. The higher oxygen affinity of HbF facilitates the transfer of O_2 from the maternal circulation across the placenta to the RBCs of the fetus.

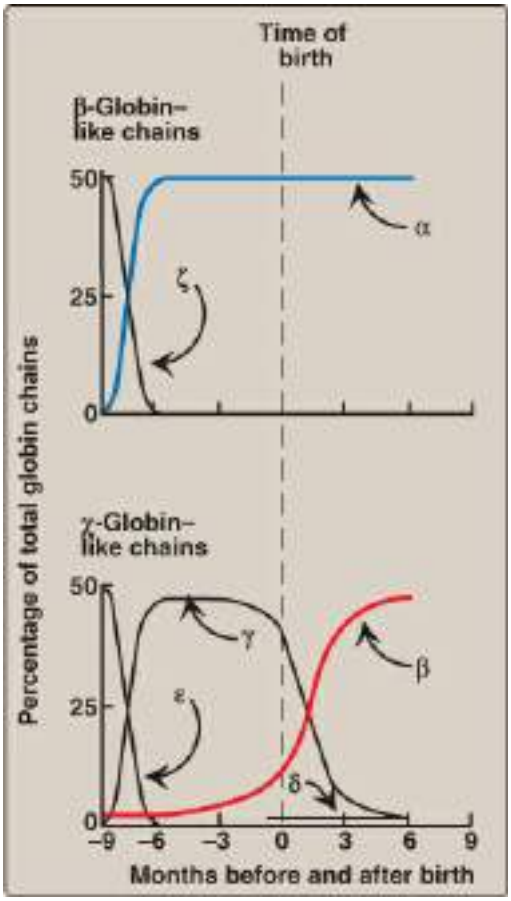


Figure 3.15
Developmental changes in globin production.

2. Hemoglobin A_2: HbA_2 is a minor component of normal adult hemoglobin, first appearing shortly before birth and, ultimately, constituting ~2% of the total hemoglobin. It is composed of two α-globin chains and two δ-globin chains ($\alpha_2\delta_2$; see Fig. 3.14).

3. Hemoglobin A_{1c}: Under physiologic conditions, sugar molecules, predominantly glucose, are added nonenzymatically to HbA in a process referred to as glycation. The extent of glycation is dependent on the plasma concentration of the hexose. The most abundant form of glycated hemoglobin is HbA_{1c}. In HbA_{1c}, glucose residues are attached to the amino groups of the N-terminal valines of the β-globin chains (Fig. 3.16). Increased amounts of HbA_{1c} are found in RBCs of patients with diabetes mellitus, because their HbA has contact with higher glucose concentrations during the 120-day lifetime of these cells (see p. 340 for a discussion of the use of HbA_{1c} levels in assessing average blood glucose levels in patients with diabetes).

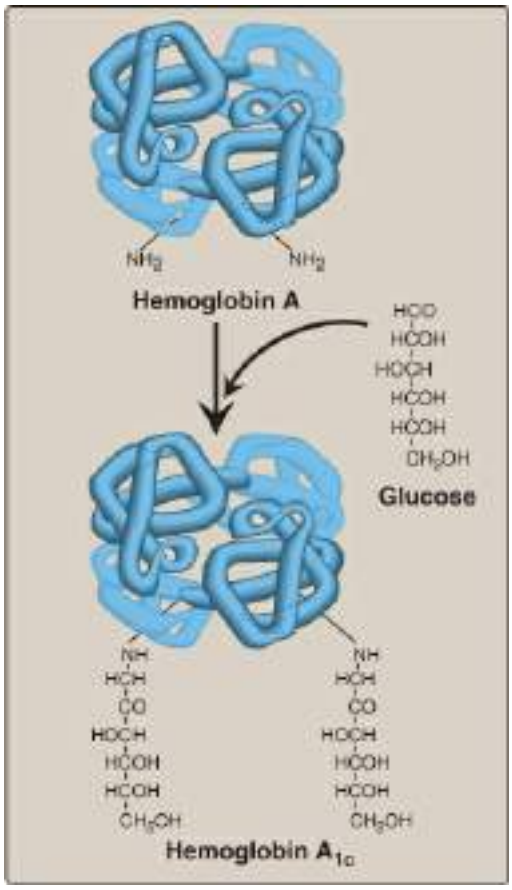


Figure 3.16
Nonenzymatic addition of glucose to hemoglobin. The nonenzymatic addition of a sugar to a protein is referred to as glycation.

III. GLOBIN GENE ORGANIZATION

To understand diseases resulting from genetic alterations in the structure or synthesis of hemoglobin, it is necessary to grasp how the hemoglobin genes, which direct the synthesis of the different globin chains, are structurally organized into gene families, and also how they are expressed. Expression of a globin gene begins in RBC precursors, where the DNA sequence encoding the gene is transcribed. Two introns are spliced out to join together three exons into the mature mRNA for translation. A more detailed description of gene expression is presented in Unit VII, Chapters 30, 31, and 32.

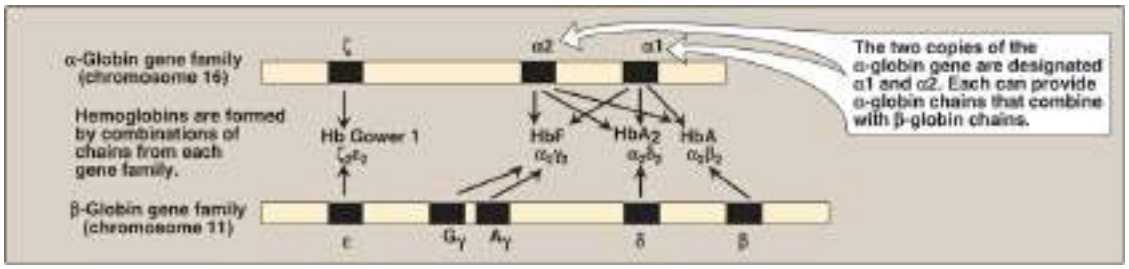


Figure 3.17
Organization of the globin gene families. Hb = hemoglobin.

A. α-Gene family

The genes coding for the α-globin and β-globin subunits of the hemoglobin chains occur in two separate gene clusters (or families) located on two different chromosomes (Fig. 3.17). The α-gene cluster on chromosome 16 contains two genes for the α-globin chains. It also contains the ζ gene that is expressed early in development as an α-globin–like component of embryonic hemoglobin. (Note: Globin gene families also contain globin-like genes that are not expressed, i.e., their genetic information is not used to produce globin chains. These are called pseudogenes.)

B. β-Gene family

The β-gene cluster contains a single gene for the β-globin chain, located on chromosome 11 (see Fig. 3.17). There are an additional four β-globin–like genes within the gene cluster: the ε gene (which, like the ζ gene, is expressed early in embryonic development), two γ genes (G_γ and A_γ that are expressed in HbF), and the δ gene that codes for the globin chain found in the minor adult hemoglobin, HbA_2.

IV. HEMOGLOBINOPATHIES

Hemoglobinopathies are defined as a group of genetic disorders caused by production of a structurally abnormal hemoglobin molecule, synthesis of insufficient quantities of normal hemoglobin, or, rarely, both. Sickle cell anemia (HbS), hemoglobin C disease (HbC), hemoglobin SC disease (HbS + HbC = HbSC), and the thalassemias are representative hemoglobinopathies that can have severe clinical consequences. The first three conditions result from production of hemoglobin with an altered amino acid sequence (a qualitative hemoglobinopathy), whereas the thalassemias are caused by decreased production of normal hemoglobin (a quantitative hemoglobinopathy).

A. Sickle cell anemia (hemoglobin S disease)

Sickle cell anemia is a genetic disorder caused by a single nucleotide substitution (a point mutation, see p. 449) in the gene for β-globin. The alteration in the amino acid sequence of HbS causes RBC morphology to form into sickle or crescent shapes, rather than the round biconcave shape of a normal RBC expressing normal HbA. This abnormal cell morphology is referred to as sickling. Sickle cell anemia is the most common inherited blood disorder in the United States, affecting 50,000 Americans. It occurs primarily in the African-American population, affecting 1 in 500 African Americans. Sickle cell anemia is an autosomal-recessive disorder. It occurs in individuals who have inherited two mutant alleles (one from each parent) that

code for synthesis of the β chains of the globin molecules. (Note: The mutant β-globin chain is designated β^S, and the resulting hemoglobin, $\alpha_2\beta^S_2$, is referred to as HbS.) An infant does not begin showing symptoms of the disease until sufficient HbF has been replaced by HbS so that sickling can occur (see p. 36). Sickle cell anemia is characterized by lifelong episodes of pain (“crises”), chronic hemolytic anemia with associated hyperbilirubinemia (see p. 316), and increased susceptibility to infections, usually beginning in infancy. (Note: The lifespan of RBCs in sickle cell anemia is <20 days, compared with 120 days for normal RBCs, hence, the anemia.) Other symptoms include acute chest syndrome, stroke, splenic and renal dysfunction, and bone changes due to marrow hyperplasia. Life expectancy is reduced (mid-40s median age). Heterozygotes, representing 1 in 12 African Americans, have one normal and one sickle cell allele. The blood cells of such heterozygotes contain both HbS and HbA, and these individuals have sickle cell trait, not sickle cell disease. They usually do not show clinical signs or symptoms (but may under conditions of extreme physical exertion with dehydration) and can have a normal life span.

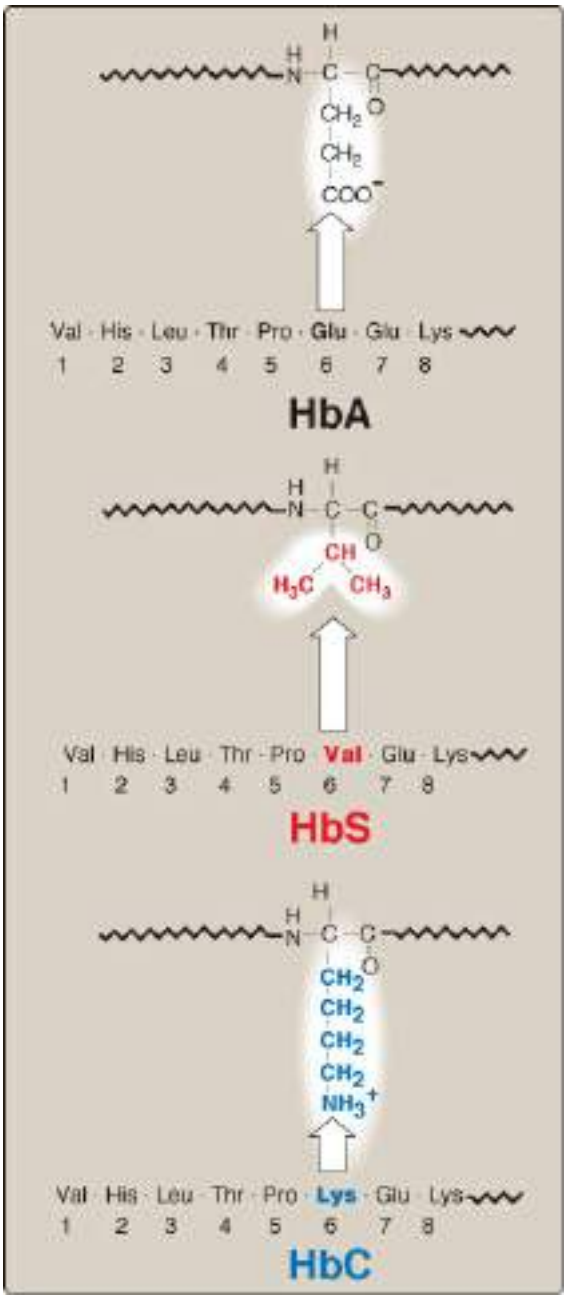


Figure 3.18
Amino acid substitutions in hemoglobin S (HbS) and hemoglobin C (HbC).

1. Amino acid substitution in HbS β chains: In a patient with sickle cell anemia, a molecule of HbS contains two normal α-globin chains and two mutant β-globin chains (β^S), in which glutamate at position six has been replaced with valine (Fig. 3.18). The resulting exchange of negatively charged polar glutamate residues for neutral nonpolar valine residues in the two β chains renders HbS less negatively charged than HbA. Therefore, during electrophoresis at alkaline pH, HbS migrates more slowly toward the anode (positive electrode) than does HbA (Fig. 3.19). Electrophoresis of hemoglobin obtained from lysed RBCs is

routinely used in the diagnosis of sickle cell trait and sickle cell anemia (or sickle cell disease). DNA analysis is also used to diagnose sickle cell anemia (see p. 544).

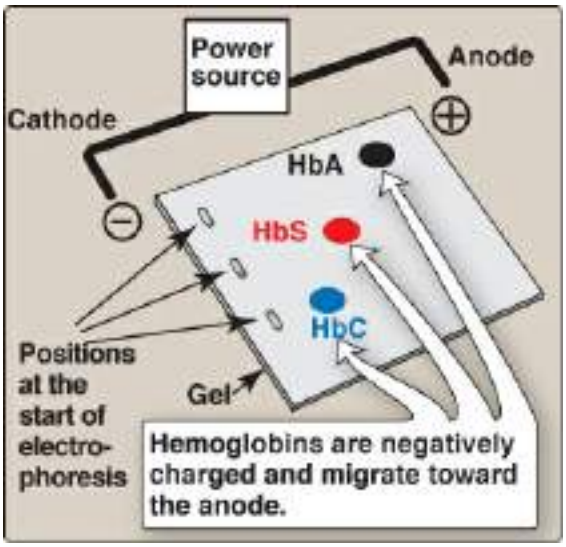


Figure 3.19
Diagram of hemoglobins HbA, HbS, and HbC after electrophoresis.

2. Sickling and tissue anoxia: The replacement of the charged glutamate with the nonpolar valine forms a hydrophobic protrusion on the β chain that fits into a complementary hydrophobic site on the β chain of another HbS molecule in the cell (Fig. 3.20). At low oxygen tension, deoxygenated HbS polymerizes inside the RBC, forming a network of insoluble fibrous polymers that stiffen and distort the cell, producing rigid, sickle-shaped RBCs. Such sickled cells frequently block the flow of blood in the narrow capillaries. This interruption in the supply of O_2 leads to localized anoxia (oxygen deprivation) in the tissue, causing pain and eventually ischemic death (infarction) of cells in the vicinity of the blockage. The anoxia also leads to an increase in deoxygenated HbS. (Note: The mean diameter of RBC is 7.5 μm, whereas that of the microvasculature is 3 to 4 μm. Compared to normal RBCs, sickled cells have a decreased ability to deform and an increased tendency to adhere to vessel walls. This makes moving through small vessels difficult, thereby causing microvascular occlusion.)

3. Variables that increase sickling: The extent of sickling and, therefore, the severity of disease are enhanced by any variable that increases the proportion of HbS in the deoxy state (i.e., reduces the oxygen affinity of HbS). These variables include decreased pO_2, increased pCO_2, decreased pH, dehydration, and an increased concentration of 2,3-BPG in RBCs.

4. Treatment: Therapy involves adequate hydration, analgesics, aggressive antibiotic therapy if infection is present, and transfusions in patients at high risk for fatal occlusion of blood vessels. Intermittent transfusions with packed RBCs

reduce the risk of stroke, but the benefits must be weighed against the complications of transfusion, which include iron overload that can result in hemosiderosis (see p. 451), blood-borne infections, and immunologic complications. Hydroxyurea (hydroxycarbamide), an antitumor drug, is therapeutically useful because it increases circulating levels of HbF, which decreases RBC sickling. This leads to decreased frequency of painful crises and reduces mortality. Stem cell transplantation is possible. (Note: The morbidity and mortality associated with sickle cell anemia have led to its inclusion in newborn screening panels to allow prophylactic antibiotic therapy to begin soon after the birth of an affected child.)

5. Possible selective advantage of the heterozygous state: The high frequency of the β^S mutation among black Africans, despite its damaging effects in the homozygous state, suggests that a selective advantage exists for heterozygous individuals. For example, heterozygotes for the sickle cell gene are less susceptible to the severe malaria caused by the parasite *Plasmodium falciparum.* This organism spends an obligatory part of its life cycle in the RBCs. One theory is that because these cells in individuals heterozygous for HbS, like those in homozygotes, have a shorter life span than normal, the parasite cannot complete the intracellular stage of its development. This may provide a selective advantage to heterozygotes living in regions where malaria is a major cause of death. For example, in Africa, the geographic distribution of sickle cell anemia is similar to that of malaria.

B. Hemoglobin C disease

Like HbS, HbC is a hemoglobin variant that has a single amino acid substitution in the sixth position of the β-globin chain (see Fig. 3.18). In HbC, however, a lysine is substituted for the glutamate (as compared with a valine substitution in HbS). (Note: This substitution causes HbC to move more slowly toward the anode than HbA or HbS does [see Fig. 3.19].) Rare patients homozygous for HbC generally have a relatively mild, chronic hemolytic anemia. They do not experience infarctive crises, and no specific therapy is required.

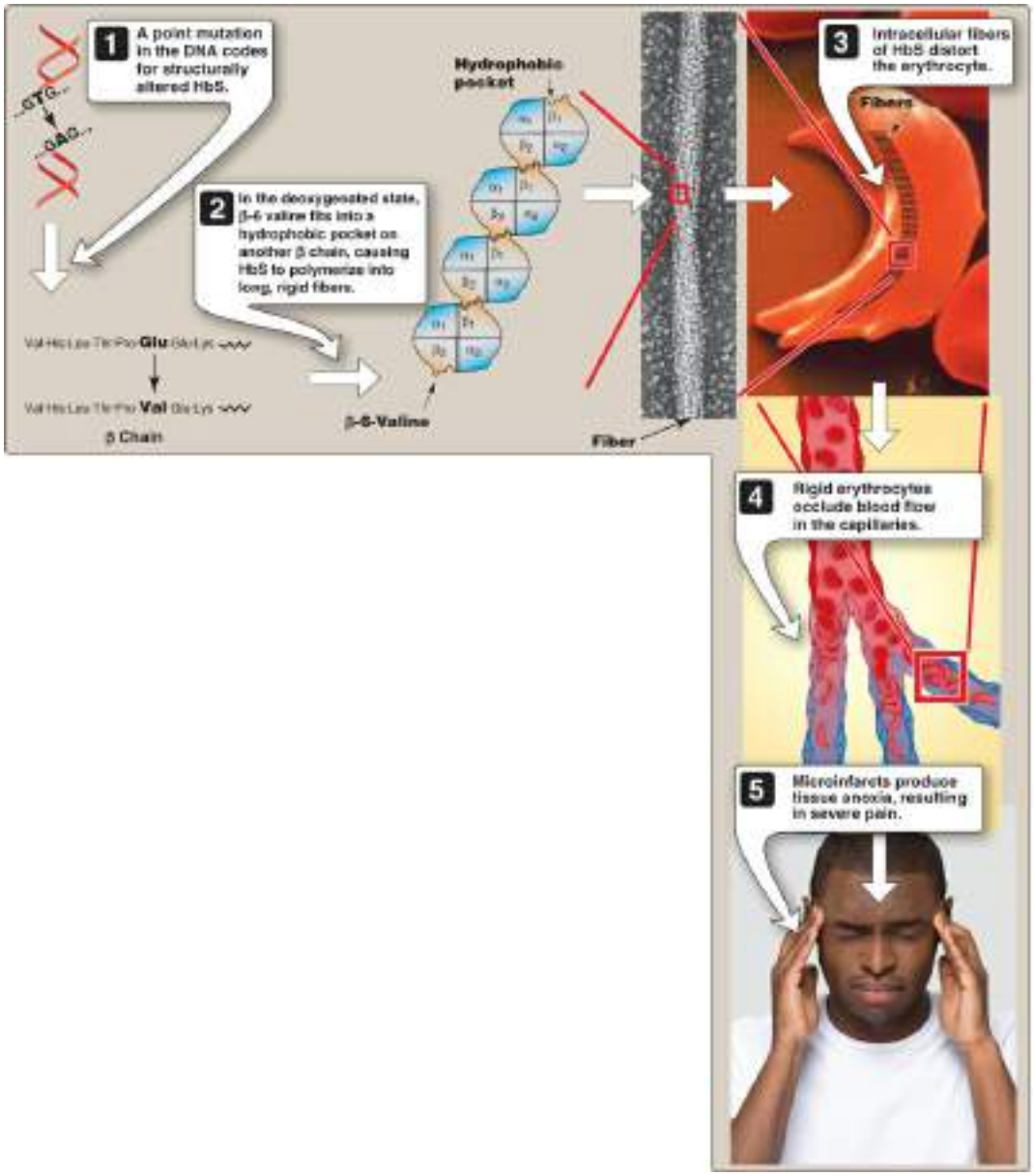


Figure 3.20
Molecular and cellular events leading to sickle cell crisis. HbS = hemoglobin S.

C. Hemoglobin SC disease

HbSC disease is another of the RBC sickling diseases. In this disease, some β-globin chains have the sickle cell mutation, whereas other β-globin chains carry the mutation found in HbC disease. (Note: Patients with HbSC disease are doubly heterozygous. They are called compound heterozygotes because both of their β-globin genes are abnormal, although different from each other.) Hemoglobin levels tend to be higher in HbSC disease than in sickle cell anemia and may even be at the low end of the normal range. The clinical course of adults with HbSC anemia differs from that of sickle cell anemia in that symptoms such as painful crises are

less frequent and less severe. However, there is significant clinical variability.

D. Methemoglobinemias

Oxidation of the heme iron in hemoglobin from Fe^{2+} to Fe^{3+} produces methemoglobin, which cannot bind O_2. This oxidation may be acquired and caused by the action of certain drugs, such as nitrates, or endogenous products such as reactive oxygen species (see p. 163). The oxidation may also result from congenital defects, for example, a deficiency of NADH-cytochrome b_5 reductase (also called NADH-methemoglobin reductase), the enzyme responsible for the conversion of methemoglobin (Fe^{3+}) to hemoglobin (Fe^{2+}), leads to the accumulation of methemoglobin (Fig. 3.21). (Note: The RBCs of newborns have approximately half the capacity of those of adults to reduce methemoglobin.) Additionally, rare mutations in the α- or β-globin chain can cause the production of HbM, an abnormal hemoglobin that is resistant to the reductase. The methemoglobinemias are characterized by “chocolate cyanosis” (a blue coloration of the skin and mucous membranes and brown-colored blood) as a result of the dark-colored methemoglobin. Symptoms are related to the degree of tissue hypoxia and include anxiety, headache, and dyspnea. In rare cases, coma and death can occur. Treatment is with methylene blue, which is oxidized as Fe^{3+} and is reduced back to Fe^{2+}.

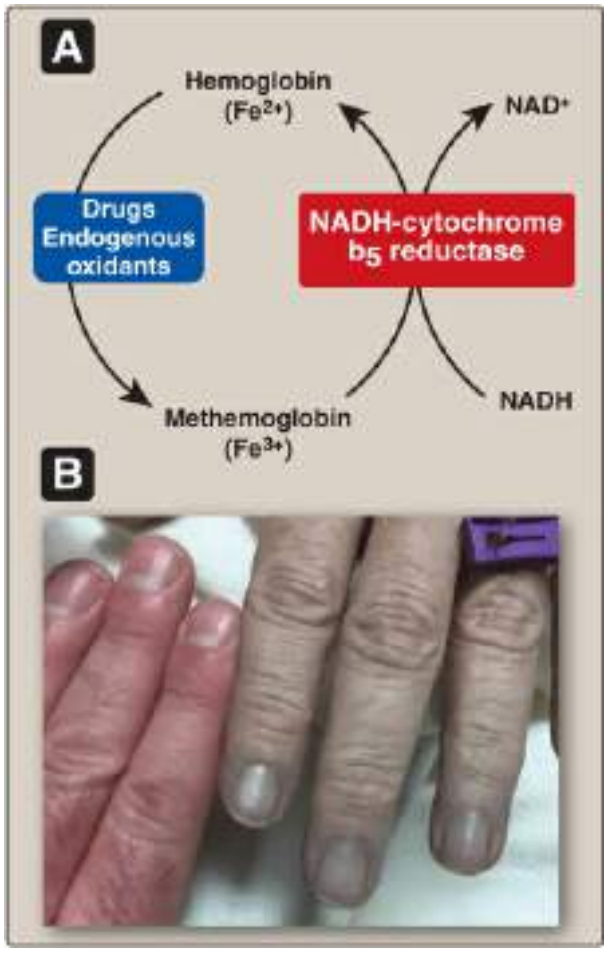


Figure 3.21
A: Formation of methemoglobin and its reduction to hemoglobin by NADH-cytochrome b_5 reductase. **B:** Methemoglobinemia.

E. Thalassemias

The thalassemias are hereditary hemolytic diseases in which an imbalance occurs in the synthesis of globin chains. As a group, they are the most common single-gene disorders in humans. Normally, synthesis of the α- and β-globin chains is coordinated, so that each α-globin chain has a β-globin chain partner. This leads to the formation of $\alpha_2\beta_2$ (HbA). In the thalassemias, the synthesis of either the α- or the β-globin chain is defective, and hemoglobin concentration is reduced. A thalassemia can be caused by a variety of mutations, including entire gene deletions, or substitutions or deletions of one of many nucleotides in the DNA. (Note: Each thalassemia can be classified as either a disorder in which no globin chains are produced (α^0- or β^0-thalassemia), or one in which some chains are synthesized but at a reduced level [α^+- or β^+-thalassemia].)

1. β-Thalassemias: In these disorders, synthesis of β-globin chains is decreased or absent, typically as a result of point mutations that affect the production of functional mRNA. However, α-globin chain synthesis is normal. Excess α-globin

chains cannot form stable tetramers and so precipitate, causing the premature death of cells initially destined to become mature RBCs. Increase in $\alpha_2\delta_2$ (HbA_2) and $\alpha_2\gamma_2$ (HbF) also occurs. There are only two copies of the β-globin gene in each cell (one on each chromosome 11). Therefore, individuals with β-globin gene defects have either β-thalassemia trait (β-thalassemia minor) if they have only one defective β-globin gene or β-thalassemia major (Cooley anemia) if both genes are defective (Fig. 3.22). Because the β-globin gene is not expressed until late in prenatal development, the physical manifestations of β-thalassemias appear only several months after birth. Those individuals with β-thalassemia minor make some β chains and usually do not require specific treatment. However, infants born with β-thalassemia major are seemingly healthy at birth but become severely anemic due to ineffective erythropoiesis, usually during the first or second year of life. Skeletal changes as a result of extramedullary hematopoiesis also are seen. These patients require regular transfusions of blood. (Note: Although this treatment is lifesaving, the cumulative effect of the transfusions is iron overload. Use of iron chelation therapy has improved morbidity and mortality.) The only curative option available is hematopoietic stem cell transplantation.

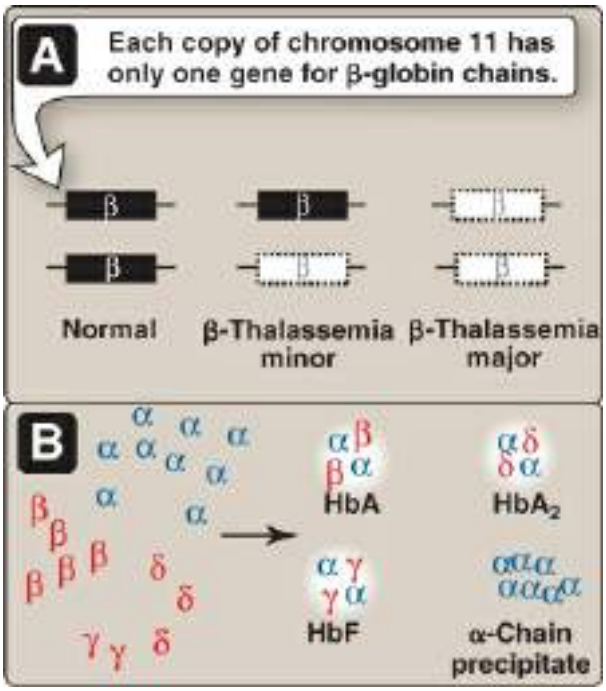


Figure 3.22
A: β-Globin gene mutations in the β-thalassemias. B: Hemoglobin (Hb) tetramers formed in β-thalassemias.

2. α-Thalassemias: In these disorders, synthesis of α-globin chains is decreased or absent, typically as a result of deletional mutations. Because each individual's genome contains four copies of the α-globin gene (two on each chromosome 16), there are several levels of α-globin chain deficiencies (Fig. 3.23). If one of

the four alleles encodes for a defective globin protein, the individual is termed a “silent” carrier of α-thalassemia, because no physical manifestations of the disease occur. If two α-globin alleles encode for defective globin proteins, the individual is designated as having α-thalassemia trait. If three α-globin alleles encode for defective globin proteins, the individual has hemoglobin H (β_4) disease, a hemolytic anemia of variable severity. If all four α-globin alleles encode for defective globin proteins, hemoglobin Bart (γ_4) disease with hydrops fetalis and fetal death results, because α-globin chains are required for the synthesis of HbF. (Note: Heterozygote advantage against malaria is seen in both α- and β-thalassemias.)

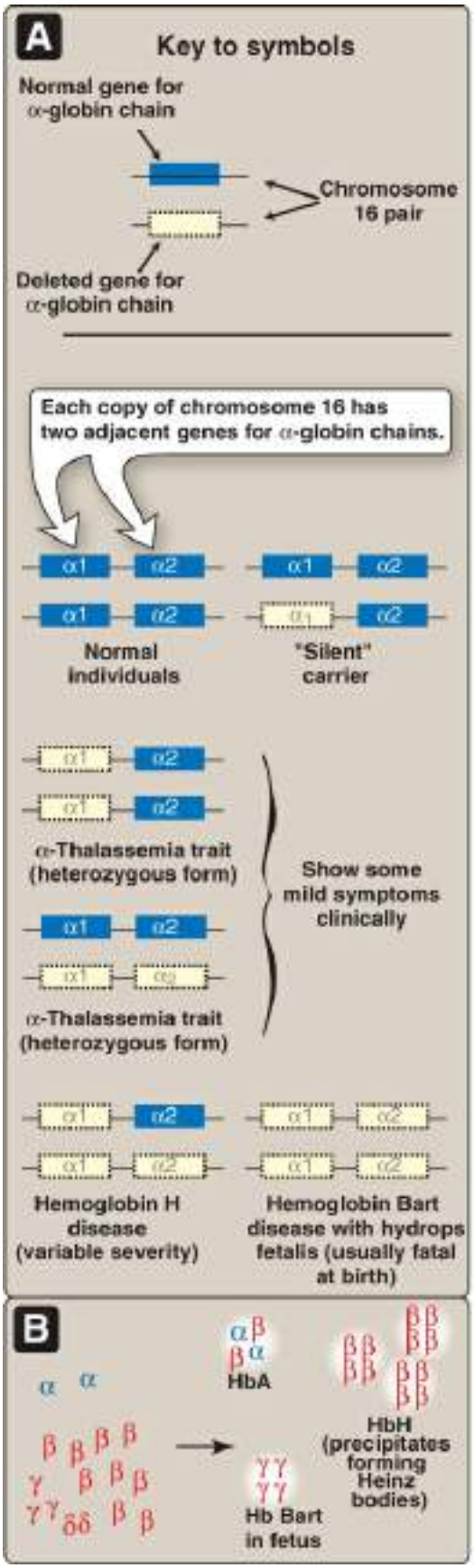
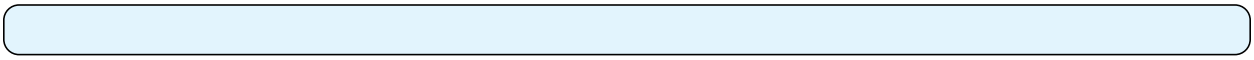


Figure 3.23
A: α-Globin gene deletions in the α-thalassemias. B: Hemoglobin (Hb) tetramers formed in α-thalassemias.



V. Chapter Summary

- **Hemoglobin A** (**HbA**), the major hemoglobin in adults, is composed of four polypeptide chains (two α chains and two β chains, $\alpha_2\beta_2$) held together by noncovalent interactions (Fig. 3.24).
- The subunits occupy different relative positions in deoxyhemoglobin compared with oxyhemoglobin. The **deoxy form** of Hb is called the "**T**," or **taut** (**tense**), **conformation**. It has a constrained structure that limits the movement of the polypeptide chains. The T form is the **low–oxygen-affinity form** of Hb.
- The binding of oxygen (O_2) to the heme iron causes rupture of some of the ionic and hydrogen bonds and movement of the dimers. This leads to a structure called the "**R**," or **relaxed**, **conformation**. The R form is the **high–oxygen-affinity form** of Hb.
- The **oxygen-dissociation curve** for Hb is **sigmoidal** in shape (in contrast to that of **myoglobin**, which is **hyperbolic**), indicating that the subunits cooperate in binding O_2. The binding of an oxygen molecule at one heme group increases the oxygen affinity of the remaining heme groups in the same Hb molecule (**cooperativity**).
- Hb's ability to bind O_2 reversibly is affected by the partial pressure of oxygen (**pO_2**), the **pH** of the environment, the partial pressure of carbon dioxide (**pCO_2**), and the availability of **2,3-bisphosphoglycerate** (**2,3-BPG**). For example, the release of O_2 from Hb is enhanced when the pH is lowered or the pCO_2 is increased (the **Bohr effect**), such as in **exercising muscle**, and the oxygen-dissociation curve of Hb is shifted to the right.
- To cope long-term with the effects of **chronic hypoxia** or **anemia**, the concentration of **2,3-BPG** in **red blood cells** increases. **2,3-BPG** binds to the Hb and decreases its oxygen affinity. It therefore also shifts the oxygen-dissociation curve to the right.
- **Fetal hemoglobin (HbF)** binds 2,3-BPG less tightly than does HbA and has a higher oxygen affinity.
- **Carbon monoxide** (**CO**) binds tightly (but reversibly) to the Hb iron, forming **carboxyhemoglobin**.
- **Hemoglobinopathies** are disorders primarily caused either by production of a structurally abnormal Hb molecule as in **sickle cell anemia** or synthesis of insufficient quantities of normal Hb subunits as in the **thalassemias** (Fig. 3.25).

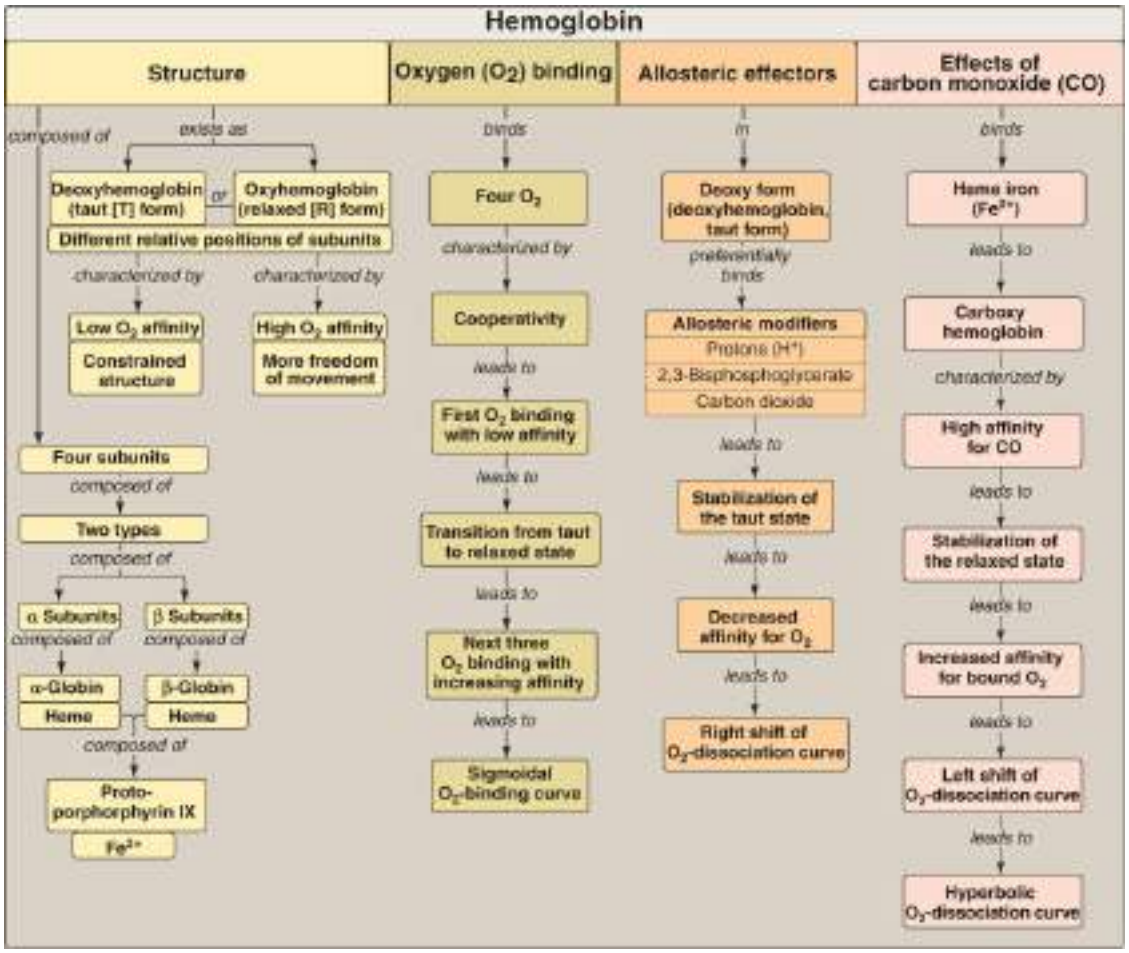


Figure 3.24
Key concept map for hemoglobin structure and function. Fe^{2+} = ferrous iron.

Study Questions

Choose the ONE best answer.

3.1 Which one of the following statements concerning the hemoglobins is correct?

A. HbA is the most abundant hemoglobin in normal adults.
B. Fetal blood has a lower affinity for oxygen than does adult blood because HbF has an increased affinity for 2,3-bisphosphoglycerate.
C. The globin chain composition of HbF is $\alpha_2\delta_2$.
D. HbA_{1c} differs from HbA by a single, genetically determined amino acid substitution.
E. HbA_2 appears early in fetal life.

Correct answer = A. HbA accounts for over 90% of the hemoglobin in a normal adult. If HbA_{1c} is included, the percentage rises to ~97%. Because 2,3-bisphosphoglycerate (2,3-BPG) reduces the affinity of hemoglobin for oxygen, the weaker interaction between 2,3-BPG and HbF results in a higher oxygen affinity for HbF relative to HbA. HbF consists of $\alpha_2\gamma_2$. HbA_{1c} is a glycated form of HbA, formed nonenzymatically in red blood cells. HbA_2 is a minor component of normal adult hemoglobin, first appearing shortly before birth and rising to adult levels (~2% of the total hemoglobin) by age 6 months.

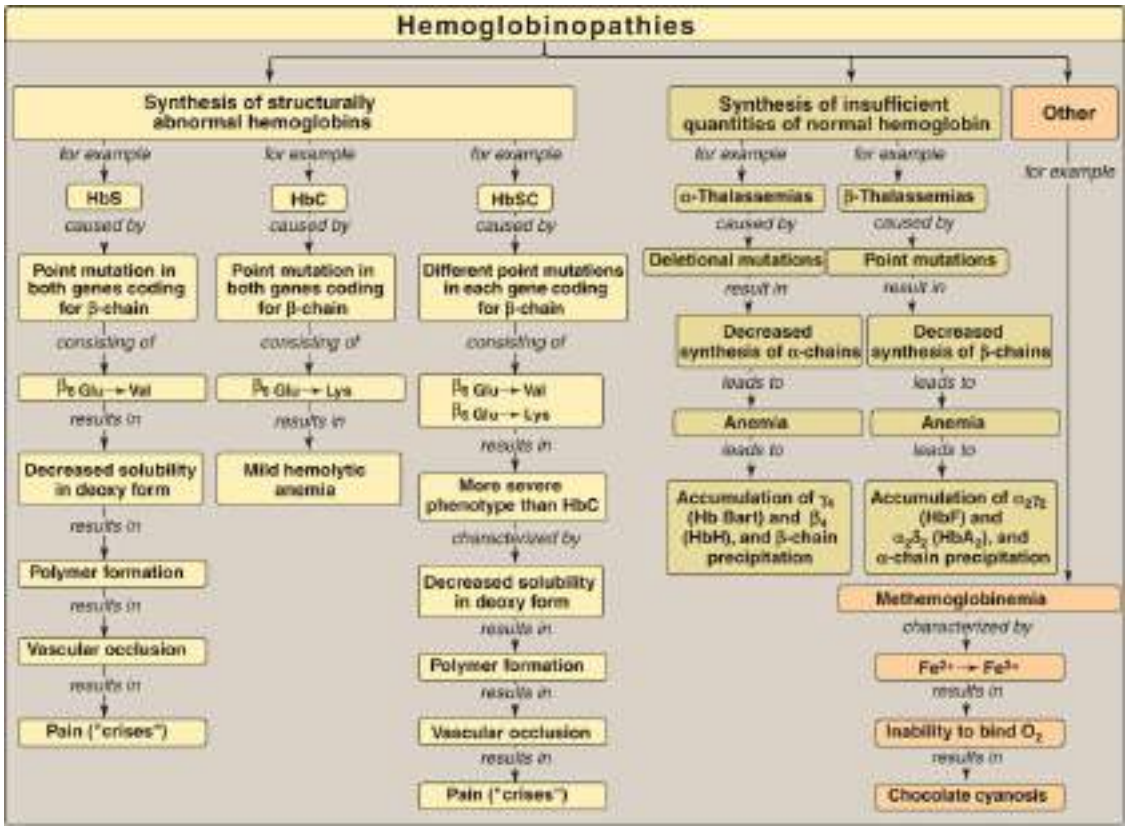


Figure 3.25
Key concept map for hemoglobinopathies. Hb = hemoglobin; Fe = iron; O_2 = oxygen.

3.2 Which one of the following statements concerning the ability of acidosis to precipitate a crisis in sickle cell anemia is correct?

A. Acidosis decreases the solubility of HbS.
B. Acidosis increases the oxygen affinity of hemoglobin.
C. Acidosis favors the conversion of hemoglobin from the taut to the relaxed conformation.
D. Acidosis shifts the oxygen-dissociation curve to the left.
E. Acidosis decreases the ability of 2,3-bisphosphoglycerate to bind to hemoglobin.

Correct answer = A. HbS is significantly less soluble in the deoxygenated form, compared with oxyhemoglobin S. Decreased pH (acidosis) causes the oxygen-dissociation curve to shift to the right, indicating decreased oxygen affinity (increased delivery). This favors the formation of the deoxy, or taut, form of hemoglobin and can precipitate a sickle cell crisis. The binding of 2,3-bisphosphoglycerate is increased, because it binds only to the deoxy form of hemoglobin.

3.3 Which one of the following statements concerning the binding of oxygen by hemoglobin is correct?

A. The Bohr effect results in a lower oxygen affinity at higher pH values.
B. Carbon dioxide increases oxygen affinity of hemoglobin.
C. The oxygen affinity of hemoglobin increases as the percentage saturation increases.
D. The hemoglobin tetramer binds four molecules of 2,3-bisphosphoglycerate.
E. Oxyhemoglobin and deoxyhemoglobin have the same affinity for protons.

Correct answer = C. The binding of oxygen at one heme group increases the oxygen affinity of the remaining heme groups in the same molecule. A rise in pH results in increased oxygen affinity. Carbon dioxide decreases oxygen affinity because it lowers the pH. Moreover, binding of carbon dioxide to the N-termini stabilizes the taut,

deoxy form. Hemoglobin binds one molecule of 2,3-bisphosphoglycerate. Deoxyhemoglobin has a greater affinity for protons than does oxyhemoglobin.

3.4 β-Lysine 82 in HbA is important for the binding of 2,3-bisphosphoglycerate. In Hb Helsinki, this basic, positively charged amino acid has been replaced by the noncharged amino acid methionine. Which of the following should be true concerning Hb Helsinki?

A. It should be stabilized in the taut, rather than the relaxed, form.
B. It should decrease oxygen delivery to tissues.
C. Hb Helsinki oxygen-dissociation curve should be shifted to the right relative to HbA.
D. It results in anemia.
E. It should decrease hemoglobin affinity to oxygen.

Correct answer = B. Substitution of positively charged lysine by neutral methionine decreases the ability of negatively charged phosphate groups in 2,3-bisphosphoglycerate (2,3-BPG) to bind the β subunits of hemoglobin. Because 2,3-BPG decreases the affinity of hemoglobin for oxygen, a reduction in 2,3-BPG should result in increased oxygen affinity and decreased oxygen (O_2) delivery to tissues. The relaxed form is the high–oxygen-affinity form of hemoglobin. Increased oxygen affinity (decreased delivery) results in a left shift in the oxygen-dissociation curve. Decreased delivery of O_2 is compensated for by increased RBC production.

3.5 A 67-year-old male presented to the emergency department with a 1-week history of angina and shortness of breath. He complained that his face and extremities had taken on a blue color. His medical history included chronic stable angina treated with isosorbide dinitrate and nitroglycerin. Blood obtained for analysis was brown. Which one of the following is the most likely diagnosis?

A. Carboxyhemoglobinemia
B. Hemoglobin SC disease
C. Methemoglobinemia
D. Sickle cell anemia
E. β-Thalassemia

Correct answer = C. Oxidation of the ferrous (Fe^{2+}) iron to the ferric (Fe^{3+}) state in the heme prosthetic group of hemoglobin forms methemoglobin. This may be caused by the action of certain drugs such as nitrates. The methemoglobinemias are characterized by chocolate cyanosis (a blue coloration of the skin and mucous membranes and chocolate-colored blood) as a result of the dark-colored methemoglobin. Symptoms are related to tissue hypoxia and include anxiety, headache, and dyspnea. In rare cases, coma and death can occur. (Note: Benzocaine, an aromatic amine used as a topical anesthetic, is a cause of acquired methemoglobinemia.)

3.6 Why is hemoglobin C disease a nonsickling disease?

In HbC, the polar glutamate is replaced by polar lysine rather than by nonpolar valine as in HbS.

3.7 What would be true about the extent of red blood cell sickling in individuals with HbS and hereditary persistence of HbF?

It would be decreased because HbF reduces HbS concentration. It also inhibits polymerization of deoxy HbS.

Fibrous Proteins 4

I. OVERVIEW

Fibrous proteins are usually folded into either extended filaments or sheet-like structures, with repeated amino acid sequences. They are relatively insoluble and provide structural or protective function in our tissues, such as in connective tissues, tendons, bone, and muscle fibers. Collagen and elastin are examples of commonly occurring, well-characterized fibrous proteins of the extracellular matrix (ECM). Collagen and elastin serve structural functions in the body, and are components of the skin, connective tissue, blood vessel walls, and the sclera and cornea of the eye. Each fibrous protein exhibits special mechanical properties, resulting from its unique structure, which is obtained by combining specific amino acids into repeated, secondary structural elements. This is in contrast to globular proteins (discussed in Chapter 3), whose shapes are the result of complex interactions between secondary, tertiary, and, sometimes, quaternary structural elements.

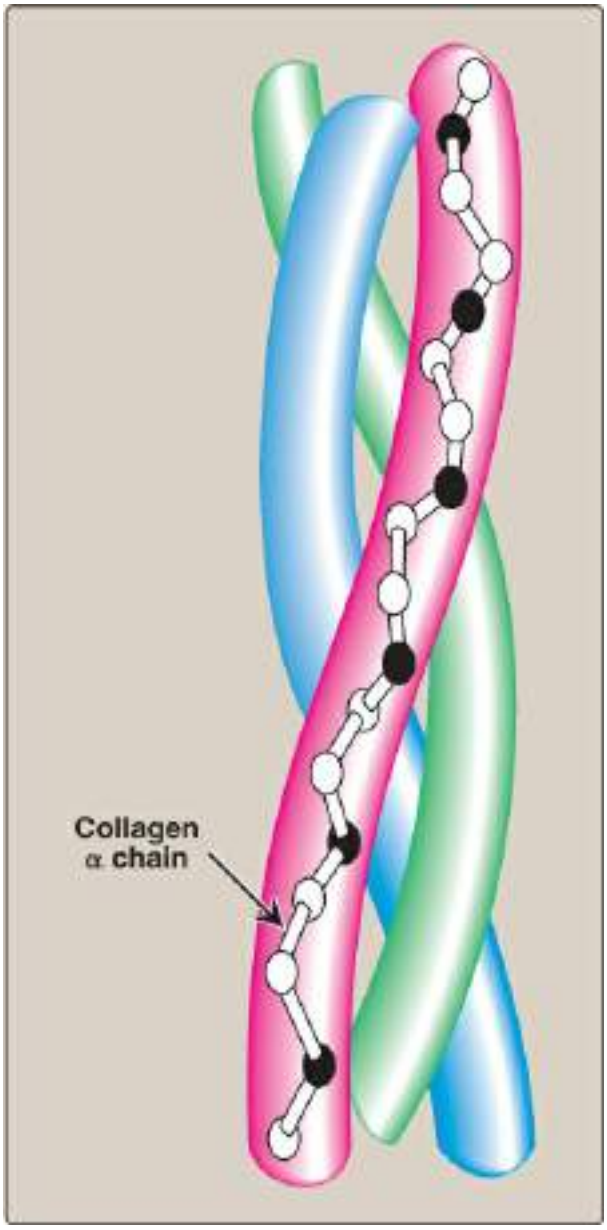


Figure 4.1
Triple-stranded helix of collagen formed from three α chains. (Note: The α chains themselves are helical in structure.)

II. COLLAGEN

Collagen is the most abundant protein in the human body. A typical collagen molecule is a long, rigid structure in which three polypeptides (referred to as α chains) are wound around one another in a rope-like triple helix (Fig. 4.1). Although this triple helix is found in all collagen molecules throughout the body, the many subtypes of collagen are further organized and dictated by the structural role collagen plays in a particular organ. In some tissues, collagen may be dispersed as a gel that gives support to the structure, such as in the ECM or the vitreous humor of the eye. In other tissues, collagen may be

bundled in tight, parallel fibers that provide great strength, as in tendons. In the cornea of the eye, collagen is stacked so as to transmit light with a minimum of scattering. Collagen of bone occurs as fibers arranged at an angle to each other so as to resist mechanical shear from any direction.

A. Types

The collagen superfamily of proteins includes >25 collagen types as well as additional proteins that have collagen-like domains. The three polypeptide α chains are held together by interchain hydrogen bonds. Variations in the amino acid sequence of the α chains result in structural components that are about the same size (~1,000 amino acids long) but with slightly different properties. These α chains are combined to form the various types of collagen found in the tissues. For example, the most common collagen, type I, contains two chains called α1 and one chain called α2 ($\alpha 1_2 \alpha 2$), whereas type II collagen contains three α1 chains ($\alpha 1_3$). The collagens can be organized into three groups, based on their location and functions in the body (Fig. 4.2).

1. Fibril-forming collagens: Types I, II, and III are the fibrillar collagens, with a rope-like structure described above for a typical collagen molecule. In the electron microscope, these linear polymers of fibrils have characteristic banding patterns, reflecting the regular staggered packing of the individual collagen molecules in the fibril (Fig. 4.3). Type I collagen fibers (composed of collagen fibrils) are found in supporting elements of high tensile strength (e.g., tendons and corneas), whereas fibers formed from type II collagen molecules are restricted to cartilaginous structures. The fibers derived from type III collagen are prevalent in more distensible tissues such as blood vessels.

2. Network-forming collagens: Types IV and VIII form a three-dimensional mesh, rather than distinct fibrils (Fig. 4.4). For example, type IV molecules assemble into a sheet or meshwork that constitutes a major part of basement membranes.

TYPE	TISSUE DISTRIBUTION
	Fibril forming
I	Skin, bone, tendon, blood vessels, cornea
II	Cartilage, intervertebral disk, vitreous body
III	Blood vessels, skin, muscle
	Network forming
IV	Basement membrane
VIII	Corneal and vascular endothelium
	Fibril associated*
IX	Cartilage
XII	Tendon, ligaments, some other tissues

Figure 4.2
The most abundant types of collagen. (Note: *Fibril-associated collagens with interrupted triple helices are known as FACIT.)

Basement membranes are thin, sheet-like structures that provide mechanical support for adjacent cells and function as a semipermeable filtration barrier to macromolecules in organs such as the kidney and the lung.

3. Fibril-associated collagens: Types IX and XII bind to the surface of collagen fibrils, linking these fibrils to one another and to other components in the ECM (Fig. 4.2).

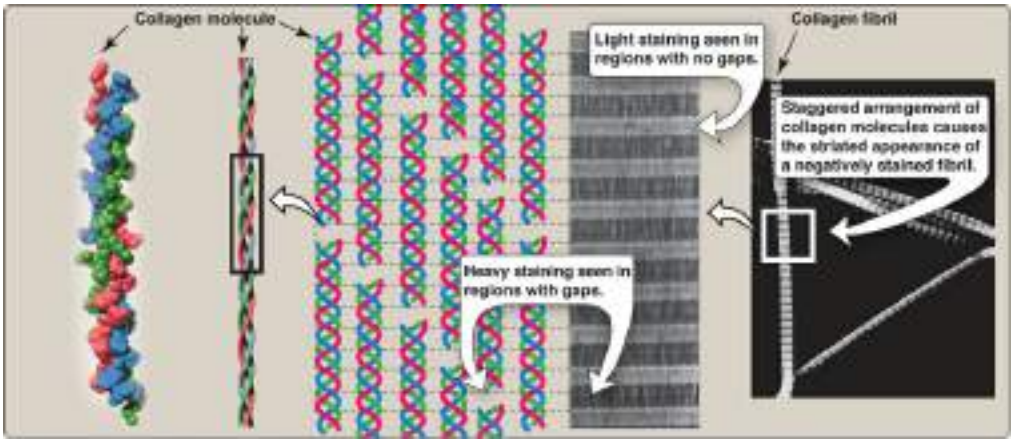


Figure 4.3
Collagen fibrils at right have a characteristic banding pattern, reflecting the regularly staggered packing of the individual collagen molecules in the fibril.

B. Structure

Unlike most globular proteins that are folded into compact structures, collagen, a fibrous protein, has an elongated, triple-helix structure that is stabilized by interchain hydrogen bonds.

1. Amino acid sequence: Collagen is rich in proline and glycine, both of which are important in the formation of the triple-stranded helix. Proline facilitates the formation of the helical conformation of each α chain because its ring structure causes “kinks” in the peptide chain. (Note: The presence of proline dictates that the helical conformation of the α chain cannot be an α helix [see p. 16].) Glycine, the smallest amino acid, is found in every third position of each polypeptide chain. Glycine fits into the restricted spaces where the three chains of the helix come together. The glycine residues are part of a repeating sequence, −Gly–X–Y–, where X is frequently proline, and Y is often hydroxyproline (but can be hydroxylysine, Fig. 4.5). Thus, most of the α chain can be regarded as a polytripeptide whose sequence can be represented as $(-\text{Gly–Pro–Hyp–})_{333}$.

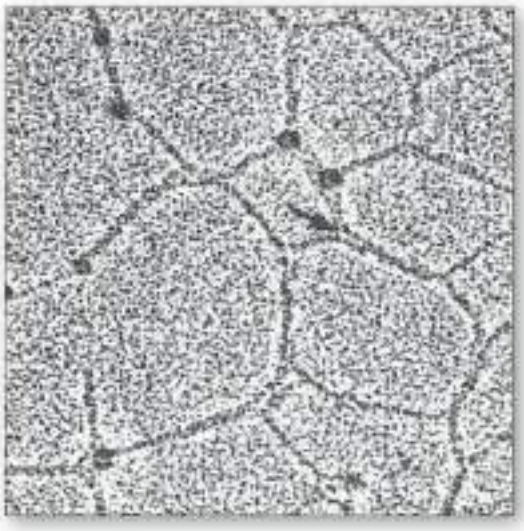


Figure 4.4
Electron micrograph of a polygonal network formed by association of collagen type IV monomers.

2. Hydroxyproline and hydroxylysine: Collagen contains hydroxyproline and hydroxylysine, which are nonstandard amino acids (see p. 1) not present in most other proteins. These unique amino acids result from the hydroxylation of some of the proline and lysine residues after their incorporation into polypeptide chains (Fig. 4.6). Therefore, hydroxylation is a posttranslational modification (see p. 509). (Note: The presence of hydroxyproline maximizes formation of interchain hydrogen bonds that stabilize the triple-helical structure.)

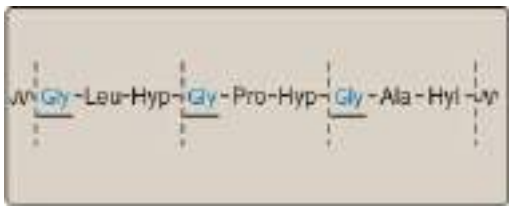


Figure 4.5
Amino acid sequence of a portion of the α1 chain of collagen. Hyp, hydroxyproline; Hyl, hydroxylysine.

3. Glycosylation: The hydroxyl group of the hydroxylysine residues of collagen may be enzymatically glycosylated. Most commonly, glucose and galactose are sequentially attached to the polypeptide chain prior to triple-helix formation (Fig. 4.7).

C. Biosynthesis

The polypeptide precursors of the collagen molecule are synthesized in fibroblasts (or in the related osteoblasts of bone and chondroblasts of cartilage). They are enzymically modified and form the triple helix, which gets secreted into the ECM.

After additional enzymic modification, the mature extracellular collagen fibrils aggregate and become cross-linked to form collagen fibers.

1. Pro-α chain formation: Collagen is one of many proteins that normally function outside of cells. Like most proteins produced for export, the newly synthesized polypeptide precursors of α chains (prepro-α chains) contain a special amino acid sequence at their amino (N)-terminal ends. This sequence acts as a signal that, in the absence of additional signals, targets the polypeptide being synthesized for secretion from the cell. The signal sequence facilitates the binding of ribosomes to the rough endoplasmic reticulum (RER) and directs the passage of the prepro-α chain into the lumen of the RER. The signal sequence is rapidly cleaved in the lumen to yield a precursor of collagen called a pro-α chain (Fig. 4.7).

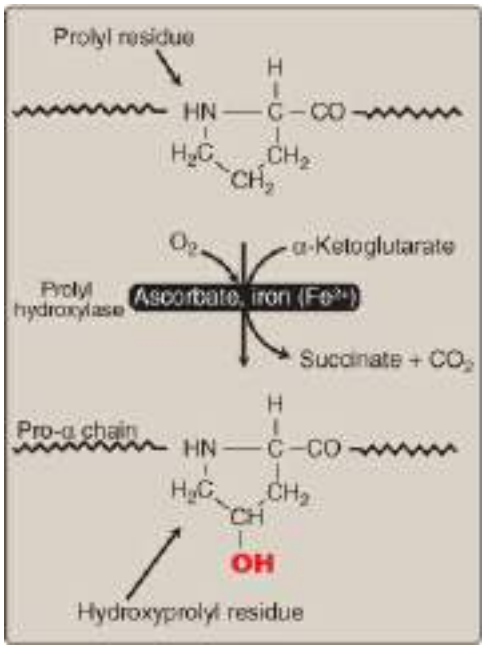


Figure 4.6
Hydroxylation of proline residues in pro-α chains of collagen by prolyl hydroxylase. (Note: Fe^{2+} (hydroxylase cofactor) is protected from oxidation to Fe^{3+} by ascorbate [vitamin C].)

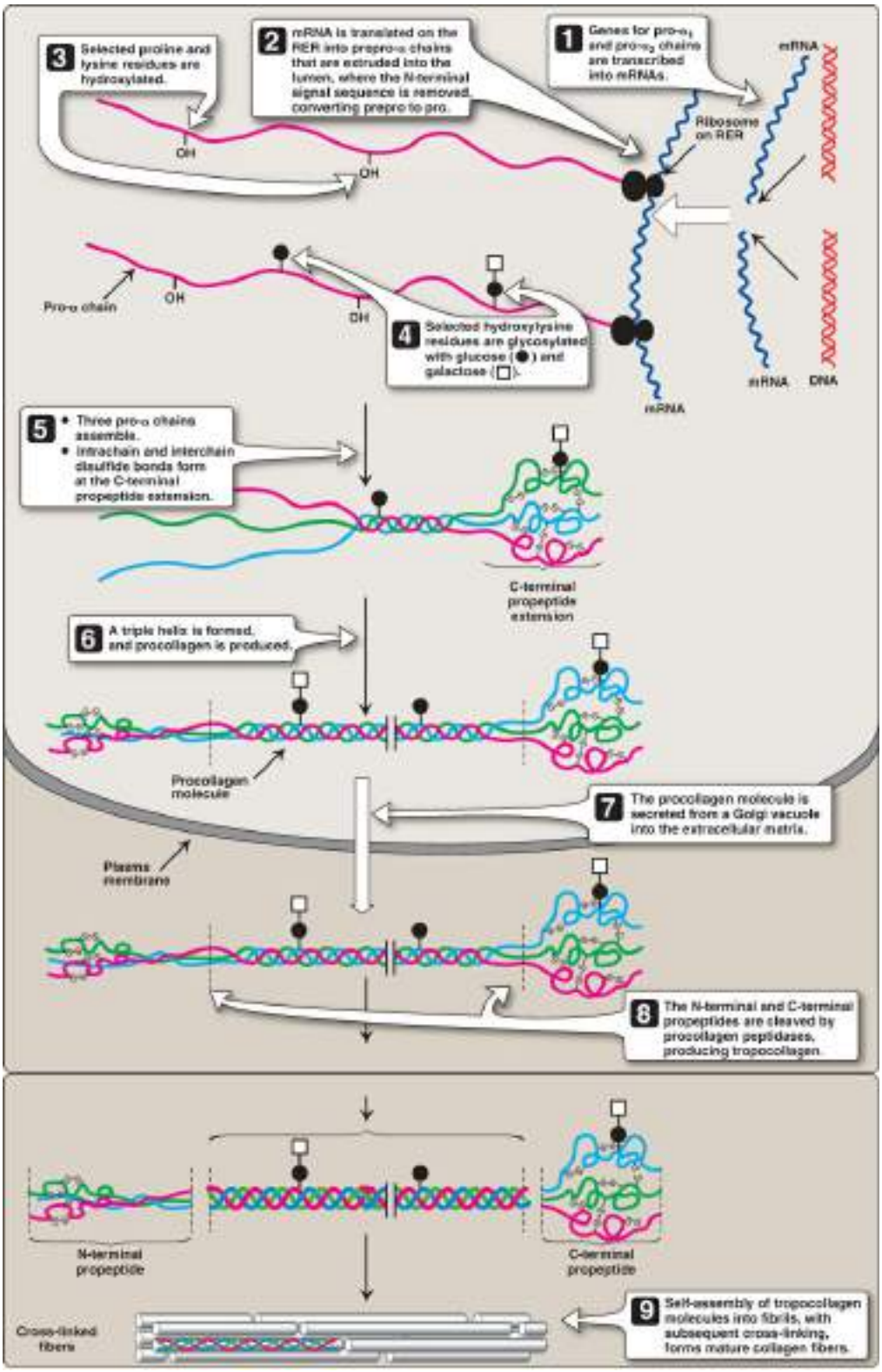


Figure 4.7
Synthesis of collagen. RER, rough endoplasmic reticulum; mRNA, messenger RNA.

2. Hydroxylation: The pro-α chains are processed by a number of enzymes within

the lumen of the RER while the polypeptides are still being synthesized (Fig. 4.7). Proline and lysine residues found in the Y-position of the –Gly–X–Y– sequence can be hydroxylated to form hydroxyproline and hydroxylysine residues. These hydroxylation reactions require molecular oxygen, ferrous iron (Fe^{2+}), and the reducing agent vitamin C (ascorbic acid, see p. 427), without which the hydroxylating enzymes, prolyl hydroxylase, and lysyl hydroxylase, are unable to function (see Fig. 4.6). In the case of ascorbic acid deficiency (and, therefore, a lack of proline and lysine hydroxylation), the formation of interchain H-bonds and the formation of a stable triple helix are impaired. Additionally, collagen fibrils cannot be cross-linked (see 7. below), greatly decreasing the tensile strength of the assembled fiber. The resulting deficiency disease is known as scurvy. Patients with scurvy often show ecchymoses (bruise-like discolorations) and petechiae on the limbs as a result of subcutaneous extravasation (leakage) of blood due to capillary fragility (Fig. 4.8). Other symptoms also include gum disease, loosening of the teeth, and poor wound healing.

3. Glycosylation: Some hydroxylysine residues are modified by glycosylation with glucose or glucosyl-galactose (Fig. 4.7).

4. Assembly and secretion: After hydroxylation and glycosylation, three pro-α chains form procollagen, a precursor of collagen that has a central region of triple helix flanked by the nonhelical N- and carboxyl (C)-terminal extensions called propeptides (Fig. 4.7). The formation of procollagen begins with formation of interchain disulfide bonds between the C-terminal extensions of the pro-α chains. This brings the three α chains into an alignment favorable for triple helix formation. The procollagen molecules move through the Golgi apparatus, where they are packaged in secretory vesicles. The vesicles fuse with the cell membrane, causing the release of procollagen molecules into the extracellular space.

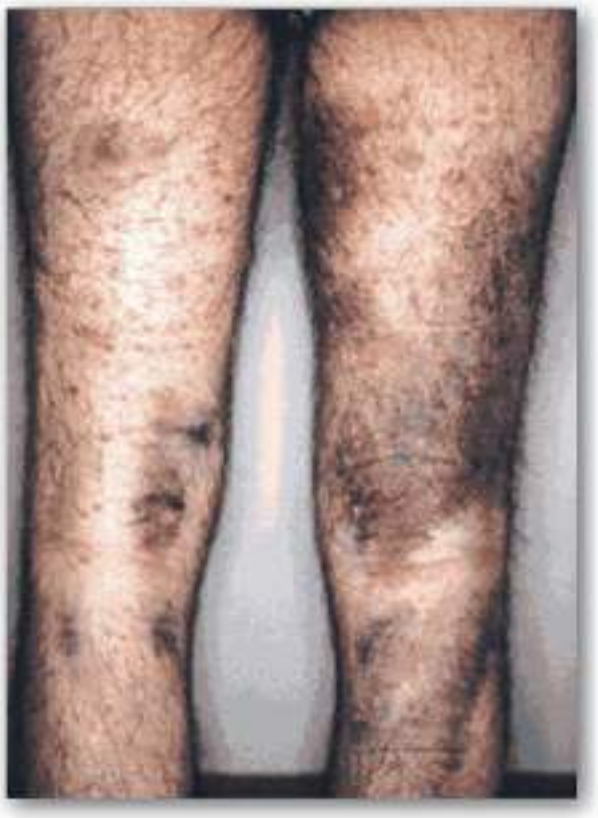


Figure 4.8
The legs of a 46-year-old man with scurvy.

5. Extracellular cleavage of procollagen molecules: After their release, the triple-helical procollagen molecules are cleaved by N- and C-procollagen peptidases, which remove the terminal propeptides, producing tropocollagen molecules.

6. Collagen fibril formation: Tropocollagen molecules spontaneously associate to form collagen fibrils. The fibrils form an ordered, parallel array, with adjacent collagen molecules arranged in a staggered pattern formed by approximately three-quarters of each molecule overlapping the neighboring molecule (Fig. 4.7).

7. Cross-link formation: The array of collagen fibril molecules serves as a substrate for lysyl oxidase. This copper-containing extracellular enzyme oxidatively deaminates some of the lysine and hydroxylysine residues in collagen. The reactive aldehydes (allysine and hydroxyallysine) that result from the deamination reactions can spontaneously condense with lysine or hydroxylysine residues in neighboring collagen molecules to form covalent cross-links and, thus, mature collagen fibers (Fig. 4.9). (Note: Cross-links can also form between two allysine residues.)

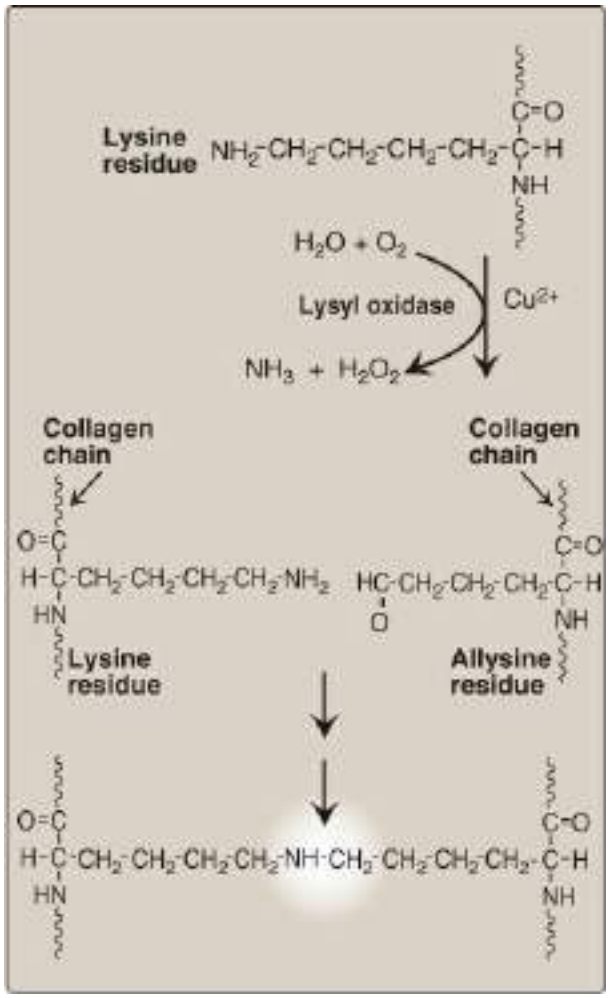


Figure 4.9
Formation of cross-links in collagen. (Note: Lysyl oxidase is irreversibly inhibited by a toxin present in seeds from *Lathyrus odoratus* [sweet pea], leading to a condition known as lathyrism that is characterized by skeletal and vascular problems.) Cu^{2+}, copper; NH_3, ammonia; H_2O_2, hydrogen peroxide.

Lysyl oxidase is one of several copper-containing enzymes. Others include ceruloplasmin (see p. 451), cytochrome c oxidase (see p. 84), dopamine hydroxylase (see p. 318), superoxide dismutase (see p. 163), and tyrosinase (see p. 303). Disruption in copper homeostasis causes copper deficiency (X-linked Menkes syndrome) or overload (Wilson disease) (see p. 449).

D. Degradation

Normal collagens fibers are highly stable molecules, having half-lives as long as several years. However, connective tissue is dynamic and is constantly being remodeled, often in response to growth or injury of the tissue. Breakdown of collagen fibers is dependent on the proteolytic action of collagenases, which are part of a large family of matrix metalloproteinases. For type I collagen, the cleavage

site is specific, generating three-quarter and one-quarter length fragments. These fragments are further degraded by other matrix proteinases.

E. Collagenopathies

Defects in any one of the many steps in collagen fiber synthesis can result in a genetic disease involving an inability of collagen to form fibers properly and, therefore, an inability to provide tissues with the needed tensile strength normally provided by collagen. More than 1,000 mutations have been identified in 23 genes coding for 13 of the collagen types. The following are examples of diseases (collagenopathies) that are the result of defective collagen synthesis.

1. Ehlers–Danlos syndrome: Ehlers–Danlos syndrome (EDS) is a heterogeneous group of connective tissue disorders that result from heritable defects in the metabolism of fibrillar collagen molecules. EDS can be caused by a deficiency of collagen-processing enzymes (e.g., lysyl hydroxylase or N-procollagen peptidase) or from mutations in the amino acid sequences of collagen types I, III, and V. The classic form of EDS, caused by defects in type V collagen, is characterized by skin extensibility and fragility and joint hypermobility (Fig. 4.10). The vascular form, due to defects in type III collagen, is the most serious form of EDS because it is associated with potentially lethal arterial rupture. (Note: The classic and vascular forms show autosomal-dominant inheritance.) Collagen that contains mutant chains may have altered structure, secretion, or distribution, and it frequently is degraded. (Note: Incorporation of just one mutant chain may result in degradation of the triple helix. This is known as a dominant-negative effect.)

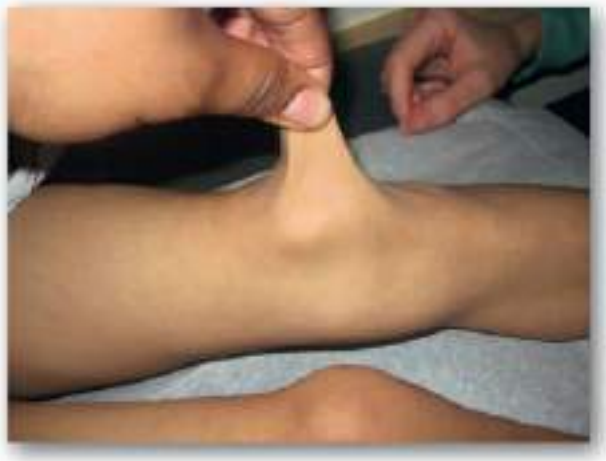


Figure 4.10
Stretchy skin of classic Ehlers–Danlos syndrome and mechanism of bisphosphonates.

2. Osteogenesis imperfecta: This syndrome, known as “brittle bone disease,” is a genetic disorder of bone fragility characterized by bones that fracture easily, with minor or no trauma (Fig. 4.11). Over 80% of cases of osteogenesis

imperfecta (OI) are caused by dominant mutations to the genes that encode the α1 or α2 chains in type I collagen. The most common mutations cause the replacement of glycine (in –Gly–X–Y–) by amino acids with bulky side chains. The resultant structurally abnormal α chains prevent the formation of the required triple-helical conformation. Phenotypic severity ranges from mild to lethal. Type I OI, the most common form, is characterized by mild bone fragility, hearing loss, and blue sclerae. Type II, the most severe form, is typically lethal in the perinatal period as a result of pulmonary complications. *In utero* fractures are seen (Fig. 4.11, left). Type III is also a severe form and is characterized by multiple fractures at birth, short stature, spinal curvature leading to a humped-back (kyphotic) appearance, and blue sclerae. Dentinogenesis imperfecta, a disorder of tooth development, may be seen in OI. OI is treated with bisphosphonates (Fig. 4.11, right), which function by inactivating osteoclasts, the cells that break down bone tissue. Bisphosphonates also increase apoptosis (cell death) of osteoclasts, and therefore inhibit the resorption of bone material. Bisphosphonates also decrease apoptosis of osteoblasts, the cells that lay down new bone matrix.

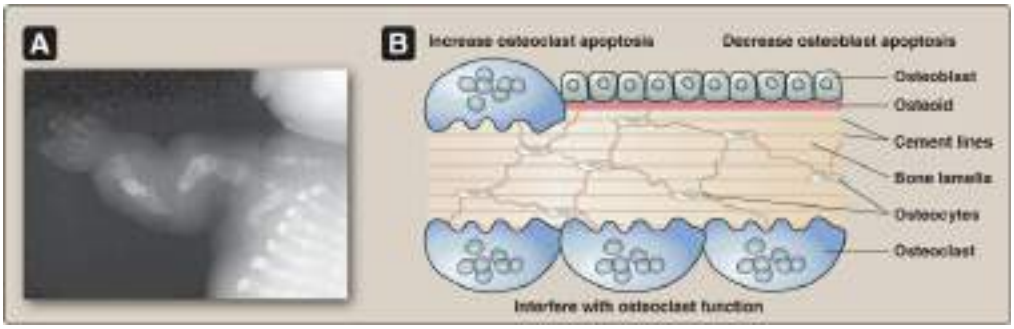


Figure 4.11
A: Lethal form (type II) of osteogenesis imperfecta in which the fractures appear *in utero*, as revealed by this radiograph of a stillborn fetus. B: Mechanism of action of bisphosphonates to treat OI patients. OI, osteogenesis imperfecta.

3. Alport syndrome: This is a group of heterogeneous inherited disorders of basement membranes of the kidney, and frequently the cochlea and the eye, characterized by glomerulonephritis, hematuria, proteinuria, hypertension, and progression to end-stage renal disease (ESRD) and hearing loss during the second to fourth decades of life. This disorder is the result of mutations in type IV collagen genes, with a genetic frequency of approximately 1 case in 5,000. The most common form inherits as X-linked autosomal dominant. The pattern of inheritance and symptoms differ depending on which type IV collagen gene is involved.

III. ELASTIN

In contrast to collagen, which forms fibers that are tough and have high tensile strength,

elastin is a fibrous protein with rubber-like properties found in connective tissue. Elastic fibers composed of elastin and glycoprotein microfibrils are found in the lungs, the walls of large arteries, and elastic ligaments. They can be stretched to several times their normal length but recoil to their original shape when the stretching force is relaxed.

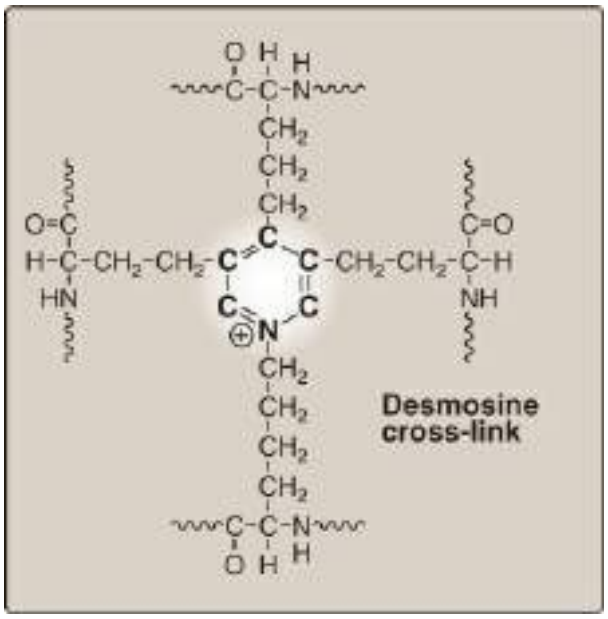


Figure 4.12
Desmosine cross-link unique to elastin.

A. Structure

Elastin is an insoluble protein polymer generated from a precursor, tropoelastin, which is a soluble polypeptide composed of ~700 amino acids that are primarily small and nonpolar (e.g., glycine, alanine, and valine). Elastin is also rich in proline and lysine but contains few hydroxyproline and hydroxylysine. Tropoelastin is secreted by the cell into the ECM. There, it interacts with specific glycoprotein microfibrils, such as fibrillin, which function as a scaffold onto which tropoelastin is deposited. Some of the lysyl side chains of the tropoelastin polypeptides are oxidatively deaminated by lysyl oxidase, forming allysine residues. Three of the allysyl side chains plus one unaltered lysyl side chain from the same or neighboring polypeptides form a desmosine cross-link (Fig. 4.12). This produces elastin, an extensively interconnected, rubbery network that can stretch and bend in any direction when stressed, giving connective tissue elasticity (Fig. 4.13). Mutations in the fibrillin-1 protein are responsible for Marfan syndrome, a connective tissue disorder characterized by impaired structural integrity in the skeleton, the eye, and the cardiovascular system. With this disease, abnormal fibrillin protein is incorporated into microfibrils along with normal fibrillin, inhibiting the formation of functional microfibrils. Patients with Marfan syndrome are frequently tall, with long slender arms, legs, fingers, and toes. They will have flexible joints and may have scoliosis. The heart and aorta are often affected as well, and there is an increased

risk for mitral valve prolapse or aortic aneurysm. (Note: Patients with Marfan syndrome, OI, or EDS may have blue sclerae due to tissue thinning that allows underlying pigment to show through.)

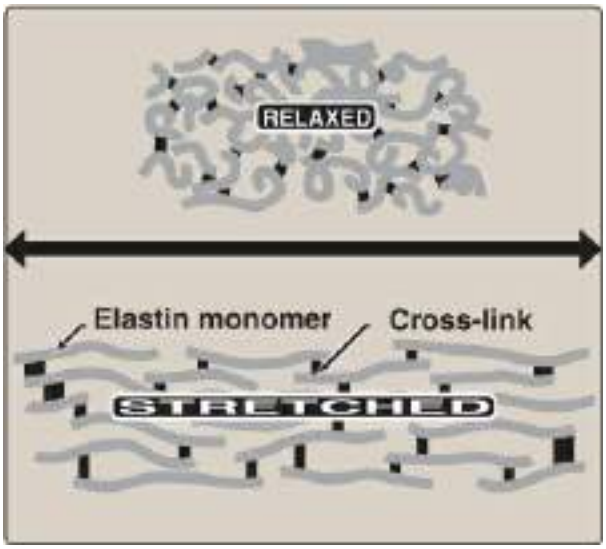


Figure 4.13
Elastin fibers in relaxed and stretched conformations.

B. α1-Antitrypsin in elastin degradation

Blood and other body fluids contain a protein, α_1-antitrypsin (AAT), which inhibits a number of proteolytic enzymes (called peptidases, proteases, or proteinases) that hydrolyze and destroy proteins. (Note: The inhibitor was originally named AAT because it inhibits the activity of trypsin, a proteolytic enzyme synthesized as trypsinogen by the pancreas [see p. 274].) AAT has the important physiologic role of inhibiting neutrophil elastase, a powerful protease that is released into the extracellular space and degrades elastin of alveolar walls as well as other structural proteins in a variety of tissues (Fig. 4.14). Most of the AAT found in plasma is synthesized and secreted by the liver. Extrahepatic synthesis also occurs.

1. α1-Antitrypsin in the lungs: In the normal lung, the alveoli are chronically exposed to low levels of neutrophil elastase released from activated and degenerating neutrophils. The proteolytic activity of elastase can destroy the elastin in alveolar walls if unopposed by the action of AAT, the most important inhibitor of neutrophil elastase (Fig. 4.14). Because lung tissue cannot regenerate, the destruction of the connective tissue of alveolar walls caused by an imbalance between the protease and its inhibitor results in pulmonary disease.

2. α1-Antitrypsin deficiency and emphysema: In the United States, ~2% to 5% of patients with emphysema are predisposed to the disease by inherited defects in AAT. A number of different mutations in the gene for AAT are known to cause a

deficiency of the protein, but one single purine base mutation (GAG to AAG, resulting in the substitution of lysine for glutamic acid at position 342 of the protein) is clinically the most widespread and severe. (Note: The mutated protein is termed the Z variant.) The mutation causes the normally monomeric AAT protein to misfold, polymerize, and aggregate within the RER of hepatocytes, resulting in decreased secretion of AAT by the liver. AAT deficiency is, therefore, a misfolded protein disease. (Note: The polymer that accumulates in hepatocytes may result in cirrhosis. Such hepatic damage is a leading cause for pediatric end-stage liver failure, which requires liver transplantation.) Because less AAT is secreted by the liver, blood levels of AAT are reduced, as is the amount of AAT that is available to lung tissues. In the United States, the AAT mutation is most common in Caucasians of Northern European ancestry. An individual must inherit two abnormal AAT alleles to be at risk for the development of emphysema. In a heterozygote, with one normal and one defective allele, the levels of AAT are sufficient to protect the alveoli from damage. (Note: Methionine 358 in AAT is required for the binding of the inhibitor to its target *proteases*. Smoking causes the oxidation and subsequent inactivation of the methionine, thereby rendering the inhibitor powerless to neutralize *elastase*. Smokers with AAT deficiency, therefore, have a considerably elevated rate of lung destruction and a poorer survival rate than nonsmokers with the deficiency.) The deficiency of *elastase* inhibitor can be treated by weekly augmentation therapy, that is, intravenous administration of AAT. The AAT diffuses from the blood into the lung, where it reaches therapeutic levels in the fluid surrounding the lung epithelial cells.

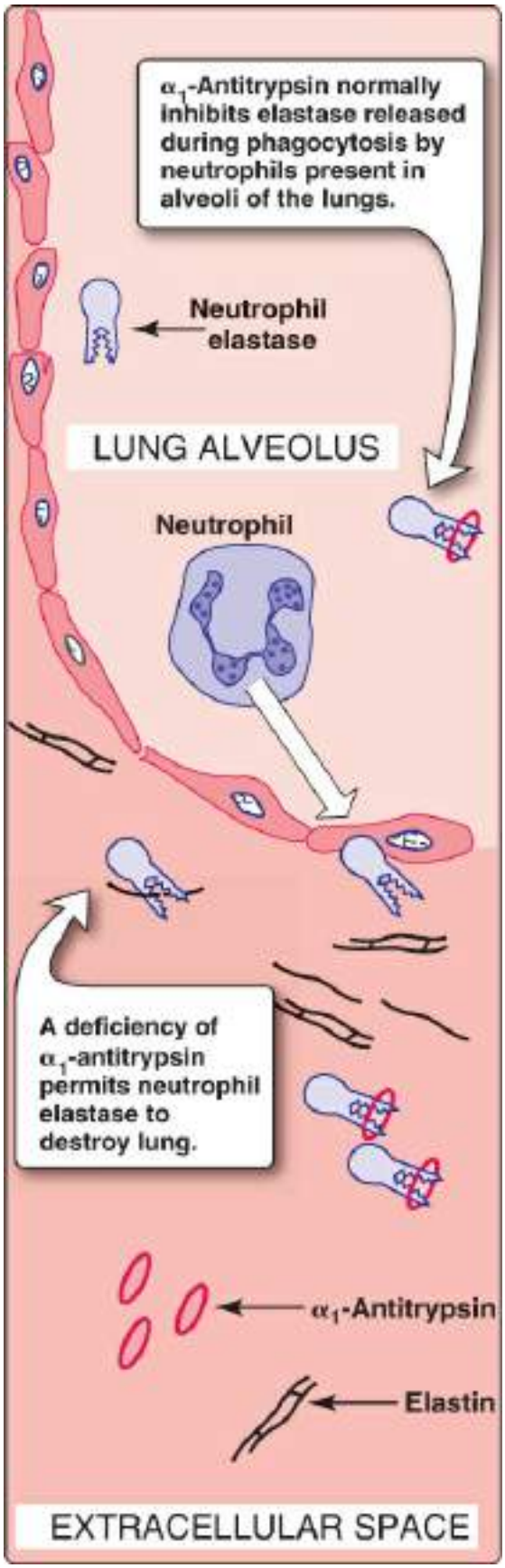


Figure 4.14
Destruction of alveolar tissue by elastase released from neutrophils activated as part of the immune response to air-borne pathogens.

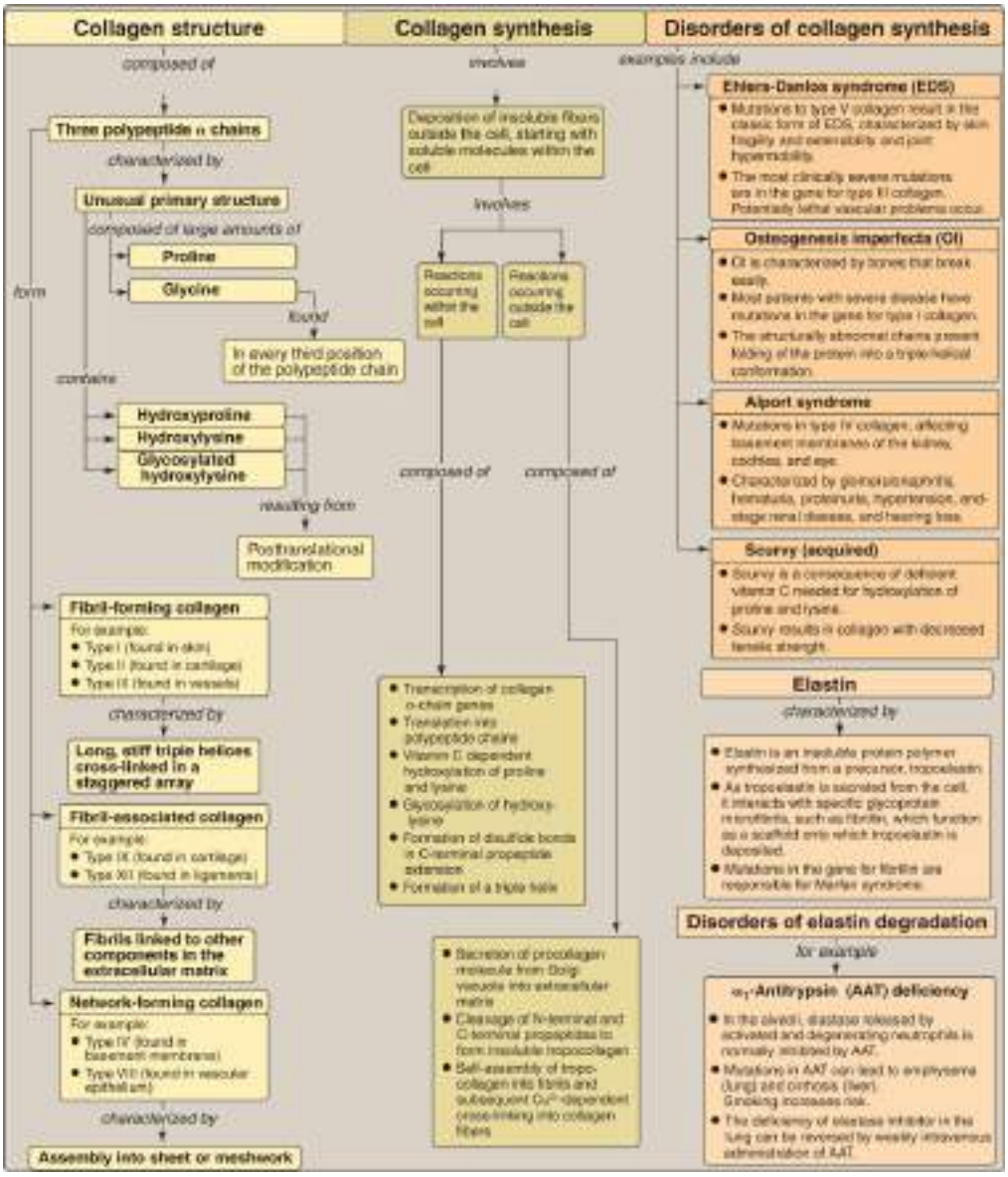


Figure 4.15
Key concept map for the fibrous proteins collagen and elastin. Cu^{2+}, copper.

IV. Chapter Summary

- Collagen and elastin are structural fibrous proteins of the extracellular matrix (Fig. 4.15).
- **Collagen** contains an abundance of **proline**, **lysine**, and **glycine**, the latter occurring at every third position in the primary structure. It also contains **hydroxyproline**, **hydroxylysine**, and **glycosylated hydroxylysine**, each formed by posttranslational modification.
- Fibrillar collagen has a long, rigid structure, in which three collagen polypeptide α chains are wound around one another in a rope-like **triple helix** stabilized by **interchain hydrogen bonds**. Diseases of fibrillar collagen synthesis affect bones, joints, skin, and blood vessels.
- **Elastin** is a connective tissue protein with rubber-like properties in tissues such as the lung. **α_1-antitrypsin** (**AAT**), produced primarily by the liver, inhibits **elastase**-catalyzed degradation of elastin in the alveolar walls. A deficiency of AAT increases elastin degradation and can cause **emphysema** and, in some cases, **cirrhosis** of the liver.

Study Questions

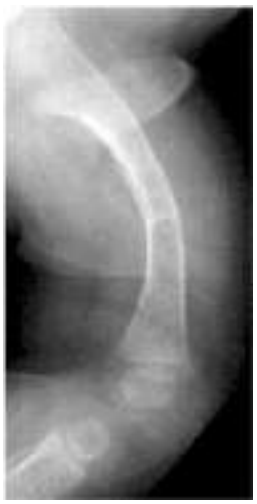
Choose the ONE best answer.

4.1 A 30-year-old female of Northern European ancestry presents with progressive dyspnea (shortness of breath). She denies the use of cigarettes. Family history reveals that her sister also has problems with her lungs. Which one of the following etiologies most likely explains this patient's pulmonary symptoms?

A. Deficiency in dietary vitamin C
B. Deficiency of α_1-antitrypsin
C. Deficiency of prolyl hydroxylase
D. Decreased elastase activity
E. Increased collagenase activity

Correct answer = B. α_1-Antitrypsin (AAT) deficiency is a genetic disorder that can cause pulmonary damage and emphysema even in the absence of cigarette use. A deficiency of AAT permits increased elastase activity to destroy elastin in the alveolar walls. AAT deficiency should be suspected when chronic obstructive pulmonary disease develops in a patient younger than age 45 years who does not have a history of chronic bronchitis or tobacco use or when multiple family members develop obstructive lung disease at an early age. Choices A, C, and E refer to collagen, not elastin.

4.2 A 7-month-old child "fell over" while crawling and now presents with a swollen leg. Imaging reveals a fracture of a bowed femur, secondary to minor trauma, and thin bones (see x-ray at right). Blue sclerae are also noted. At age 1 month, the infant had multiple fractures in various states of healing (right clavicle, right humerus, and right radius). A careful family history has ruled out nonaccidental trauma (child abuse) as a cause of the bone fractures. Which pairing of a defective (or deficient) molecule and the resulting pathology best fits this clinical description?



A. Elastin and emphysema
B. Fibrillin and Marfan disease
C. Type I collagen and osteogenesis imperfecta
D. Type V collagen and Ehlers–Danlos syndrome
E. Vitamin C and scurvy

Correct answer = C. The child most likely has osteogenesis imperfecta. Most cases arise from a defect in the genes encoding type I collagen. Bones in affected patients are thin, osteoporotic, often bowed, and extremely prone to fracture. Pulmonary problems are not seen in this child. Individuals with Marfan syndrome have impaired structural integrity of the skeleton, eyes, and cardiovascular system. Defects in type V collagen cause the classic form of Ehlers–Danlos syndrome characterized by skin extensibility and fragility and joint hypermobility. Scurvy caused by vitamin C deficiency is characterized by capillary fragility.

4.3 What is the differential basis of the liver and lung pathology seen in α_1-antitrypsin deficiency?

With α_1-antitrypsin (AAT) deficiency, the cirrhosis that can result is due to polymerization and retention of AAT in the liver, its site of synthesis. The alveolar damage is due to the retention-based deficiency of AAT (a serine protease inhibitor) in the lung such that elastase (a serine protease) is unopposed.

4.4 How and why is proline hydroxylated in collagen?

Proline is hydroxlyated by prolyl hydroxylase, an enzyme of the endoplasmic reticulum that requires oxygen, ferrous iron, and vitamin C. Hydroxylation increases interchain hydrogen bond formation, strengthening the triple helix of collagen. Vitamin C deficiency impairs hydroxylation.

4.5 A 60-year-old homeless male presents to the emergency room complaining of progressive fatigue, leg pain, and generalized weakness. He has bloody stools, shortness of breath, easy bruising, leg swelling, and a red rash on his arms and legs. He is taking no medications. On further questioning he reveals that his diet consists entirely of bread, canned meat, and beer. Closer examination of the rashes on his legs reveals corkscrew hairs and subepidermal red blood cell extravasation surrounding the hair follicles. What is the underlying problem in this patient?

A. Mutation of type V collagen
B. Mutation of type I collagen
C. Decreased prolyl hydroxylase and lysyl hydroxylase activity
D. Decreased circulating AAT levels
E. Mutation of fibrillin

Correct answer = C. The patient has scurvy, caused by a vitamin C deficiency. Vitamin C is required for prolyl hydroxylase and lysyl hydroxylase activity. Hydroxylation of proline and lysine residues in the –Gly-X-Y- sequence of collagen is essential for interchain H-bond formation and a stable collagen triple helix. A mutation in type V collagen is characteristic of EDS. A mutation in type I collagen is characteristic of OI. Decreased circulating AAT levels are the basis of AAT deficiency, which results in possible pulmonary damage and emphysema symptoms, or pediatric end-stage liver failure. A mutation in fibrillin is characteristic of Marfan syndrome.

Enzymes 5

I. OVERVIEW

Virtually all reactions in the body are mediated by enzymes, which are protein catalysts, usually within cells, that increase the rate of reactions without being changed in the overall process. Among the many biologic reactions that are energetically possible, enzymes selectively channel reactants or substrates, into useful pathways. Thus, enzymes direct all metabolic events. This chapter examines the nature of these catalytic molecules and their mechanisms of action.

II. NOMENCLATURE

Each enzyme is assigned two names. The first is its short, recommended name, convenient for everyday use. The second is the more complete systematic name, which is used when an enzyme must be identified without ambiguity.

A. Recommended name

Most commonly used enzyme names have the suffix “-ase” attached to the substrate of the reaction, such as glucosidase and urease. Names of other enzymes include a description of the action performed, for example, lactate dehydrogenase (LDH) and adenylyl cyclase. Some enzymes retain their original trivial names, which give no hint of the associated enzymatic reaction, for example, trypsin and pepsin.

B. Systematic name

In the systematic naming system, enzymes are divided into six major classes (Fig. 5.1), each with numerous subgroups. For a given enzyme, the suffix -ase is attached to a fairly complete description of the chemical reaction catalyzed, including the names of all the substrates, for example, lactate:nicotinamide adenine dinucleotide (NAD^+) oxidoreductase. (Note: Each enzyme is also assigned a classification number. Lactate:NAD^+ oxidoreductase is 1.1.1.27.) The systematic names are unambiguous and informative but are frequently too cumbersome to be of general use.

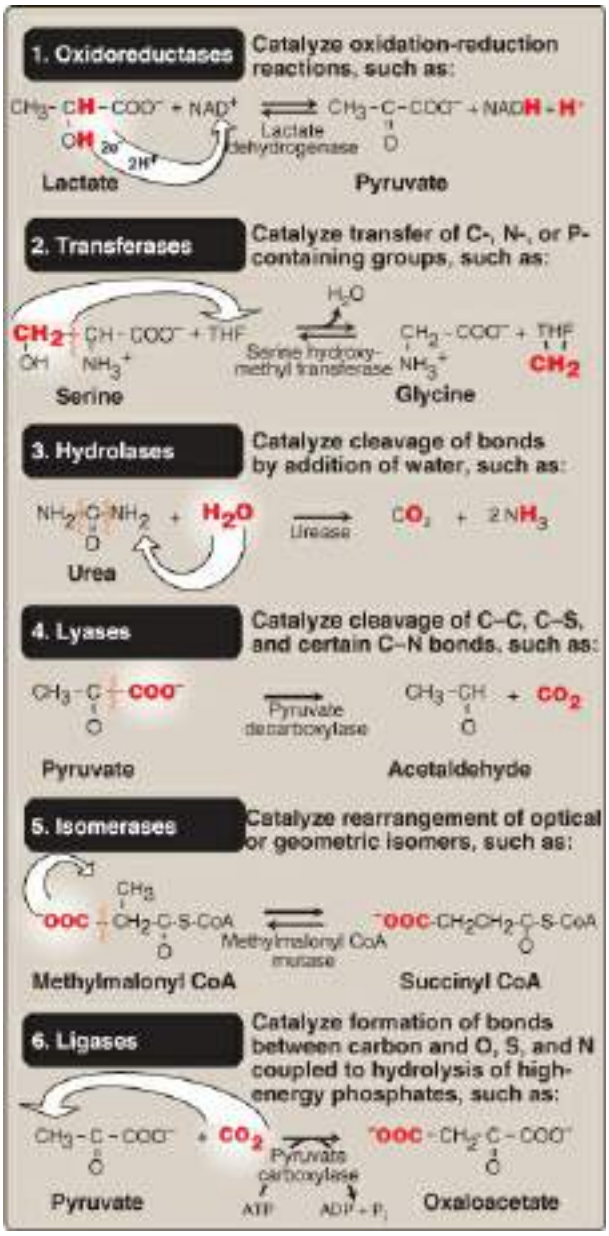


Figure 5.1
The six major classes of enzymes with examples. NAD(H), nicotinamide adenine dinucleotide; THF, tetrahydrofolate; CoA, coenzyme A; CO_2, carbon dioxide; NH_3, ammonia; ADP, adenosine diphosphate; P_i, inorganic phosphate.

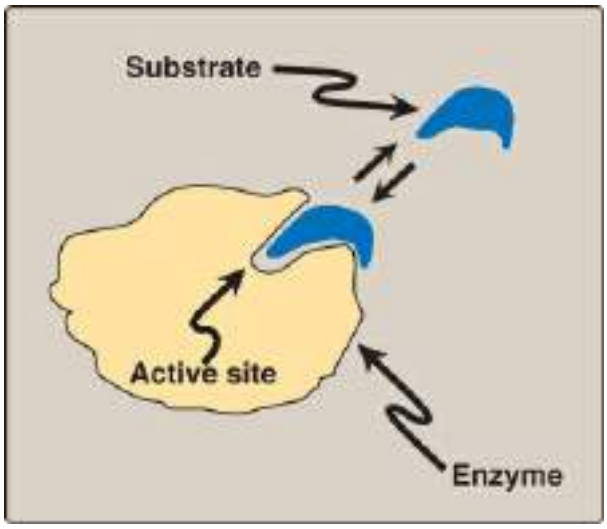


Figure 5.2
Schematic representation of an enzyme with one active site binding a substrate molecule.

Potentially confusing enzyme nomenclature includes enzymes with similar names but different functions or mechanisms. For example, synthetases require ATP, while synthases do not require ATP. Phosphatases use water to remove a phosphate group, while phosphorylases use inorganic phosphate to break a bond and generate a phosphorylated product. Dehydrogenases (using NAD^+ or flavin adenine dinucleotide, FAD) accept electrons in a redox reaction. Oxidases use oxygen as the acceptor, with no oxygen atoms incorporated into the substrate, while oxygenases do incorporate oxygen atoms into their substrates.

III. PROPERTIES

An enzyme is an efficient, specific protein catalyst that combines with a substrate at the enzyme active site and performs chemistry on that substrate to convert it to product. Without enzymes most biochemical reactions would not occur quickly enough to have physiologic importance in the human body. While enzymes increase the velocity of a chemical reaction they are not consumed during the reaction. (Note: Some ribonucleic acids [RNAs] can catalyze reactions that affect phosphodiesterase and peptide bonds. RNAs with catalytic activity are called ribozymes and are much less common than protein catalysts.)

A. Active site

Enzyme molecules contain a special pocket or cleft called the active site which is formed by folding of the protein. The active site contains amino acid residues whose side chains participate in substrate binding and catalysis (Fig. 5.2). The substrate first binds the enzyme, forming an enzyme–substrate (ES) complex. Binding is thought to cause a conformational change in the enzyme (induced fit model) that allows a rapid conversion of the ES to enzyme–product (EP) complex that subsequently dissociates to free enzyme and product.

B. Efficiency

Enzyme-catalyzed reactions are highly efficient, proceeding from 10^3 to 10^8 times faster than uncatalyzed reactions. The number of substrate molecules converted to product per enzyme molecule per second is called the turnover number, or k_{cat}, and typically is 10^2 to 10^4 second^{-1}. (Note: k_{cat} is the rate constant for the conversion of ES to E + P.)

C. Specificity

Enzymes are highly specific and are capable of interacting with one or a very few substrates and can catalyze only one type of chemical reaction. The set of enzymes synthesized within a cell determines which reactions occur in that cell.

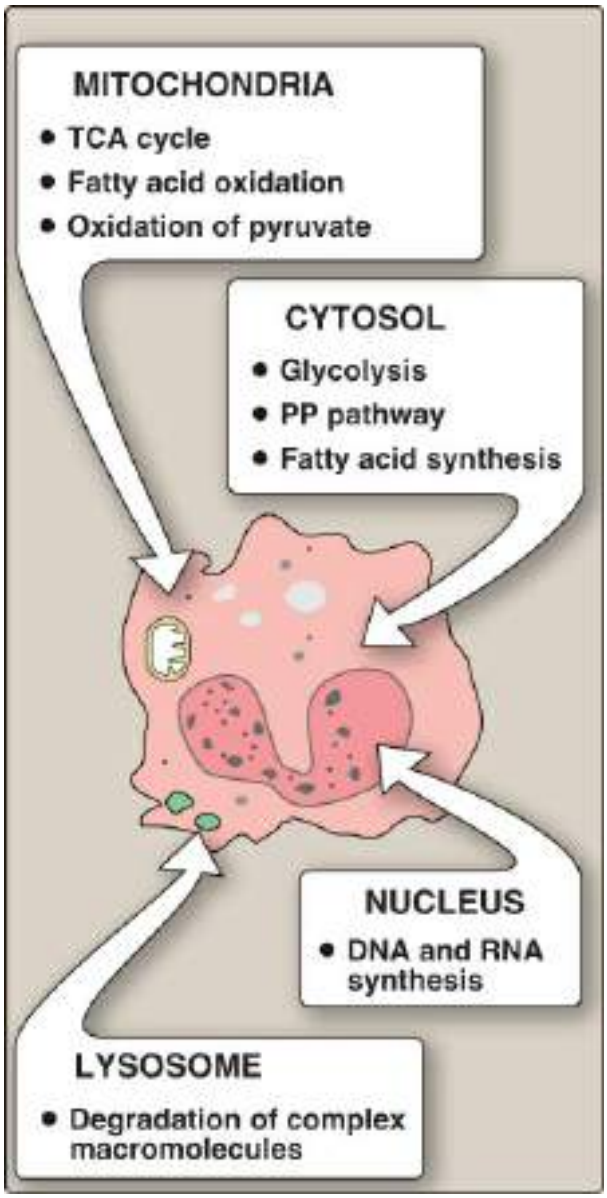


Figure 5.3
The intracellular location of some important biochemical pathways. TCA, tricarboxylic acid; PP, pentose phosphate.

D. Holoenzymes, apoenzymes, cofactors, and coenzymes

Some enzymes require nonprotein components to have enzymatic activity. The term **holoenzyme** refers to the protein component of the enzyme along with its nonprotein component, whereas the enzyme without its nonprotein moiety is termed an **apoenzyme** and is inactive. For enzymes that require nonprotein components, those components must be present for the enzyme to function in catalysis.

If the nonprotein moiety is a metal ion, such as zinc (Zn^{2+}) or iron (Fe^{2+}), it is called a **cofactor**. If it is a small organic molecule, it is termed a **coenzyme.** Coenzymes or cosubstrates only transiently associate with the enzyme and dissociate from the enzyme in an altered state (for example, NAD^+). If the coenzyme is permanently associated with the enzyme and returned to its original form, it is called a **prosthetic group** (for example, FAD). Coenzymes commonly are derived from vitamins. For example, NAD^+ contains niacin, and FAD contains riboflavin.

E. Regulation

Enzyme activity can often be increased or decreased, so that the rate of product formation responds to the present cellular needs.

F. Location within the cell

Most enzymes function inside cells, within the confines of plasma membranes. Many enzymes are localized in specific organelles within the cell (Fig. 5.3). Such compartmentalization serves to isolate the reaction substrate or product from other competing reactions. This provides a favorable environment for the reaction and organizes the thousands of enzymes present in the cell into purposeful pathways.

IV. MECHANISM OF ENZYME ACTION

The mechanism of enzyme action can be viewed from two different perspectives. The first treats catalysis in terms of energy changes that occur during the reaction. That is, enzymes provide an alternate, energetically favorable reaction pathway different from the uncatalyzed reaction. The second perspective describes how the active site chemically facilitates catalysis.

A. Energy changes occurring during the reaction

Virtually all chemical reactions have an energy barrier separating the reactants and the products. This barrier, called the activation energy (E_a), is the energy difference between that of the reactants and a high-energy intermediate, the transition state

(T*), which is formed during the conversion of reactant to product. Figure 5.4 shows the changes in energy during the conversion of a molecule of reactant A to product B as it proceeds through the transition state.

$$A \rightleftarrows T^* \rightleftarrows B$$

1. Activation energy: The peak of energy in Figure 5.4 is the difference in free energy between the reactant and T*, in which the high-energy, short-lived intermediate is formed during the conversion of reactant to product. Because of the high E_a, the rates of uncatalyzed chemical reactions are often slow.

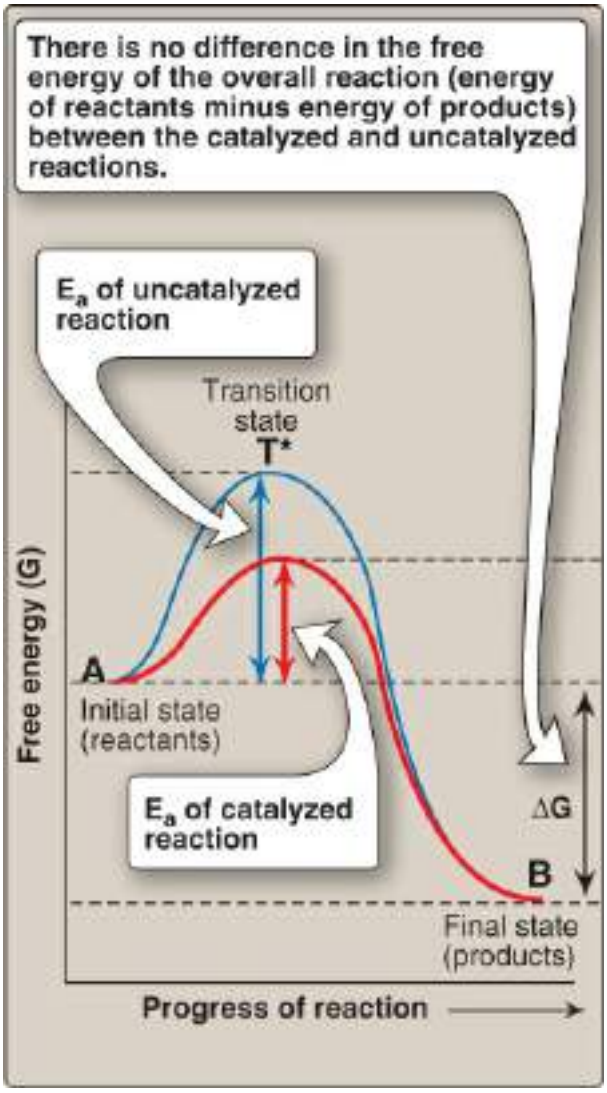


Figure 5.4
Effect of an enzyme on the activation energy (E_a) of a reaction. ΔG, change in free energy.

2. Rate of reaction: For molecules to react, they must contain sufficient energy to overcome the energy barrier of the transition state. In the absence of an enzyme, only a small proportion of a population of molecules may possess

enough energy to achieve the transition state between reactant and product. The rate of reaction is determined by the number of such energized molecules. In general, the lower the E_a, the more molecules have sufficient energy to pass through the transition state and, therefore, the faster the rate of the reaction.

3. Alternate reaction pathway: An enzyme allows a reaction to proceed rapidly under conditions prevailing in the cell by providing an alternate reaction pathway with a lower E_a (see Fig. 5.4). The enzyme does not change the free energies of the reactants (substrates) or products and, therefore, does not change the equilibrium of the reaction. It does, however, accelerate the rate by which equilibrium is reached.

B. Active site chemistry

The active site is not a passive receptacle for binding the substrate but, rather, is a complex molecular machine that can employs diverse chemical mechanisms to facilitate the conversion of substrate to product. A number of factors are responsible for the catalytic efficiency of enzymes, including the following examples.

1. Transition-state stabilization: The active site often acts as a flexible molecular template that binds the substrate and initiates its conversion to the transition state, a structure in which the bonds are not like those in the substrate or the product (see T* at the top of the curve in Fig. 5.4). By stabilizing the transition state, the enzyme greatly increases the concentration of the reactive intermediate that can be converted to product and, thus, accelerates the reaction. (Note: The transition state cannot be isolated.)

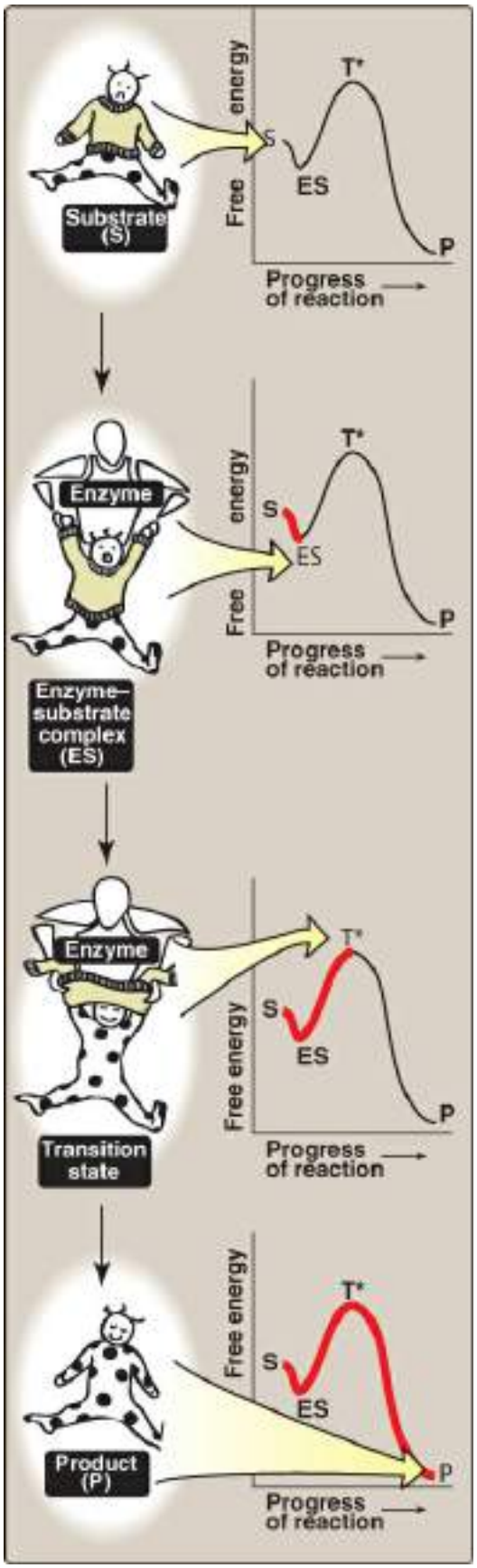


Figure 5.5
Schematic representation of energy changes accompanying formation of an enzyme–substrate complex and subsequent formation of a transition state.

2. Catalysis: The active site can provide catalytic groups that enhance the probability that the transition state is formed. In some enzymes, these groups can participate in general acid–base catalysis in which amino acid residues provide or accept protons. In other enzymes, catalysis may involve the transient formation of a covalent ES complex.

> The mechanism of action of chymotrypsin, an enzyme of protein digestion in the intestine, includes general base, general acid, and covalent catalysis. A histidine at the active site of the enzyme gains (general base) and loses (general acid) protons, mediated by the pK of histidine in proteins being close to physiologic pH. Serine at the active site forms a transient covalent bond with the substrate.

3. Transition-state visualization: The enzyme-catalyzed conversion of substrate to product can be depicted as being similar to removing a sweater (chemical group) from an uncooperative infant (substrate) (Fig. 5.5). The process has a high E_a because the only reasonable strategy for removing the garment requires that both arms being fully extended over the head, an unlikely posture to be adopted without a catalyst. We can envision a parent acting as an enzyme, first coming in contact with the baby (forming ES) and then guiding the baby's arms into an extended, vertical position, analogous to the transition state. This posture (conformation) of the baby facilitates the removal of the sweater, forming the disrobed baby, which represents product. (Note: The substrate bound to the enzyme [ES] is at a slightly lower energy than unbound substrate [S] and explains the small dip in the curve at ES.)

V. FACTORS AFFECTING REACTION VELOCITY

Enzymes can be isolated from cells and their properties studied in a test tube, that is, *in vitro*. Different enzymes show different responses to changes in substrate concentration, temperature, and pH. This section describes factors that influence the reaction velocity of enzymes. Enzymatic responses to these factors give us valuable clues as to how enzymes function in living cells, that is, *in vivo*.

A. Substrate concentration

1. Maximal velocity: The rate or velocity of a reaction (v) is the number of substrate molecules converted to product per unit time. Velocity is usually expressed as μmol of product formed per second. The rate of an enzyme-catalyzed reaction increases with substrate concentration until a maximal velocity (V_{max}) is reached (Fig. 5.6). The leveling off of the reaction rate at high substrate concentrations

reflects the saturation with substrate of all available binding sites on the enzyme molecules present.

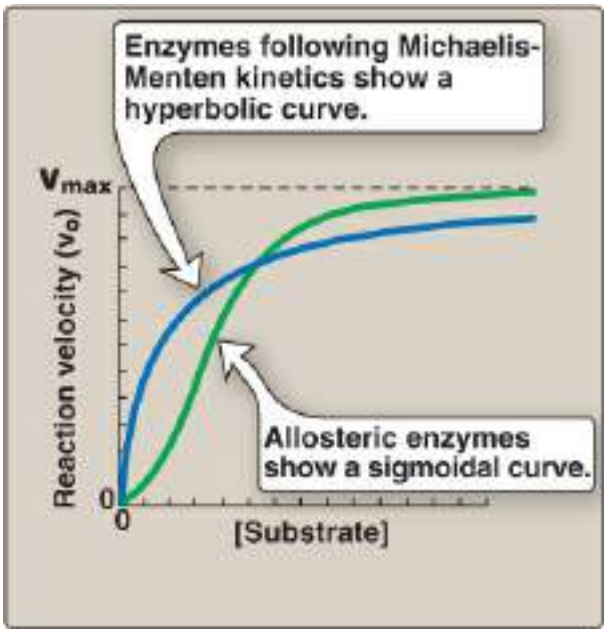


Figure 5.6
Effect of substrate concentration on reaction velocity.

2. Shape of the enzyme kinetics curve: Most enzymes follow Michaelis–Menten kinetics (see p. 62), in which a plot of initial reaction velocity (v_o) against substrate concentration is hyperbolic (similar in shape to that of the oxygen-dissociation curve of myoglobin; see Chapter 3). In contrast, allosteric enzymes do not follow Michaelis–Menten kinetics and instead show a sigmoidal curve (see Fig. 5.6) that is similar in shape to the oxygen-dissociation curve of hemoglobin.

B. Temperature

1. Velocity increase with temperature: The reaction velocity increases with temperature until a peak velocity is reached (Fig. 5.7). This increase is the result of the increased number of substrate molecules having sufficient energy to pass over the energy barrier and form the products of the reaction.

2. Velocity decrease with higher temperature: Further elevation of the temperature causes a decrease in reaction velocity as a result of temperature-induced denaturation of the enzyme (see Fig. 5.7).

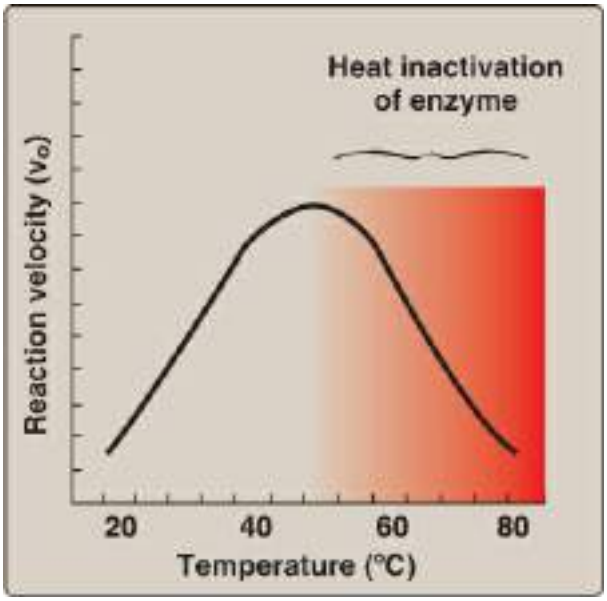


Figure 5.7
Effect of temperature on an enzyme-catalyzed reaction.

Normal body temperature is 37°C. The optimum temperature for most human enzymes is between 35° and 40°C. Human enzymes start to denature at temperatures above 40°C, but thermophilic bacteria found in hot springs have optimum temperatures of 70°C.

C. pH

1. pH effect on active site ionization: The concentration of protons ($[H^+]$) affects reaction velocity in several ways. First, the catalytic process usually requires that the enzyme and substrate have specific chemical groups in either an ionized or unionized state in order to interact. For example, catalytic activity may require that an amino group of the enzyme be in the protonated form ($-NH_3^+$). Because this group is deprotonated at alkaline pH, the rate of the reaction declines.

2. pH effect on enzyme denaturation: Extremes of pH can also lead to denaturation of the enzyme, because the structure of the catalytically active protein molecule depends on the ionic character of the amino acid side chains.

3. Variable pH optimum: The pH at which maximal enzyme activity is achieved is different for different enzymes and often reflects the $[H^+]$ at which the enzyme functions in the body. For example, pepsin, a digestive enzyme in the stomach, is maximally active at pH 2, whereas other enzymes, designed to work at neutral pH, are denatured by such an acidic environment (Fig. 5.8).

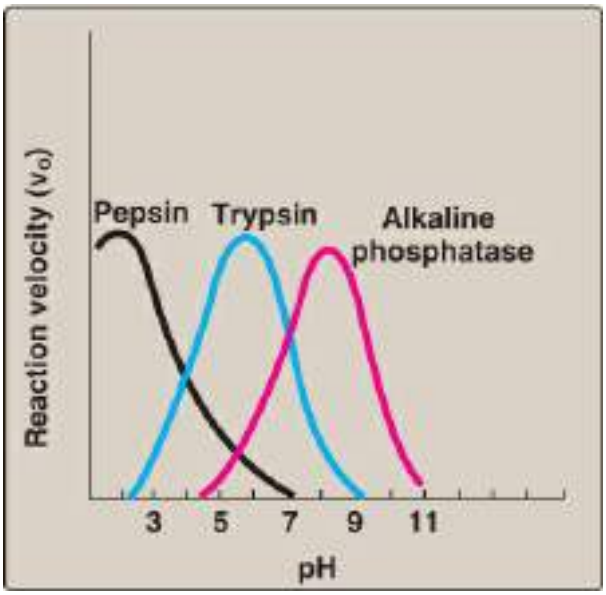


Figure 5.8
Effect of pH on enzyme-catalyzed reactions.

VI. MICHAELIS–MENTEN KINETICS

In a paper published in 1913, Leonor Michaelis and Maud Menten proposed a model that accounts for most features of many enzyme-catalyzed reactions. In this model, the enzyme reversibly combines with its substrate to form an ES complex that subsequently yields product, regenerating the free enzyme. The reaction model, involving one substrate molecule, is represented below:

$$E + S \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} ES \xrightarrow{K_2} E + P$$

Where S is the substrate;
E is the enzyme;
ES is the enzyme–substrate complex;
P is the product;
k_1, k_{-1}, and k_2 (or, k_{cat}) are rate constants.

A. Michaelis–Menten equation

The Michaelis–Menten equation describes how reaction velocity varies with substrate concentration:

$$V_o = \frac{V_{max}[S]}{K_m + [S]}$$

Where v_o = initial reaction velocity;
V_{max} = maximal velocity = $k_{cat}[E]_{Total}$;

K_m = Michaelis constant = $(k_{-1} + k_2)/k_1$;
[S] = substrate concentration;

The following assumptions are made in deriving the Michaelis–Menten rate equation.

1. Enzyme and substrate relative concentrations: The substrate concentration ([S]) is much greater than the concentration of enzyme so that the percentage of total substrate bound by the enzyme at any one time is small.
2. Steady-state assumption: The concentration of the ES complex does not change with time (the steady-state assumption), that is, the rate of formation of ES is equal to that of the breakdown of ES (to E + S and to E + P). In general, an intermediate in a series of reactions is said to be in steady state when its rate of synthesis is equal to its rate of degradation.
3. Initial velocity: Initial reaction velocities (v_o) are used in the analysis of enzyme reactions. This means that the rate of the reaction is measured as soon as enzyme and substrate are mixed. At that time, the concentration of product is very small, and therefore, the rate of the reverse reaction from product to substrate can be ignored.

B. Important conclusions

1. K_m characteristics: K_m, the Michaelis constant, is characteristic of an enzyme and its particular substrate and reflects the affinity of the enzyme for that substrate. K_m is numerically equal to the substrate concentration at which the reaction velocity is equal to one half V_{max}. K_m does not vary with enzyme concentration.
    a. Small K_m: A numerically small (low) K_m reflects a high affinity of the enzyme for substrate, because a low concentration of substrate is needed to half-saturate the enzyme—that is, to reach a velocity that is one half V_{max} (Fig. 5.9).
    b. Large K_m: A numerically large (high) K_m reflects a low affinity of enzyme for substrate because a high concentration of substrate is needed to half saturate the enzyme.
2. Velocity relationship to enzyme concentration: The rate of the reaction is directly proportional to the enzyme concentration because [S] is not limiting. For example, if the enzyme concentration is halved, the initial rates of the reaction (v_o) and that of V_{max} are reduced to half that of the original.

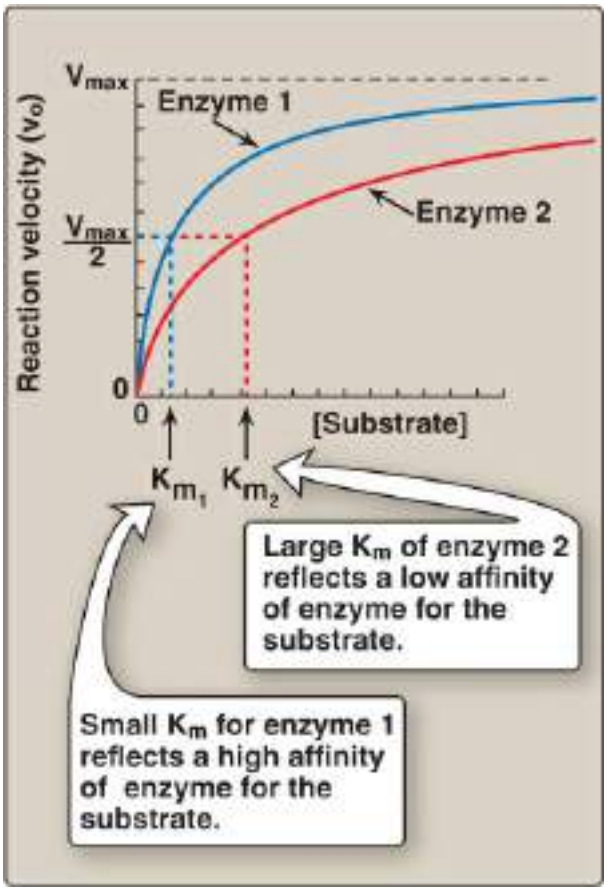


Figure 5.9
Effect of substrate concentration on reaction velocities for two enzymes: enzyme 1 with a small Michaelis constant (K_m) and enzyme 2 with a large K_m. V_{max}, maximal velocity.

3. Reaction order: When [S] is much less (<<) than K_m, the velocity of the reaction is approximately proportional to the substrate concentration (Fig. 5.10). The rate of reaction is then said to be first order with respect to substrate. When [S] is much greater (>>) than K_m, the velocity is constant and equal to V_{max}. The rate of reaction is then independent of substrate concentration because the enzyme is saturated with substrate and is said to be zero order with respect to substrate concentration (see Fig. 5.10).

C. Lineweaver–Burk plot

When v_o is plotted against [S], it is not always possible to determine when V_{max} has been achieved because of the gradual upward slope of the hyperbolic curve at high substrate concentrations. However, as Hans Lineweaver and Dean Burk first described in 1934, if $1/v_o$ is plotted versus 1/[S], then a straight line is obtained (Fig. 5.11). This plot, the Lineweaver–Burk plot, also called a double-reciprocal plot, can be used to calculate K_m and V_{max} as well as to determine the mechanism of action of enzyme inhibitors.

The equation describing the Lineweaver–Burk plot is:

$$\frac{1}{V_o} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$

where the intercept on the x axis is equal to $-1/K_m$, and the intercept on the y axis is equal to $1/V_{max}$. (Note: The slope = K_m/V_{max}.)

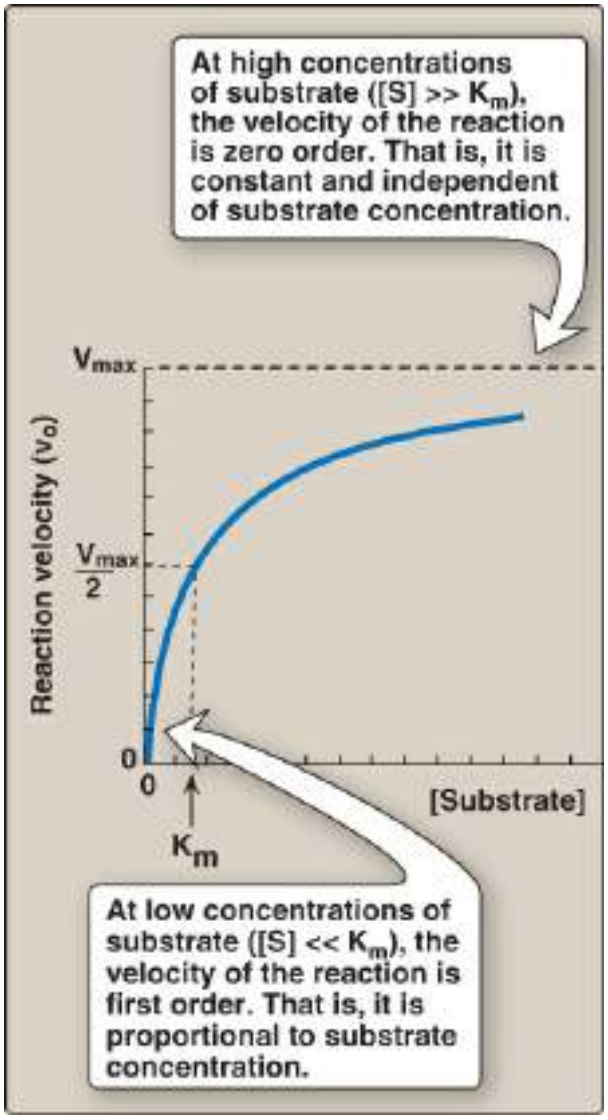


Figure 5.10
Effect of substrate concentration on reaction velocity for an enzyme-catalyzed reaction. V_{max}, maximal velocity; K_m, Michaelis constant.

VII. ENZYME INHIBITION

Any substance that can decrease the velocity of an enzyme-catalyzed reaction is considered to be an **inhibitor**. Inhibitors can be reversible or irreversible. Irreversible

inhibitors bind to enzymes through covalent bonds. Lead, for example, can act as an irreversible inhibitor of some enzymes. It forms covalent bonds with the sulfhydryl side chain of cysteine in proteins. Ferrochelatase, an enzyme involved in heme synthesis, is irreversibly inhibited by lead. Reversible inhibitors bind to enzymes through noncovalent bonds forming an enzyme–inhibitor complex. Dilution of the enzyme–inhibitor complex results in dissociation of the reversibly bound inhibitor and recovery of enzyme activity. The two most commonly encountered types of reversible inhibition are competitive and noncompetitive.

A. Competitive inhibition

This type of inhibition occurs when the inhibitor binds reversibly to the same site that the substrate would normally occupy and, therefore, competes with the substrate for binding to the enzyme active site.

1. Effect on V_{max}: The effect of a competitive inhibitor is reversed by increasing the concentration of substrate. At a sufficiently high [S], the reaction velocity reaches the V_{max} observed in the absence of inhibitor, that is, V_{max} is unchanged in the presence of a competitive inhibitor (Fig. 5.12).
2. Effect on K_m: A competitive inhibitor increases the apparent K_m for a given substrate. This means that, in the presence of a competitive inhibitor, more substrate is needed to achieve half V_{max}.

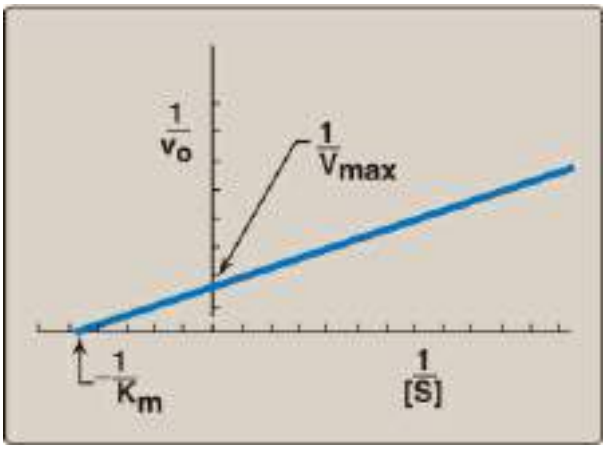


Figure 5.11
Lineweaver–Burk plot. v_o, initial reaction velocity; V_{max}, maximal velocity; K_m, Michaelis constant; [S], substrate concentration.

3. Effect on the Lineweaver–Burk plot: Competitive inhibition shows a characteristic Lineweaver–Burk plot in which the plots of the inhibited and uninhibited reactions intersect on the y axis at $1/V_{max}$ (V_{max} is unchanged). The inhibited and uninhibited reactions show different x-axis intercepts, indicating that the apparent K_m is increased in the presence of the competitive inhibitor

because $-1/K_m$ moves closer to zero from a negative value (see Fig. 5.12). (Note: An important group of competitive inhibitors are the transition state analogs, stable molecules that approximate the structure of the transition state, and, therefore, bind the enzyme more tightly than does the substrate.)

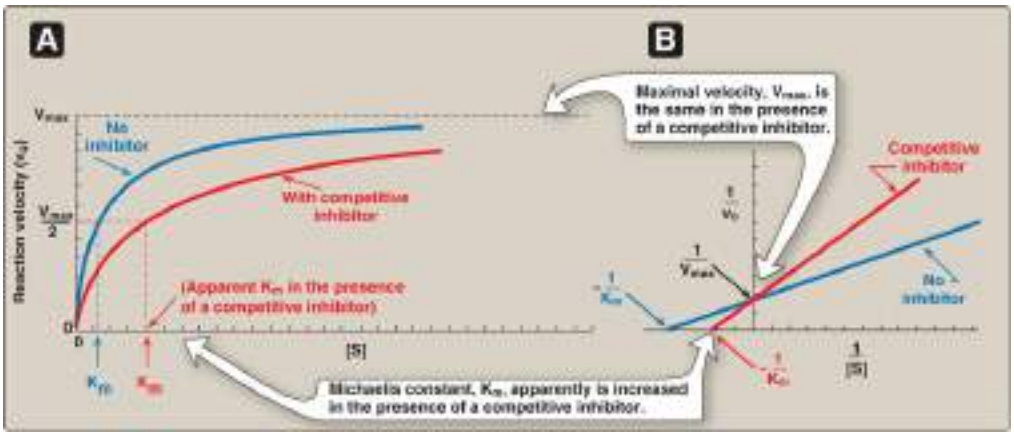


Figure 5.12
A: Effect of a competitive inhibitor on the reaction velocity versus substrate concentration ([S]) plot. B: Lineweaver–Burk plot of competitive inhibition of an enzyme. (Note: The slope increases if inhibitor concentration increases.)

4. Statin drugs as examples of competitive inhibitors: Statin drugs are cholesterol-lowering agents that competitively inhibit the rate-limiting (slowest) step in cholesterol biosynthesis. This reaction is catalyzed by hydroxymethylglutaryl coenzyme A reductase (HMG CoA reductase; see Chapter 19). Statins, such as atorvastatin and pravastatin, are structural analogs of the natural substrate for this enzyme and compete effectively to inhibit HMG CoA reductase. By doing so, they inhibit *de novo* cholesterol synthesis (Fig. 5.13).

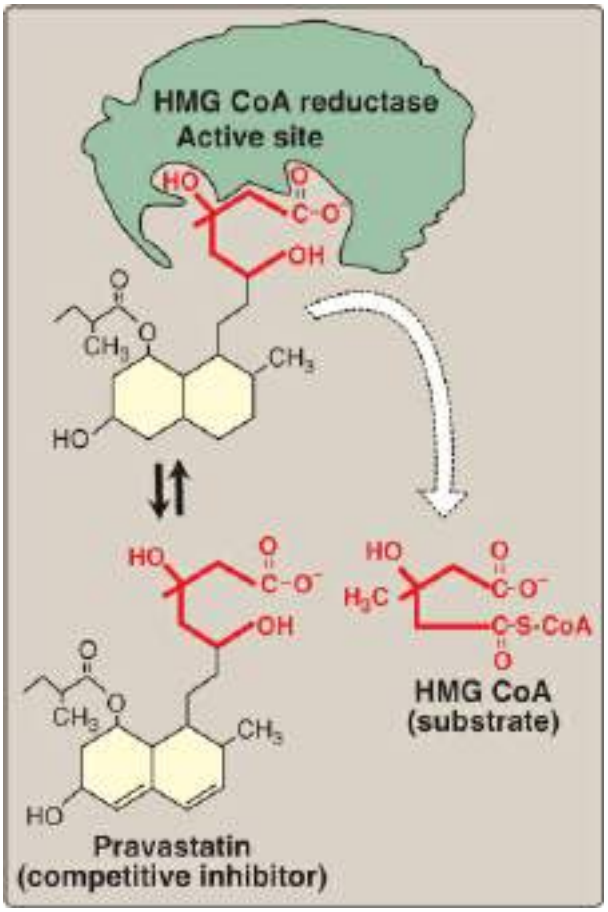


Figure 5.13
Pravastatin competes with hydroxymethylglutaryl coenzyme A (HMG CoA) for the active site of HMG CoA reductase.

B. Noncompetitive inhibition

This type of inhibition is recognized by its characteristic effect causing a decrease in V_{max} (Fig. 5.14). Noncompetitive inhibition occurs when the inhibitor and substrate bind at different sites on the enzyme. The noncompetitive inhibitor can bind either free enzyme or the ES complex, thereby preventing the reaction from occurring (Fig. 5.15).

1. Effect on V_{max}: Effects of a noncompetitive inhibitor cannot be overcome by increasing the concentration of substrate. Therefore, noncompetitive inhibitors decrease the apparent V_{max} of the reaction.

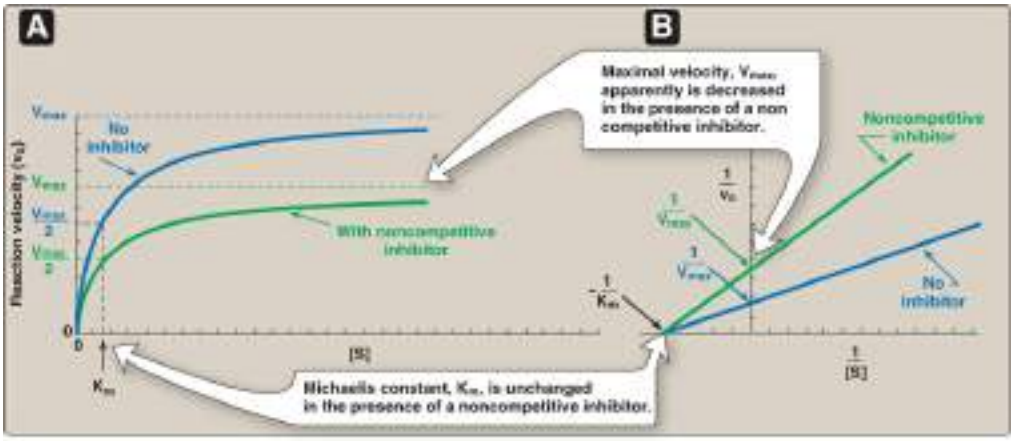


Figure 5.14
A: Effect of a noncompetitive inhibitor on the reaction velocity versus substrate concentration ([S]) plot. B: Lineweaver–Burk plot of noncompetitive inhibition of an enzyme. (Note: The slope increases if inhibitor concentration increases.)

2. Effect on K_m: Noncompetitive inhibitors do not interfere with the binding of substrate to enzyme. Therefore, the enzyme shows the same K_m in the presence or absence of the noncompetitive inhibitor, that is, K_m is unchanged in the presence of a noncompetitive inhibitor.

3. Effect on Lineweaver–Burk plot: Noncompetitive inhibition is readily differentiated from competitive inhibition by plotting $1/v_o$ versus $1/[S]$ and noting that the apparent V_{max} decreases in the presence of a noncompetitive inhibitor, whereas K_m is unchanged (see Fig. 5.14).

C. Enzyme inhibitors as drugs

At least half of the 10 most commonly prescribed drugs in the United States act as enzyme inhibitors. For example, the widely prescribed β-lactam antibiotics, such as penicillin and amoxicillin, act by inhibiting enzymes involved in bacterial cell wall synthesis. Drugs may also act by inhibiting extracellular reactions. This is illustrated by angiotensin-converting enzyme (ACE) inhibitors. They lower blood pressure by blocking plasma ACE that cleaves angiotensin I to form the potent vasoconstrictor, angiotensin II. These drugs, which include captopril, enalapril, and lisinopril, cause vasodilation and, therefore, a reduction in blood pressure. Aspirin, a nonprescription drug, irreversibly inhibits prostaglandin and thromboxane synthesis by inhibiting cyclooxygenase.

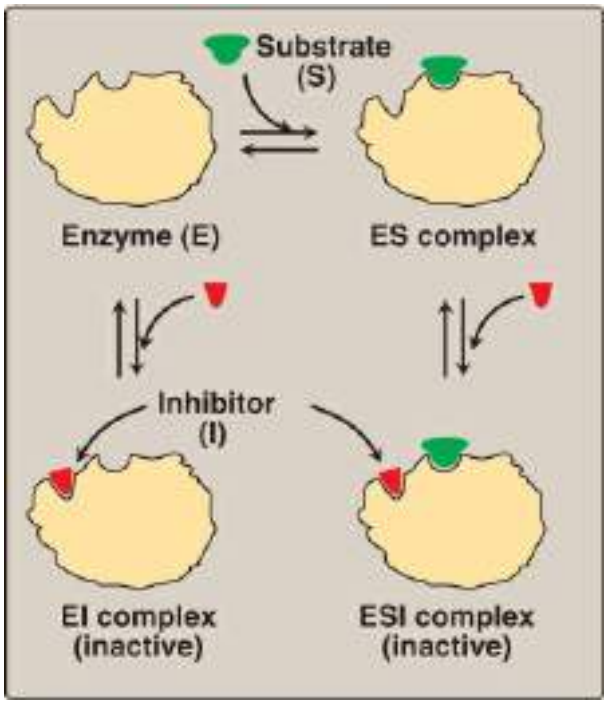


Figure 5.15
A noncompetitive inhibitor binding to both free enzyme and enzyme–substrate (ES) complex.

VIII. ENZYME REGULATION

The regulation of the reaction velocity of enzymes is essential if an organism is to coordinate its numerous metabolic processes. The rates of most enzymes are responsive to changes in substrate concentration, because the intracellular level of many substrates is in the range of the K_m. Thus, an increase in substrate concentration prompts an increase in reaction rate, which tends to return the concentration of substrate toward normal. In addition, some enzymes with specialized regulatory functions respond to allosteric effectors and/or covalent modification or they show altered rates of enzyme synthesis (or degradation) when physiologic conditions are changed.

A. Allosteric enzymes

Allosteric enzymes do not follow Michaelis–Menten kinetics but are regulated by molecules called **effectors** that bind to them noncovalently at a site other than the active site. These enzymes are almost always composed of multiple subunits, and the regulatory (allosteric) site that binds the effector is distinct from the substrate-binding site and may be located on a subunit that is not itself catalytic.

Effectors that inhibit enzyme activity are termed negative effectors, whereas those that increase enzyme activity are called positive effectors. Positive and negative effectors can affect the affinity of the enzyme for its substrate ($K_{0.5}$), modify the

maximal catalytic activity of the enzyme (V_{max}), or both (Fig. 5.16). Note that allosteric enzymes frequently catalyze the committed step, often the rate-limiting step, early in a pathway.

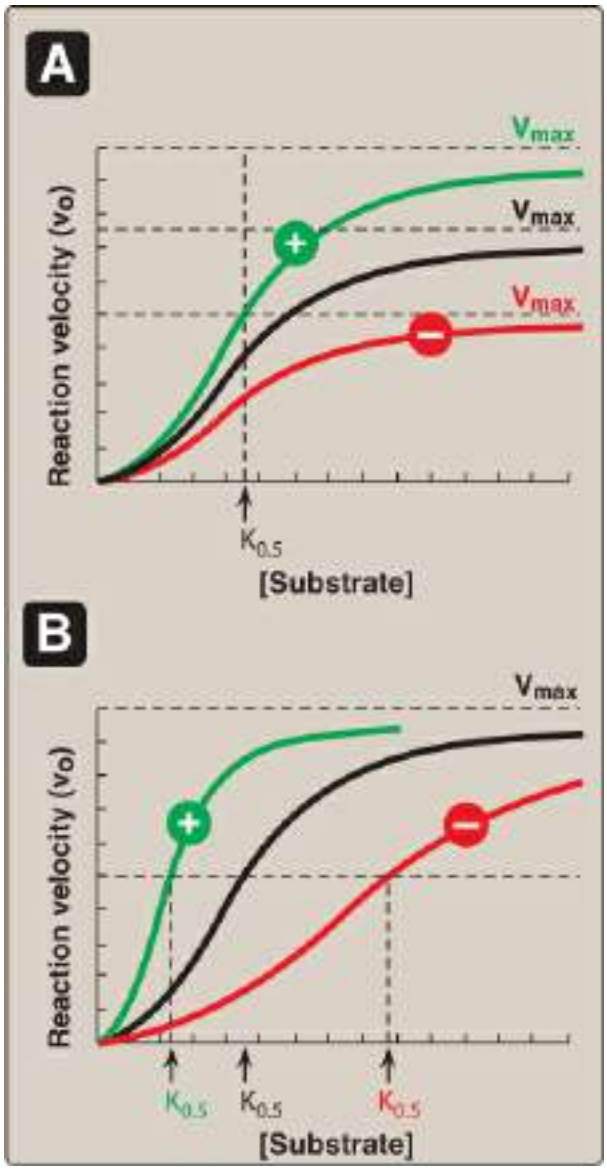


Figure 5.16
Effects of negative or positive effectors on an allosteric enzyme. **A:** Maximal velocity (V_{max}) is altered. **B:** The substrate concentration that gives half maximal velocity ($K_{0.5}$) is altered.

1. Homotropic effectors: When the substrate itself serves as an effector, the effect is said to be homotropic, or same as the substrate. Most often, an allosteric substrate functions as a positive effector. In such a case, the presence of a substrate molecule at one site on the enzyme enhances the catalytic properties of the other substrate-binding sites. That is, their binding sites cooperate with each other for substrate binding and are said to exhibit cooperativity. These

enzymes show a sigmoidal curve when v_o is plotted against substrate concentration, as shown in Figure 5.16. This contrasts with the hyperbolic curve characteristic of enzymes following Michaelis–Menten kinetics, as previously discussed. (Note: The concept of cooperativity of substrate binding is analogous to the binding of oxygen to hemoglobin [see Chapter 3].)

2. Heterotropic effectors: When the effector is a different molecule than the substrate, it is said to be heterotropic. For example, consider the feedback inhibition shown in Figure 5.17. The enzyme that converts D to E has an allosteric site that binds the end product, G. If the concentration of G increases (e.g., because it is not used as rapidly as it is synthesized), the first irreversible step unique to the pathway is typically inhibited. Feedback inhibition provides the cell with appropriate amounts of a product it needs by regulating the flow of substrate molecules through the pathway that synthesizes that product. Heterotropic effectors are commonly encountered. For example, the glycolytic enzyme phosphofructokinase-1 is allosterically inhibited by citrate, which is not a substrate for the enzyme.

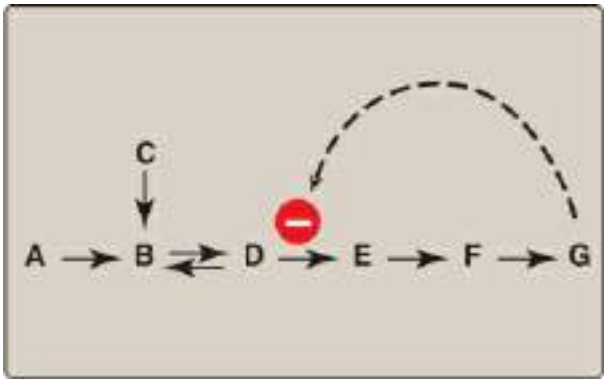


Figure 5.17
Feedback inhibition of a metabolic pathway.

B. Covalent modification

Many enzymes are regulated by covalent modification, most often by the addition or removal of phosphate groups from specific serine, threonine, or tyrosine residues of the enzyme. Protein phosphorylation is recognized as one of the primary ways in which cellular processes are regulated.

1. Phosphorylation and dephosphorylation: Phosphorylation reactions are catalyzed by a family of enzymes called protein kinases that catalyze the addition of a phosphate group to its protein or enzyme substrate, using ATP as the phosphate donor. Phosphoprotein phosphatases are enzymes that cleave phosphate groups from phosphorylated proteins and enzymes (Fig. 5.18).

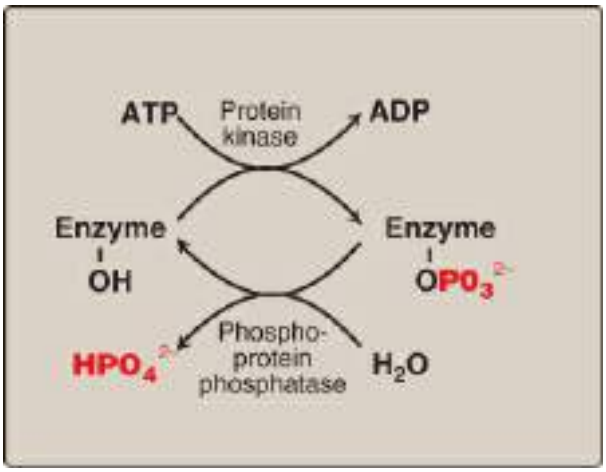


Figure 5.18
Covalent modification by the addition and removal of phosphate groups. (Note: HPO_4^{2-} may be represented as P_i and PO_3^{2-} as P.) ADP, adenosine diphosphate.

2. Enzyme response to phosphorylation: Depending on the specific enzyme, the phosphorylated form of an enzyme may be more or less active than the unphosphorylated enzyme. For example, hormone-mediated phosphorylation of glycogen phosphorylase, an enzyme that degrades glycogen, increases its activity, whereas phosphorylation of glycogen synthase, an enzyme that synthesizes glycogen, decreases its activity (see Chapter 11).

C. Enzyme synthesis

The regulatory mechanisms described above can modify the activity of existing enzyme molecules. However, cells can also regulate the amount of enzyme present by altering the rate of enzyme degradation or, more typically, the rate of enzyme synthesis. The increase (induction) or decrease (repression) of enzyme synthesis leads to an alteration in the total population of active sites. Enzymes subject to regulation of synthesis are often those that are needed at only one stage of development or under selected physiologic conditions. For example, elevated levels of insulin as a result of high blood glucose levels cause an increase in the synthesis of key enzymes involved in glucose metabolism (see Chapter 23).

In contrast, enzymes that are in constant use are usually not regulated by altering the rate of enzyme synthesis. Alterations in enzyme levels as a result of induction or repression of protein synthesis are slow (hours to days), compared with allosterically or covalently regulated changes in enzyme activity, which occur in seconds to minutes. Table 5.1 summarizes the common ways that enzyme activity is regulated.

Table 5.1 Mechanisms for Regulating Enzyme Activity

Regulator Event	Typical Effector	Results	Time Required for Change

Substrate availability	Substrate	Change in velocity (V_o)	Immediate
Product inhibition	Reaction product	Change in V_{max} and/or K_m	Immediate
Allosteric control	Pathway end product	Change in V_{max} and/or $K_{0.5}$	Immediate
Covalent modification	Another enzyme	Change in V_{max} and/or K_m	Immediate to minutes
Synthesis or degradation of enzyme	Hormone or metabolite	Change in the amount of enzyme	Hours to days

Note: Inhibition by pathway end product is also referred to as feedback inhibition.

IX. ENZYMES IN HUMAN BLOOD

While most enzymes function intracellularly, enzymes can be found outside cells in fluids including blood plasma, the fluid portion of blood. Enzymes that appear in blood plasma of healthy persons can be classified into two major groups. First, a relatively small group of enzymes are actively secreted into the blood by certain cell types. For example, the liver secretes zymogens (inactive precursors) of the protease enzymes involved in blood coagulation. Such proteases can be activated and have enzymatic function in the blood. Second, enzymes are released from cells during normal cell turnover. These enzymes almost always function only intracellularly and have no ability to catalyze reactions in blood plasma. In healthy individuals, the levels of these enzymes are fairly constant and represent a steady state in which the rate of release from damaged cells into the plasma is balanced by an equal rate of removal from the plasma. Increased blood plasma levels of these enzymes may indicate tissue damage and cell death that is greater than cell death from normal turnover (Fig. 5.19).

> Blood plasma is the fluid, noncellular fraction of blood. Laboratory assays of enzyme activity most often use serum, which is the fluid obtained by centrifugation of whole blood after it has been allowed to coagulate. Plasma is a physiologic fluid, whereas serum is a fluid prepared in the laboratory from a patient's whole blood sample.

A. Blood plasma enzyme levels in disease states

Many diseases cause tissue damage that includes the rupture of plasma membranes and lysis of cells in the tissue. As a result, the damaged cells release their contents into fluids, including the blood plasma, causing an increased concentration of the enzymes in the plasma. These enzymes are normally intracellular and cannot catalyze reactions when present outside their normal cellular location. However, these enzymes are routinely measured in patient's blood samples for diagnostic purposes. The level of specific enzyme activity in the plasma frequently correlates with the extent of tissue damage. Therefore, determining the extent of elevation of a particular enzyme activity in the blood plasma is often useful in evaluating the extent of tissue damage, response to therapies, and the prognosis for the patient.

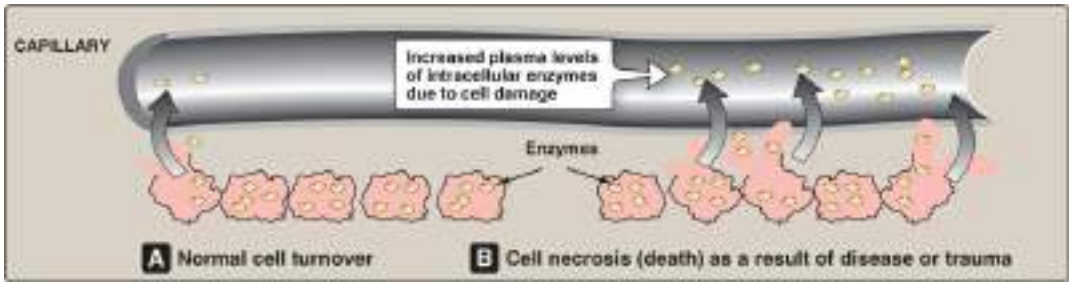


Figure 5.19
Release of enzymes from normal **(A)** and diseased or traumatized **(B)** cells.

Table 5.2 Some Clinically Useful Enzymes

Enzyme	Abbreviation	Main Tissue Source(s)	Useful to Assess
Alanine aminotransferase	ALT	Liver	Liver damage or disease
Alkaline phosphatase	ALP	Liver, bone	Liver and bone diseases
Amylase	Amylase	Pancreas	Pancreatic diseases
Aspartate aminotransferase	AST	Liver, muscle	Liver and muscle diseases
Creatine kinase	CK	Muscle	Muscle damage or disease
Gamma glutamyl transferase	GGT	Liver, bile duct	Hepatobiliary disease (obstructive jaundice)
Lipase	Lipase	Pancreas	Pancreatic diseases
Lactate dehydrogenase	LDH	Red blood cells, liver, muscle—most cells	General marker of cell death; particularly in hemolysis, hepatic or muscle diseases
5’ Nucleotidase	5’NT	Liver	Hepatobiliary disease (obstructive jaundice)

Appearance of these enzymes in blood can indicate damage to cells in the tissue where the enzyme normally functions.

B. Plasma enzymes as diagnostic tools

Some enzymes show relatively high activity in only one or a few tissues (Table 5.2). Therefore, the presence of increased levels of these enzymes in blood plasma reflects damage to the corresponding tissue. For example, the enzyme alanine aminotransferase (ALT) is one of many enzymes that are abundant in the liver. The appearance of elevated levels of ALT in plasma signals possible damage to hepatic tissue. Measurement of ALT released into a patient’s blood from dying cells is part of the liver function test panel. Increases in plasma levels of enzymes with a wide tissue distribution provide a less specific indication of the site of cellular injury and limits their diagnostic value.

C. Isoenzymes

Isoenzymes are variant forms of a particular enzyme that all catalyze the same

reaction but have slightly different physical properties because of genetically determined differences in amino acid sequence. For this reason, isoenzymes may contain different numbers of charged amino acids, which allows them to be separated from each other by electrophoresis (the movement of charged particles in an electric field) (Fig. 5.20).

Different organs commonly contain characteristic proportions of different isoenzymes. LDH is found in relatively high concentration in most tissues; five isoenzyme forms of LDH exist, LD 1–5, with LD5 prevalent in liver and skeletal muscle, LD2 in red blood cells and LD1 in myocardial muscle for example. The pattern of isoenzymes found in the blood plasma may, therefore, serve as a means of identifying the site of tissue damage. The plasma levels of various isoenzyme forms of LDH and of creatine kinase (CK) vary under different disease states.

1. Isoenzyme quaternary structure: Isoenzymes of a given enzyme often contain different subunits in various combinations. For example, LDH occurs as five isoenzymes and each exists as a tetramer, containing four subunits (combinations of subunits called H and M for heart and skeletal muscle where they were first discovered) such that LD1 = HHHH, LD2 (HHHM), LD3 (HHMM), LD4 (HMMM), and LD5 (MMMM). CK occurs as three isoenzymes. Each CK isoenzyme is a dimer composed of two polypeptide subunits (called B and M subunits for brain and skeletal muscle) associated in one of three combinations: CK1 = BB, CK2 = MB, and CK3 = MM. Each CK isoenzyme shows a characteristic electrophoretic mobility (see Fig. 5.20). (Note: Virtually all CK in the brain is the BB isoform, whereas it is MM in skeletal muscle. In cardiac muscle, there a majority of CK is MM, but the presence of CK MB is unique to myocardium.)

2. Historical use in diagnosis of myocardial infarction: Measurement of blood levels of isoenzymes with cardiac specificity (biomarkers) had an important use in the diagnosis of MI prior to the advent of testing for cardiac proteins known as troponins (see below). Because myocardial muscle is the only tissue that contains >5% of the total CK activity as the CK MB (CK2) isoenzyme, its appearance in blood plasma is virtually specific for damage to myocardial muscle and is seen after an acute myocardial infarction (MI or heart attack). Following an acute MI, CK MB appears in a patient's blood plasma within 4 to 8 hours following onset of chest pain, reaches a peak of activity at ~24 hours, and returns to baseline after 48 to 72 hours (Fig. 5.21).

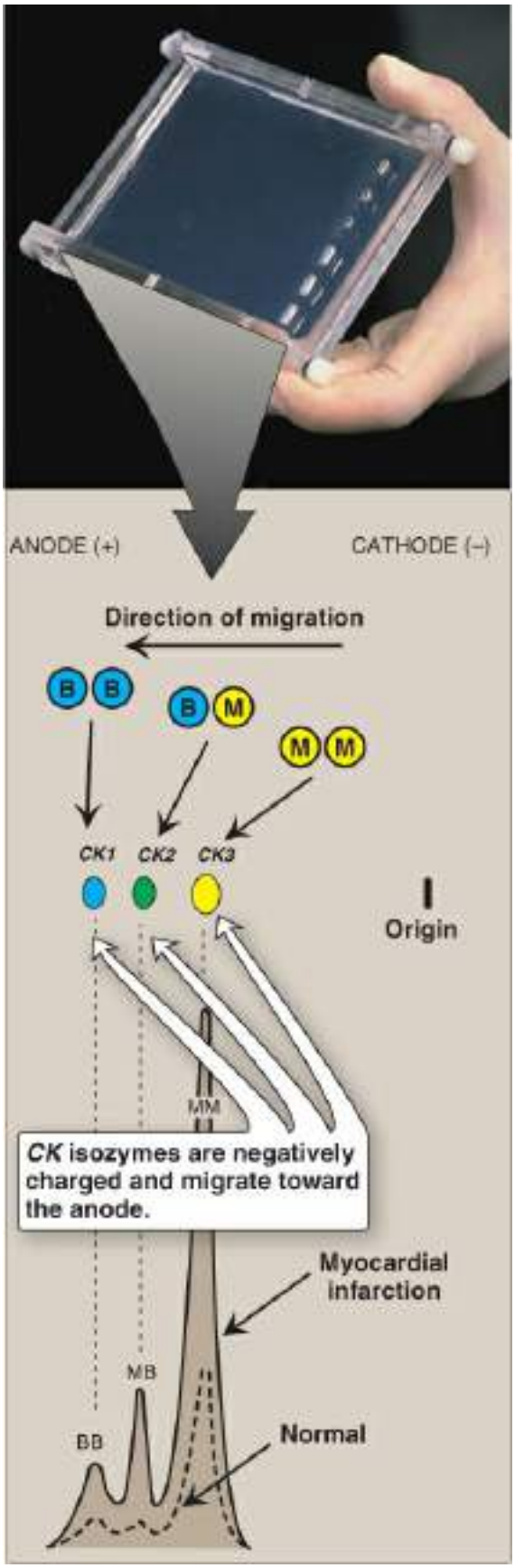


Figure 5.20
Subunit composition, electrophoretic mobility, and enzyme activity of creatine kinase (CK) isoenzymes.

Clinical Application 5.1: Diagnostic Use of Troponins

Troponins T (TnT) and I (TnI) are regulatory proteins involved in muscle contractility. Cardiac-specific isoforms (cTn) of troponins are released into the plasma in response to cardiac damage. and there is a highly sensitive and specific indication of damage to cardiac tissue. cTn appear in plasma within 4 to 6 hours after an MI, peak in 24 to 36 hours, and remain elevated for 3 to 10 days. Elevated cTn, in combination with the clinical presentation and characteristic changes in the ECG, are currently considered the “gold standard” in the diagnosis of an MI. While the appearance characteristics of cTN in blood plasma after an acute MI are similar to those of CK MB, the change from baseline to peak values is much greater for cTN (see Fig. 5.21).

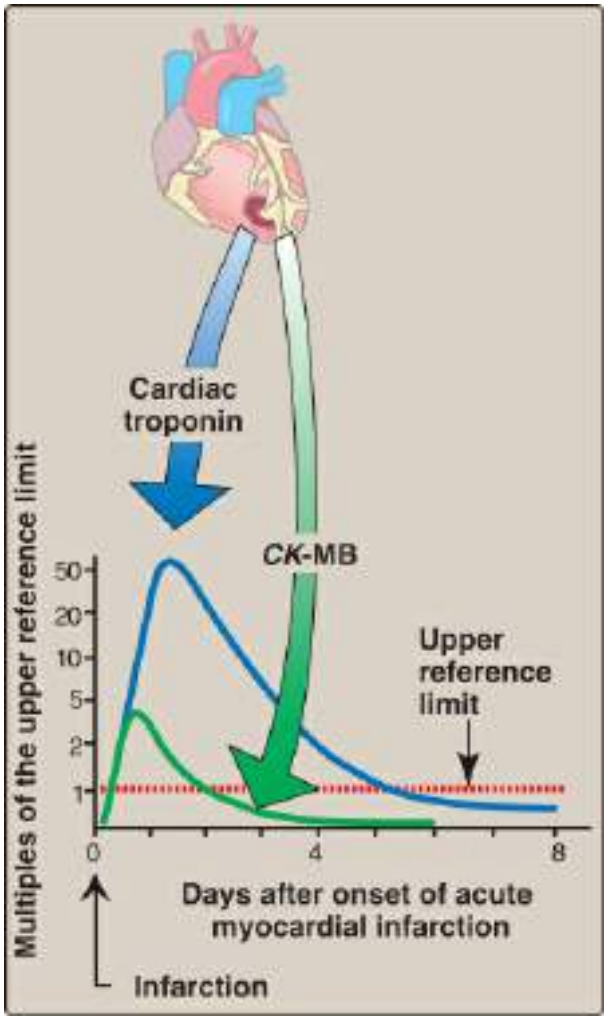


Figure 5.21
Appearance of creatine kinase isozyme CK-MB and cardiac troponin in plasma after an myocardial infarction. (Note: Either cardiac troponin T or I may be measured.)

X. Chapter Summary

- Enzymes are **protein catalysts** that increase the velocity of a chemical reaction by providing an alternate reaction pathway with a lower activation energy (Fig. 5.22).
- Enzymes contain a specialized cleft called the **active site**, which binds the substrate, forming an **ES complex**, with conversion to product (ES → EP → E + P).
- Most enzymes show **Michaelis–Menten kinetics**, and a plot of the **initial reaction velocity** ($\mathbf{v_o}$) against **[S]** has a **hyperbolic** shape; **allosteri**c enzymes show a **sigmoidal** curve.
- A **Lineweaver–Burk** or double-reciprocal plot of 1/v and 1/[S] transforms the hyperbolic shaped curve to a straight line and allows easier determination of $\mathbf{V_{max}}$ (maximal velocity) and $\mathbf{K_m}$ (Michaelis constant, which reflects affinity for substrate).
- An **inhibitor** is any substance that can decrease the velocity of an enzyme-catalyzed reaction.
- The two most common types of enzyme inhibition are **competitive**, which **increase** the **apparent** $\mathbf{K_m}$ and **noncompetitive** which **decreases** the **apparent** $\mathbf{V_{max}}$.
- **Allosteric enzymes** are composed of subunits and are regulated by **effectors** that bind noncovalently at a site other than the active site.
- **Positive** allosteric effectors increase enzyme activity and **negative** effectors decrease enzyme activity.
- Enzymes can also be regulated by **covalent modification**, most often via phosphorylation catalyzed by protein kinases while phosphoprotein phosphatases remove phosphate groups.
- Regulation can also occur by changes in the rate of synthesis or degradation.
- Since most enzymes function intracellularly, their appearance in blood plasma can indicate damage to a corresponding tissue, giving enzymes a diagnostic value in medicine.

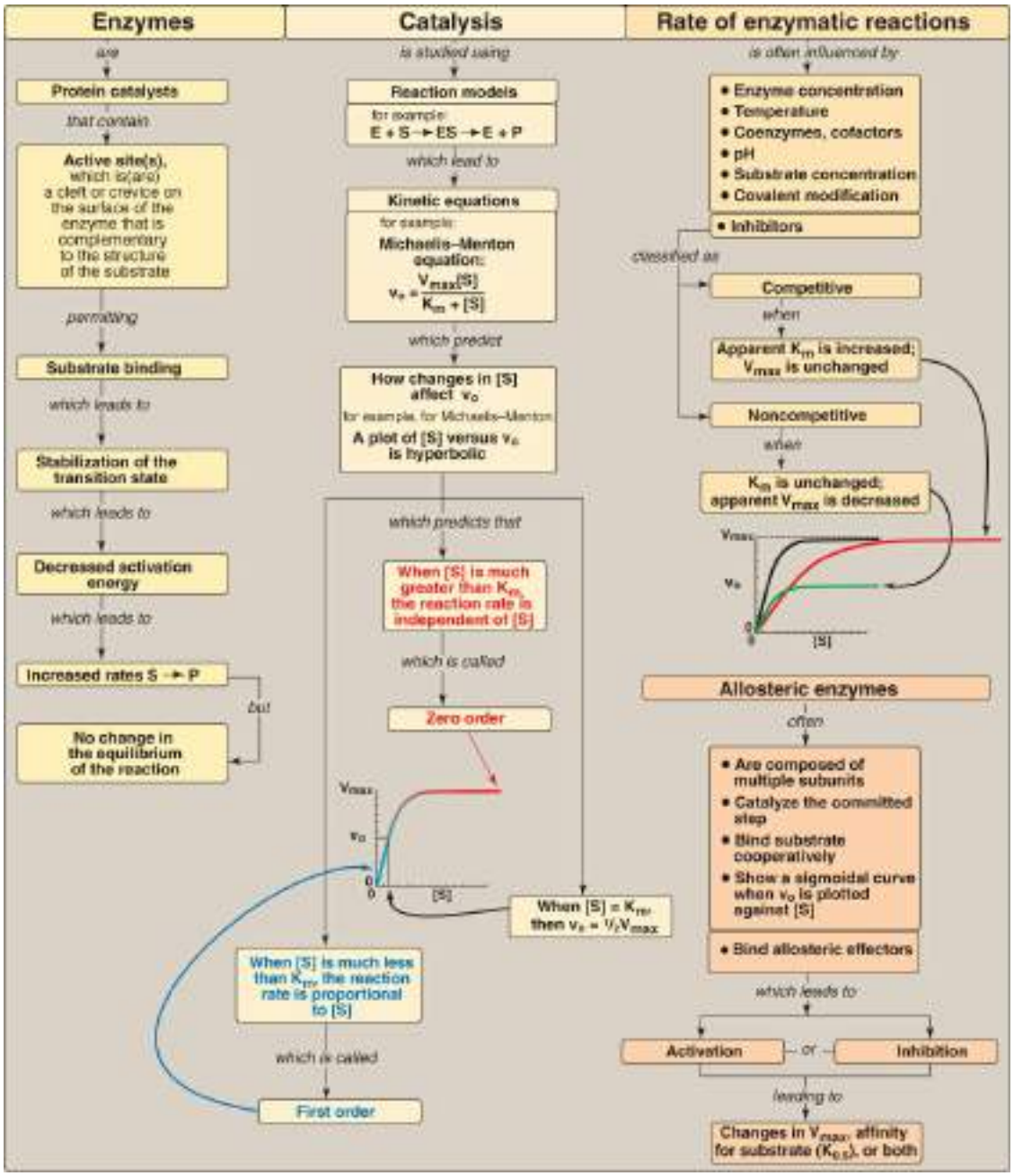


Figure 5.22
Key concept map for the enzymes. S, substrate; [S], substrate concentration; P, product; E, enzyme; v_o, initial velocity; V_{max}, maximal velocity; K_m, Michaelis constant; $K_{0.5}$, substrate concentration that gives half maximal velocity.

Study Questions

Choose the ONE best answer.

5.1 In cases of ethylene glycol poisoning and its characteristic metabolic acidosis, treatment involves correction of the acidosis, removal of any remaining ethylene glycol, and administration of an inhibitor of alcohol dehydrogenase (ADH), the enzyme that oxidizes ethylene glycol to the organic acids that cause the acidosis.

Ethanol (grain alcohol) frequently is the inhibitor given to treat ethylene glycol poisoning. Results of experiments using ADH with and without ethanol are shown to the right. Based on these data, what type of inhibition is caused by the ethanol?

A. Competitive
B. Feedback
C. Irreversible
D. Noncompetitive

Substrate Concentration with Ethanol (mM)	Rate of Reaction (mol/l/sec)	Substrate Concentration without Ethanol	Rate of Reaction (mol/l/sec)
5	3.0×10^{-7}	5	8.0×10^{-7}
10	5.0×10^{-7}	10	1.2×10^{-6}
20	1.0×10^{-6}	20	1.8×10^{-6}
40	1.6×10^{-6}	40	1.9×10^{-6}
80	2.0×10^{-6}	80	2.0×10^{-6}

Correct answer = A. A competitive inhibitor increases the apparent K_m for a given substrate. This means that, in the presence of a competitive inhibitor, more substrate is needed to achieve one half V_{max}. The effect of a competitive inhibitor is reversed by increasing substrate concentration ([S]). At a sufficiently high [S], the reaction velocity reaches the V_{max} observed in the absence of inhibitor.

5.2 Alcohol dehydrogenase (ADH) requires oxidized nicotinamide adenine dinucleotide (NAD^+) for catalytic activity. In the reaction catalyzed by ADH, an alcohol is oxidized to an aldehyde as NAD^+ is reduced to NADH and dissociates from the enzyme. The NAD^+ is functioning as a/an:

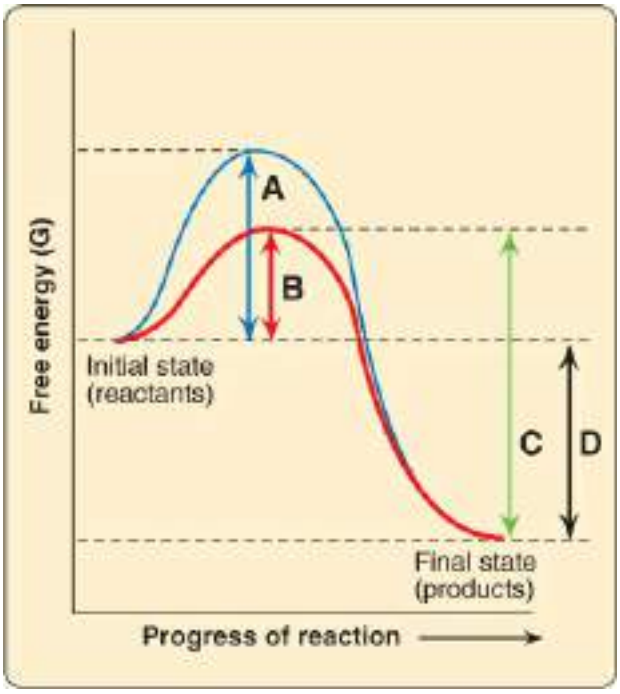
A. apoenzyme
B. coenzyme–cosubstrate
C. coenzyme–prosthetic group
D. cofactor
E. heterotropic effector

Correct answer = B. Coenzymes–cosubstrates are small organic molecules that associate transiently with an enzyme and leave the enzyme in a changed form. Coenzyme–prosthetic groups are small organic molecules that associate permanently with an enzyme and are returned to their original form on the enzyme. Cofactors are metal ions. Heterotropic effectors are not substrates.

For Questions 5.3 and 5.4, use the graph below that shows the changes in free energy when a reactant is converted to a product in the presence and absence of an enzyme. Select the letter that best represents:

5.3 The activation energy of the catalyzed forward reaction.

5.4 The free energy of the reaction.



Correct answers = B; D. Enzymes (protein catalysts) provide an alternate reaction pathway with a lower activation energy. However, they do not change the free energy of the reactant or product. A is the activation energy of the uncatalyzed reaction. C is the activation energy of the catalyzed reverse reaction.

5.5 If a noncompetitive inhibitor is included in the reaction of an enzyme with its substrate then:

A. addition of sufficient concentrations of substrate will overcome the inhibition.
B. Km will be decreased owing to reduced enzyme–substrate affinity.
C. inhibitor and substrate will bind to different sites on the enzyme.
D. the curve when plotting velocity versus [substrate] will become sigmoidal.
E. Vmax will remain the same as for the uninhibited reaction.

Correct answer = C; Noncompetitive inhibitors do not bind to the enzyme active site but to other binding sites on the enzyme. Substrates bind to enzyme active sites. Because binding is to a different site, the addition of substrate will not overcome the inhibition. Km will remain the same since substrate will continue to bind to the active site, however will be an inactive complex when a noncompetitive inhibitor is also bound. For enzymes that follow Michaelis–Menten kinetics, the hyperbolic shape curve will be shift to a lower Vmax in the presence of a noncompetitive inhibitor.

5.6 In an enzyme-catalyzed reaction, Protein Q is the substrate for Enzyme X, a kinase. The product of this reaction will be:

A. ATP
B. dephosphorylated Enzyme X
C. phosphorylated Enzyme X
D. dephosphorylated Protein Q
E. phosphorylated Protein Q

Correct answer = E; The substrate of a kinase will be converted to its phosphorylated form as a result of the reaction. ATP is used as a phosphate donor and will be hydrolyzed to ADP during the course of the reaction. The kinase enzyme itself will not be altered by the reaction.

5.7 Sulfa drugs can be effective in limiting bacterial infections in humans while not producing toxic effects within human cells. To account for these characteristics, sulfa drugs most likely act as a(n):

A. allosteric effector increasing catalytic action of a bacterial enzyme.
B. antimetabolite that interrupts replication in all types of dividing cells.
C. competitive inhibitor of an enzyme required by bacteria but not human cells.
D. noncompetitive inhibitors of several regulatory steps in glycolysis.
E. positive allosteric effector of an enzyme in bacterial cell wall synthesis.

Correct answer = C; Acting as a competitive inhibitor of an enzyme required only by bacteria, sulfa drugs and other antibiotics can halt bacterial growth without damaging human cells. Agents such as antimetabolites that interrupt all dividing cells would harm host human cells as well as bacteria. Most drugs that act as enzyme inhibitors are competitive, and are designed to resemble the substrate structurally so to be able to bind to the active site. Both human and bacterial cells undergo glycolysis. Allosteric effectors that increase an enzyme's catalytic function are positive effectors. Positive allosteric effectors enhance enzyme activity and do not inhibit it.

UNIT II:
Bioenergetics and Carbohydrate Metabolism

Bioenergetics and Oxidative Phosphorylation 6

I. OVERVIEW

Bioenergetics describes the transfer and utilization of energy in biologic systems and concerns the initial and final energy states of the reaction components. Bioenergetics makes use of a few basic ideas from the field of thermodynamics, particularly the concept of free energy. Because changes in free energy provide a measure of the energetic feasibility of a chemical reaction, they allow prediction of whether a reaction or process can take place. In short, bioenergetics predicts if a process is possible, whereas kinetics measures the reaction rate.

II. FREE ENERGY

The direction and extent to which a chemical reaction proceeds are determined by the degree to which two factors change during the reaction. These are enthalpy (ΔH, a measure of the change [Δ] in heat content of the reactants and products) and entropy (ΔS, a measure of the change in randomness or disorder of the reactants and products), as shown in Figure 6.1. Neither of these thermodynamic quantities by itself is sufficient to determine whether a chemical reaction will proceed spontaneously in the direction it is written. However, when combined mathematically (see Fig. 6.1), enthalpy and entropy can be used to define a third quantity, free energy (G), which predicts the direction in which a reaction will spontaneously proceed.

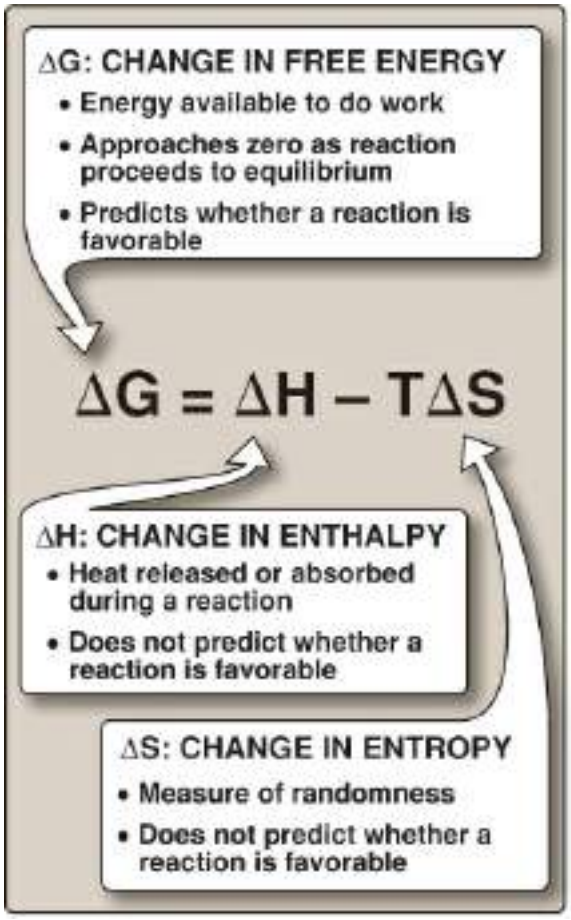


Figure 6.1
Relationship between changes in free energy (G), enthalpy (H), and entropy (S). T is the absolute temperature in Kelvin (K), where K = °C + 273.

III. FREE ENERGY CHANGE

The **change in free energy** is represented in two ways, ΔG and ΔG^0. The first, ΔG (without the superscript "0"), represents the change in free energy and, thus, the direction of a reaction at any specified concentration of products and reactants. ΔG, then, is a variable. This contrasts with the standard free energy change, ΔG^0 (with the superscript "0"), which is the energy change when reactants and products are at a concentration of 1 mol/l. (Note: The concentration of protons $[H^+]$ is assumed to be 10^{-7}mol/l [i.e., pH = 7]. This may be shown by a prime sign [′], e.g., $\Delta G^{0\prime}$.) Although ΔG^0, a constant, represents energy changes at these nonphysiologic concentrations of reactants and products, it is nonetheless useful in comparing the energy changes of different reactions. Furthermore, $\Delta G°$ can readily be determined from measurement of the equilibrium constant.

A. ΔG and reaction direction

The sign of ΔG can be used to predict the direction of a reaction at constant

temperature and pressure. Consider the reaction:

$$A \rightleftarrows B$$

If ΔG is negative, the reaction is considered exergonic with a net loss of energy. In this case, the reaction proceeds spontaneously as written, with A converted to B (Fig. 6.2A). If ΔG is positive, the reaction is endergonic with a net gain of energy. Energy must be added to the system in order for the reaction from B to A to take place (Fig. 6.2B). In cases where ΔG = 0, the reaction is in equilibrium. Note that when a reaction is proceeding spontaneously (ΔG is negative), the reaction will continue until ΔG reaches zero and equilibrium is established.

B. ΔG of the forward and reverse reactions

The free energy of the forward reaction (A → B) is equal in magnitude but opposite in sign to that of the reverse reaction (B → A). For example, if ΔG of the forward reaction is −5 kcal/mol, then that of the back reaction is +5 kcal/mol. (Note: ΔG can also be expressed in kilojoules per mole or kJ/mol[1 kcal = 4.2 kJ].)

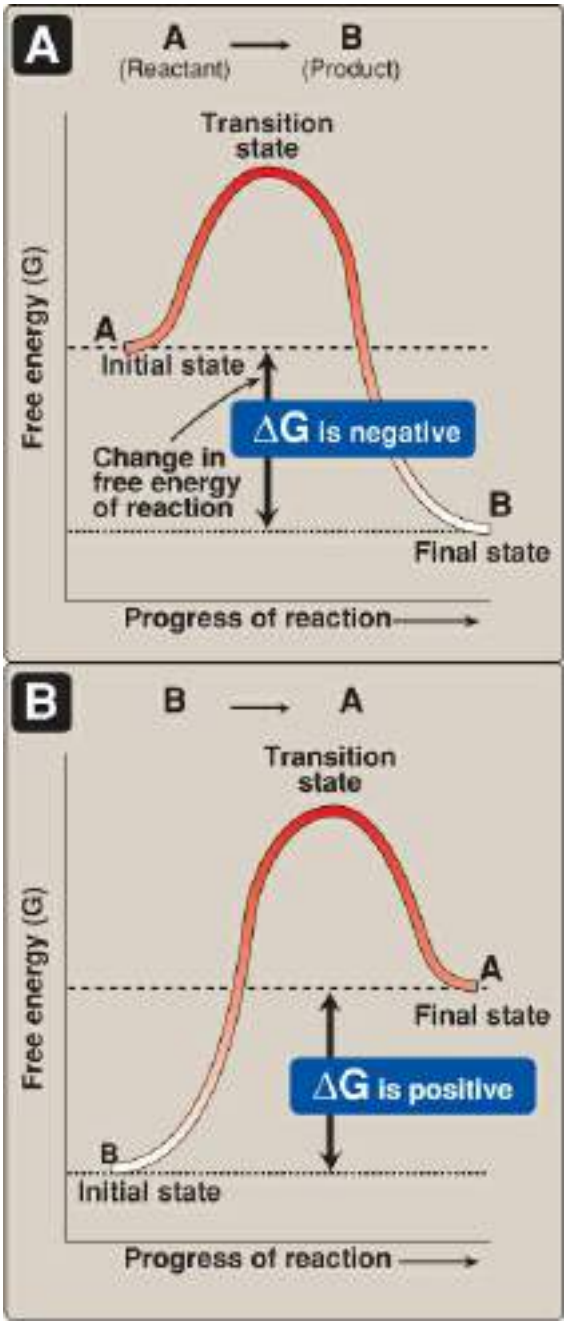


Figure 6.2
Change in free energy (ΔG) during a reaction. **A:** The product has a lower free energy (G) than the reactant. **B:** The product has a higher free energy than the reactant.

C. ΔG and reactant and product concentrations

The ΔG of the reaction A → B depends on the concentrations of the reactant and of the product. At constant temperature and pressure, the following relationship can be derived:

$$\Delta G = \Delta G^0 + RT \ln \frac{[B]}{[A]}$$

where ΔG^0 is the standard free energy change (see D. below)
R is the gas constant (1.987 cal/mol K)
T is the absolute temperature (K)
[A] and [B] are the actual concentrations of the reactant and product
ln represents the natural logarithm.

A reaction with a positive ΔG° can proceed in the forward direction if the ratio of products to reactants ([B]/[A]) is sufficiently small (i.e., the ratio of reactants to products is large) to make ΔG negative. For example, consider the reaction:

Glucose 6-phosphate ⇄ fructose 6-phosphate

Figure 6.3A shows reaction conditions in which the concentration of reactant, glucose 6-phosphate, is high compared with the concentration of product, fructose 6-phosphate. This means that the ratio of the product to reactant is small, and RT ln([fructose 6-phosphate]/[glucose 6-phosphate]) is large and negative, causing ΔG to be negative despite ΔG^0 being positive. Thus, the reaction can proceed in the forward direction.

D. Standard free energy change

The standard free energy change, ΔG^0, is equal to the free energy change, ΔG, under standard conditions, when reactants and products are at 1 mol/l concentrations (Fig. 6.3B). Under these conditions, the natural logarithm of the ratio of products to reactants is zero (ln1 = 0), and, therefore, the equation shown at the bottom of the previous page becomes:

$$\Delta G = \Delta G^0 + 0$$

1. ΔG^0 and reaction direction: Under standard conditions, ΔG° can be used to predict the direction a reaction proceeds because, under these conditions, ΔG^0 is equal to ΔG. However, ΔG° cannot predict the direction of a reaction under physiologic conditions because it is composed solely of constants (R, T, and K_{eq} [see 2. below]) and is not, therefore, altered by changes in product or substrate concentrations.

2. Relationship between ΔG^0 and K_{eq}: In a reaction A ⇄ B, a point of equilibrium is reached at which no further net chemical change takes place. In this state, the ratio of [B] to [A] is constant, regardless of the actual concentrations of the two compounds:

$$K_{eq} = \frac{[B]_{eq}}{[A]_{eq}}$$

where K_{eq} is the equilibrium constant, and $[A]_{eq}$ and $[B]_{eq}$ are the concentrations of A and B at equilibrium. If the reaction A ⇄ B is allowed to reach equilibrium at constant temperature and pressure, then, at equilibrium, the overall ΔG is zero (Fig. 6.3C). Therefore,

$$\Delta G = 0 = \Delta G^0 + RT \ln \frac{[B]_{eq}}{[A]_{eq}}$$

where the actual concentrations of A and B are equal to the equilibrium concentrations of reactant and product ($[A]_{eq}$ and $[B]_{eq}$), and their ratio is equal to the K_{eq}. Thus,

$$\Delta G^0 = -RT \ln K_{eq}$$

This equation allows some simple predictions:

If $K_{eq} = 1$, then $\Delta G^0 = 0$
If $K_{eq} > 1$, then $\Delta G^0 < 0$
If $K_{eq} < 1$, then $\Delta G^0 > 0$

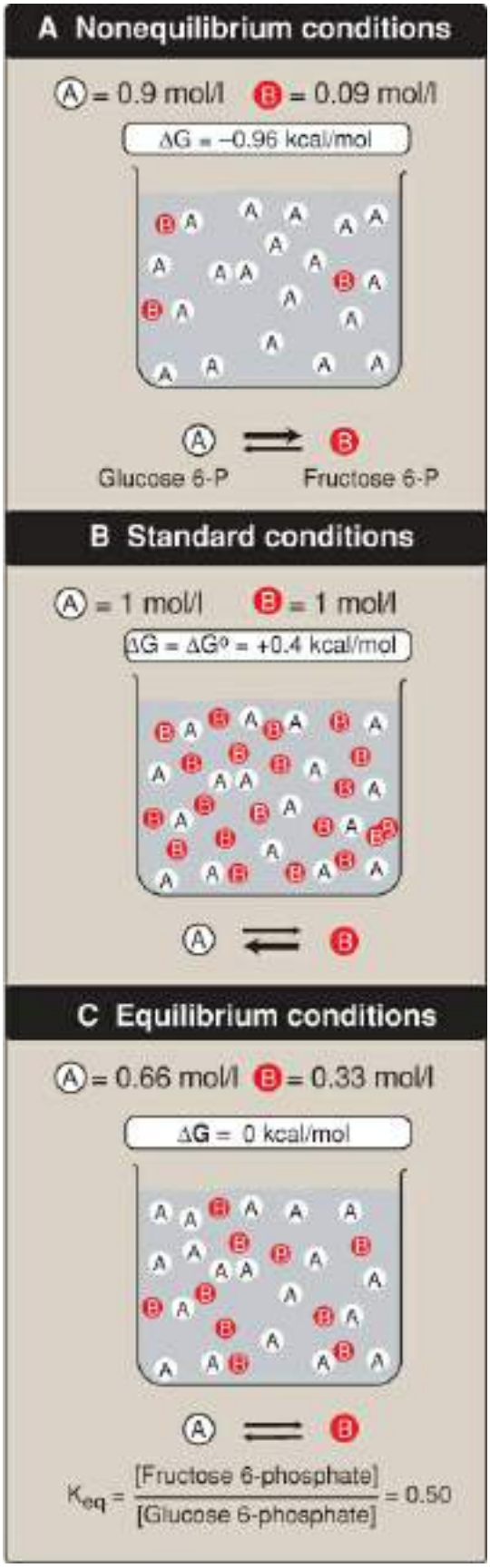


Figure 6.3
Free energy change (ΔG) of a reaction depends on the concentration of reactant and product. For the conversion of glucose 6-phosphate to fructose 6-phosphate, ΔG is negative when the ratio of reactant to product is large (top, panel A), is positive under standard conditions (middle, panel B), and is zero at equilibrium (bottom, panel C). ΔG^0 = standard free energy change.

3. ΔG^0s of two consecutive reactions: The ΔG^0s are additive in any sequence of consecutive reactions, as are the ΔGs. For example:

Glucose + ATP	→ glucose 6-phosphate + adenosine diphosphate (ADP)	$\Delta G^0 = -4{,}000$ cal/mol
Glucose 6-phosphate	→ fructose 6-phosphate	$\Delta G^0 = +400$ cal/mol
Glucose + ATP	→ fructose 6-phosphate + ADP	$\Delta G^0 = -3{,}600$ cal/mol

4. ΔGs of a pathway: The additive property of ΔG is very important in biochemical pathways through which substrates must pass in a particular direction (e.g., A → B → C → D → …). As long as the sum of the ΔGs of the individual reactions is negative, the pathway can proceed as written, even if some of the individual reactions of the pathway have a positive ΔG. However, the actual rates of the reactions depend on the lowering of activation energies (E_a) by the enzymes that catalyze the reactions.

IV. ATP: AN ENERGY CARRIER

Reactions with a large positive ΔG, are made possible by coupling the endergonic movement of ions with a second, spontaneous process with a large negative ΔG such as the exergonic hydrolysis of ATP (see p. 96). Figure 6.4 shows a mechanical model of energy coupling. The simplest example of energy coupling in biologic reactions occurs when the energy-requiring and the energy-yielding reactions share a common intermediate.

A. Common intermediates

Two chemical reactions have a common intermediate when they occur sequentially in that the product of the first reaction is a substrate for the second. For example, given the reactions

$$A + B \rightarrow C + D$$

$$D + X \rightarrow Y + Z$$

D is the common intermediate and can serve as a carrier of chemical energy between the two reactions. (Note: The intermediate may be linked to an enzyme.) Many coupled reactions use ATP to generate a common intermediate. These reactions may involve the transfer of a phosphate group from ATP to another molecule. Other reactions involve the transfer of phosphate from an energy-rich intermediate to ADP, forming ATP.

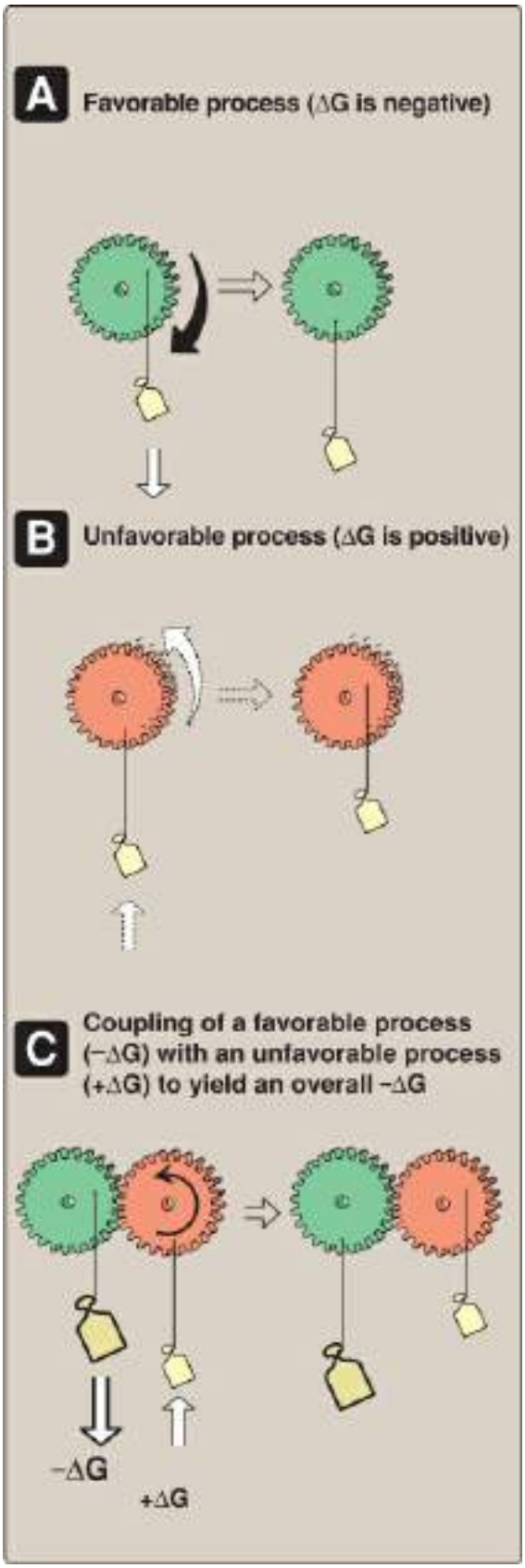


Figure 6.4
Mechanical model of the coupling of favorable and unfavorable processes. **A:** Gear with weight attached spontaneously turns in the direction that achieves the lowest energy state. **B:** The reverse movement is energetically unfavorable (not spontaneous). **C:** The energetically favorable movement can drive the unfavorable one. ΔG = change in free energy.

B. Energy carried by ATP

ATP consists of a molecule of adenosine to which three phosphate groups are attached (Fig. 6.5). Removal of one phosphate produces ADP, and removal of two phosphates produces adenosine monophosphate (AMP). For ATP, the ΔG^0 of hydrolysis is approximately −7.3 kcal/mol for each of the two terminal phosphate groups. Because of this large negative ΔG^0 of hydrolysis, ATP is called a high-energy phosphate compound. (Note: Adenine nucleotides are interconverted [2 ADP ⇄ ATP + AMP] by adenylate kinase.)

V. ELECTRON TRANSPORT CHAIN

Energy-rich molecules, such as glucose, are metabolized by a series of oxidation reactions ultimately yielding carbon dioxide and water (H_2O) (Fig. 6.6). The metabolic intermediates of these reactions donate electrons to specific coenzymes, nicotinamide adenine dinucleotide (NAD^+) and flavin adenine dinucleotide (FAD), to form the energy-rich reduced forms, NADH and flavin adenine dinucleotide ($FADH_2$). These reduced coenzymes can, in turn, each donate a pair of electrons to a specialized set of electron carriers, collectively called the electron transport chain (ETC), described in this section. As electrons are passed down the ETC, they lose much of their free energy. This energy is used to move H^+ across the inner mitochondrial membrane, creating a H^+ gradient that drives the production of ATP from ADP and inorganic phosphate (P_i). The coupling of electron transport with ATP synthesis is called oxidative phosphorylation, sometimes denoted as OXPHOS. It proceeds continuously in all tissues that contain mitochondria. Note that the free energy not trapped as ATP is used to drive ancillary reactions such as transport of calcium ions into mitochondria and to generate heat.

A. Mitochondrial electron transport chain

The ETC (except for cytochrome c) is located in the inner mitochondrial membrane and is the final common pathway by which electrons derived from different fuels of the body flow to oxygen (O_2), reducing it to H_2O (see Fig. 6.6).

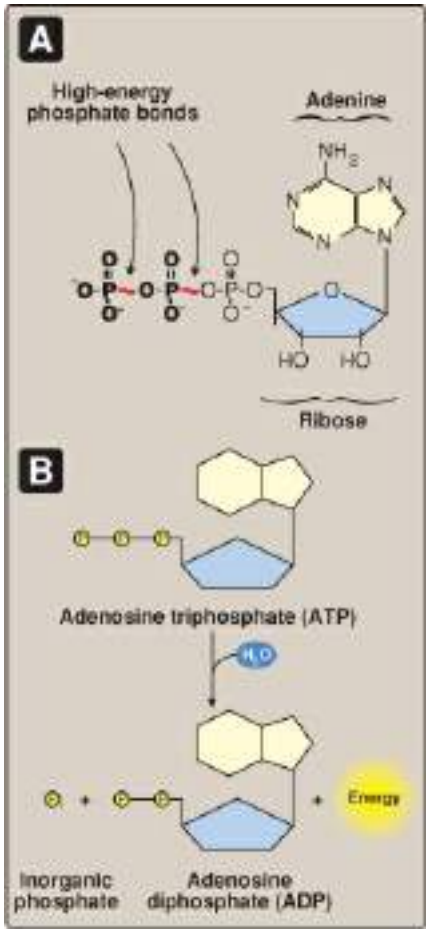


Figure 6.5
A: Adenosine triphosphate (ATP). **B:** Hydrolysis of ATP.

1. Mitochondrial membranes: Mitochondria have an outer and an inner membrane separated by the intermembrane space. The outer membrane contains specialized channels formed by the protein porin, making it freely permeable to most ions and small molecules. The inner membrane is a specialized structure that is impermeable to most small ions, including H^+, and small molecules such as ATP, ADP, pyruvate, and other metabolites important to mitochondrial function (Fig. 6.7). Transport proteins are required to move ions or molecules across this membrane. The inner mitochondrial membrane is unusually rich in proteins, over half of which are directly involved in oxidative phosphorylation. It also contains convolutions, called cristae, which greatly increase its surface area.

2. Mitochondrial matrix: The gel-like solution of the interior of the mitochondria is called the matrix and it is also rich in proteins. These include the enzymes responsible for the oxidation of pyruvate, amino acids, and fatty acids (by β-oxidation) as well as those of the tricarboxylic acid (TCA) cycle. The synthesis of glucose, urea, and heme occurs partially in the matrix of mitochondria. In addition, the matrix contains NAD^+ and FAD (the oxidized forms of the two

coenzymes that are required as electron acceptors), and ADP and P_i, which are used to produce ATP. (Note: The matrix also contains mitochondrial deoxyribonucleic acid [mtDNA], ribonucleic acid [mtRNA], and ribosomes.)

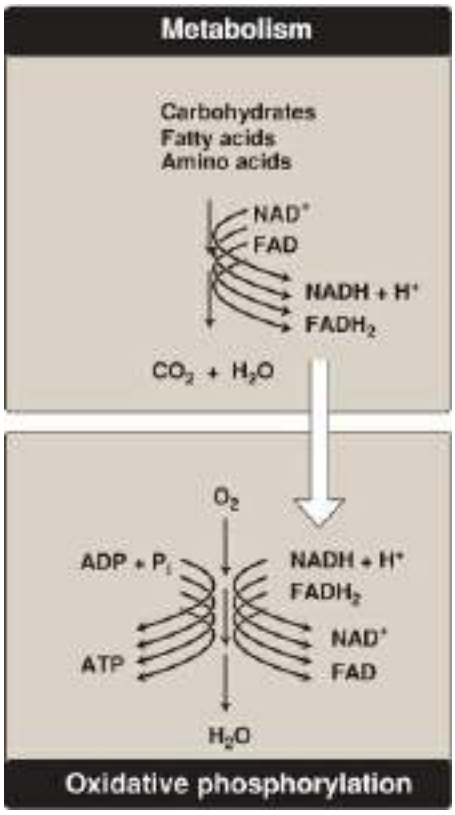


Figure 6.6
The metabolic breakdown of energy-yielding molecules. NAD(H) = nicotinamide adenine dinucleotide; FAD(H_2) = flavin adenine dinucleotide; ADP = adenosine diphosphate; P_i = inorganic phosphate; CO_2 = carbon dioxide.

B. Organization

The inner mitochondrial membrane contains four separate protein complexes, called Complexes I, II, III, and IV that each contain part of the ETC (Fig. 6.8). These complexes accept or donate electrons to the relatively mobile electron carrier coenzyme Q (CoQ) and **cytochrome c**. Each carrier in the ETC can receive electrons from an electron donor and can subsequently donate electrons to the next acceptor in the chain. The electrons ultimately combine with O_2 and H^+ to form H_2O. This requirement for O_2 makes the electron transport process the respiratory chain, which accounts for the greatest portion of the body's use of O_2.

C. Reactions

With the exception of CoQ, which is a lipid-soluble quinone, all members of the ETC are proteins. These may function as enzymes as is the case with the flavin-

containing dehydrogenases, may contain iron as part of an iron–sulfur (Fe–S) center, may contain iron as part of the porphyrin prosthetic group of heme as in the cytochromes, or may contain copper (Cu) as does the cytochrome a + a_3 complex.

1. NADH formation: NAD^+ is reduced to NADH by dehydrogenases that remove two hydrogen atoms from their substrate. (Note: For examples of these reactions, see the discussion of the dehydrogenases of the TCA cycle, p. 123.) Both electrons but only one H^+ (i.e., a hydride ion [$:H^-$]) are transferred to the NAD^+, forming NADH plus a free H^+.

2. NADH dehydrogenase: The free H^+ plus the hydride ion carried by NADH are transferred to NADH dehydrogenase, a protein complex (Complex I) embedded in the inner mitochondrial membrane. Complex I has a tightly bound molecule of flavin mononucleotide (FMN), a coenzyme structurally related to FAD that accepts the two hydrogen atoms (two electrons + two H^+), becoming $FMNH_2$. NADH dehydrogenase also contains peptide subunits with Fe–S centers (Fig. 6.9). At Complex I, electrons move from NADH to FMN to the iron of the Fe–S centers and then to CoQ. As electrons flow, they lose energy. This energy is used to pump four H^+ across the inner mitochondrial membrane, from the matrix to the intermembrane space.

3. Succinate dehydrogenase: At Complex II, electrons from the succinate dehydrogenase–catalyzed oxidation of succinate to fumarate move from the coenzyme, $FADH_2$, to an Fe–S protein, and then to CoQ. (Note: Because no energy is lost in this process, no H^+ are pumped at Complex II.)

4. Coenzyme Q: CoQ, also known as ubiquinone, is a quinone derivative with a long, hydrophobic isoprenoid tail, made from an intermediate of cholesterol synthesis (see Chapter 18). CoQ is a mobile electron carrier and can accept electrons from NADH dehydrogenase (Complex I), from succinate dehydrogenase (Complex II) and from other mitochondrial dehydrogenases, such as glycerol 3-phosphate dehydrogenase and acyl CoA dehydrogenases. CoQ transfers electrons to Complex III (cytochrome bc_1). Thus, a function of CoQ is to link the flavoproteindehydrogenases to the cytochromes.

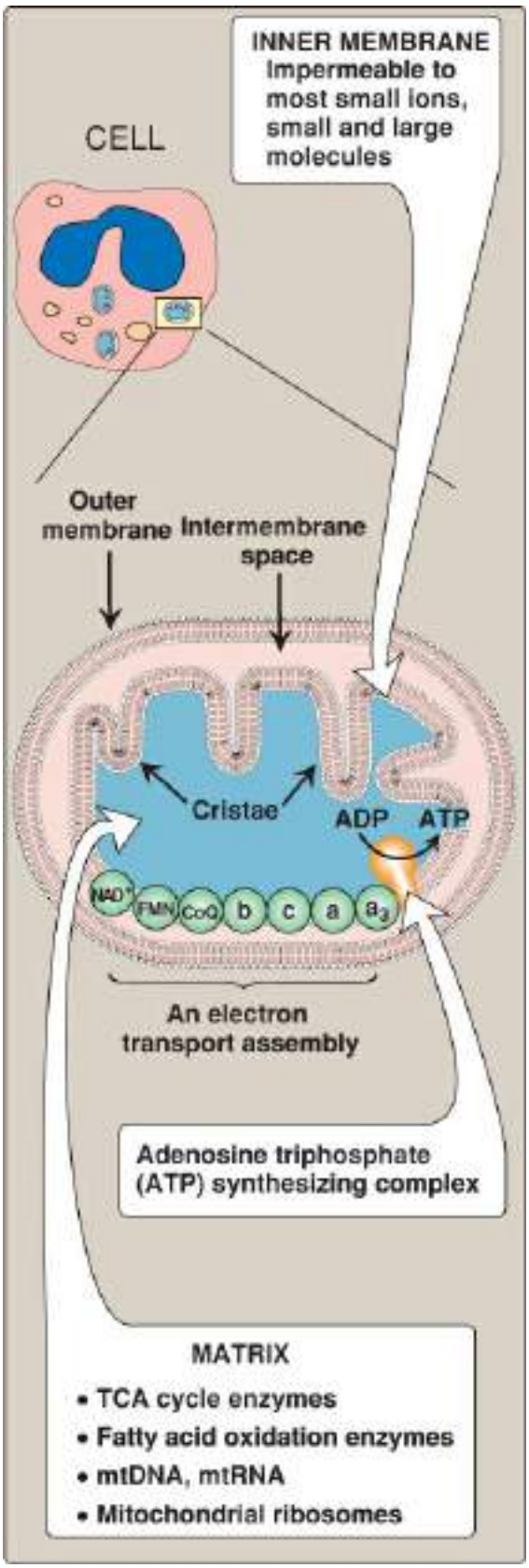


Figure 6.7

Structure of a mitochondrion showing schematic representation of the electron transport chain and the ATP synthesizing complex on the inner membrane. (Note: Unlike the inner membrane, the outer membrane is highly permeable, and the milieu of the intermembrane space is like that of the cytosol.) mt = mitochondrial; RNA = ribonucleic acid; ADP = adenosine diphosphate; TCA = tricarboxylic acid.

5. Cytochromes: The remaining members of the ETC are cytochrome proteins. Each contains a heme group which is a porphyrin ring plus iron. Unlike the heme groups of hemoglobin, the cytochrome iron is reversibly converted from its ferric (Fe^{3+}) to its ferrous (Fe^{2+}) form as a normal part of its function as an acceptor and donor of electrons. Electrons are passed along the chain from cytochrome bc_1 (Complex III), to cytochrome c, and then to cytochromes a + a_3 ([Complex IV], see Fig. 6.8). As electrons flow, four H^+ are pumped across the inner mitochondrial membrane at Complex III and two at Complex IV. (Note: Cytochrome c is located in the intermembrane space, loosely associated with the outer face of the inner membrane. As seen with CoQ, cytochrome c is a mobile electron carrier.)

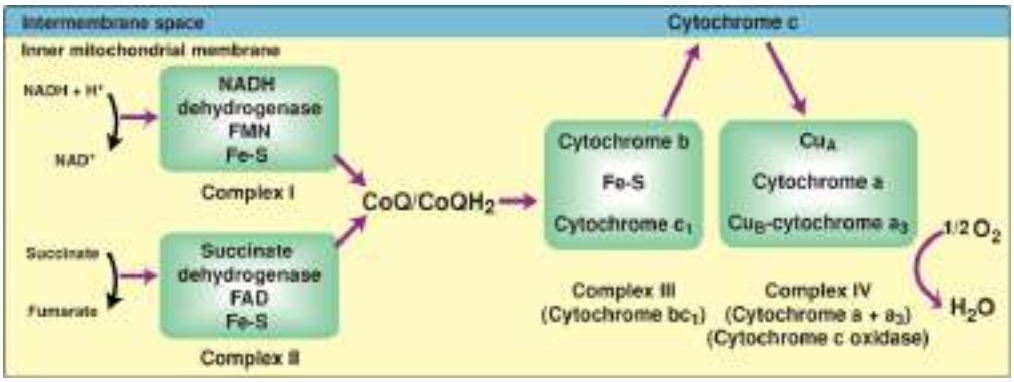


Figure 6.8
Electron transport chain. Electron flow is shown by *magenta arrows*. NAD(H) = nicotinamide adenine dinucleotide; FMN = flavin mononucleotide; FAD = flavin adenine dinucleotide; Fe-S = iron–sulfur; CoQ = coenzyme Q; Cu = copper.

6. Cytochrome a + a_3: Because this cytochrome complex (Complex IV) is the only electron carrier in which the heme iron has an available coordination site that can react directly with O_2, it also is called cytochrome c oxidase. At Complex IV, the transported electrons, O_2, and free H^+ are brought together, and O_2 is reduced to H_2O (see Fig. 6.8). (Note: Four electrons are required to reduce one molecule of O_2 to two molecules of H_2O.) Cytochrome c oxidase contains Cu atoms that are required for this complicated reaction to occur. Electrons move from Cu_A to cytochrome a to cytochrome a_3 (in association with Cu_B) to O_2.

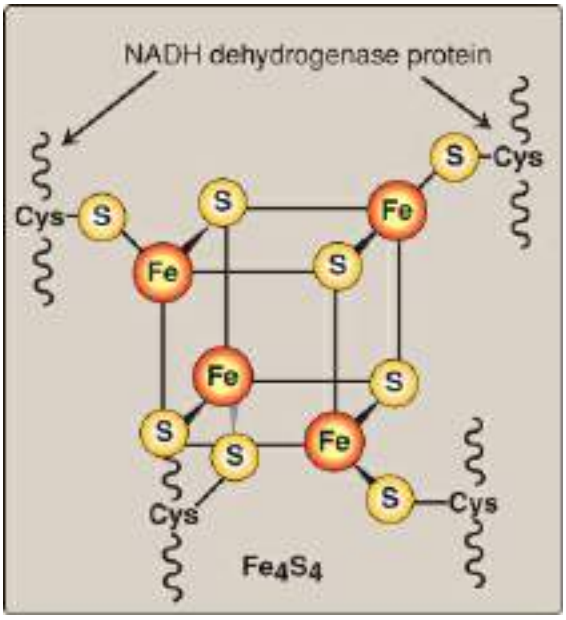


Figure 6.9
Iron–sulfur (Fe–S) center of Complex I. (Note: Complexes II and III also contain Fe–S centers.) NADH = nicotinamide adenine dinucleotide; Cys = cysteine.

7. Site-specific inhibitors: Inhibitors of specific sites in the ETC have been identified and are illustrated in Figure 6.10. These respiratory inhibitors prevent the passage of electrons by binding to a component of the chain, blocking the oxidation–reduction reaction. Therefore, all electron carriers before the block are fully reduced, whereas those located after the block are oxidized. (Note: Inhibition of the ETC inhibits ATP synthesis because these processes are tightly coupled.)

> Leakage of electrons from the ETC produces reactive oxygen species (ROS), such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH). ROS damages DNA and proteins and cause lipid peroxidation. Enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase are cellular defenses against ROS (see p. 163).

D. Free energy release during electron transport

The free energy released as electrons is transferred along the ETC from an electron donor (reducing agent or reductant) to an electron acceptor (oxidizing agent or oxidant) is used to pump H^+ at Complexes I, III, and IV. (Note: The electrons can be transferred as hydride ions to NAD^+; as hydrogen atoms to FMN, CoQ, and FAD; or as electrons to cytochromes.)

1. Redox pairs: Oxidation (loss of electrons) of one substance is always accompanied by reduction (gain of electrons) of a second. For example, Figure 6.11 shows the oxidation of NADH to NAD^+ by NADH dehydrogenase at

Complex I, accompanied by the reduction of FMN, the prosthetic group, to $FMNH_2$. Such redox reactions can be written as the sum of two separate half reactions, one an oxidation and the other a reduction (see Fig. 6.11). NAD^+ and NADH form a redox pair, as do FMN and $FMNH_2$. Redox pairs differ in their tendency to lose electrons. This tendency is a characteristic of a particular redox pair and can be quantitatively specified by a constant, E_0 (the standard reduction potential), with units in volts.

2. Standard reduction potential: The E_0 of various redox pairs can be ordered from the most negative E_0 to the most positive. The more negative the E_0 of a redox pair, the greater the tendency of the reductant member of that pair to lose electrons. The more positive the E_0, the greater the tendency of the oxidant member of that pair to accept electrons. Therefore, electrons flow from the pair with the more negative E_0 to that with the more positive E_0. The E_0 values for some members of the ETC are shown in Figure 6.12. (Note: The components of the chain are arranged in order of increasingly positive E_0 values.)

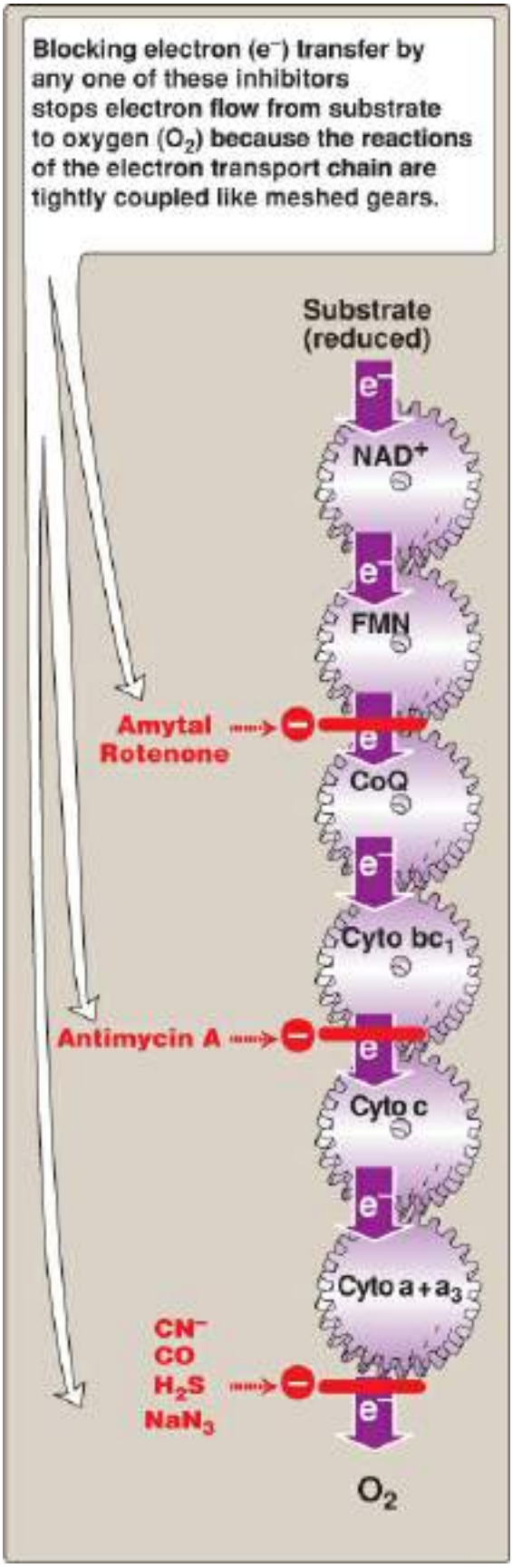


Figure 6.10
Site-specific inhibitors of electron transport shown using a mechanical model for the coupling of oxidation–reduction reactions. (Note: Normal direction of electron flow is illustrated.) NAD^+ = nicotinamide adenine dinucleotide; FMN = flavin mononucleotide; CoQ = coenzyme Q; Cyto = cytochrome; CN^- = cyanide; CO = carbon monoxide; H_2S = hydrogen sulfide; NaN_3 = sodium azide.

3. Relationship of ΔG^0 to ΔE_0: The ΔG^0 is related directly to the magnitude of the change in E_0:

$$\Delta G^0 = -nF\,\Delta E_0,$$

where n = number of electrons transferred (1 for a cytochrome, 2 for NADH, $FADH_2$, and CoQ)
F = Faraday constant (23.1 kcal/volt mol)
ΔE_0 = E_0 of the electron-accepting pair minus the E_0 of the electron-donating pair
ΔG^0 = change in the standard free energy

4. ΔG^0 of ATP: The $\Delta G°$ for the phosphorylation of ADP to ATP is +7.3 kcal/mol. The transport of a pair of electrons from NADH to O_2 through the ETC releases 52.6 kcal. Therefore, more than sufficient energy is available to produce three ATP from three ADP and three P_i (3 × 7.3 = 21.9 kcal/mol), sometimes expressed as a P/O ratio (ATP made per O atom reduced) of 3:1. The remaining calories are used for ancillary reactions or released as heat. (Note: The P:O for $FADH_2$ is 2:1 because Complex I is bypassed.)

VI. PHOSPHORYLATION OF ADP TO ATP

The transfer of electrons down the ETC is energetically favored because NADH is a strong electron donor and O_2 is an avid electron acceptor. However, the flow of electrons does not directly result in ATP synthesis.

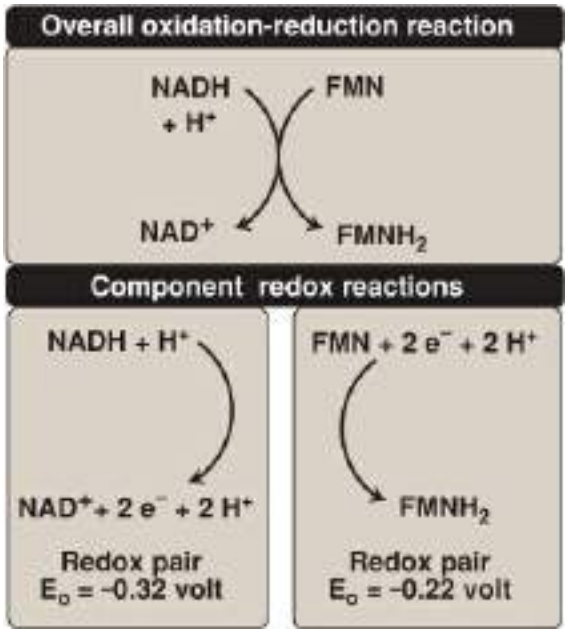


Figure 6.11
Oxidation of NADH by FMN, separated into two component half reactions. NAD(H) = nicotinamide adenine dinucleotide; FMN(H_2) = flavin mononucleotide; e^- = electron; H^+ = proton; E0 = standard reduction potential.

A. Chemiosmotic hypothesis

The chemiosmotic hypothesis (also known as the Mitchell hypothesis) explains how the free energy generated by the transport of electrons by the ETC is used to produce ATP from ADP + P_i.

1. Proton pump: Electron transport is coupled to ADP phosphorylation by the pumping of H^+ across the inner mitochondrial membrane, from the matrix to the intermembrane space, at Complexes I, III, and IV. For each pair of electrons transferred from NADH to O_2, 10 H^+ are pumped. This creates an electrical gradient (with more positive charges on the cytosolic side of the membrane than on the matrix side) and a pH (chemical) gradient (the cytosolic side of the membrane is at a lower pH than the matrix side), as shown in Figure 6.13. The energy (proton-motive force) generated by these gradients is sufficient to drive ATP synthesis. Thus, the H^+ gradient serves as the common intermediate that couples oxidation to phosphorylation.

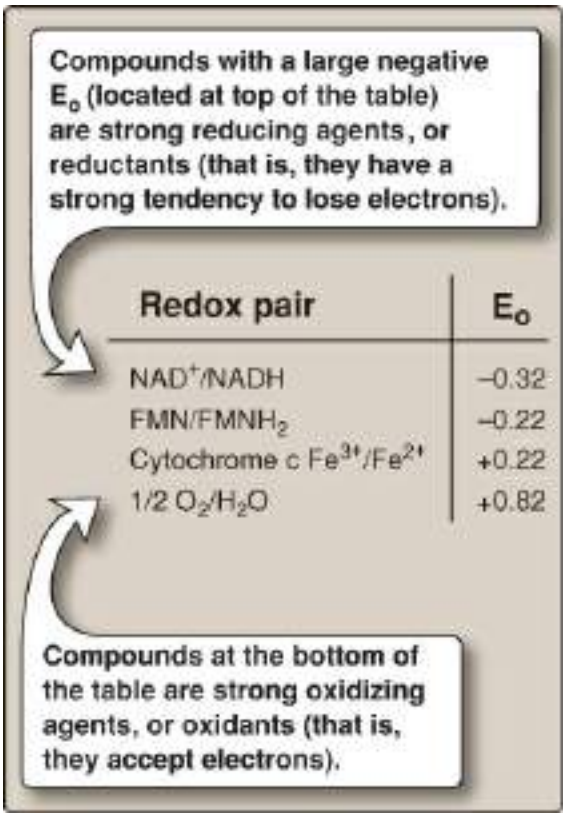


Figure 6.12
Standard reduction potentials (E_0) of some reactions. NAD(H) = nicotinamide adenine dinucleotide; FMN(H_2) = flavin mononucleotide; Fe = iron.

2. ATP synthase: The multisubunit enzyme ATP synthase ([Complex V], Fig. 6.14) synthesizes ATP using the energy of the H^+ gradient. It contains a membrane domain (F_o) that spans the inner mitochondrial membrane and an extramembranous domain (F_1) that appears as a sphere that protrudes into the mitochondrial matrix (see Fig. 6.13). The chemiosmotic hypothesis proposes that after H^+ have been pumped to the cytosolic side of the inner mitochondrial membrane, they reenter the matrix by passing through a H^+ channel in the F_o domain, driving rotation of the c ring of F_o and, at the same time, dissipating the pH and electrical gradients. Rotation in F_o causes conformational changes in the three β subunits of F_1 that allow them to bind ADP + P_i, phosphorylate ADP to ATP, and release ATP. One complete rotation of the c ring produces three ATP. (Note: ATP synthase is also called F_1/F_o-ATPase because the enzyme can also catalyze the hydrolysis of ATP to ADP and P_i.)

a. Coupling in oxidative phosphorylation: In normal mitochondria, ATP synthesis is coupled to electron transport through the H^+ gradient. Increasing (or decreasing) one process has the same effect on the other. For example, hydrolysis of ATP to ADP and P_i in energy-requiring reactions increases the

availability of substrates for ATP synthase and, thus, increases H^+ flow through the enzyme. Electron transport and H^+ pumping by the ETC increase to maintain the H^+ gradient and allow ATP synthesis.

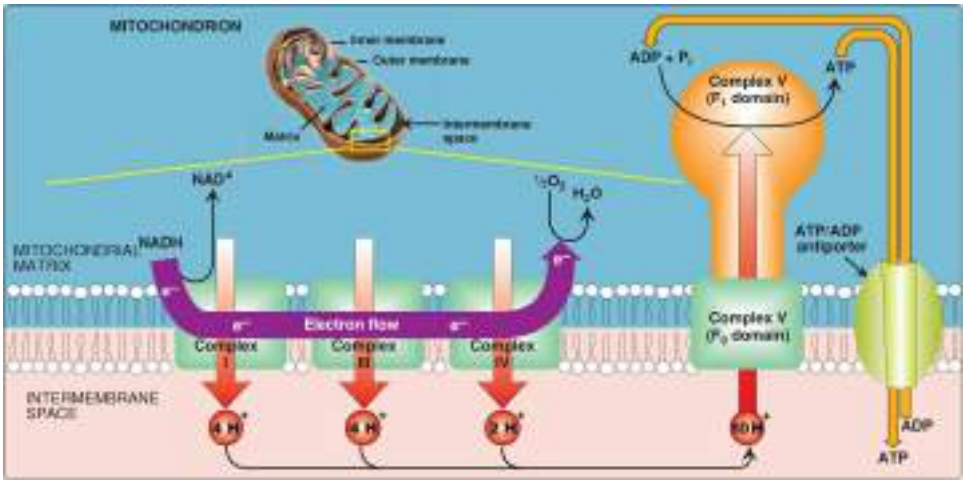


Figure 6.13
Electron transport chain shown in association with proton (H^+) pumping. Ten H^+ are pumped for each nicotinamide adenine dinucleotide (NADH) oxidized. (Note: $H^+$are not pumped at Complex II.) e^- = electron; complex V = ATP synthase.

b. Oligomycin: This drug binds to the F_o (hence the letter "o") domain of ATP synthase, closing the H^+ channel and preventing reentry of H^+ into the matrix, thereby inhibiting phosphorylation of ADP to ATP. Because the pH and electrical gradients cannot be dissipated in the presence of this phosphorylation inhibitor, electron transport stops because of the difficulty of pumping any more H^+ against the steep concentration gradient. This dependency of cellular respiration on the ability to phosphorylate ADP to ATP is known as respiratory control and is the consequence of the tight coupling of these processes.

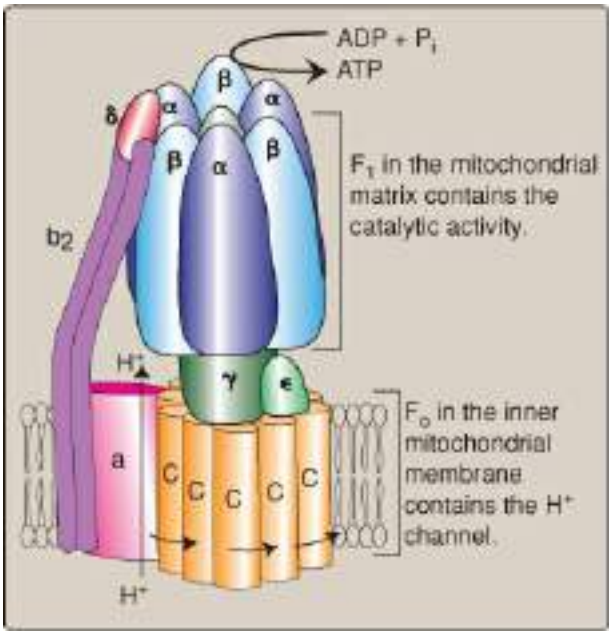


Figure 6.14
ATP synthase (F_1F_o-ATPase). (Note: The cring of vertebrates contains eight subunits. One complete turn of the ring is driven by eight H^+(protons) moving through the F_o domain. The resulting conformational changes in the three β subunits of the F_1 domain allow phosphorylation of three adenosine diphosphates[ADP] to three ATP.) P_i = inorganic phosphate.

c. Uncoupling proteins: Uncoupling proteins (UCPs) occur in the inner mitochondrial membrane of mammals, including humans. These proteins form channels that allow H^+ to reenter the mitochondrial matrix without energy being captured as ATP (Fig. 6.15). The energy is released as heat, and the process is called nonshivering thermogenesis. UCP1, also called thermogenin, is responsible for heat production in the mitochondria-rich brown adipocytes of mammals. (Note: Cold causes catecholamine-dependent activation of UCP1 expression.) In brown fat, unlike the more abundant white fat, ~90% of its respiratory energy is used for thermogenesis in infants in response to cold. Thus, brown fat is involved in energy expenditure, whereas white fat is involved in energy storage. (Note: Brown fat depots have recently been shown to be present in adults.)

d. Synthetic uncouplers: Electron transport and phosphorylation of ADP can also be uncoupled by compounds that shuttle H^+ across the inner mitochondrial membrane, dissipating the gradient. The classic example is 2,4-dinitrophenol, a lipophilic H^+ carrier (ionophore) that readily diffuses through the mitochondrial membrane (Fig. 6.16). This uncoupler causes electron transport to proceed at a rapid rate without establishing a H^+ gradient, much as do the UCP. Again, energy is released as heat rather than being used to synthesize ATP. (Note: In high doses, aspirin and other salicylates uncouple oxidative phosphorylation, explaining the fever that

accompanies toxic overdoses of these drugs.)

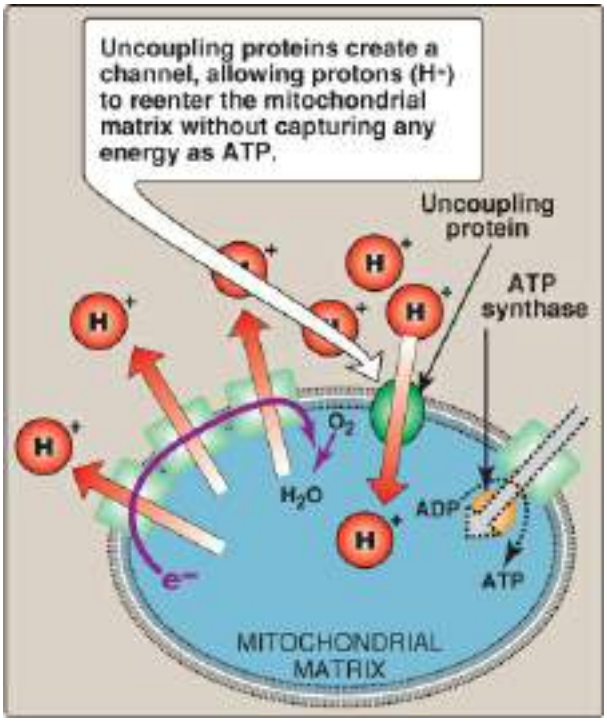


Figure 6.15
Transport of protons across the mitochondrial membrane by an uncoupling protein. ADP = adenosine diphosphate; e^- = electrons.

B. Membrane transport systems

The inner mitochondrial membrane is impermeable to most charged or hydrophilic substances. However, it contains numerous transport proteins that permit passage of certain molecules from the cytosol to the mitochondrial matrix.

1. ATP and ADP transport: The inner membrane requires specialized carriers to transport ADP and P_i from the cytosol (where ATP is hydrolyzed to ADP in many energy-requiring reactions) into mitochondria, where ATP can be resynthesized. An adenine nucleotide antiporter imports one ADP from the cytosol into the matrix, while exporting one ATP from the matrix into the cytosol (see Fig. 6.13). A symportercotransports P_i and H^+ from the cytosol into the matrix.

2. Reducing equivalent transport: The inner mitochondrial membrane lacks an NADH transporter, and NADH produced in the cytosol (e.g., in glycolysis; see p. 111) cannot directly enter the mitochondrial matrix. However, reducing equivalents of NADH are transported from the cytosol into the matrix using substrate shuttles. In the glycerol 3-phosphate shuttle (Fig. 6.17A), two electrons are transferred from NADH to dihydroxyacetone phosphate by cytosolic glycerol 3-phosphate dehydrogenase. The glycerol 3-phosphate

produced is oxidized by the mitochondrial isozyme as FAD is reduced to $FADH_2$. CoQ of the ETC oxidizes the $FADH_2$. Therefore, the glycerol 3-phosphate shuttle results in the synthesis of two ATP for each cytosolic NADH oxidized. This contrasts with the malate–aspartate shuttle (Fig. 6.17B), which produces NADH (rather than $FADH_2$) in the mitochondrial matrix, thereby yielding three ATP for each cytosolic NADH oxidized by malate dehydrogenase as oxaloacetate is reduced to malate. A transport protein moves malate into the mitochondrial matrix.

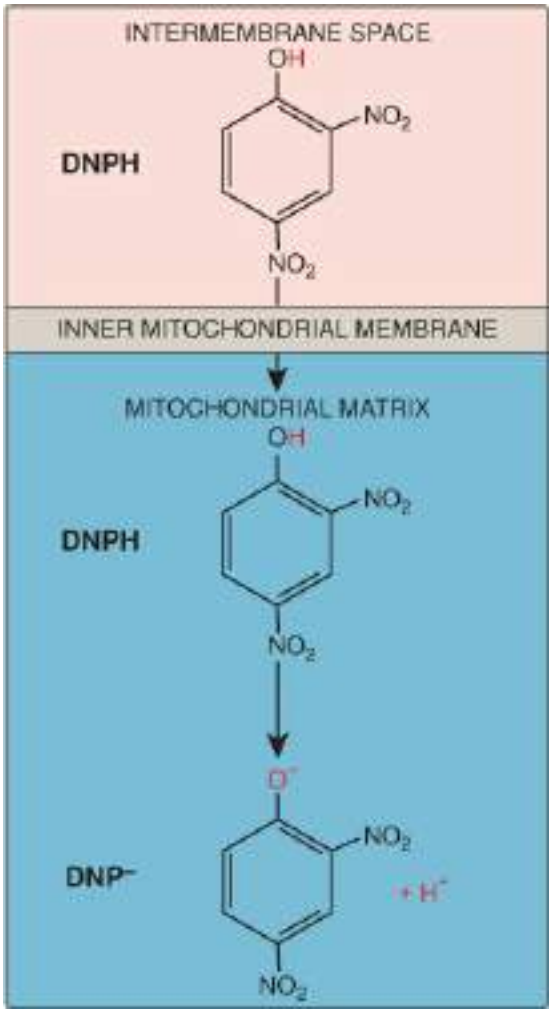


Figure 6.16
2,4-Dinitrophenol (DNP), a proton (H^+) carrier, shown in its reduced (DNPH) and oxidized (DNP^-) forms.

C. Inherited defects in oxidative phosphorylation

Thirteen of the ~90 polypeptides required for oxidative phosphorylation are encoded by mtDNA and synthesized in mitochondria, whereas the remaining proteins are encoded by nuclear DNA, synthesized in the cytosol, and then transported into

mitochondria. Defects in oxidative phosphorylation are more likely a result of alterations in mtDNA, which has a mutation rate about 10 times greater than that of nuclear DNA. Cells in tissues with high ATP requirements include those in brain, nerves, retina, skeletal and heart muscle, and the liver are particularly vulnerable. Impairments in oxidative phosphorylation usually cause lactic acidosis, particularly in the muscles, central nervous system, and retina. Clinical manifestations of oxidative phosphorylation disorders include seizures, ophthalmoplegia, muscle weakness, and cardiomyopathy (Table 6.1). Some medications are known to affect mitochondrial function and these should be avoided in persons with mitochondrial disorders. (Note: mtDNA is maternally inherited because mitochondria from the sperm do not survive the fertilization process and only those from the oocyte survive in the developing embryo and into the adult individual.)

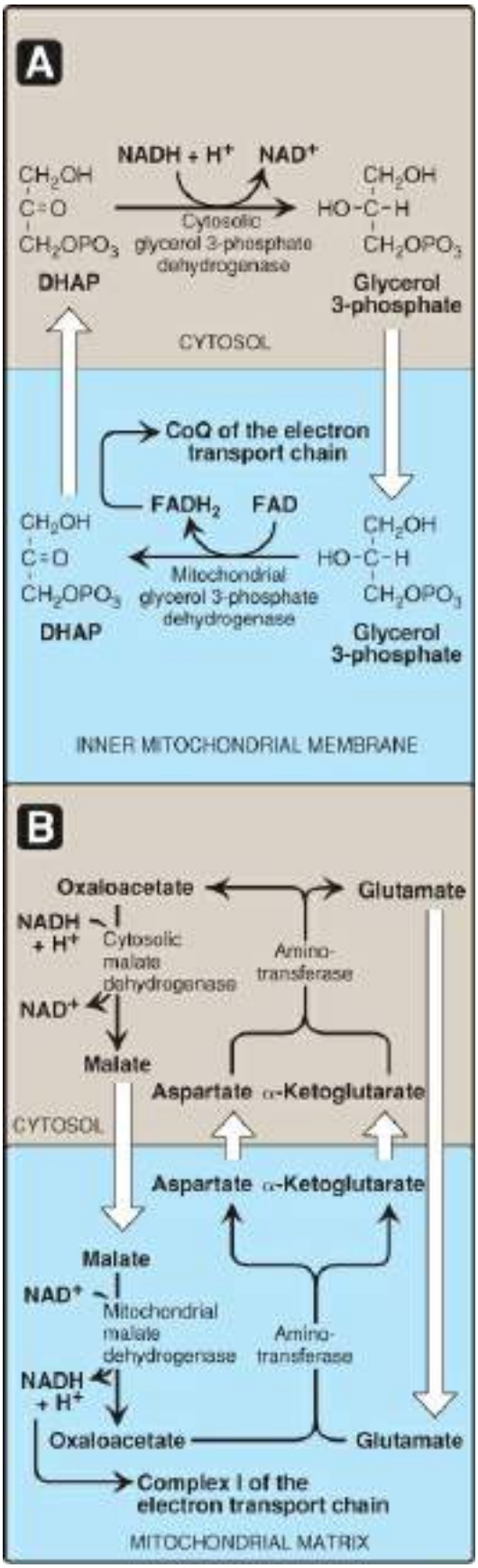


Figure 6.17
Substrate shuttles for the transport of reducing equivalents across the inner mitochondrial membrane. **A:** Glycerol 3-phosphate shuttle. **B:** Malate–aspartate shuttle. DHAP = dihydroxyacetone phosphate; NAD(H) = nicotinamide adenine dinucleotide; H^+ = proton; FAD(H_2) = flavin adenine dinucleotide; CoQ = coenzyme Q.

Table 6.1 Disorders of Mitochondrial Oxidative Phosphorylation

Disease	Characteristics
Kearns–Sayre syndrome	• Weakness or paralysis of eye muscles with drooping eyelids (ptosis), vision loss, cardiac conduction defects, unsteadiness when walking (ataxia), muscle weakness in limbs, kidney problems, deterioration of cognitive function (dementia), and short stature • Features appear before age 20 • Caused by mutation in mtDNA
Leber hereditary optic neuropathy (LHON)	• Bilateral central vision loss caused by retinal detachment • Onset usually in the patient's 20s or 30s • Caused by mitochondrial inheritance along maternal line, however four times more males are affected than females
Leigh disease	• Severe neurologic disorder that manifests in first year of life. Progressive swallowing problems, poor weight gain, hypotonia, weakness, ataxia, nystagmus, and optic atrophy accompany lactic acidosis • Death usually occurs between ages 2 and 3 yrs from respiratory failure • Caused by mutations in nuclear or mtDNA
Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS)	• Progressive neurodegeneration • Repeated episodes of lactic acidosis and myopathy • Cells often contain mutant and wild-typemtDNA and expression is variable
Myoclonic epilepsy with ragged-red fibers (MERRF)	• Progressive condition • Uncontrolled muscle contractions, dementia, ataxia, and myopathy • Caused by mutation in mtDNA; expression of disease is variable
Neuropathy, ataxia, and retinitis pigmentosa (NARP)	• Progressive condition • Sensory neuropathy with numbness or tingling in the extremities, muscle weakness, ataxia and vision loss, cognitive decline, and seizures • Caused by mutation in mtDNA altering ATP synthase and reducing ability to make ATP

D. Mitochondria and apoptosis

The process of apoptosis or programmed cell death may be initiated through the intrinsic or mitochondrial-mediated pathway in response to irreparable damage within the cell. During this process, channel proteins (Bax or Bak) are inserted in the outer mitochondrial membrane and allow cytochrome c to leave the intermembrane space and enter the cytosol. There, cytochrome c, in association with proapoptotic factors to form a structure called the apoptosome which then activates a family of proteolytic enzymes (the caspases), causing cleavage of key proteins and resulting in the morphologic and biochemical changes characteristic of apoptosis.*

VII. Chapter Summary

- The **change** in **free energy** (ΔG) occurring during a reaction predicts the **direction** in which that reaction will spontaneously proceed (Fig. 6.18).
- If ΔG is **negative**, then the reaction is **spontaneous** as written. If ΔG is **positive**, then the reaction is **not spontaneous**. If ΔG = **0**, then the reaction is in **equilibrium**.
- The ΔG of the forward reaction is equal in magnitude but opposite in sign to that of the reverse reaction.
- Reactions with a large, positive ΔG are made possible by **coupling** with those that have a large, negative ΔG such as **ATP hydrolysis**.
- The reduced coenzymes **NADH** and **$FADH_2$** each donate a pair of electrons to a specialized set of **electron carriers**, consisting of **FMN**, Fe–S center**s**, **CoQ**, and a series of heme-containing **cytochromes**, collectively called the **ETC**.
- This pathway is present in the **inner mitochondrial membrane** and is the final common pathway by which electrons derived from different fuels of the body flow to O_2, which has a large, positive **reduction potential** (**E_0**), reducing it to water.
- The terminal cytochrome, **cytochrome c oxidase**, is the only cytochrome able to bind O_2.
- Electron transport results in the **pumping of protons** (**H^+**) across the inner mitochondrial membrane from the matrix to the intermembrane space, 10 H^+ per NADH oxidized.
- This process creates **electrical** and **pH gradients** across the inner mitochondrial membrane. After H^+ have been transferred to the cytosolic side of the membrane, they reenter the matrix by passing through the **F_o** H^+ channel in **ATP synthase** (**Complex V**), dissipating the pH and electrical gradients and causing conformational changes in the **F_1** β subunits of the synthase that result in the synthesis of ATP from ADP + inorganic phosphate.
- **Electron transport** and **phosphorylation** are tightly coupled in **oxidative phosphorylation**. These processes can be uncoupled by **UCP1** of the inner mitochondrial membrane of brown adipocytes and by synthetic compounds such as **2,4-dinitrophenol** and **aspirin**, all of which dissipate the H^+ gradient.
- In uncoupled mitochondria, the energy produced by electron transport is released as **heat** rather than being used to synthesize ATP.
- Defects in oxidative phosphorylation are usually a result of alterations in mtDNA. Impairments in oxidative phosphorylation usually cause **lactic acidosis**, particularly in the muscles, central nervous system, and retina. Clinical manifestations of oxidative phosphorylation disorders include seizures, ophthalmoplegia, muscle weakness, and cardiomyopathy.
- The release of **cytochrome c** from mitochondria into the cytosol stimulates generation of the apoptosome and subsequent activation of proteolyticcaspases that results in **apoptotic cell death**.

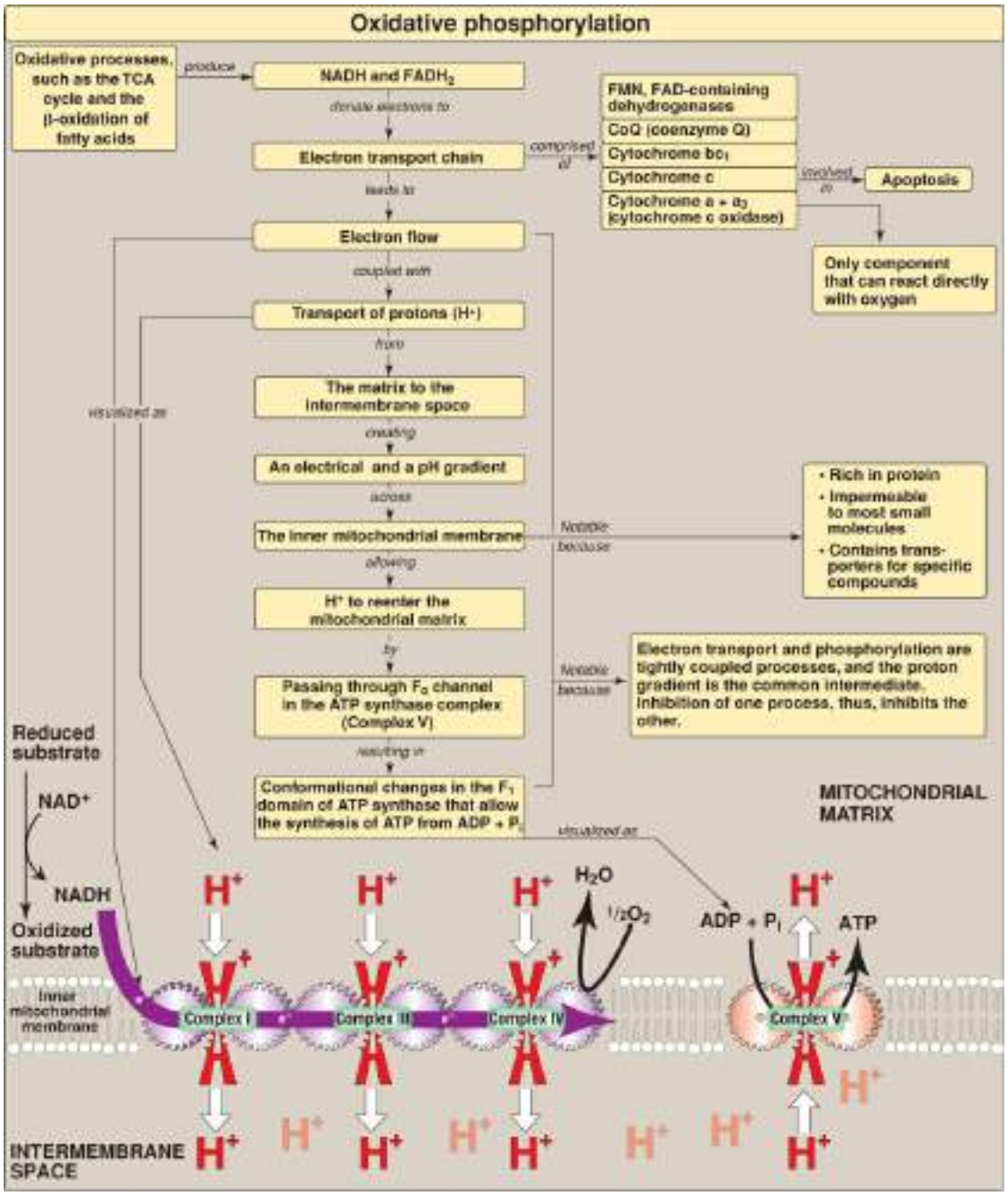


Figure 6.18

Key concept map for oxidative phosphorylation (OXPHOS). (Note: Electron [e^-] flow and ATP synthesis are shown as sets of interlocking gears to emphasize coupling.) TCA = tricarboxylic acid; NAD(H) = nicotinamide adenine dinucleotide; FAD(H_2) = flavin adenine dinucleotide; FMN = flavin mononucleotide; ADP = adenosine diphosphate.

Study Questions

Choose the ONE best answer.

6.1 2,4-Dinitrophenol (DNP), an uncoupler of oxidative phosphorylation, was used as a weight-loss agent in the

1930s. Reports of fatal overdoses led to its discontinuation in 1939. Which of the following would most likely be true concerning individuals taking 2,4-DNP?

A. ATP levels in the mitochondria are greater than normal.
B. Body temperature is elevated as a result of hypermetabolism.
C. Cyanide has no effect on electron flow.
D. The H+ gradient across the inner mitochondrial membrane is greater than normal.
E. The rate of electron transport is abnormally low.

Correct answer = B. When phosphorylation is uncoupled from electron flow, a decrease in the proton gradient across the inner mitochondrial membrane and, therefore, impaired ATP synthesis are expected. In an attempt to compensate for this defect in energy capture, metabolism and electron flow to oxygen are increased. This hypermetabolism will be accompanied by elevated body temperature because the energy in fuels is largely wasted, appearing as heat. The electron transport chain will still be inhibited by cyanide.

6.2 Which of the following has the strongest tendency to gain electrons?

A. Coenzyme Q
B. Cytochrome c
C. Flavin adenine dinucleotide
D. Nicotinamide adenine dinucleotide
E. Oxygen

Correct answer = E. Oxygen is the terminal acceptor of electrons in the electron transport chain (ETC). Electrons flow down the ETC to oxygen because it has the highest (most positive) reduction potential (E_0). The other choices precede oxygen in the ETC and have lower E_0 values.

6.3 Explain why and how the malate–aspartate shuttle moves nicotinamide adenine dinucleotide reducing equivalents from the cytosol to the mitochondrial matrix.

There is no transporter for nicotinamide adenine dinucleotide (NADH) in the inner mitochondrial membrane. However, cytoplasmic NADH can be oxidized to NAD^+ by malate dehydrogenase as oxaloacetate (OAA) is reduced to malate. The malate is transported across the inner membrane to the matrix where the mitochondrial isozyme of malate dehydrogenase oxidizes it to OAA as mitochondrial NAD^+ is reduced to NADH. This NADH can be oxidized by Complex I of the electron transport chain, generating three ATP through the coupled processes of oxidative phosphorylation.

6.4 Carbon monoxide (CO) binds to and inhibits Complex IV of the electron transport chain. What effect, if any, should this respiratory inhibitor have on phosphorylation of adenosine diphosphate (ADP) to ATP?

Inhibition of electron transport by respiratory inhibitors such as CO results in an inability to maintain the proton (H^+) gradient. Therefore, phosphorylation of ADP to ATP is inhibited, as are ancillary reactions such as calcium uptake by mitochondria, because they also require the H^+ gradient.

6.5 Persons with defects in oxidative phosphorylation most often developed their condition from

A. acquired damage to autosomal genes.
B. inheritance of a mutation on mtDNA.
C. mutations inherited from their father.
D. X-linked inheritance from their mother.

Correct answer = B. Defects in oxidative phosphorylation are more likely a result of alterations in mtDNA, which has a mutation rate about 10 times greater than that of nuclear DNA. Mitochondria and mtDNA are inherited exclusively from the mother. X-linked inheritance is of nuclear DNA, not mtDNA.

*For further discussion of apopotosis, see *LIR Cell and Molecular Biology*, 2nd Edition, Chapter 23.

Introduction to Carbohydrates 7

I. OVERVIEW

Carbohydrates are the most abundant organic molecules in nature. They have a wide range of functions, including providing a significant fraction of the dietary calories for most organisms, acting as a storage form of energy in the body, and serving as cell membrane components that mediate some forms of intercellular communication. Carbohydrates also serve as a structural component of many organisms, including the cell walls of bacteria, the exoskeleton of insects, and the fibrous cellulose of plants. The empiric formula for many of the simpler carbohydrates is $(CH_2O)_n$, where $n \geq 3$, hence the name “hydrate of carbon.”

II. CLASSIFICATION AND STRUCTURE

Monosaccharides or simple sugars can be classified according to the number of carbon atoms they contain. Examples of some monosaccharides commonly found in humans are listed in Figure 7.1. They can also be classified by the type of carbonyl group they contain. Carbohydrates with an aldehyde as their carbonyl group are called aldoses, whereas those with a keto as their carbonyl group are called ketoses (Fig. 7.2). For example, glyceraldehyde is an aldose, whereas dihydroxyacetone is a ketose. Carbohydrates that have a free carbonyl group have the suffix -ose. (Note: Ketoses have an additional “ul” in their suffix such as xylulose. There are exceptions, such as fructose, to this rule.) Monosaccharides can be linked by glycosidic bonds to create larger structures (Fig. 7.3). Disaccharides contain two monosaccharide units, oligosaccharides contain 3 to 10 monosaccharide units, and polysaccharides contain more than 10 monosaccharide units and can be hundreds of sugar units in length.

GENERIC NAMES		EXAMPLES
3 Carbons:	trioses	Glyceraldehyde
4 Carbons:	tetroses	Erythrose
5 Carbons:	pentoses	Ribose
6 Carbons:	hexoses	Glucose
7 Carbons:	heptoses	Sedoheptulose
9 Carbons:	nonoses	Neuraminic acid

Figure 7.1
Examples of monosaccharides found in humans, classified according to the number of carbons they contain.

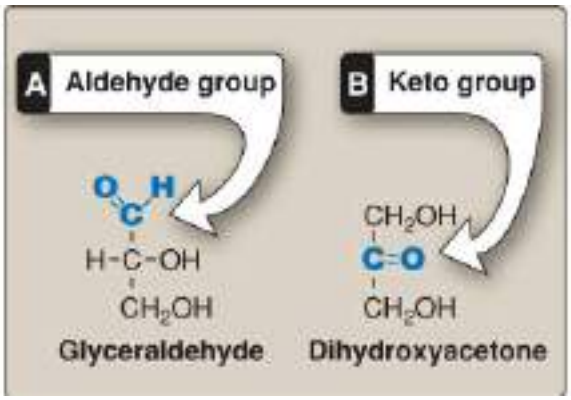


Figure 7.2
Examples of an aldose (**A**) and a ketose (**B**) sugar.

A. Isomers and epimers

Compounds that have the same chemical formula but different structures are isomers of each other. For example, fructose, glucose, mannose, and galactose all have the same chemical formula, $C_6H_{12}O_6$, with different structures. Carbohydrate isomers that differ in configuration around only one specific carbon atom (with the exception of the carbonyl carbon, see C. 1. below) are defined as epimers of each other. For example, glucose and galactose are C-4 epimers because their structures differ only in the position of the –OH (hydroxyl) group at carbon 4. (Note: The carbons in sugars are numbered beginning at the end that contains the carbonyl carbon [i.e., the aldehyde or keto group], as shown in Fig. 7.4.) Glucose and mannose are C-2 epimers. However, because galactose and mannose differ in the position of –OH groups at two carbons (carbons 2 and 4), they are isomers rather than epimers (see Fig. 7.4).

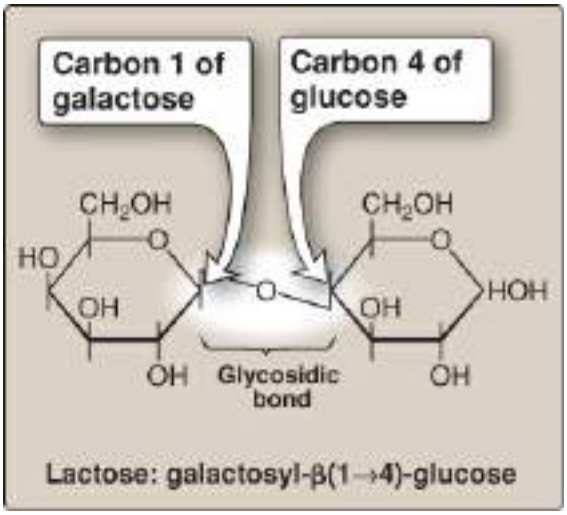


Figure 7.3
A glycosidic bond between two hexoses producing a disaccharide.

B. Enantiomers

A special type of isomerism is found in the pairs of structures that are mirror images of each other. These mirror images are called enantiomers, and the two members of the pair are designated as a D- and an L-sugar (Fig. 7.5). The vast majority of the sugars in humans are D-isomers. In the D-isomeric form, the –OH group on the asymmetric carbon (a carbon linked to four different atoms or groups) farthest from the carbonyl carbon is on the right, whereas in the L-isomer, it is on the left. Most enzymes are specific for either the D or the L form, but enzymes known as isomerases are able to interconvert D- and L-isomers.

C. Monosaccharide cyclization

Less than 1% of each of the monosaccharides with five or more carbons exists in the open-chain (acyclic) form in solution. Rather, they are predominantly found in a ring or cyclic form, in which the aldehyde (or keto) group has reacted with a hydroxyl group on the same sugar, making the carbonyl carbon (carbon 1 for an aldose, carbon 2 for a ketose) asymmetric. This asymmetric carbon is referred to as the anomeric carbon.

1. Anomers: Creation of an anomeric carbon (the former carbonyl carbon) generates a new pair of isomers, the α and β configurations of the sugar (e.g., α-D-glucopyranose and β-D-glucopyranose), as shown in Figure 7.6, that are anomers of each other. (Note: In the α configuration, the –OH group on the anomeric carbon projects to the same side as the ring in a modified Fischer projection formula [see Fig. 7.6A] and is trans to the CH_2OH group in a Haworth projection formula [see Fig. 7.6B]. The α and β forms are not mirror images, and they are referred to as diastereomers.) Enzymes are able to distinguish between these two structures and use one or the other preferentially. For example, glycogen is synthesized from α-D-glucopyranose, whereas cellulose is synthesized from β-D-glucopyranose. The cyclic α and β anomers of a sugar in solution spontaneously (but slowly) form an equilibrium mixture, a process known as mutarotation (see Fig. 7.6). (Note: For glucose, the α form makes up 36% of the mixture.)

2. Reducing sugars: If the hydroxyl group on the anomeric carbon of a cyclized sugar is not linked to another compound by a glycosidic bond (see E. below), the ring can open. The sugar can act as a reducing agent and is termed a reducing sugar. Such sugars can react with chromogenic agents (e.g., the Benedict reagent) causing the reagent to be reduced and colored as the aldehyde group of the acyclic sugar is oxidized to a carboxyl group. All monosaccharides, but not all disaccharides, are reducing sugars. (Note: Fructose, a ketose, is a reducing sugar because it can be isomerized to an aldose.)

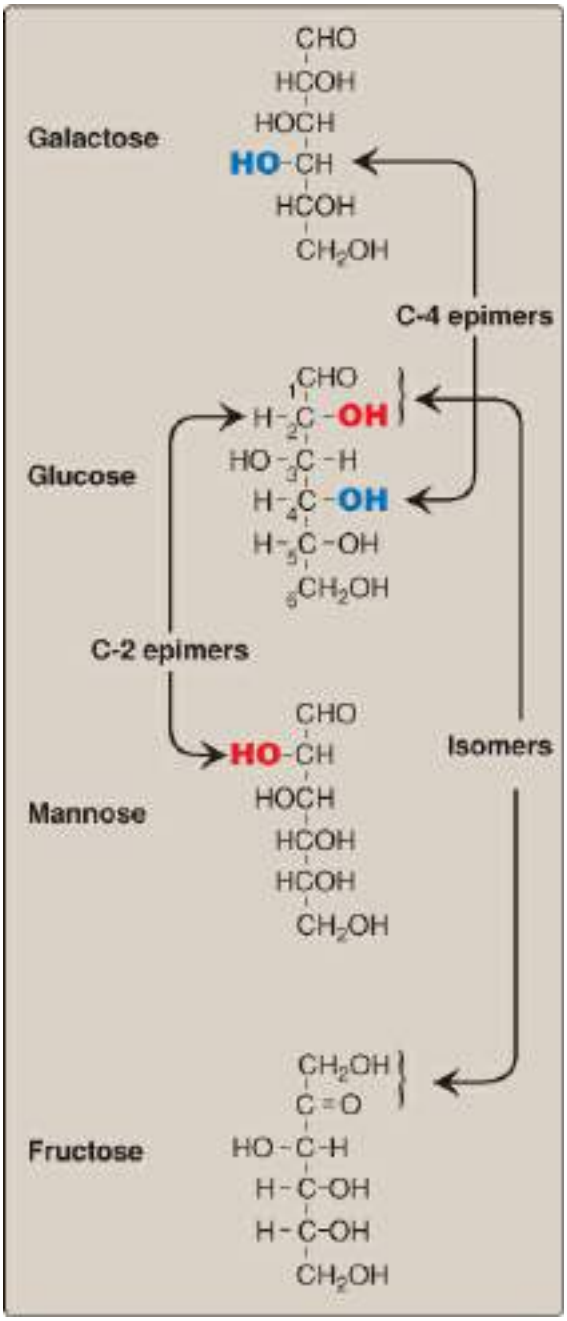


Figure 7.4
Carbon-2 (C-2) and C-4 epimers and an isomer of glucose.

A colorimetric test can detect a reducing sugar in urine. A positive result is indicative of an underlying pathology (because sugars are not normally present in urine) and can be followed up by more specific tests to identify the reducing sugar.

D. Monosaccharide joining

Monosaccharides can be joined to form disaccharides, oligosaccharides, and

polysaccharides. Important disaccharides include lactose (galactose + glucose), sucrose (glucose + fructose), and maltose (glucose + glucose). Important polysaccharides include branched glycogen (from animal sources) and starch (plant sources) and unbranched cellulose (plant sources). Each is a polymer of glucose.

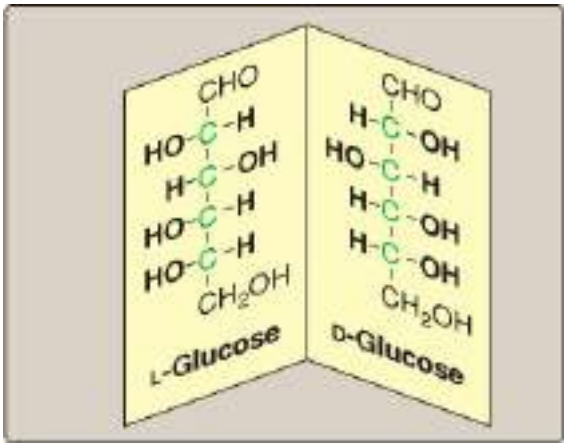


Figure 7.5
Enantiomers (mirror images) of glucose. Designation of D and L is by comparison to a triose, glyceraldehyde. (Note: The asymmetric carbons are shown in *green*.)

E. Glycosidic bonds

The bonds that link sugars are called glycosidic bonds. They are formed by enzymes known as glycosyltransferases that use nucleotide sugars (activated sugars) such as uridine diphosphate glucose as substrates. Glycosidic bonds between sugars are named according to the numbers of the connected carbons and with regard to the position of the anomeric hydroxyl group of the first sugar involved in the bond. If this anomeric hydroxyl is in the α configuration, then the linkage is an α-bond. If it is in the β configuration, then the linkage is a β-bond. Lactose, for example, is synthesized by forming a glycosidic bond between carbon 1 of β-galactose and carbon 4 of glucose. Therefore, the linkage is a β(1→4) glycosidic bond (see Fig. 7.3). (Note: Because the anomeric end of the glucose residue is not involved in the glycosidic linkage, it [and, therefore, lactose] remains a reducing sugar.)

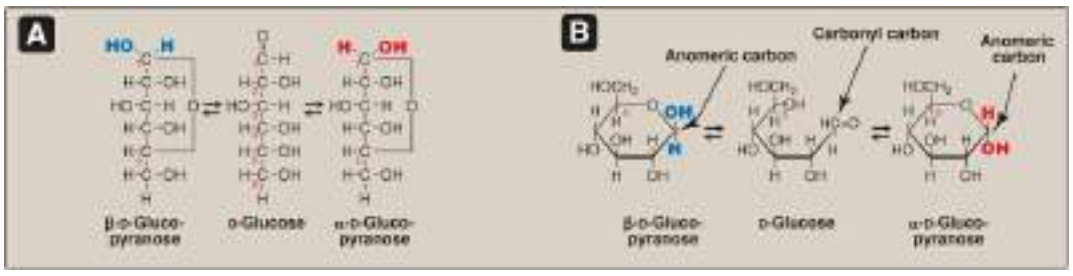


Figure 7.6
A: The interconversion (mutarotation) of the α and β anomeric forms of glucose shown as modified Fischer projection formulas. **B:** The interconversion shown as Haworth projection formulas. (Note: A

sugar with a six-membered ring [5 C + 1 O] is termed a pyranose, whereas one with a five-membered ring [4 C + 1 O] is a furanose. Virtually all glucose in solution is in the pyranose form.)

F. Carbohydrate linkage to noncarbohydrates

Carbohydrates can be attached by glycosidic bonds to noncarbohydrate structures, including purine and pyrimidine bases in nucleic acids, aromatic rings such as those found in steroids, proteins, and lipids. If the group on the noncarbohydrate molecule to which the sugar is attached is an $–NH_2$ group, then the bond is called an N-glycosidic link. If the group is an –OH, then the bond is an O-glycosidic link (Fig. 7.7). (Note: All sugar-sugar glycosidic bonds are O-type linkages.)

III. DIETARY CARBOHYDRATE DIGESTION

The principal sites of dietary carbohydrate digestion are the mouth and intestinal lumen. This digestion is rapid and is catalyzed by enzymes known as glycoside hydrolases (glycosidases) that hydrolyze glycosidic bonds (Fig. 7.8). Because little monosaccharide is present in diets of mixed animal and plant origin, the enzymes are primarily endoglycosidases that hydrolyze polysaccharides and oligosaccharides and disaccharidases that hydrolyze tri- and disaccharides into their reducing sugar components. Glycosidases are usually specific for the structure and configuration of the glycosyl residue to be removed as well as for the type of bond to be broken. The final products of carbohydrate digestion are the monosaccharides glucose, galactose, and fructose that are absorbed by cells (enterocytes) of the small intestine.

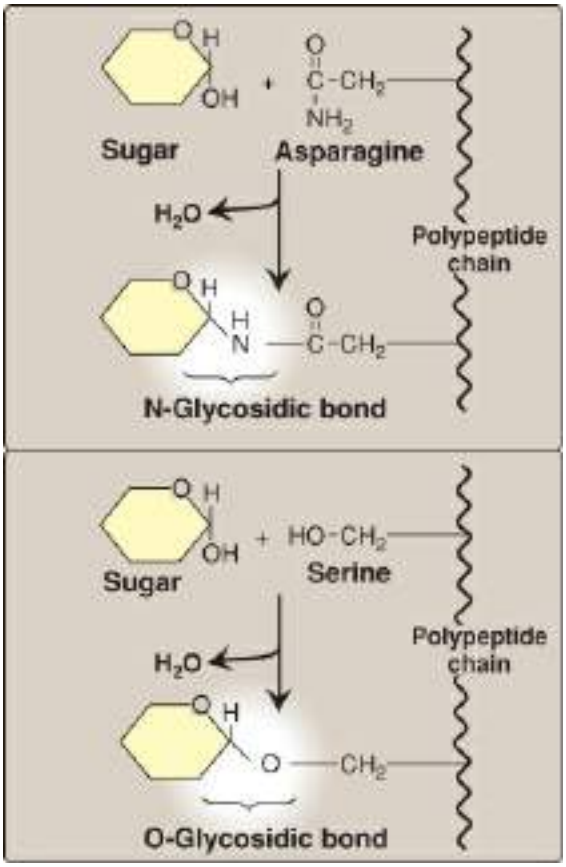


Figure 7.7
Examples of N- and O-glycosidic bonds in glycoproteins.

A. Salivary α-amylase

The major dietary polysaccharides consumed by humans are of plant (starch, composed of amylose and amylopectin) and animal (glycogen) origin. During mastication or chewing, salivary α-amylase acts briefly on dietary starch and glycogen, hydrolyzing random α(1→4) bonds. (Note: There are both α[1→4]- and β[1→4]-endoglucosidases in nature, but humans do not produce the latter. Therefore, we are unable to digest cellulose, a carbohydrate of plant origin containing β[1→4] glycosidic bonds between glucose residues.) Because branched amylopectin and glycogen also contain α(1→6) bonds, which α-amylase cannot hydrolyze, the digest resulting from its action contains a mixture of short, branched and unbranched oligosaccharides known as dextrins (Fig. 7.9). (Note: Disaccharides are also present as they, too, are resistant to amylase.) Carbohydrate digestion halts temporarily in the stomach, because the high acidity inactivates salivary α-amylase.

B. Pancreatic α-amylase

When the acidic stomach contents reach the small intestine, they are neutralized by bicarbonate secreted by the pancreas, and pancreatic α-amylase continues the

process of starch digestion.

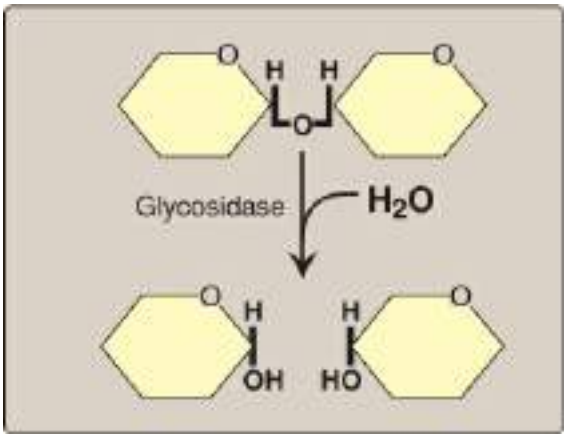


Figure 7.8
Hydrolysis of a glycosidic bond.

C. Intestinal disaccharidases

The final digestive processes occur primarily at the mucosal lining of the duodenum and upper jejunum and include the action of several disaccharidases (see Fig. 7.9). For example, isomaltase cleaves the α(1→6) bond in isomaltose, and maltase cleaves the α(1→4) bond in maltose and maltotriose, each producing glucose. Sucrase cleaves the α(1→2) bond in sucrose, producing glucose and fructose, and lactase (β-galactosidase) cleaves the β(1→4) bond in lactose, producing galactose and glucose. (Note: The substrates for isomaltase are broader than its name suggests, and it hydrolyzes the majority of maltose.) Trehalose, an α(1→1) disaccharide of glucose found in mushrooms and other fungi, is cleaved by trehalase. These enzymes are transmembrane proteins of the brush border on the luminal (apical) surface of the enterocytes.

> Sucrase and isomaltase are enzymatic activities of a single protein cleaved into two functional subunits that remain associated in the cell membrane and form the sucrase–isomaltase (SI) complex. In contrast, maltase is one of two enzymic activities of the single membrane protein maltase—glucoamylase (MGA) that does not get cleaved. Its second enzymic activity, glucoamylase, cleaves α(1→4) glycosidic bonds in dextrins.

D. Intestinal absorption of monosaccharides

The upper jejunum absorbs the bulk of the monosaccharide products of digestion. However, different sugars have different mechanisms of absorption (Fig. 7.10). For example, galactose and glucose are taken into enterocytes by secondary active transport that requires a concurrent uptake (symport) of sodium (Na^+) ions. The transport protein is the sodium-dependent glucose cotransporter 1 (SGLT-1). (Note: Sugar transport is driven by the Na^+ gradient created by the Na^+-potassium [K^+]

ATPase that moves Na^+ out of the enterocyte and K^+ in [see Fig. 7.10].) Fructose absorption utilizes an energy- and Na^+-independent monosaccharide transporter (GLUT-5). All three monosaccharides are transported from the enterocytes into the portal circulation by yet another transporter, GLUT-2. (Note: See pp. 106 and 107 for a discussion of these transporters.)

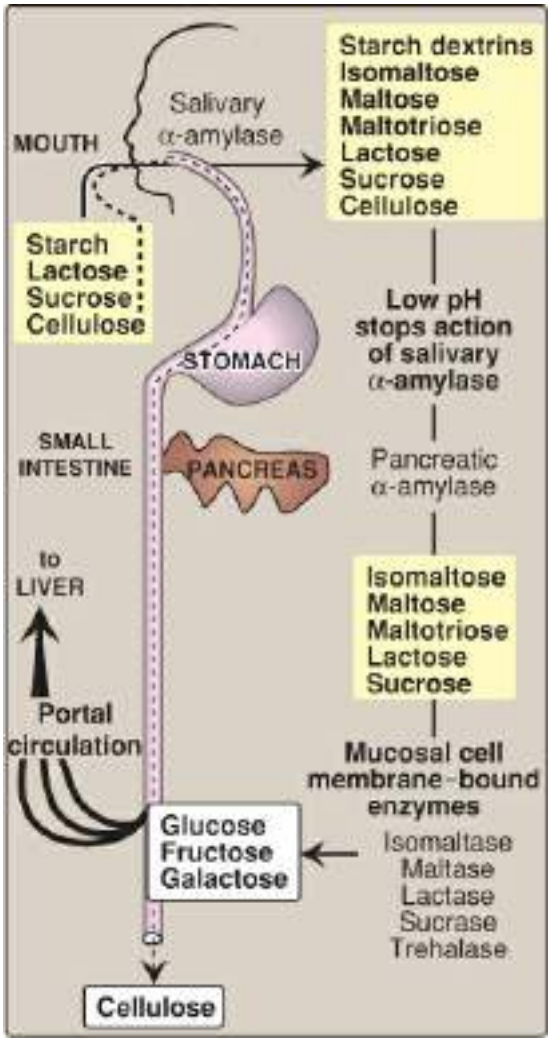


Figure 7.9
Digestion of carbohydrates. (Note: Indigestible cellulose enters the colon and is excreted.)

E. Abnormal degradation of disaccharides

The overall process of carbohydrate digestion and absorption is so efficient in healthy individuals that ordinarily all digestible dietary carbohydrate is absorbed by the time the ingested material reaches the lower jejunum. However, because only monosaccharides are absorbed, any deficiency (genetic or acquired) in a specific disaccharidase activity of the intestinal mucosa causes the passage of undigested carbohydrate into the large intestine. As a consequence of the presence of this osmotically active material, water is drawn from the mucosa into the large intestine,

causing osmotic diarrhea. This is reinforced by the bacterial fermentation of the remaining carbohydrate to two- and three-carbon compounds (which are also osmotically active) plus large volumes of carbon dioxide and hydrogen gas (H_2), causing abdominal cramps, diarrhea, and flatulence.

1. Digestive enzyme deficiencies: Genetic deficiencies of the individual disaccharidases result in disaccharide intolerance. Alterations in disaccharide degradation can also be caused by a variety of intestinal diseases, malnutrition, and drugs that injure the mucosa of the small intestine. For example, brush border enzymes are rapidly lost in normal individuals with severe diarrhea, causing a temporary, acquired enzyme deficiency. Therefore, patients suffering or recovering from such a disorder cannot drink or eat significant amounts of dairy products or sucrose without exacerbating the diarrhea.

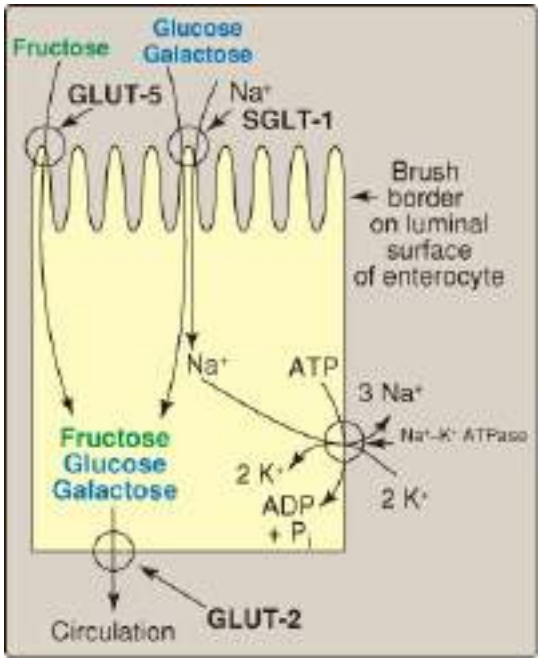


Figure 7.10
Absorption by enterocytes of the monosaccharide products of carbohydrate digestion. GLUT = glucose transporter; SGLT-1 = sodium (Na^+)-dependent glucose cotransporter. K^+ = potassium.

2. Lactose intolerance: Over 60% of the world's adults experience lactose malabsorption because they lack the enzyme lactase (Fig. 7.11). Individuals of Northern European heritage are most likely to maintain the ability to digest lactose into adulthood. Up to 90% of adults of African or Asian descent are lactase deficient. Consequently, they are less able to metabolize lactose than are individuals of Northern European origin. The age-dependent loss of lactase activity starting at approximately age 2 years represents a reduction in the amount of enzyme produced. It is thought to be caused by small variations in the DNA sequence of a region on chromosome 2 that controls expression of the

gene for lactase, also on chromosome 2. Treatment for this disorder is to reduce consumption of milk; eat yogurt and some cheeses (bacterial action and aging process decrease lactose content) as well as green vegetables, such as broccoli, to ensure adequate calcium intake; use lactase-treated products; or take lactase in pill form prior to eating. Rare cases of congenital lactase deficiency are known.

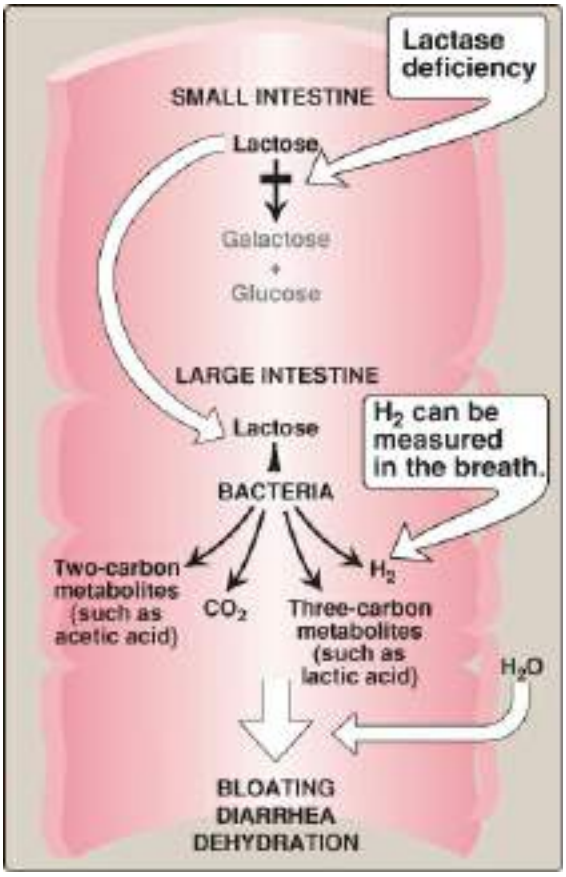


Figure 7.11
Abnormal lactose metabolism. CO_2 = carbon dioxide; H_2 = hydrogen gas.

3. Sucrase–isomaltase deficiency: SI deficiency results in intolerance of ingested sucrose. This condition was considered quite rare, more common in the Inuit people of Alaska and Greenland; now, up to 9% of Americans of European descent are estimated to be affected by a form of SI deficiency.

Initially considered to be exclusively an autosomal-recessive disorder, those with one mutation (carriers) sometimes express disease manifestations. Now, 25 different mutations in the human sucrose gene are known. Individuals homozygous for mutations express congenital SI deficiency and experience osmotic diarrhea, mild steatorrhea, irritability, and vomiting after consuming sucrose. Heterozygous carriers often have symptoms including chronic diarrhea, abdominal pain, and bloating. Treatment includes the dietary

restriction of sucrose and enzyme replacement therapy.

4. Diagnosis of enzyme deficiencies: Identification of a specific enzyme deficiency can be obtained by performing oral tolerance tests with the individual disaccharides. Measurement of H_2 in the breath is a reliable test for determining the amount of ingested carbohydrate not absorbed by the body, but which is metabolized instead by the intestinal flora (see Fig. 7.11).

IV. Chapter Summary

- **Monosaccharides** (Fig. 7.12) containing an aldehyde group are called **aldoses**, and those with a keto group are called **ketoses**.
- **Disaccharides**, **oligosaccharides**, and **polysaccharides** consist of monosaccharides linked by **glycosidic bonds**.
- Compounds with the same chemical formula but different structures are called **isomers**.
- Two monosaccharide isomers differing in configuration around one specific carbon atom (not the carbonyl carbon) are defined as **epimers**.
- In **enantiomers** (mirror images), the members of the sugar pair are designated as **D-** and **L-isomers**. When the aldehyde group on an acyclic sugar is oxidized as a chromogenic agent is reduced, that sugar is a **reducing sugar**.
- When a sugar cyclizes, an **anomeric carbon** is created from the carbonyl carbon of the aldehyde or keto group. The sugar can have two configurations, forming α or **β anomers**.
- A sugar can have its anomeric carbon linked to an $–NH_2$ or an –OH group on another structure through **N-** and **O-glycosidic bonds**, respectively.
- **Salivary α-amylase** initiates digestion of **dietary polysaccharides** (e.g., starch or glycogen), producing oligosaccharides. **Pancreatic α-amylase** continues the process. The final digestive processes occur at the **mucosal lining** of the **small intestine**.
- Several disaccharidases (e.g., **lactase** [**β-galactosidase**], **sucrase**, **isomaltase**, and **maltase**) produce monosaccharides (glucose, galactose, and fructose). These enzymes are **transmembrane proteins** of the luminal **brush border** of **intestinal mucosal cells** (**enterocytes**).
- Absorption of the monosaccharides requires specific **transporters**. If carbohydrate degradation is deficient (as a result of heredity, disease, or drugs that injure the intestinal mucosa), undigested carbohydrate will pass into the large intestine, where it can cause **osmotic diarrhea**.
- Bacterial fermentation of the material produces large volumes of carbon dioxide and hydrogen gas, causing abdominal cramps, diarrhea, and flatulence. **Lactose intolerance**, primarily caused by the age-dependent loss of **lactase** (**adult-type hypolactasia**), is by far the most common of these deficiencies.

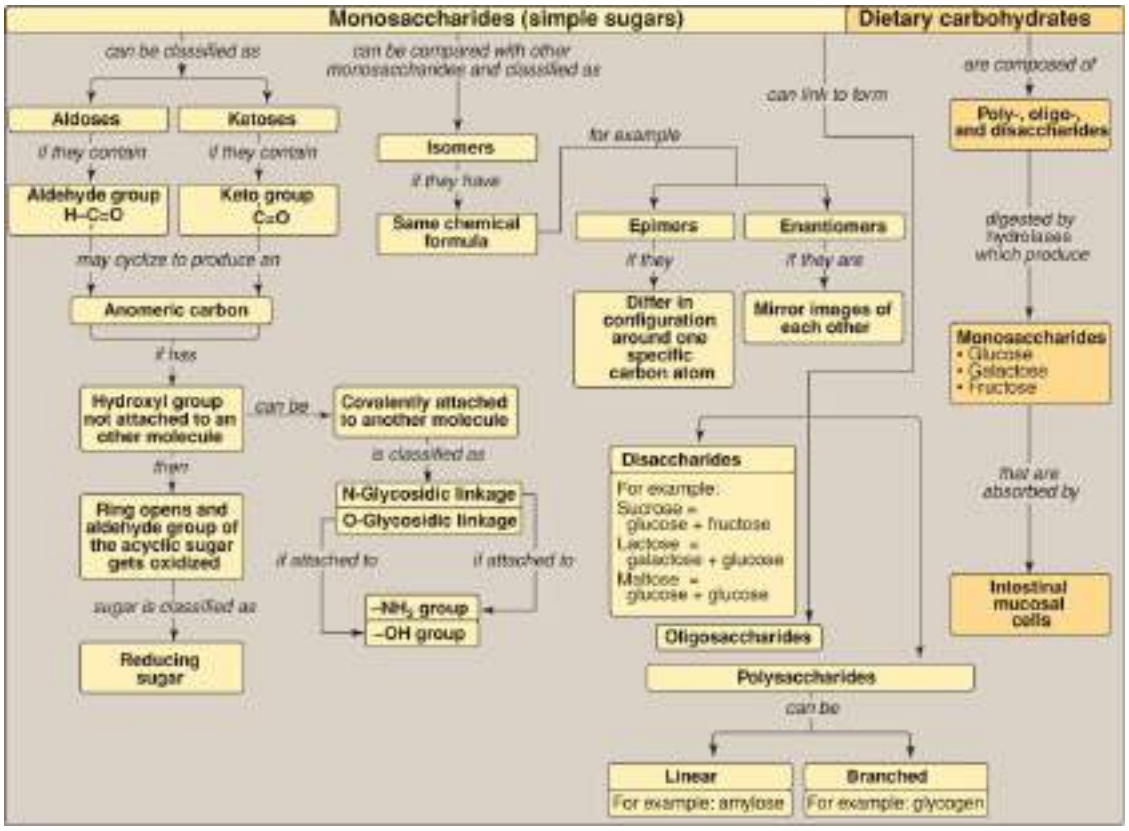


Figure 7.12
Key concept map for the classification and structure of monosaccharides and the digestion of dietary carbohydrates.

Study Questions

Choose the ONE best answer.

7.1 Glucose is

A. a C-4 epimer of galactose.
B. a ketose and usually exists as a furanose ring in solution.
C. produced from dietary starch by the action of α-amylase.
D. utilized in biologic systems only in the L-isomeric form.

Correct answer = A. Because glucose and galactose differ only in configuration around carbon 4, they are C-4 epimers that are interconvertible by the action of an epimerase. Glucose is an aldose sugar that typically exists as a pyranose ring in solution. Fructose, however, is a ketose with a furanose ring. α-Amylase does not produce monosaccharides. The D-isomeric form of carbohydrates is the form typically found in biologic systems, in contrast to amino acids that typically are found in the L-isomeric form.

7.2 A 28-year-old male presents in the office with a chief complaint of recurrent bloating and diarrhea. His eyes are sunken, and the physician notes additional signs of dehydration. The patient's temperature is normal. He explains that the most recent episode occurred last night soon after he had ice cream for dessert. This clinical picture is most likely caused by a deficiency in the activity of:

A. isomaltase.
B. lactase.
C. pancreatic α-amylase.

D. salivary α-amylase.
E. sucrase.

Correct answer = B. The physical symptoms suggest a deficiency in an enzyme responsible for carbohydrate degradation. The symptoms observed following the ingestion of dairy products suggest that the patient is deficient in lactase as a result of the age-dependent reduction in expression of the enzyme.

7.3 Routine examination of the urine of an asymptomatic pediatric patient showed a positive reaction with Clinitest (a copper reduction method of detecting reducing sugars) but a negative reaction with the glucose oxidase test for detecting glucose. Using these data, show on the chart below which of the sugars could (YES) or could not (NO) be present in the urine of this individual.

Sugar	Yes	No
Fructose		
Galactose		
Glucose		
Lactose		
Sucrose		
Xylulose		

Each of the listed sugars, except for sucrose and glucose, could be present in the urine of this individual. Clinitest is a nonspecific test that produces a change in color if urine is positive for reducing substances such as reducing sugars (fructose, galactose, glucose, lactose, xylulose). Because sucrose is not a reducing sugar, it is not detected by Clinitest. The glucose oxidase test will detect only glucose, and it cannot detect other sugars. The negative glucose oxidase test coupled with a positive reducing sugar test means that glucose cannot be the reducing sugar in the patient's urine.

7.4 Explain why α-glucosidase inhibitors such as acarbose and miglitol, which are taken with meals, can be used in the treatment of some patients with diabetes mellitus. What effect should these drugs have on the digestion of lactose?

α-Glucosidase inhibitors slow the production of glucose from dietary carbohydrates, thereby reducing the postprandial rise in blood glucose and facilitating better blood glucose control in diabetic patients. These drugs have no effect on lactose digestion because the disaccharide lactose contains a β-glycosidic bond, not an α-glycosidic bond.

Introduction to Metabolism and Glycolysis 8

I. METABOLISM OVERVIEW

In Chapter 5, individual enzyme reactions were analyzed to explain the mechanisms of catalysis. However, in cells, these reactions rarely occur in isolation. Instead, they are organized into multistep sequences called pathways, such as that of glycolysis (Fig. 8.1). In a pathway, the product of one reaction serves as the substrate of the subsequent reaction. Most pathways can be classified as either **catabolic** (degradative) or **anabolic** (synthetic). Catabolic pathways break down complex molecules, such as proteins, polysaccharides, and lipids, to a few simple molecules (e.g., carbon dioxide, ammonia, and water). Anabolic pathways form complex end products from simple precursors, for example, the synthesis of the polysaccharide glycogen from glucose. Different pathways can intersect, forming an integrated and purposeful network of chemical reactions. Metabolism is the sum of all the chemical changes occurring in a cell, a tissue, or the body. Metabolites are intermediate products of metabolism. The next several chapters focus on the central metabolic pathways that are involved in synthesizing and degrading carbohydrates, lipids, and amino acids.

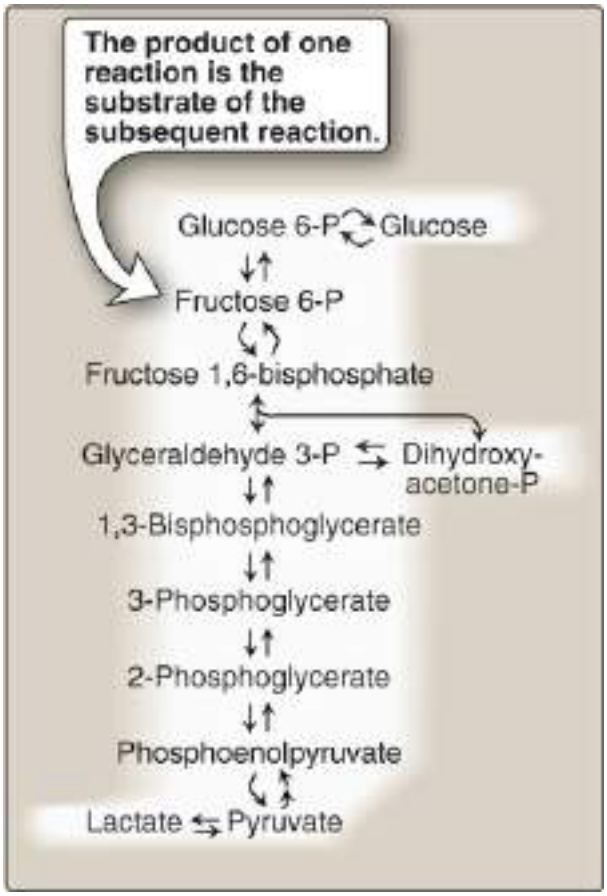


Figure 8.1
Glycolysis, an example of a metabolic pathway. (Note: Pyruvate to phosphoenolpyruvate requires two reactions.) *Curved reaction arrows* (↻) indicate forward and reverse reactions that are catalyzed by different enzymes. P = phosphate.

A. Metabolic map

Metabolism is best understood by examining its component pathways. Each pathway is composed of multienzyme sequences, and each enzyme, in turn, may exhibit important catalytic or regulatory features. A metabolic map containing the important central pathways of energy metabolism is presented in Figure 8.2. This "big picture" view of metabolism is useful in tracing connections between pathways, visualizing the purposeful movement of metabolites and depicting the effect on the flow of intermediates if a pathway is inhibited or blocked, for example, by a drug or an inherited deficiency of an enzyme. Throughout the next three units of this book, each pathway under discussion will be repeatedly featured as part of the major metabolic map shown in Figure 8.2.

B. Catabolic pathways

Catabolic reactions serve to capture chemical energy in the form of ATP from the degradation of energy-rich fuel molecules. ATP generation by degradation of complex molecules occurs in three stages, as shown in Figure 8.3. (Note: Catabolic

pathways are typically oxidative and require oxidized coenzymes such as nicotinamide adenine dinucleotide [NAD^+].) Catabolism also allows molecules in the diet or nutrient molecules stored in cells, to be converted into basic building blocks needed for the synthesis of complex molecules. Catabolism, then, is described as a convergent process in which a wide variety of molecules are transformed into a few common end products.

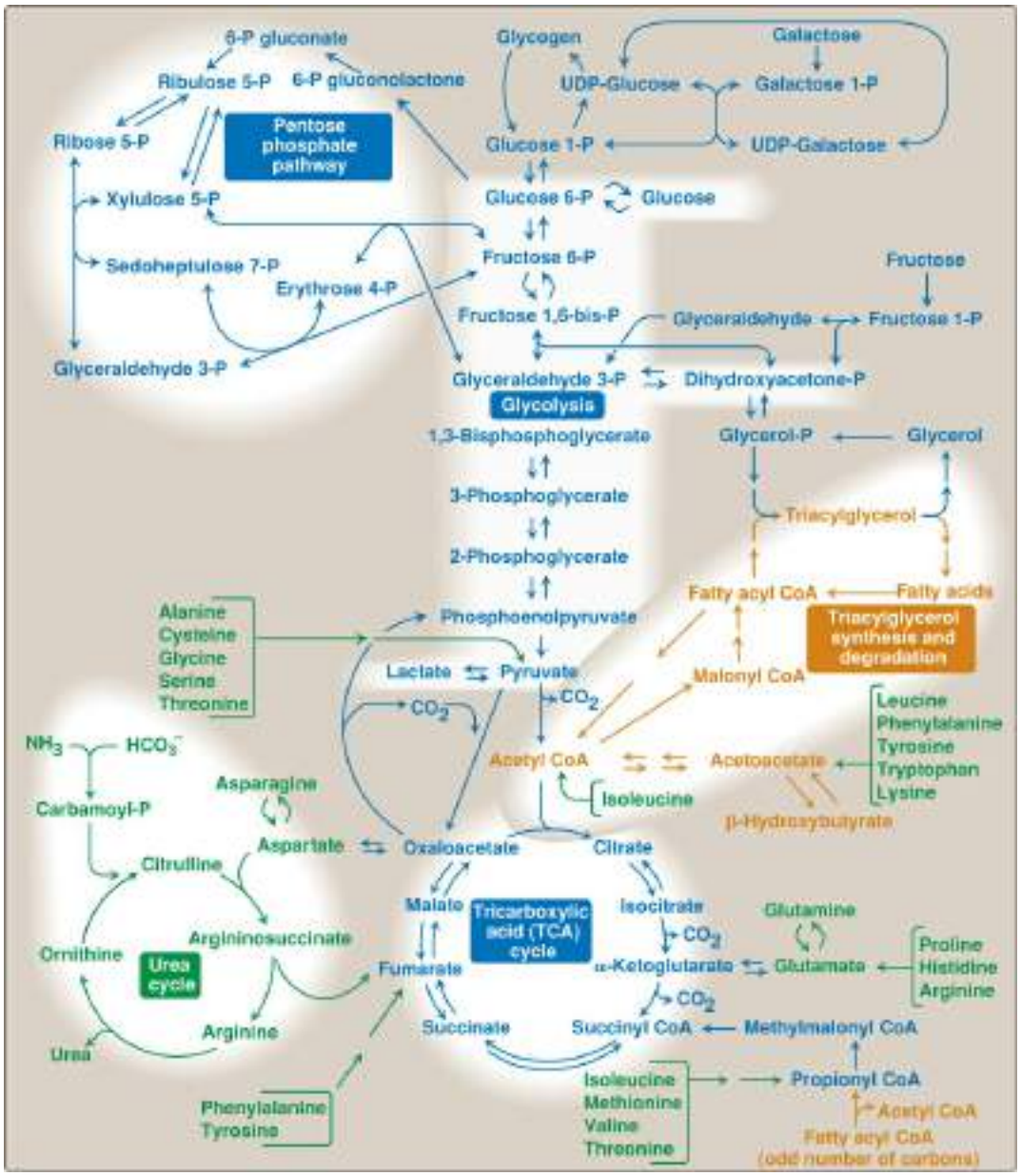


Figure 8.2
Important reactions of intermediary metabolism. Several important pathways to be discussed in later chapters are highlighted. *Curved reaction arrows* (⇄) indicate forward and reverse reactions that are catalyzed by different enzymes. The *straight arrows* (⇆) indicate forward and reverse reactions that are catalyzed by the same enzyme. *Blue text* = intermediates of carbohydrate metabolism; *brown text* = intermediates of lipid metabolism; *green text* = intermediates of protein metabolism.

UDP = uridine diphosphate; P = phosphate; CoA = coenzyme A; CO_2 = carbon dioxide; HCO_3^- = bicarbonate; NH_3 = ammonia.

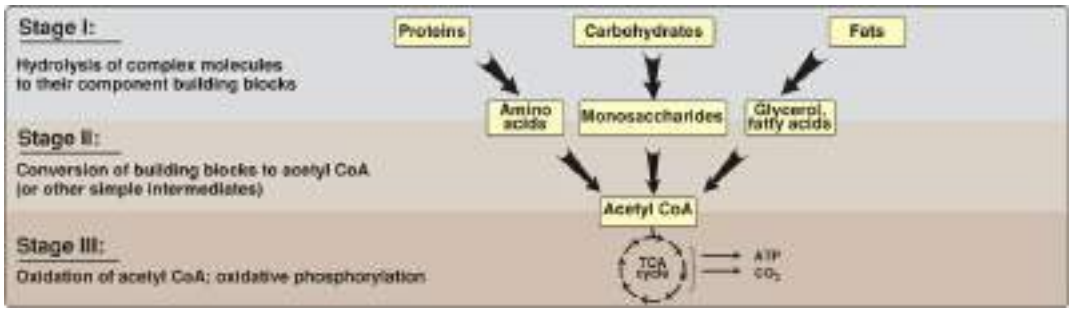


Figure 8.3
Three stages of catabolism. CoA = coenzyme A; TCA = tricarboxylic acid; CO_2 = carbon dioxide.

1. Hydrolysis of complex molecules: In the first stage, complex molecules are broken down into their component building blocks. For example, proteins are degraded to amino acids, polysaccharides to monosaccharides, and fats (triacylglycerols) to free fatty acids and glycerol.
2. Conversion of building blocks to simple intermediates: In the second stage, these diverse building blocks are further degraded to acetyl coenzyme A (CoA) and a few other simple molecules. Some energy is captured as ATP, but the amount is small compared with the energy produced during the third stage of catabolism.
3. Oxidation of acetyl CoA: The tricarboxylic acid (TCA) cycle (see Chapter 9) is the final common pathway in the oxidation of fuel molecules that produce acetyl CoA. Oxidation of acetyl CoA generates large amounts of ATP via oxidative phosphorylation as electrons flow from NADH and flavin adenine dinucleotide ($FADH_2$) to oxygen ([O_2], see Chapter 6).

C. Anabolic pathways

In contrast to catabolism, anabolism is a divergent process in which a few biosynthetic precursors (such as amino acids) form a wide variety of polymeric, or complex, products (such as proteins [Fig. 8.4]). Anabolic reactions require energy (are endergonic), which is generally provided by the hydrolysis of ATP to adenosine diphosphate (ADP) and inorganic phosphate (P_i). (Note: Catabolic reactions generate energy [are exergonic].) Anabolic reactions often involve chemical reductions in which the reducing power is most frequently provided by the electron donor NADPH (phosphorylated NADH, see Chapter 13).

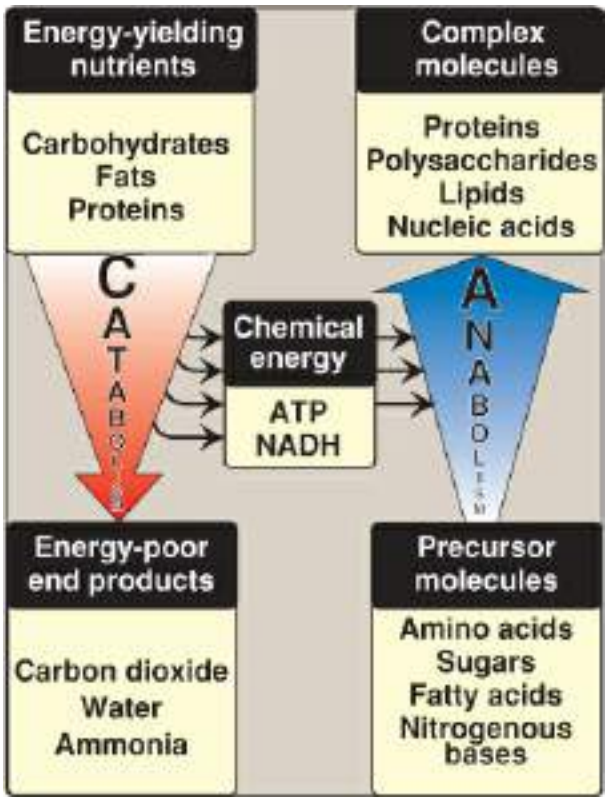


Figure 8.4
Comparison of catabolic and anabolic pathways. NADH = nicotinamide adenine dinucleotide.

II. METABOLISM REGULATION

The pathways of metabolism must be coordinated so that the production of energy or the synthesis of end products meets the needs of the cell. Furthermore, individual cells function as part of a community of interacting tissues, not in isolation. Thus, a sophisticated communication system has evolved to coordinate the functions of the body. Regulatory signals that inform an individual cell of the metabolic state of the body as a whole include hormones, neurotransmitters, and the availability of nutrients. These, in turn, influence signals generated within the cell (Fig. 8.5).

A. Intracellular communication

The rate of a metabolic pathway can respond to regulatory signals that arise from within the cell. For example, the rate may be influenced by the availability of substrates, product inhibition, or alterations in the levels of allosteric activators or inhibitors. These intracellular signals typically elicit rapid responses and are important for the moment-to-moment regulation of metabolism.

B. Intercellular communication

The ability to respond to intercellular signals is essential for the development

and survival of organisms. Signaling between cells provides for long-range integration of metabolism and usually results in a response, such as a change in gene expression that is slower than is seen with intracellular signals. Communication between cells can be mediated, for example, by surface-to-surface contact and, in some tissues, by formation of gap junctions, allowing direct communication between the cytoplasms of adjacent cells. However, for energy metabolism, the most important route of communication is chemical signaling between cells by blood-borne hormones or by neurotransmitters.

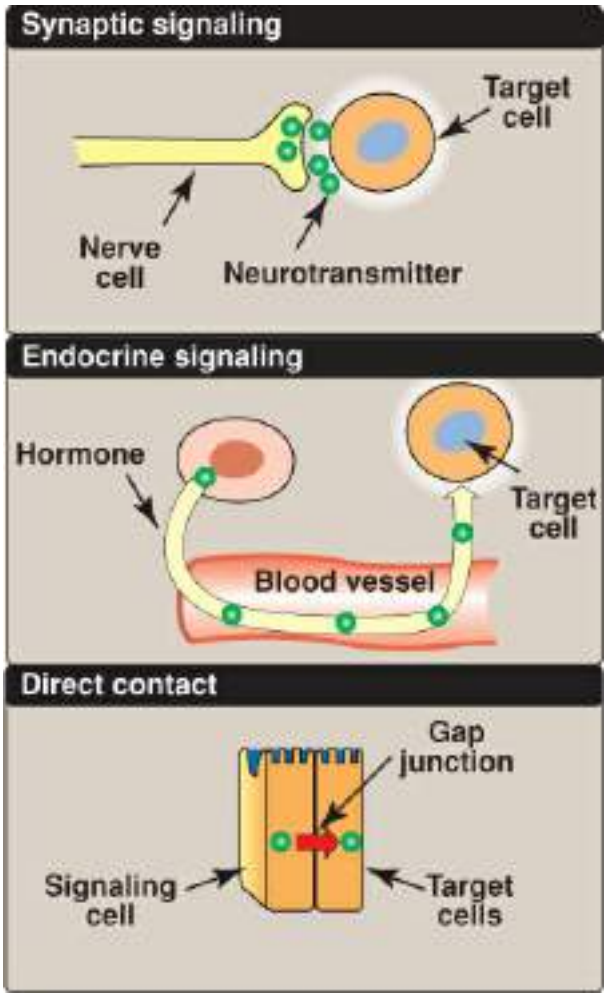


Figure 8.5
Some commonly used mechanisms for transmission of regulatory signals between cells.

C. G protein–linked receptors and second messenger systems

Hormones and neurotransmitters can be thought of as signals and their receptors as signal detectors. Receptors are proteins often found embedded in the plasma membranes of their target cells. They respond to a ligand bound to them by initiating a series of reactions that ultimately result in specific intracellular responses. Many receptors that regulate metabolism are linked to

intracellular GTP-binding proteins called G proteins and are known as G protein–coupled receptors (GPCRs). This type of receptor regulates production of molecules referred to as second messengers, which are so named because they intervene between the original extracellular messenger (the neurotransmitter or hormone) and the ultimate intracellular effect. Second messengers are part of the cascade of events that converts (transduces) ligand binding into a response.

Two of the most widely recognized second messenger systems regulated by G proteins are the phospholipase C system that involves calcium and phosphatidylinositol system and the adenylyl cyclase (adenylate cyclase) system, which is particularly important in regulating the pathways of intermediary metabolism. Both systems are initiated by the binding of hormone ligands, such as epinephrine or glucagon, to specific GPCR embedded with the plasma membrane of the target cell that will respond to the hormone.

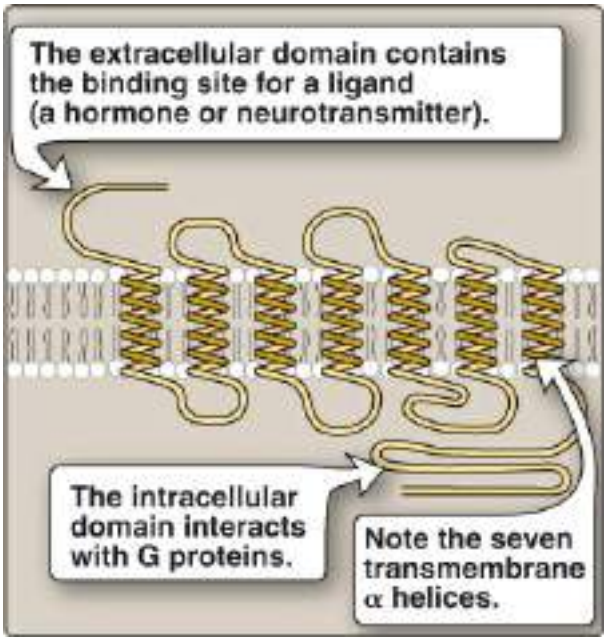


Figure 8.6
Structure of a typical G protein–coupled receptor of the plasma membrane.

GPCRs are characterized by an extracellular ligand-binding domain, seven transmembrane α helices, and an intracellular domain that interacts with heterotrimeric G proteins composed of α, β, and γ subunits (Fig. 8.6). (Note: Insulin, another key regulator of metabolism, does not signal via GPCRs but instead acts via a receptor with tyrosine kinase activity [see Chapter 23].[a])

D. Adenylyl cyclase

Binding of the hormone ligand by some GPCRs, including the β- and α_2-

adrenergic receptors, triggers either an increase or a decrease in the activity of adenylyl cyclase. This is a membrane-bound enzyme that converts ATP to 3′,5′-adenosine monophosphate (cyclic AMP, or cAMP) when active.

1. Guanosine triphosphate–dependent regulatory proteins or G proteins: The effect of the activated, occupied GPCR on second messenger formation is mediated by specialized heterotrimeric G proteins (α, β, and γ subunits) found on the inner face of the plasma membrane. G proteins are named because their α subunit binds guanosine triphosphate (GTP) when activated. In the inactive form of a G protein, the α subunit is bound to GDP (Fig. 8.7). Ligand binding causes a conformational change in the receptor, triggering replacement of this GDP with GTP. The GTP-bound form of the α subunit dissociates from the βγ subunits and moves to the membrane-bound adenylyl cyclase enzyme, affecting its enzyme activity. Many molecules of active Gα protein are formed by one activated receptor. (Note: The ability of a hormone or neurotransmitter to stimulate or inhibit adenylyl cyclase depends on the type of Gα protein that is linked to the receptor. One type, designated G_S, stimulates adenylyl cyclase [see Fig. 8.7] whereas Gi inhibits adenylyl cyclase [not shown].)

   Activated adenylyl cyclase converts adenosine triphosophate (ATP) to the second messenger cAMP or cyclic adenosine monophosphate. cAMP then activates the serine/threonine protein kinase known as protein kinase A (PKA), described below. The actions of the Gα–GTP complex are short-lived because Gα has an inherent GTPase activity, resulting in the rapid hydrolysis of GTP to GDP. This causes inactivation of Gα, its dissociation from adenylyl cyclase, and its reassociation with the βγ dimer.

> Toxins from *Vibrio cholerae* (cholera) and *Bordetella pertussis* (whooping cough) cause inappropriate activation of adenylyl cyclase through covalent modification (ADP-ribosylation) of different G proteins that interact with adenylyl cyclase. With cholera toxin, the GTPase activity of $G\alpha_s$ is inhibited in intestinal cells. With whooping cough, the pertussis toxin inactivates $G\alpha_i$ in respiratory tract cells. The result in both situations is increased adenylyl cyclase activity and excess production of the second messenger, cAMP.

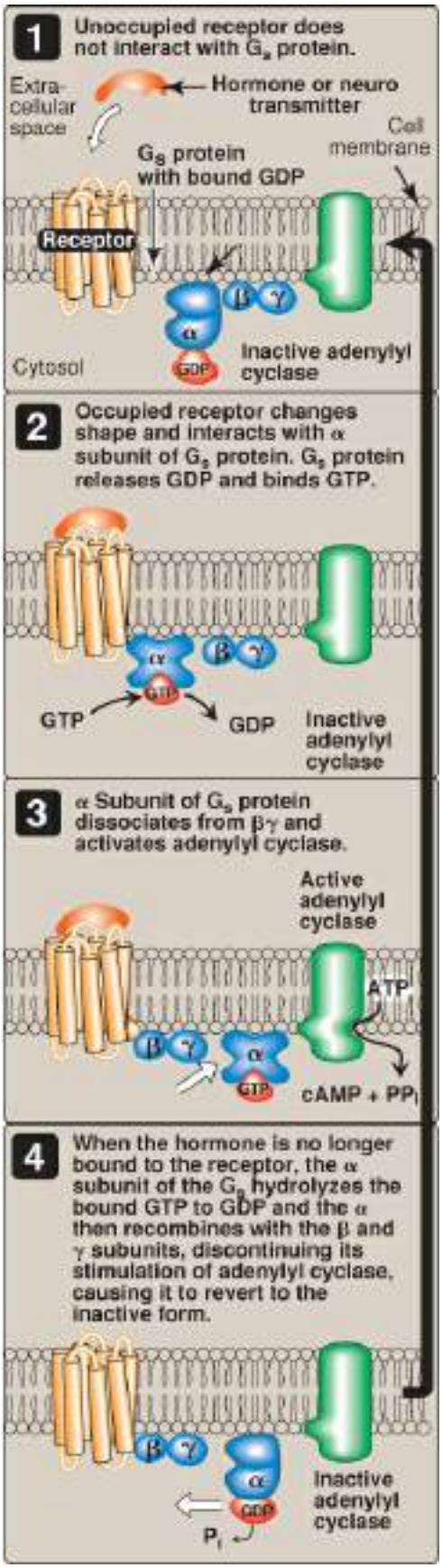


Figure 8.7
The recognition of chemical signals by certain membrane receptors triggers an increase (or, less often, a decrease) in the activity of adenylyl cyclase. GDP and GTP = guanosine di- and triphosphates; cAMP = cyclic adenosine monophosphate.

2. Protein kinases: The next step in the cAMP second messenger system is the activation of a family of enzymes called cAMP-dependent protein kinases, including PKA, as shown in Figure 8.8. cAMP activates PKA by binding to its two regulatory subunits, causing the release of its two catalytically active subunits. Active PKA is a serine/threonine kinase because it functions to transfer phosphate from ATP to specific serine or threonine residues of its specific protein substrates. The phosphorylated proteins may act directly on the cell's ion channels or, if enzymes, may become activated or inhibited. (Note: Not all types of protein kinases are cAMP dependent, e.g., protein kinase C, activated in response to phospholipase C signaling, is calcium dependent.)

3. Protein phosphatases: The phosphate groups added to proteins by protein kinases are removed by phosphoprotein phosphatases, enzymes that hydrolytically cleave phosphate esters (see Fig. 8.8). Actions of phosphatases ensure that changes in protein activity induced by phosphorylation are not permanent.

4. cAMP hydrolysis: cAMP is rapidly hydrolyzed to 5′-AMP by cAMP phosphodiesterase that cleaves the cyclic 3′,5′-phosphodiester bond. 5′-AMP is not an intracellular signaling molecule. Therefore, the effects of neurotransmitter- or hormone-mediated increases of cAMP are rapidly terminated if the extracellular signal is removed. (Note: cAMP phosphodiesterase is inhibited by caffeine, a methylxanthine derivative.)

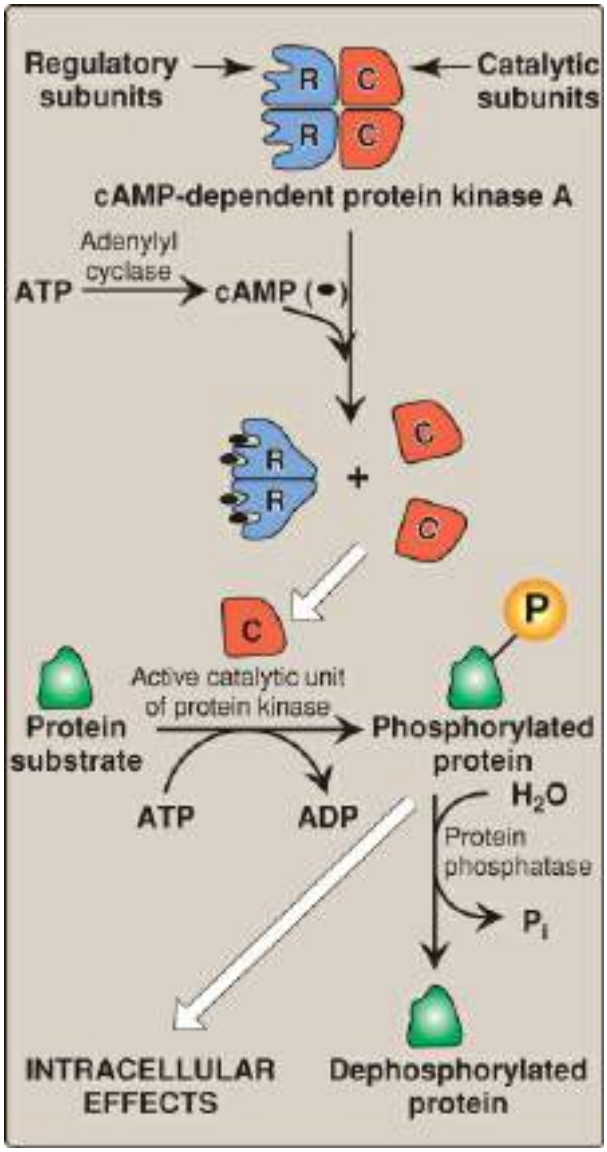


Figure 8.8
Actions of cyclic adenosine monophosphate (cAMP). P = phosphate; ADP = adenosine diphosphate; P_i = inorganic phosphate.

III. GLYCOLYSIS OVERVIEW

The glycolytic pathway is used by all tissues for the oxidation of glucose to provide energy (as ATP) and intermediates for other metabolic pathways. Glycolysis is at the hub of carbohydrate metabolism because virtually all sugars, whether arising from the diet or from catabolic reactions in the body, can ultimately be converted to glucose (Fig. 8.9A). Pyruvate is the end product of glycolysis in cells with mitochondria and an adequate supply of O_2. This series of 10 reactions is called aerobic glycolysis because O_2 is required to reoxidize the NADH formed during the oxidation of glyceraldehyde 3-phosphate (Fig. 8.9B). Aerobic glycolysis sets the stage for the oxidative

decarboxylation of pyruvate to acetyl CoA, a major fuel of the TCA cycle. Alternatively, pyruvate is reduced to lactate as NADH is oxidized to NAD^+ (Fig. 8.9C). This conversion of glucose to lactate is called anaerobic glycolysis because it can occur without the participation of O_2. Anaerobic glycolysis allows the production of ATP in tissues that lack mitochondria (e.g., red blood cells [RBCs] and parts of the eye) or in cells deprived of sufficient O_2 (hypoxia).

IV. GLUCOSE TRANSPORT INTO CELLS

Glucose cannot diffuse directly into cells but enters by one of two transport systems: a sodium (Na^+)- and ATP-independent transport system or a Na^+- and ATP-dependent cotransport system.

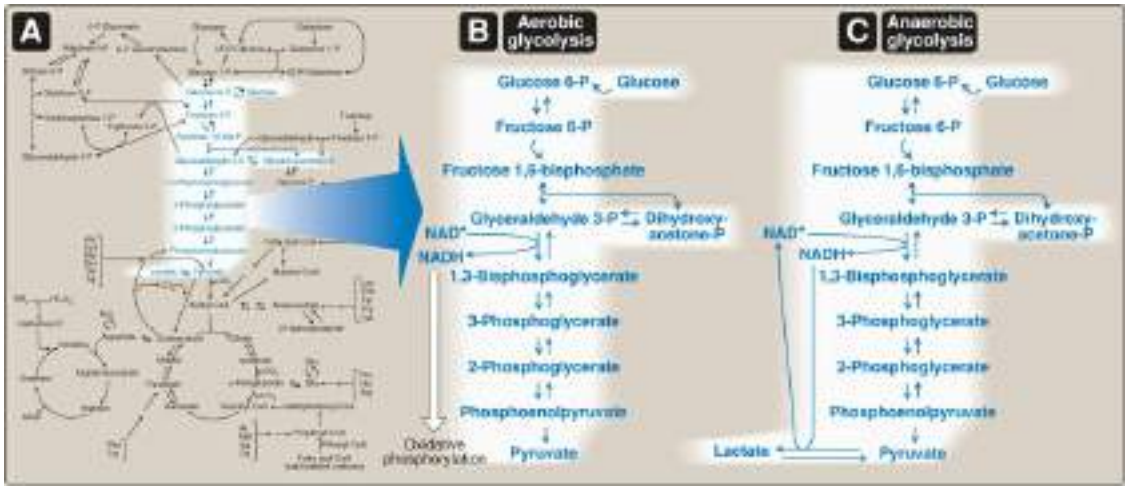


Figure 8.9
A: Glycolysis shown as one of the essential pathways of energy metabolism. **B:** Reactions of aerobic glycolysis. **C:** Reactions of anaerobic glycolysis. NAD(H) = nicotinamide adenine dinucleotide; P = phosphate.

A. Sodium- and ATP-independent transport system

This passive system is mediated by a family of 14 glucose transporter (GLUT) isoforms found in cell membranes. They are designated GLUT-1 to GLUT-14. These monomeric protein transporters exist in the membrane in two conformational states (Fig. 8.10). Extracellular glucose binds to the transporter, which then alters its conformation, transporting glucose across the cell membrane via facilitated diffusion. Because GLUTs transport one molecule at a time, they are uniporters.[b]

1. Tissue specificity: GLUT display a tissue-specific pattern of expression (See Table 8.1 for examples of some GLUTs). For example, GLUT-1 is abundant in most tissues, whereas GLUT-4 is abundant in muscle and adipose tissue, and GLUT-5 transports fructose. (Note: The number of GLUT-4 transporters active in these tissues is increased by insulin [see p. 345 for a discussion of insulin

and glucose transport].) GLUT-2 is abundant in the liver, kidneys, and pancreatic β cells. The other GLUT isoforms also have tissue-specific distributions.

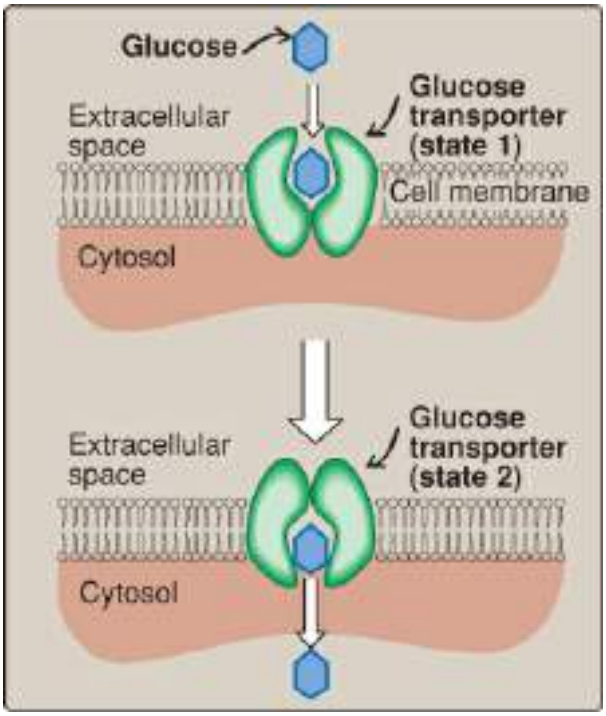


Figure 8.10
Schematic representation of the facilitated transport of glucose through a cell membrane.
(Note: Glucose transporter proteins are monomeric and contain 12 transmembrane α helices.)

2. Specialized functions: In facilitated diffusion, transporter-mediated glucose movement is down a concentration gradient (i.e., from a high concentration to a lower one, therefore requiring no energy). For example, GLUT-1, GLUT-3, and GLUT-4 are primarily involved in glucose uptake from the blood. In contrast, GLUT-2, in the liver and kidneys, can either transport glucose into these cells when blood glucose levels are high or transport glucose from these cells when blood glucose levels are low (e.g., during fasting). GLUT-5 is unusual in that it is the primary transporter for fructose (not glucose) in the small intestine and the testes.

Table 8.1 Tissue Distribution of Selected GLUTs

	Location	Function	K_m (mM)
GLUT-1	Most tissues	Basal glucose uptake	1
GLUT-2	Liver, kidneys, pancreas	Removes excess glucose from blood	15–20
GLUT-3	Most tissues	Basal glucose uptake	1
GLUT-4	Muscle and fat	Removes excess glucose from blood	5
GLUT-5	Small intestine, testes	Transport of fructose	10

B. Sodium- and ATP-dependent cotransport of glucose

This type of glucose cotransport with sodium occurs in the epithelial cells of the intestine, the renal tubules, and the choroid plexus. This is an energy-requiring process that transports glucose against (up) its concentration gradient, from low extracellular concentrations to higher intracellular concentrations while Na^+ is transported down its electrochemical gradient. There is a much higher extracellular than intracellular concentration of Na^+, which is the result of the Na^+–K^{++} ATPase. The Na^+ concentration gradient powers the transport of glucose against its concentration gradient; ATP hydrolysis is an indirect energy source because it is necessary to establish the Na^+ gradient. (see also Fig. 7.10). Because this secondary active transport of glucose requires the concurrent uptake (symport) of Na^+, the transporter is a sodium-dependent glucose cotransporter (SGLT). (Note: The choroid plexus, part of the blood–brain barrier, also contains GLUT-1.)[c]

Sodium-dependent glucose cotransporter protein 2 (SGLT2) functions in the kidneys, and is the major transporter for glucose reabsorption back into the blood. Gliflozins are SGLT2 inhibitors, which reduce reabsorption of glucose in the kidney, and therefore lower blood sugar. SGLT2 inhibitors are used to treat hyperglycemia in people with type II diabetes.

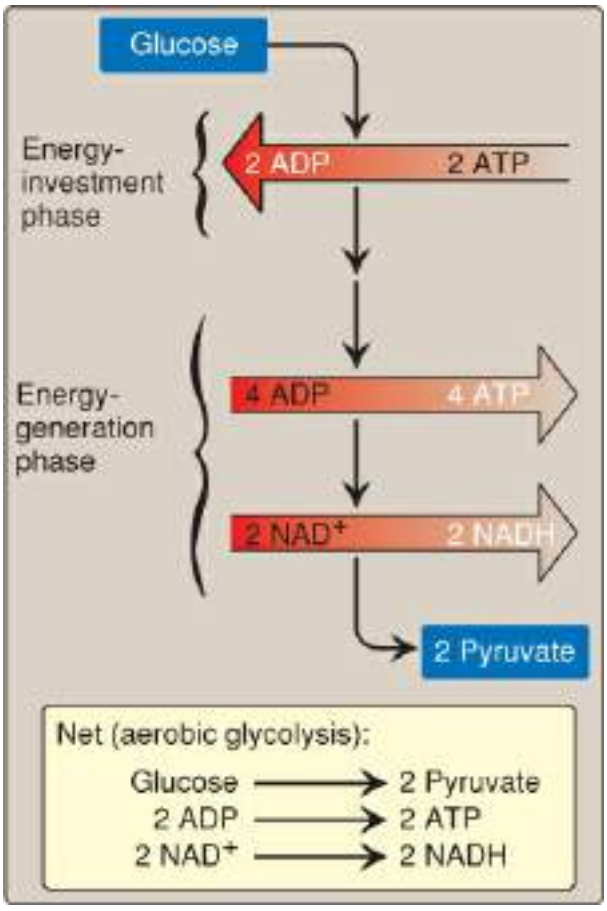


Figure 8.11
Two phases of aerobic glycolysis. NAD(H) = nicotinamide adenine dinucleotide; ADP = adenosine diphosphate.

V. GLYCOLYSIS REACTIONS

The conversion of glucose to pyruvate occurs in two stages (Fig. 8.11). The first five reactions of glycolysis correspond to an energy-investment phase in which the phosphorylated forms of intermediates are synthesized at the expense of ATP. The subsequent reactions of glycolysis constitute an energy-generation phase in which a net of two molecules of ATP are formed by substrate-level phosphorylation per glucose molecule metabolized.

A. Glucose phosphorylation

Phosphorylated sugar molecules do not readily penetrate cell membranes because there are no specific transmembrane carriers for these compounds and because they are too polar to diffuse through the lipid core of membranes. Therefore, the irreversible phosphorylation of glucose (Fig. 8.12) effectively traps the sugar as cytosolic glucose 6-phosphate and commits it to further metabolism in the cell. Mammals have four isozymes (I–IV) of the enzyme hexokinase that catalyze the phosphorylation of glucose to glucose 6-phosphate.

1. Hexokinases I–III: In most tissues, glucose phosphorylation is catalyzed by one of these isozymes of hexokinase, which is one of three regulatory enzymes of glycolysis (along with phosphofructokinase [PFK] and pyruvate kinase [PK]). They are inhibited by the reaction product glucose 6-phosphate, which accumulates when further metabolism of this hexose phosphate is reduced. Hexokinases I–III have a low Michaelis constant (K_m) and, therefore, a high affinity (see p. 63) for glucose. This permits the efficient phosphorylation and subsequent metabolism of glucose even when tissue concentrations of glucose are low (Fig. 8.13). However, because these isozymes have a low maximal velocity ([V_{max}], see p. 61) for glucose, they do not sequester (trap) cellular phosphate in the form of phosphorylated glucose or phosphorylate more glucose than the cell can use. (Note: These isozymes have broad substrate specificity and are able to phosphorylate several hexoses in addition to glucose.)

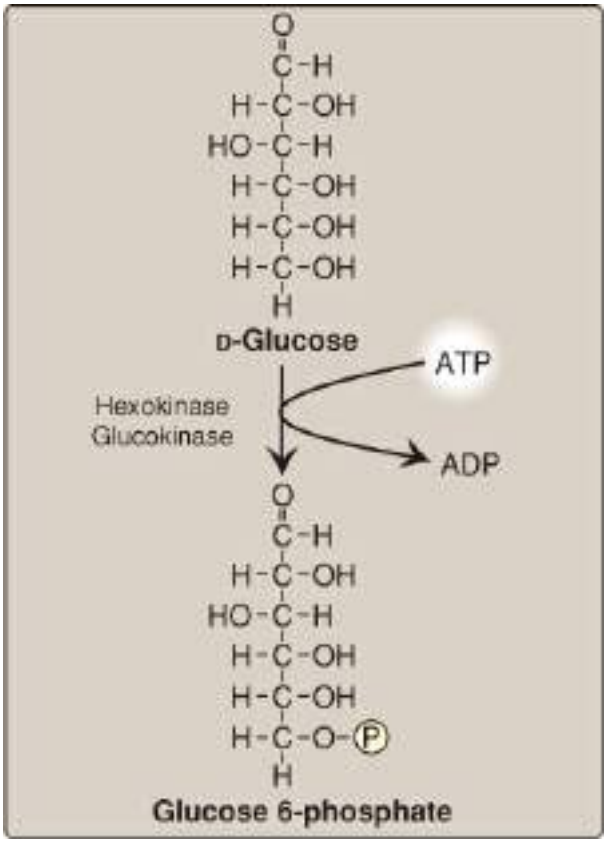


Figure 8.12
Energy-investment phase: phosphorylation of glucose. (Note: Kinases utilize ATP complexed with a divalent metal ion, most typically magnesium.) ADP = adenosine diphosphate; Ⓟ = phosphate.

2. Hexokinase IV: In liver parenchymal cells and pancreatic β cells, glucokinase (the hexokinase IV isozyme) is the predominant enzyme responsible for glucose phosphorylation. In β cells, glucokinase functions as a glucose sensor, determining the threshold for insulin secretion (see p. 343). (Note: Hexokinase IV also serves as a glucose sensor in hypothalamic neurons, playing a key role in the adrenergic response to hypoglycemia [see p. 350].) In the liver, the enzyme facilitates glucose phosphorylation during hyperglycemia. Despite the popular but misleading name glucokinase, the sugar specificity of the enzyme is similar to that of other hexokinase isoenzymes.

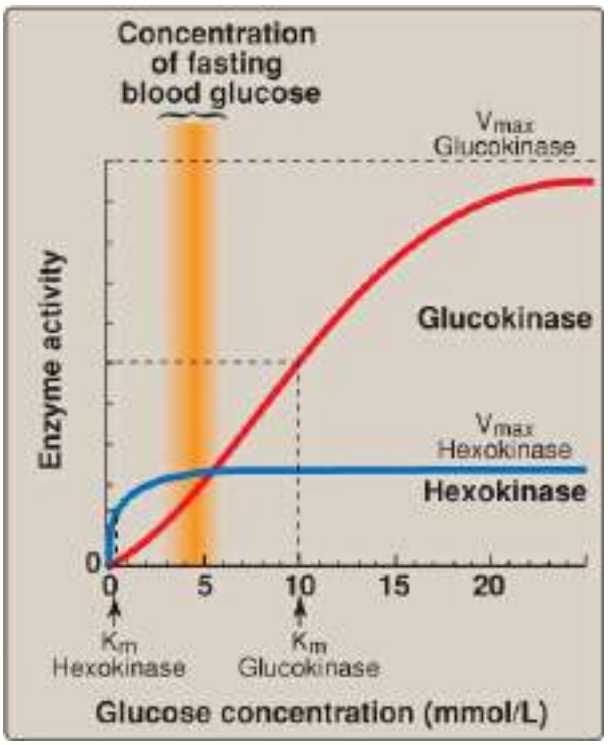


Figure 8.13
Effect of glucose concentration on the rate of phosphorylation catalyzed by hexokinase and glucokinase. K_m = Michaelis constant; V_{max} = maximal velocity.

a. Kinetics: Glucokinase differs from hexokinases I–III in several important properties. For example, it has a much higher K_m, requiring a higher glucose concentration for half-saturation (see Fig. 8.13). Thus, glucokinase functions only when the intracellular concentration of glucose in the hepatocyte is elevated such as during the brief period following consumption of a carbohydrate-rich meal, when high levels of glucose are delivered to the liver via the portal vein. Glucokinase has a high V_{max}, allowing the liver to effectively remove the flood of glucose delivered by the portal blood. This prevents large amounts of glucose from entering the systemic circulation following such a meal, thereby minimizing hyperglycemia during the absorptive period. (Note: GLUT-2 ensures that blood glucose equilibrates rapidly across the hepatocyte membrane.)

b. Regulation: Glucokinase activity is not directly inhibited by glucose 6-phosphate as are the other hexokinases. Instead, it is indirectly inhibited by fructose 6-phosphate (which is in equilibrium with glucose 6-phosphate, a product of glucokinase) and is indirectly stimulated by glucose (a substrate of glucokinase). Regulation is achieved by reversible binding to the hepatic protein glucokinase regulatory protein (GKRP). In the presence of fructose 6-phosphate, glucokinase binds tightly to GKRP and is translocated to the nucleus, thereby rendering the enzyme inactive (Fig. 8.14). When glucose

levels in the blood (and also in the hepatocyte, as a result of GLUT-2) increase, glucokinase is released from GKRP, and the enzyme reenters the cytosol where it phosphorylates glucose to glucose 6-phosphate. (Note: GKRP is a competitive inhibitor of glucose use by glucokinase.)

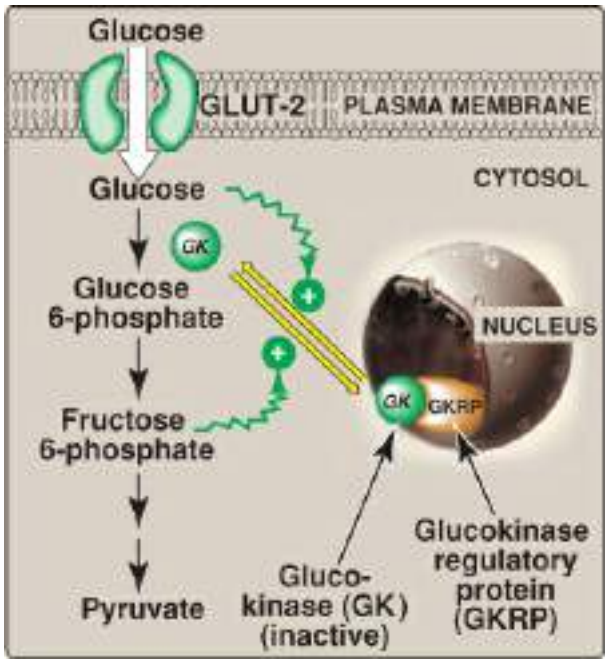


Figure 8.14
Regulation of glucokinase activity by glucokinase regulatory protein. GLUT = glucose transporter.

Glucokinase functions as a glucose sensor in blood glucose homeostasis. Inactivating mutations of glucokinase are the cause of a rare form of diabetes, maturity-onset diabetes of the young type 2 (MODY 2) that is characterized by impaired insulin secretion and hyperglycemia.

B. Glucose 6-phosphate isomerization

The isomerization of glucose 6-phosphate to fructose 6-phosphate is catalyzed by phosphoglucose isomerase (Fig. 8.15). The reaction is readily reversible and is not a rate-limiting or regulated step.

C. Fructose 6-phosphate phosphorylation

The irreversible phosphorylation reaction catalyzed by PFK-1 is the most important control point and the rate-limiting and committed step of glycolysis (Fig. 8.16). PFK-1 is controlled by the available concentrations of the substrates ATP and fructose 6-phosphate as well as by other regulatory molecules.

1. Regulation by intracellular energy levels: PFK-1 is inhibited allosterically by

elevated levels of ATP, which act as an energy-rich signal indicating an abundance of high-energy compounds. Elevated levels of citrate, an intermediate in the TCA cycle (see p. 122), also inhibit PFK-1. (Note: Inhibition by citrate favors the use of glucose for glycogen synthesis [see p. 138].) Conversely, PFK-1 is activated allosterically by high concentrations of AMP, which signal that the cell's energy stores are depleted.

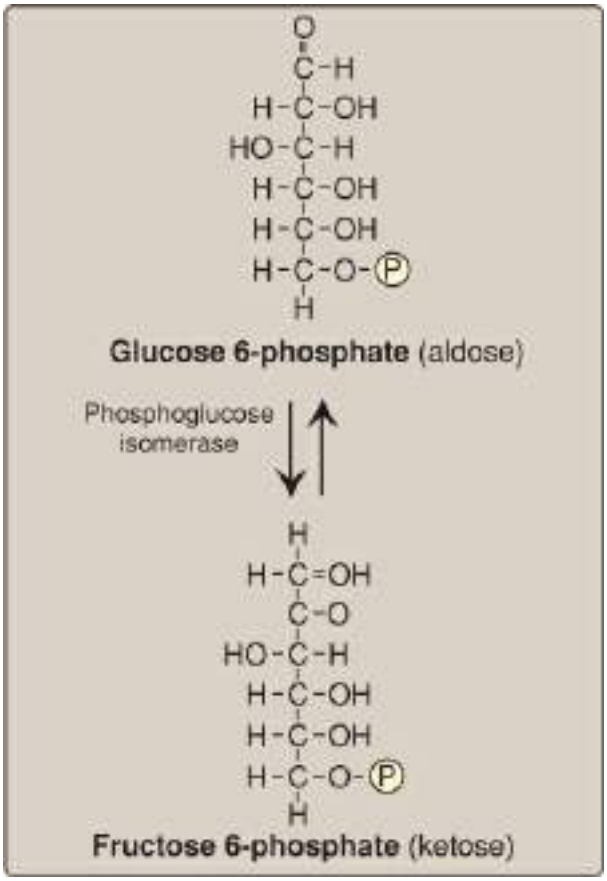


Figure 8.15
Aldose-ketose isomerization of glucose 6-phosphate to fructose 6-phosphate. Ⓟ = phosphate.

2. Regulation by fructose 2,6-bisphosphate: Fructose 2,6-bisphosphate is the most potent activator of PFK-1 (see Fig. 8.16) and is able to activate the enzyme even when ATP levels are high. It is formed from fructose 6-phosphate by PFK-2. Unlike PFK-1, PFK-2 is a bifunctional protein that has both the kinase activity that produces fructose 2,6-bisphosphate and the phosphatase activity that dephosphorylates fructose 2,6-bisphosphate to fructose 6-phosphate. In the liver isozyme, phosphorylation of PFK-2 inactivates the kinase domain and activates the phosphatase domain (Fig. 8.17). The opposite is seen in the cardiac isozyme. Skeletal PFK-2 is not covalently regulated. (Note: Fructose 2,6-bisphosphate is an inhibitor of fructose 1,6-bisphosphatase, an enzyme of gluconeogenesis. The reciprocal actions of fructose 2,6-bisphosphate on glycolysis [activation] and gluconeogenesis [inhibition] ensure that both

pathways are not fully active at the same time, preventing a futile cycle of glucose oxidation to pyruvate followed by glucose resynthesis from pyruvate.)

a. During the well-fed state: Decreased levels of glucagon and elevated levels of insulin (such as occur following a carbohydrate-rich meal) cause an increase in hepatic fructose 2,6-bisphosphate (PFK-2 is dephosphorylated) and, thus, in the rate of glycolysis (see Fig. 8.17). Therefore, fructose 2,6-bisphosphate acts as an intracellular signal of glucose abundance.

b. During fasting: By contrast, the elevated levels of glucagon and low levels of insulin that occur during fasting (see p. 364) cause a decrease in hepatic fructose 2,6-bisphosphate (PFK-2 is phosphorylated). This results in inhibition of glycolysis and activation of gluconeogenesis.

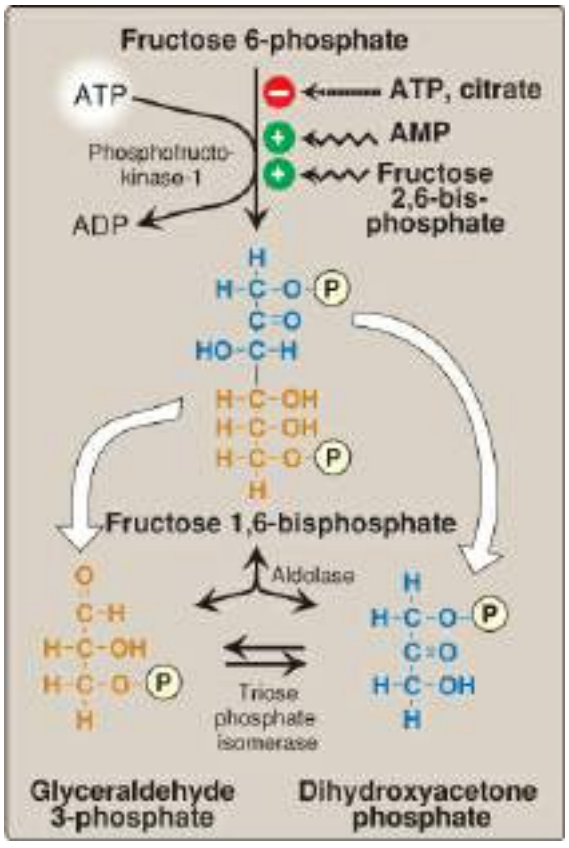


Figure 8.16
Energy-investment phase (continued): conversion of fructose 6-phosphate to triose phosphates. Ⓟ = phosphate; AMP and ADP = adenosine mono- and diphosphates.

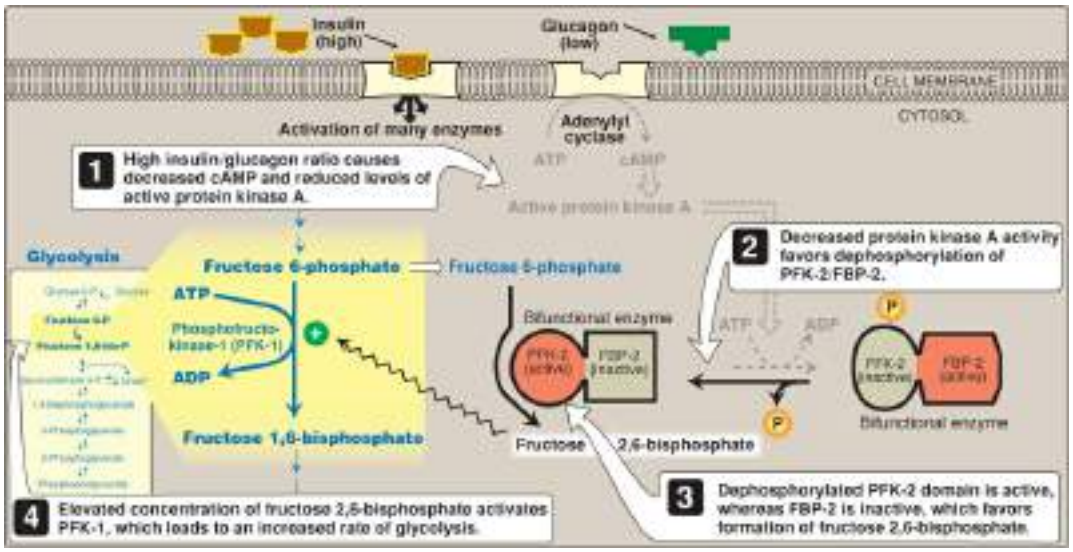


Figure 8.17
Effect of elevated insulin concentration on the intracellular concentration of fructose 2,6-bisphosphate in the liver. PFK-2 = phosphofructokinase-2; FBP-2 = fructose 2,6-bisphosphatase; AMP and ADP = adenosine mono- and diphosphates; cAMP = cyclic AMP; (P) = phosphate.

D. Fructose 1,6-bisphosphate cleavage

Aldolase cleaves fructose 1,6-bisphosphate to dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (Fig. 8.16). The reaction is reversible and not regulated. (Note: Aldolase B, the hepatic isoform, also cleaves fructose 1-phosphate and functions in dietary fructose metabolism.)

E. Dihydroxyacetone phosphate isomerization

Triose phosphate isomerase interconverts DHAP and glyceraldehyde 3-phosphate (Fig. 8.16). DHAP must be isomerized to glyceraldehyde 3-phosphate for further metabolism by the glycolytic pathway. This isomerization results in the net production of two molecules of glyceraldehyde 3-phosphate from the cleavage products of fructose 1,6-bisphosphate. (Note: DHAP is utilized in triacylglycerol synthesis.)

F. Glyceraldehyde 3-phosphate oxidation

The conversion of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate (1,3-BPG) by glyceraldehyde 3-phosphate dehydrogenase is the first oxidation–reduction reaction of glycolysis (Fig. 8.18). (Note: Because there is a limited amount of NAD^+ in the cell, the NADH formed by the dehydrogenase reaction must be oxidized for glycolysis to continue. Two major mechanisms for oxidizing NADH to NAD^+ are the reduction of pyruvate to lactate by lactate dehydrogenase [LDH] anaerobic, and the electron transport chain ([ETC] aerobic). Because NADH cannot cross the inner mitochondrial membrane, the ETC requires the malate–aspartate and glycerol 3-phosphate substrate shuttles to move NADH reducing equivalents

into the mitochondrial matrix.)

1. 1,3-Bisphosphoglycerate synthesis: The oxidation of the aldehyde group of glyceraldehyde 3-phosphate to a carboxyl group is coupled to the attachment of P_i to the carboxyl group. This phosphate group, linked to carbon 1 of the 1,3-BPG product by a high-energy bond, conserves much of the free energy produced by the oxidation of glyceraldehyde 3-phosphate. This high-energy phosphate drives ATP synthesis in the next reaction of glycolysis.

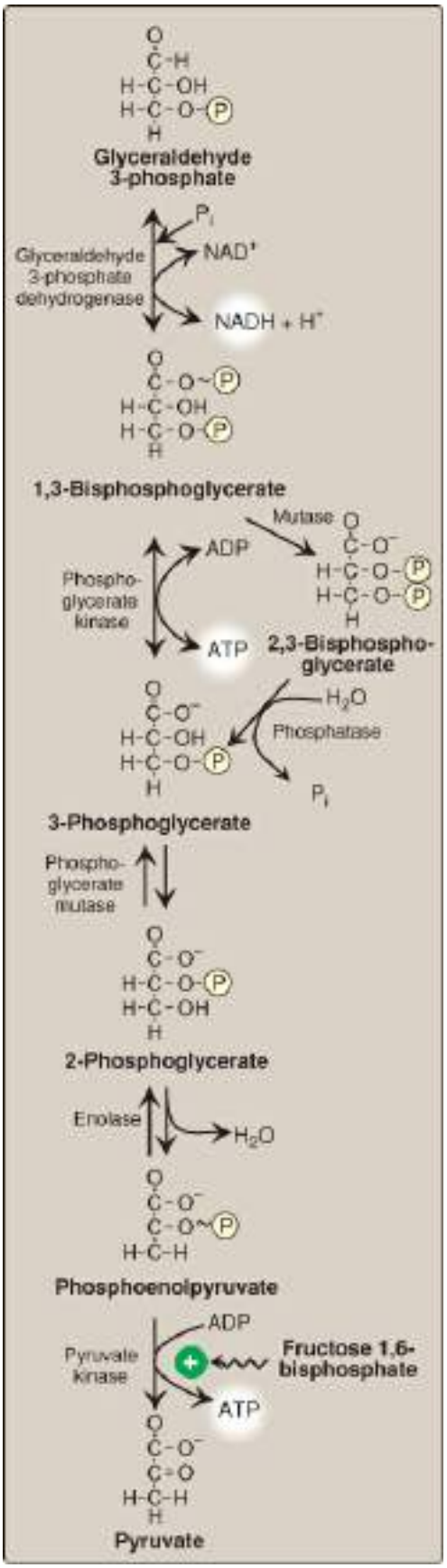


Figure 8.18
Energy-generating phase: conversion of glyceraldehyde 3-phosphate to pyruvate. NAD(H) = nicotinamide adenine dinucleotide; Ⓟ = phosphate; P_i = inorganic phosphate; ~ = high-energy bond; ADP = adenosine diphosphate.

Clinical Application 8.1: Arsenic Poisoning

The toxicity of arsenic is due primarily to the inhibition by trivalent arsenic (arsenite) of enzymes such as the pyruvate dehydrogenase complex (PDHC), which require lipoic acid as a coenzyme (see p. 121). However, pentavalent arsenic (arsenate) can prevent net ATP and NADH production by glycolysis without inhibiting the pathway itself. It does so by competing with P_i as a substrate for glyceraldehyde 3-phosphate dehydrogenase, forming a complex that spontaneously hydrolyzes to form 3-phosphoglycerate (see Fig. 8.18). By bypassing the synthesis of and phosphate transfer from 1,3-BPG, the cell is deprived of energy usually obtained from the glycolytic pathway. (Note: Arsenate also competes with P_i binding to the F_1 domain of ATP synthase resulting in formation of ADP-arsenate that is rapidly hydrolyzed.)

2. 2,3-Bisphosphoglycerate synthesis in RBC: Some of the 1,3-BPG is converted to 2,3-BPG by the action of bisphosphoglycerate mutase (Fig. 8.18). 2,3-BPG, which is found in only trace amounts in most cells, is present at high concentration in RBC and serves to increase O_2 delivery. 2,3-BPG is hydrolyzed by a phosphatase to 3-phosphoglycerate, which is also an intermediate in glycolysis (Fig. 8.18). In the RBC, glycolysis is modified by inclusion of these shunt reactions.

G. 3-Phosphoglycerate synthesis and ATP production

When 1,3-BPG is converted to 3-phosphoglycerate, the high-energy phosphate group of 1,3-BPG is used to synthesize ATP from ADP (Fig. 8.18). This reaction is catalyzed by phosphoglycerate kinase, which, unlike most other kinases, is physiologically reversible. Because two molecules of 1,3-BPG are formed from each glucose molecule, this kinase reaction replaces the two ATP molecules consumed by the earlier formation of glucose 6-phosphate and fructose 1,6-bisphosphate. (Note: This reaction is an example of substrate-level phosphorylation, in which the energy needed for the production of a high-energy phosphate comes from a substrate rather than from the ETC [see J. below for other examples].)

H. Phosphate group shift

The shift of the phosphate group from carbon 3 to carbon 2 of phosphoglycerate by phosphoglycerate mutase is freely reversible.

I. 2-Phosphoglycerate dehydration

The dehydration of 2-phosphoglycerate by enolase redistributes the energy within the substrate, forming phosphoenolpyruvate (PEP), which contains a high-energy

enol phosphate (Fig. 8.18). The reaction is reversible, despite the high-energy nature of the product. (Note: Fluoride inhibits enolase, and water fluoridation reduces lactate production by mouth bacteria, decreasing dental caries.)

J. Pyruvate synthesis and ATP production

The conversion of PEP to pyruvate, catalyzed by PK, is the third irreversible reaction of glycolysis. The high-energy enol phosphate in PEP is used to synthesize ATP from ADP and is another example of substrate-level phosphorylation (Fig. 8.18).

1. Feedforward regulation: PK is activated by fructose 1,6-bisphosphate, the product of the PFK-1 reaction. This feedforward (instead of the more usual feedback) regulation has the effect of linking the two kinase activities: increased PFK-1 activity results in elevated levels of fructose 1,6-bisphosphate, which activates PK. (Note: PK is inhibited by ATP.)

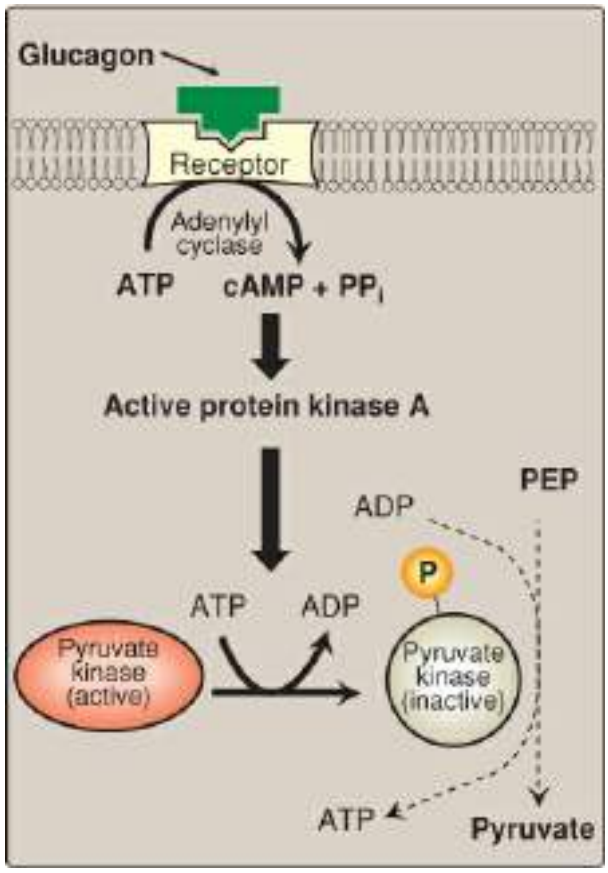


Figure 8.19
Covalent modification of hepatic pyruvate kinase results in inactivation of the enzyme. cAMP = cyclic adenosine monophosphate; PEP = phosphoenolpyruvate; P = phosphate; PP_i = pyrophosphate; ADP = adenosine diphosphate.

2. Covalent regulation in the liver: Phosphorylation by cAMP-dependent PKA leads

to inactivation of the hepatic isozyme of PK (Fig. 8.19). When blood glucose levels are low, elevated glucagon increases the intracellular level of cAMP, which causes the phosphorylation and inactivation of PK in the liver only. Therefore, PEP is unable to continue in glycolysis and, instead, enters the gluconeogenesis pathway. This partly explains the observed inhibition of hepatic glycolysis and stimulation of gluconeogenesis by glucagon. Dephosphorylation of PK by a phosphatase results in reactivation of the enzyme.

3. Pyruvate kinase deficiency: Because mature RBCs lack mitochondria, they are completely dependent on glycolysis for ATP production. ATP is required to meet the metabolic needs of RBCs and to fuel the ion pumps necessary for the maintenance of the flexible, biconcave shape that allows them to squeeze through narrow capillaries. The anemia observed in glycolytic enzyme deficiencies is a consequence of the reduced rate of glycolysis, leading to decreased ATP production by substrate-level phosphorylation. The resulting alterations in the RBC membrane lead to changes in cell shape and, ultimately, to phagocytosis by cells of the mononuclear phagocyte system, particularly splenic macrophages. The premature death and lysis of RBC result in mild-to-severe hemolytic anemia, with the severe form requiring regular transfusions. Among patients with rare genetic defects of glycolytic enzymes, the majority has a deficiency in PK. (Note: Liver PK is encoded by the same gene as the RBC isozyme. However, liver cells show no effect because they can synthesize more PK and can also generate ATP by oxidative phosphorylation.) Severity depends both on the degree of enzyme deficiency (generally 5% to 35% of normal levels) and on the extent to which RBC compensate by synthesizing increased levels of 2,3-BPG (see p. 32). Almost all individuals with PK deficiency have a mutant enzyme that shows altered kinetics or decreased stability (Fig. 8.20). Individuals heterozygous for PK deficiency have resistance to the most severe forms of malaria.

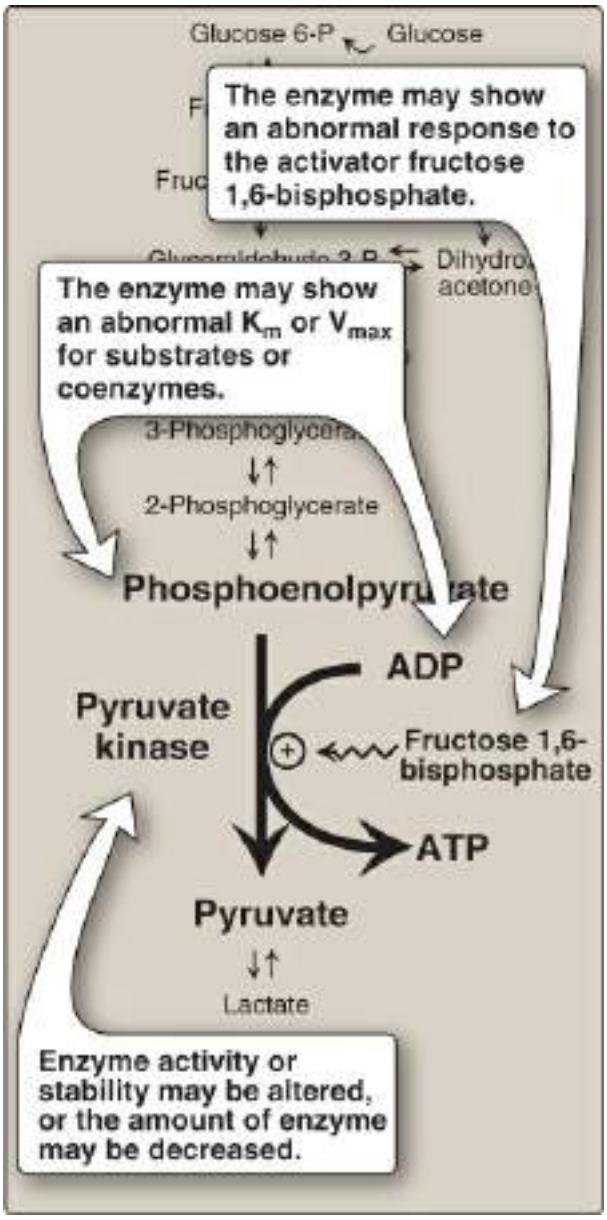


Figure 8.20
Alterations observed with various mutant forms of pyruvate kinase. K_m = Michaelis constant; V_{max} = maximal velocity; ADP = adenosine diphosphate.

The tissue-specific expression of PK in RBC and the liver results from the use of different start sites in transcription (see p. 473) of the gene that encodes the enzyme.

K. Pyruvate reduction to lactate

Lactate, formed from pyruvate by LDH, is the final product of anaerobic glycolysis in eukaryotic cells (Fig. 8.21). Reduction to lactate is the major fate for pyruvate in tissues that are poorly vascularized (e.g., the lens and cornea of the eye and the

kidney medulla) or in RBC that lack mitochondria.

1. Lactate formation in muscle: In exercising skeletal muscle, NADH production (by glyceraldehyde 3-phosphate dehydrogenase and by the three NAD^+-linked dehydrogenases of the TCA cycle, see also Chapter 9) exceeds the oxidative capacity of the ETC. This results in an elevated $NADH/NAD^+$ ratio, favoring reduction of pyruvate to lactate by LDH. Therefore, during intense exercise, lactate accumulates in muscle, causing a drop in the intracellular pH, potentially resulting in cramps. Much of this lactate eventually diffuses into the bloodstream and can be used by the liver to make glucose.

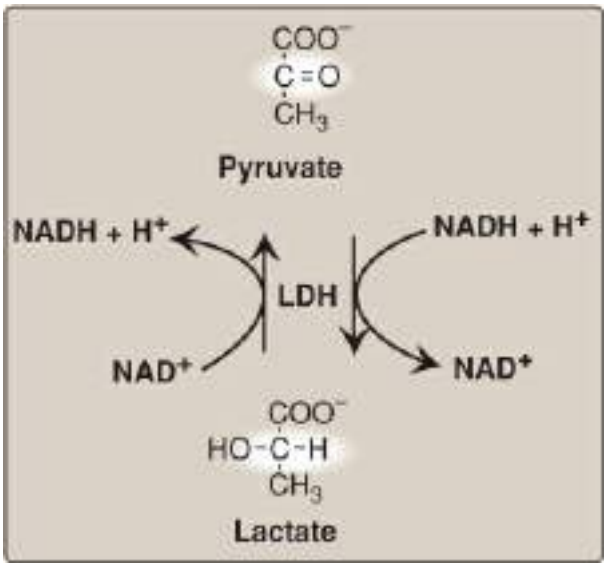


Figure 8.21
Interconversion of pyruvate and lactate by lactate dehydrogenase (LDH). NAD(H) = nicotinamide adenine dinucleotide.

2. Lactate utilization: The direction of the LDH reaction depends on the relative intracellular concentrations of pyruvate and lactate and on the ratio of $NADH/NAD^+$. For example, in the liver and heart, this ratio is lower than in exercising muscle. Consequently, the liver and heart oxidize lactate (obtained from the blood) to pyruvate. In the liver, pyruvate is either converted to glucose by gluconeogenesis or converted to acetyl CoA that is oxidized in the TCA cycle. Heart muscle exclusively oxidizes lactate to carbon dioxide and water via the TCA cycle.

3. Lactic acidosis: Elevated concentrations of lactate in the plasma, termed lactic acidosis (a type of metabolic acidosis), occur when there is a collapse of the circulatory system, such as with myocardial infarction, pulmonary embolism, and uncontrolled hemorrhage, or when an individual is in shock. The failure to bring adequate amounts of O_2 to the tissues results in impaired oxidative phosphorylation and decreased ATP synthesis. To survive, the cells rely on anaerobic glycolysis for generating ATP, producing lactic acid as the end

product. (Note: Production of even meager amounts of ATP may be lifesaving during the period required to reestablish adequate blood flow to the tissues.) The additional O_2 required to recover from a period when O_2 availability has been inadequate is termed the O_2 debt. (Note: The O_2 debt is often related to patient morbidity or mortality. In many clinical situations, measuring the blood levels of lactic acid allows the rapid, early detection of O_2 debt in patients and the monitoring of their recovery.)

L. Energy yield from glycolysis

Despite the production of some ATP by substrate-level phosphorylation during glycolysis, the end product, pyruvate or lactate, still contains most of the energy originally contained in glucose. The TCA cycle is required to release that energy completely.

1. Anaerobic glycolysis: A net of two molecules of ATP are generated for each molecule of glucose converted to two molecules of lactate (Fig. 8.22). There is no net production or consumption of NADH.

2. Aerobic glycolysis: The generation of ATP is the same as in anaerobic glycolysis (i.e., a net gain of two ATP per molecule of glucose). Two molecules of NADH are also produced per molecule of glucose. Ongoing aerobic glycolysis requires the oxidation of most of this NADH by the ETC, producing three ATP for each NADH molecule entering the chain (see p. 85). (Note: NADH cannot cross the inner mitochondrial membrane, and substrate shuttles are required.)

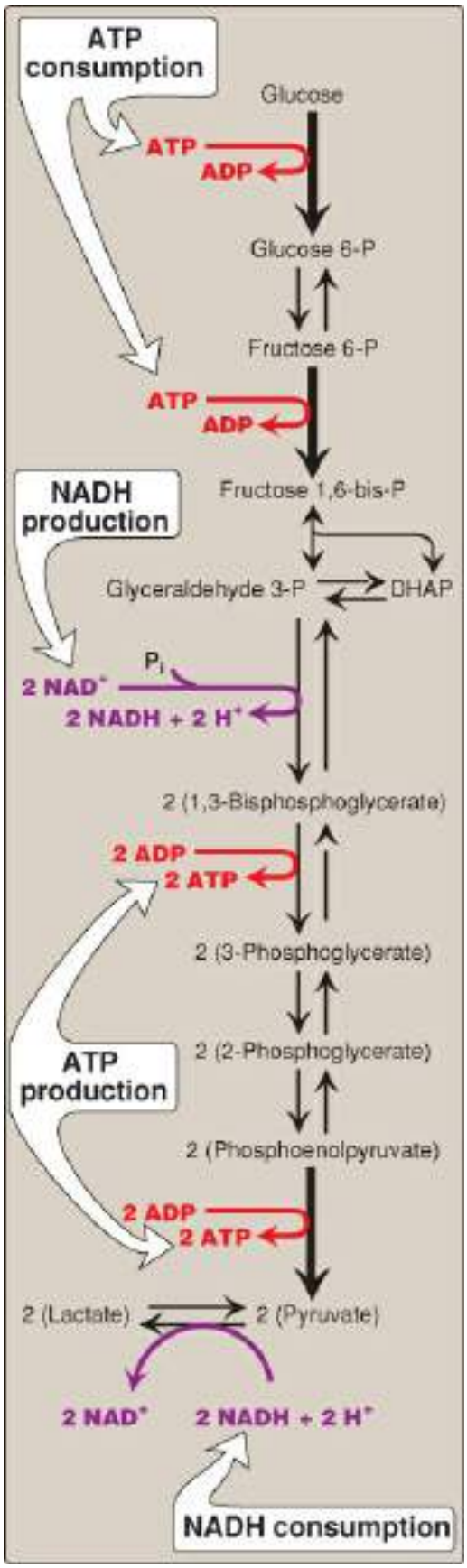


Figure 8.22
Summary of anaerobic glycolysis. Reactions involving the production or consumption of ATP or nicotinamide adenine dinucleotide (NADH) are indicated. The three irreversible reactions of glycolysis are shown with *thick arrows*. DHAP = dihydroxyacetone phosphate; ADP = adenosine diphosphate; P = phosphate.

VI. HORMONAL REGULATION

Regulation of the activity of the irreversible glycolytic enzymes by allosteric activation/inhibition or covalent phosphorylation/dephosphorylation is short term (i.e., the effects occur over minutes or hours). Superimposed on these effects on the activity of preexisting enzyme molecules are the long-term hormonal effects on the number of new enzyme molecules. These hormonal effects can result in 10- to 20-fold increases in enzyme synthesis that typically occur over hours to days.

Regular consumption of meals rich in carbohydrate or administration of insulin initiates an increase in the amount of glucokinase, PFK-1, and PK in the liver (Fig. 8.23). The change reflects an increase in gene transcription, resulting in increased enzyme synthesis. Increased availability of these three enzymes favors the conversion of glucose to pyruvate, a characteristic of the absorptive state. (Note: The transcriptional effects of insulin and carbohydrate [specifically glucose] are mediated by the transcription factors sterol regulatory element–binding protein-1c and carbohydrate response element–binding protein, respectively. These factors also regulate transcription of genes involved in fatty acid synthesis.) Conversely, gene expression of the three enzymes is decreased when plasma glucagon is high and insulin is low (e.g., as seen in fasting or diabetes).

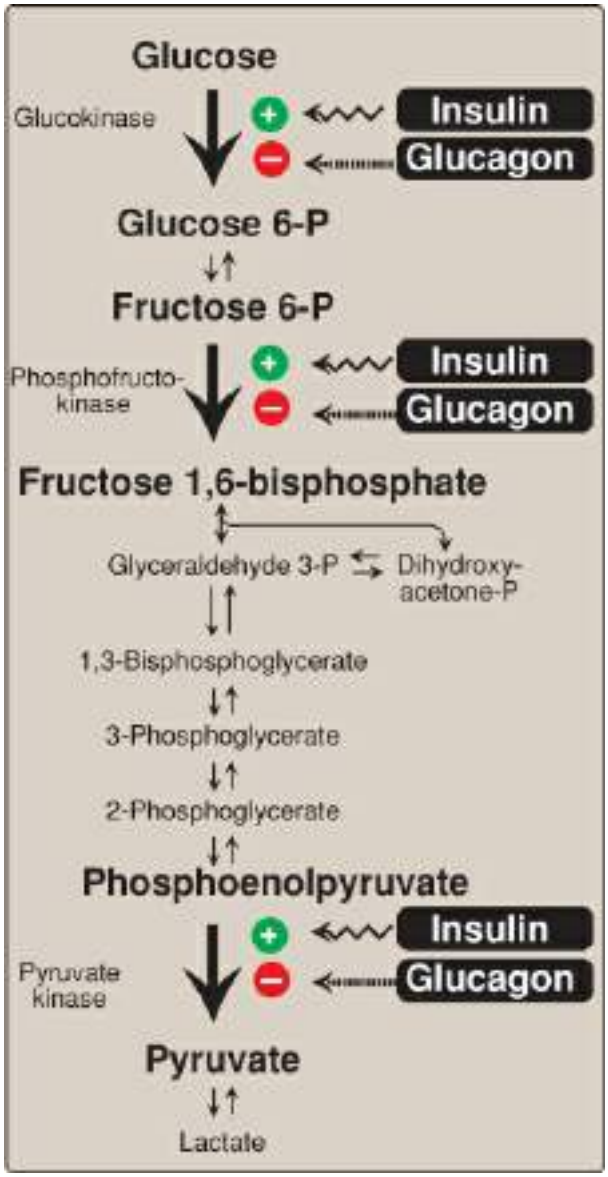


Figure 8.23
Effect of insulin and glucagon on the expression of key enzymes of glycolysis in the liver. P = phosphate.

VII. ALTERNATE FATES OF PYRUVATE

Pyruvate can be metabolized to products other than lactate.

A. Oxidative decarboxylation to acetyl CoA

Oxidative decarboxylation of pyruvate by the PDHC is an important pathway in tissues with a high oxidative capacity such as cardiac muscle (Fig. 8.24). PDHC irreversibly converts pyruvate, the end product of aerobic glycolysis, into acetyl CoA, a TCA cycle substrate and the carbon source for fatty acid synthesis.

B. Carboxylation to oxaloacetate

Carboxylation of pyruvate to oxaloacetate by pyruvate carboxylase is a biotin-dependent reaction (Fig. 8.24). This irreversible reaction is important because it replenishes the TCA cycle intermediate and provides substrate for gluconeogenesis.

C. Reduction to ethanol (microorganisms)

The reduction of pyruvate to ethanol occurs by the two reactions summarized in Figure 8.24. The decarboxylation of pyruvate to acetaldehyde by thiamine-requiring pyruvate decarboxylase occurs in yeast and certain other microorganisms but not in humans.

VIII. Chapter Summary

- Most **pathways** can be classified as either **catabolic** (degrade complex molecules to a few simple products with **ATP production**) or **anabolic** (synthesize complex end products from simple precursors with **ATP hydrolysis**).
- **Intercellular** signaling provides for the integration of metabolism. The primary route of this communication is **chemical signaling** (e.g., by **hormones** or **neurotransmitters**).
- **Second messenger molecules** are regulated in response to GPCR and transduce a chemical signal to appropriate intracellular responders.
- **Adenylyl cyclase is** a cell membrane enzyme regulated by GPCR that catalyzes synthesizes **cyclic cAMP** in response to hormones **glucagon** and **epinephrine**.
- The cAMP produced activates **PKA**, which phosphorylates a variety of enzymes, on serine/threonine residues, causing their activation or deactivation.
- Phosphorylation is reversed by **phosphoprotein phosphatases**.
- **Aerobic glycolysis**, in which **pyruvate** is the end product, occurs in cells with mitochondria and an adequate supply of oxygen ([O_2], Fig. 8.25).
- **Anaerobic glycolysis**, in which **lactic acid** is the end product, occurs in cells that lack mitochondria and in cells deprived of sufficient O_2.
- Glucose is passively transported across membranes by **GLUTs** which have tissue-specific distributions.
- The oxidation of glucose to pyruvate (**glycolysis**, see Fig. 8.25) occurs through an **energy-investment** phase in which phosphorylated intermediates are synthesized at the expense of ATP and an **energy-generation** phase in which ATP is produced by **substrate-level phosphorylation**.
- **Hexokinase** has a **high affinity** (**low K_m**) and a **low maximal velocity** (**V_{max}**) for glucose and is inhibited by glucose 6-phosphate. **Glucokinase** has a high K_m and a high V_{max} for glucose. It is regulated indirectly by fructose 6-phosphate (inhibits) and glucose (activates) via **GKRP**.
- Glucose 6-phosphate is isomerized to **fructose 6-phosphate**, which is phosphorylated to **fructose 1,6-bisphosphate** by **PFK-1**. A total of **two ATPs** are used during this phase of glycolysis.
- Fructose 1,6-bisphosphate is cleaved to form two trioses that are further metabolized by the glycolytic pathway, forming pyruvate. During this phase, **four ATP** and **two NADHs** are produced per glucose molecule.
- The final step in pyruvate synthesis from **PEP** is catalyzed by **PK**. **PK deficiency** accounts for the majority of all inherited defects in glycolytic enzymes. Effects are restricted to **RBC** and present as mild-to-severe **chronic**, **hemolytic anemia**.
- Glycolytic gene **transcription** is enhanced by insulin and glucose.

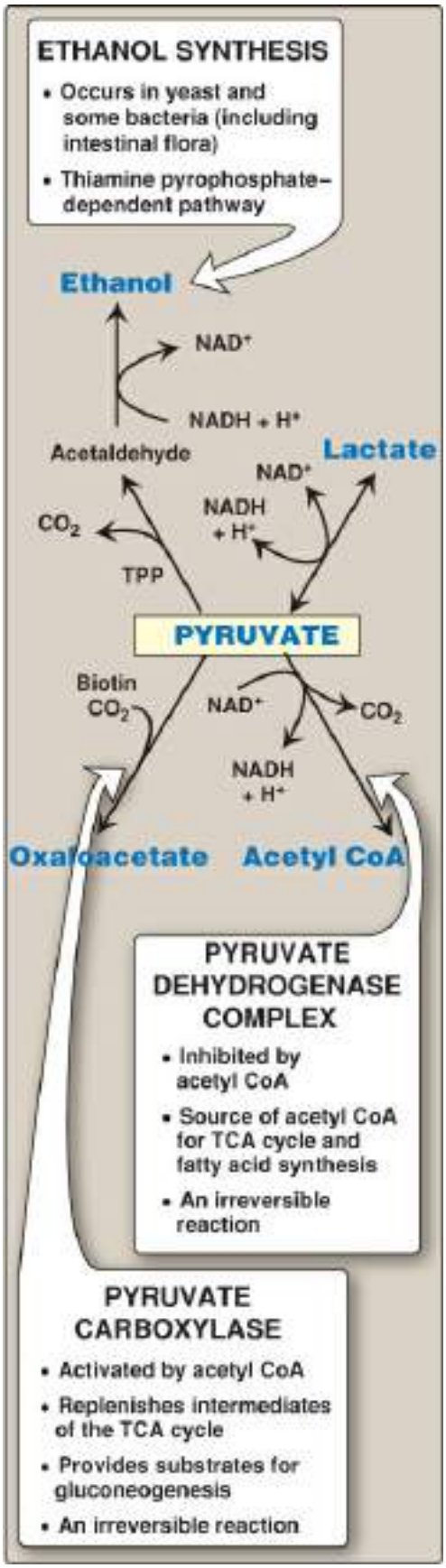


Figure 8.24
Summary of the metabolic fates of pyruvate. TPP = thiamine pyrophosphate. TCA = tricarboxylic acid; NAD(H) = nicotinamide adenine dinucleotide; CoA = coenzyme A; CO_2 = carbon dioxide.

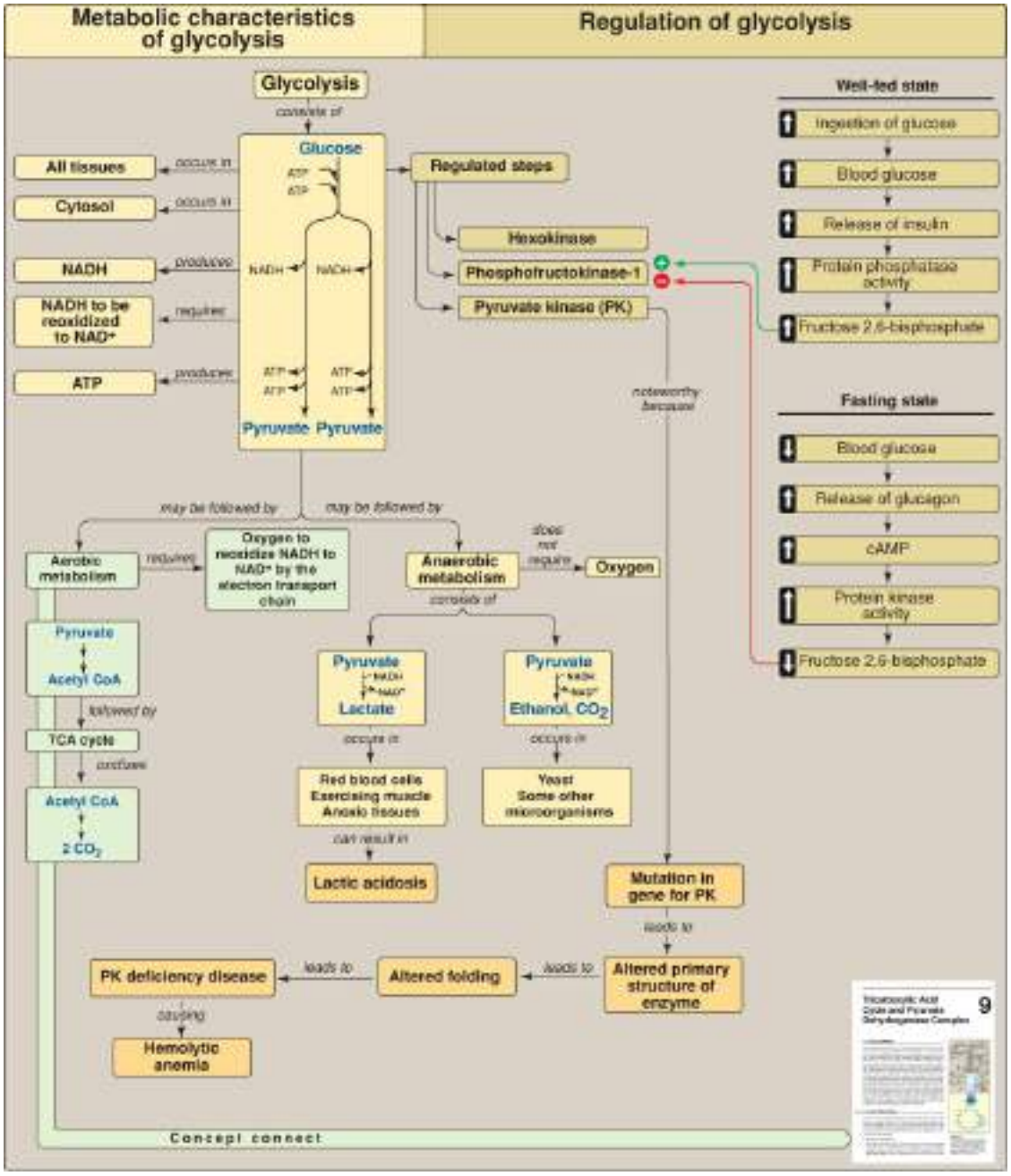


Figure 8.25
Key concept map for glycolysis. NAD(H) = nicotinamide adenine dinucleotide; cAMP = cyclic adenosine monophosphate; CoA = coenzyme A; TCA = tricarboxylic acid; CO_2 = carbon dioxide.

Study Questions

Choose the ONE best answer.

8.1 Which of the following best describes the activity level and phosphorylation state of the listed hepatic enzymes in an individual who consumed a carbohydrate-rich meal about an hour ago? PFK-1 = phosphofructokinase-1; PFK-2 = phosphofructokinase-2; Ⓟ = phosphorylated.

Choice	PFK-1		PFK-2		Pyruvate Kinase	
	Activity	Ⓟ	Activity	Ⓟ	Activity	Ⓟ
A.	Low	No	Low	No	Low	No
B.	High	Yes	Low	Yes	Low	Yes
C.	High	No	High	No	High	No
D.	High	Yes	High	Yes	High	Yes

Correct answer = C. Immediately following a meal, blood glucose levels and hepatic uptake of glucose increase. The glucose is phosphorylated to glucose 6-phosphate and used in glycolysis. In response to the rise in blood glucose, the insulin/glucagon ratio increases. As a result, the kinase domain of PFK-2 is dephosphorylated and active. Its product, fructose 2,6-bisphosphate, allosterically activates PFK-1. (PFK-1 is not covalently regulated.) Active PFK-1 produces fructose 1,6-bisphosphate that is a feedforward activator of pyruvate kinase. Hepatic pyruvate kinase is covalently regulated, and the rise in insulin favors dephosphorylation and activation.

8.2 Which of the following statements is true for anabolic pathways only?

A. Their irreversible (nonequilibrium) reactions are regulated.
B. They are called cycles if they regenerate an intermediate.
C. They are convergent and generate a few simple products.
D. They are synthetic and require energy.
E. They typically require oxidized coenzymes.

Correct answer = D. Anabolic processes are synthetic and energy requiring (endergonic). Statements A and B apply to both anabolic and catabolic processes, whereas C and E apply only to catabolic processes.

8.3 Compared with the resting state, vigorously contracting skeletal muscle shows:

A. decreased AMP/ATP ratio.
B. decreased levels of fructose 2,6-bisphosphate.
C. decreased $NADH/NAD^+$ ratio.
D. increased oxygen availability.
E. increased reduction of pyruvate to lactate.

Correct answer = E. Vigorously contracting skeletal muscle shows an increase in the reduction of pyruvate to lactate compared with resting muscle. The levels of reduced nicotinamide adenine dinucleotide (NADH) increase and exceed the oxidative capacity of the electron transport chain. Consequently, the levels of adenosine monophosphate (AMP) increase. The concentration of fructose 2,6-bisphosphate is not a key regulatory factor in skeletal muscle.

8.4 Choose the correct statement. Glucose transport into:

A. brain cells is through active transport.
B. intestinal mucosal cells requires insulin.
C. liver cells involves a glucose transporter.
D. most cells is through simple diffusion.

Correct answer = C. Glucose uptake in the liver, brain, muscle, and adipose tissue is down a concentration

gradient, and the transport is facilitated by tissue-specific glucose transporters (GLUTs). In adipose and muscle tissues, insulin is required for glucose uptake. Moving glucose against a concentration gradient requires energy and is seen with the sodium-dependent glucose cotransporter 1 (SGLT1) of intestinal mucosal cells. Except for some gasses, membrane transport into cells does not occur via simple diffusion. All glucose transport utilizes GLUT transport proteins.

8.5 Given that the K_m of glucokinase for glucose is 10 mM, whereas that of hexokinase is 0.1 mM, which isozyme will more closely approach V_{max} at the normal blood glucose concentration of 5 mM?

Correct answer = Hexokinase. K_m (Michaelis constant) is that substrate concentration that gives one half V_{max} (maximal velocity). When blood glucose concentration is 5 mM, hexokinase (K_m = 0.1 mM) will be saturated, but glucokinase (K_m = 10 mM) will not.

8.6 In patients with pertussis infection and whooping cough, $G\alpha_i$ is inhibited. How does this lead to a rise in cyclic adenosine monophosphate (cAMP)?

Correct answer = G proteins of the $G\alpha_i$ type inhibit adenylyl cyclase (AC) when their associated G protein–coupled receptor is bound by ligand. If $G\alpha_i$ is inhibited by pertussis toxin, AC production of cAMP is inappropriately activated.

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[a] For more information on GPCR signaling and second messengers, see *LIR Cell and Molecular Biology*, 2nd ed.

[b] For more information on glucose transport, see *LIR Cell and Molecular Biology*, 2nd Ed., Chapter 15.

[c] For further information, see *LIR Cell and Molecular Biology*, 2nd Editions, Chapters 14 and 15.

Tricarboxylic Acid Cycle and Pyruvate Dehydrogenase Complex 9

I. CYCLE OVERVIEW

The **tricarboxylic acid cycle** (TCA cycle) can also be referred to as the citric acid cycle or the Krebs cycle, and plays several roles in metabolism. It is the final pathway where the oxidative catabolism of carbohydrates, amino acids, and fatty acids converge, their carbon skeletons being converted to carbon dioxide (CO_2), as shown in Figure 9.1. This oxidation provides energy for the production of the majority of ATP in most animals, including humans. Because the TCA cycle occurs totally in mitochondria, it is in close proximity to the electron transport chain ([ETC]), which oxidizes the reduced coenzymes nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$) produced by the cycle. The TCA cycle is an aerobic pathway, because oxygen (O_2) is required as the final electron acceptor. Reactions such as the catabolism of some amino acids generate intermediates of the cycle and are called anaplerotic (from the Greek for "filling up") reactions. The TCA cycle also provides intermediates for a number of important anabolic reactions, such as glucose formation from the carbon skeletons of some amino acids and the synthesis of some amino acids (see Chapter 20 Section V) and heme (see Chapter 21 Section II B). Therefore, this cycle should not be viewed as a closed system but, instead, as an open one with compounds entering and leaving as required.

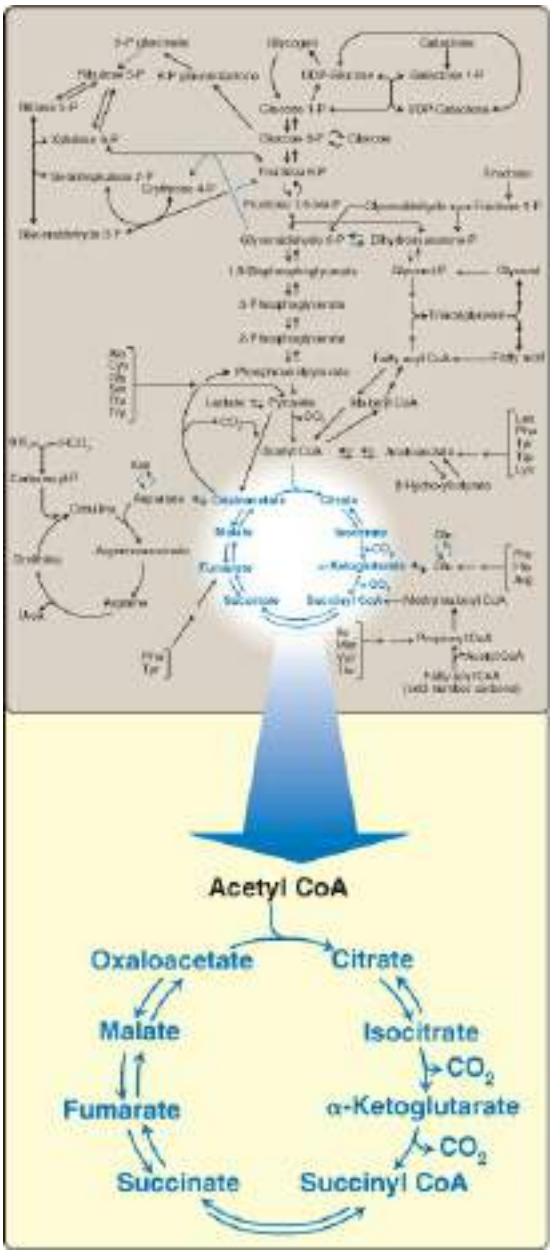


Figure 9.1
The tricarboxylic acid cycle shown as a part of the essential pathways of energy metabolism. (Note: See Fig. 8.2, for a more detailed map of metabolism.) CO_2 = carbon dioxide; CoA = coenzyme A.

II. CYCLE REACTIONS

In the TCA cycle, oxaloacetate (OAA) is first condensed with an acetyl group from **acetyl coenzyme A** (CoA) and then is regenerated as the cycle is completed (see Fig. 9.1). Two carbons enter the cycle as acetyl CoA and two leave as CO_2. Therefore, the entry of one acetyl CoA into one round of the TCA cycle does not lead to the net production or consumption of intermediates.

A. Acetyl CoA production

The major source of acetyl CoA for the TCA cycle is the oxidative decarboxylation of **pyruvate** by the multienzyme **pyruvate dehydrogenase complex** (PDH complex, or PDHC). However, the PDHC (described below) is not a component of the TCA cycle. Pyruvate, the end product of glycolysis, is transported from the cytosol into the mitochondrial matrix by the pyruvate mitochondrial carrier of the inner mitochondrial membrane. In the matrix, the PDHC converts pyruvate to acetyl CoA. (Note: Fatty acid oxidation is another source of acetyl CoA [see Chapter 16 Section IV].)

1. PDHC component enzymes: The PDHC is a protein aggregate of multiple copies of three enzymes, pyruvate decarboxylase ([E1] sometimes called PDH), dihydrolipoyl transacetylase (E2), and dihydrolipoyl dehydrogenase (E3). Each catalyzes a part of the overall reaction (Fig. 9.2). Their physical association links the reactions in proper sequence without the release of intermediates. In addition to the enzymes participating in the conversion of pyruvate to acetyl CoA, the PDHC also contains two regulatory enzymes, pyruvate dehydrogenase kinase (PDH kinase) and pyruvate dehydrogenase phosphatase (PDH phosphatase).

2. Coenzymes: The PDHC contains five coenzymes that act as carriers or oxidants for the intermediates of the reactions shown in Figure 9.2. E1 requires thiamine pyrophosphate (TPP), E2 requires lipoic acid and CoA, and E3 requires FAD and NAD^+. (Note: TPP, lipoic acid, and FAD are tightly bound to the enzymes and function as coenzymes–prosthetic groups [see p. 58].)

> Deficiencies of thiamine or niacin can cause serious central nervous system problems. This is because brain cells are unable to produce sufficient ATP via the TCA cycle if the PDHC is inactive. Wernicke–Korsakoff, an encephalopathy-psychosis syndrome due to thiamine deficiency, may be seen in persons with alcohol use disorder.

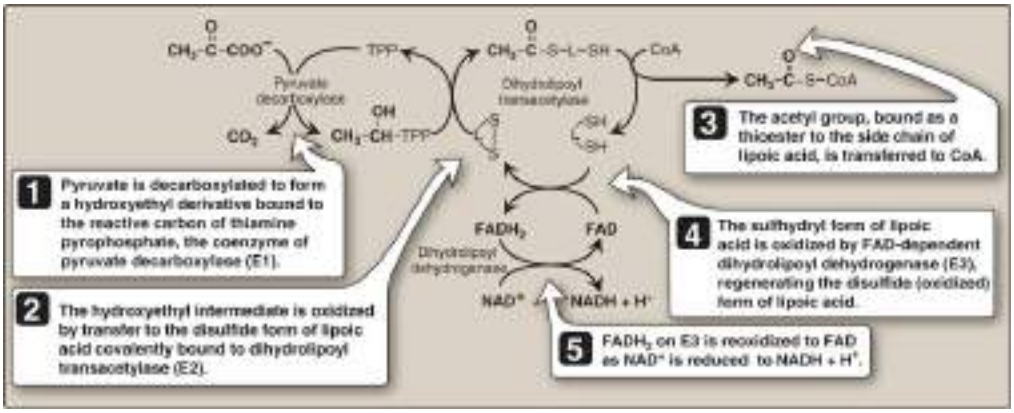


Figure 9.2
Mechanism of action of the enzymes (E) of the pyruvate dehydrogenase complex. (Note: All the coenzymes of the complex, except for lipoic acid, are derived from vitamins. TPP is from

thiamine, FAD from riboflavin, NAD from niacin, and CoA from pantothenic acid.) CO_2 = carbon dioxide; TPP = thiamine pyrophosphate; L = lipoic acid; CoA = coenzyme A; FAD(H_2) and NAD(H) = flavin and nicotinamide adenine dinucleotides; ~ = high-energy bond.

3. Regulation: Covalent modifications by the two regulatory enzymes of the PDHC alternately activate and inactivate E1. PDH kinase phosphorylates and inactivates E1, whereas PDH phosphatase dephosphorylates and activates E1 (Fig. 9.3). The kinase itself is allosterically activated by ATP, acetyl CoA, and NADH. Therefore, in the presence of these high-energy products, the PDHC is turned off. (Note: It is actually the rise in the ATP/ADP [adenosine diphosphate], NADH/NAD^+, or acetyl CoA/CoA ratios that affects enzymic activity.)

Pyruvate is a potent inhibitor of PDH kinase. Therefore, if pyruvate concentrations are elevated, E1 will be maximally active. Calcium (Ca^{2+}) is a strong activator of PDH phosphatase, stimulating E1 activity. This is particularly important in skeletal muscle, where Ca^{2+} release during contraction stimulates the PDHC and, thus, energy production. (Note: Although covalent regulation by the kinase and phosphatase is primary, the PDHC is also subject to product [NADH and acetyl CoA] inhibition.)

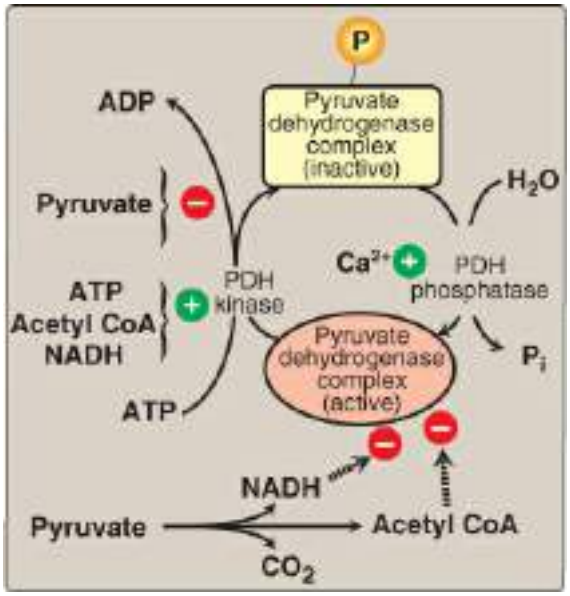


Figure 9.3
Regulation of pyruvate dehydrogenase (PDH) complex. P = phosphate (⇢ denotes product inhibition.)

4. Deficiency: A deficiency of the α subunits of the tetrameric E1 component of the PDHC, although very rare, is the most common biochemical cause of congenital lactic acidosis. The deficiency results in a decreased ability to convert pyruvate to acetyl CoA, causing pyruvate to be shunted to lactate via lactate dehydrogenase (see p. 113). This creates particular problems for the brain, which relies on the TCA cycle for most of its energy and is particularly sensitive

to acidosis. Symptoms are variable and include neurodegeneration, muscle spasticity, and, in the neonatal-onset form, early death. The gene for the α-subunit is located on the X chromosome. Inheritance of just one X chromosome with the mutation results in disease; the inheritance pattern is X-linked dominant, with both males and females affected. Although there is no proven treatment for PDHC deficiency, dietary restriction of carbohydrate and supplementation with thiamine may reduce symptoms in select patients.

Leigh syndrome (subacute necrotizing encephalomyelopathy) is a rare, progressive, neurodegenerative disorder caused by defects in mitochondrial ATP production, primarily as a result of mutations in genes that encode proteins of the PDHC, the ETC, or ATP synthase. Both nuclear and mitochondrial DNA can be affected.

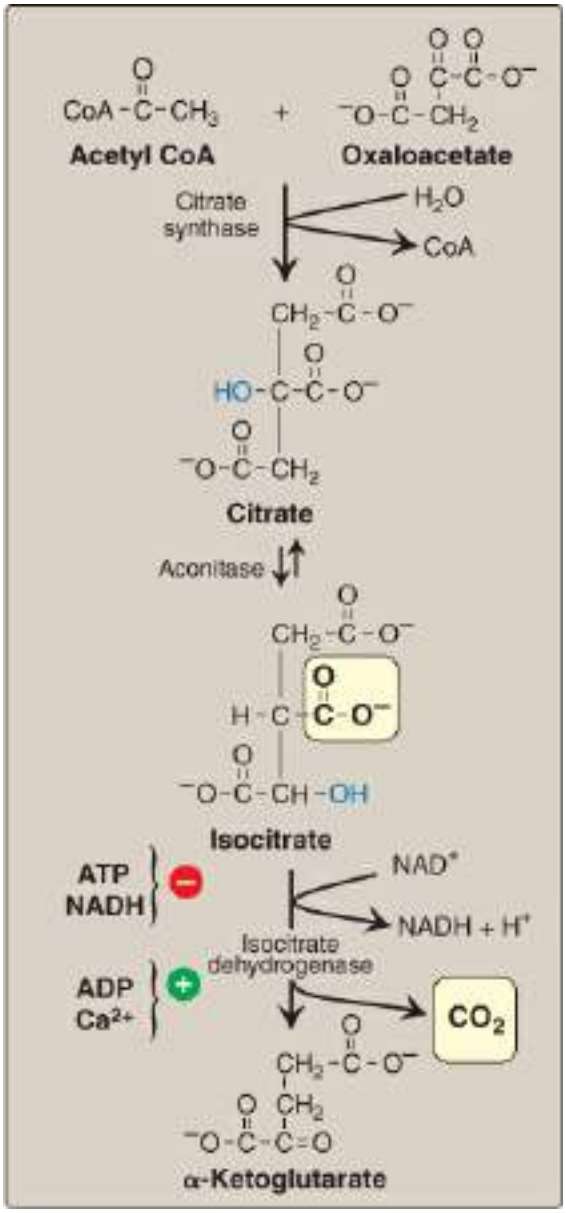


Figure 9.4
Formation of α-ketoglutarate from acetyl coenzyme A (CoA) and oxaloacetate. NAD(H) = nicotinamide adenine dinucleotide; CO_2 = carbon dioxide.

5. Arsenic poisoning: As previously described (see p. 111), pentavalent arsenic (arsenate) can interfere with glycolysis at the glyceraldehyde 3-phosphate step, thereby decreasing ATP production. However, arsenic poisoning is due primarily to inhibition of enzyme complexes that require lipoic acid as a coenzyme, including PDH, α-ketoglutarate dehydrogenase (see E. below), and branched-chain α-keto acid dehydrogenase (see Chapter 20 Section III). Arsenite (the trivalent form of arsenic) forms a stable complex with the thiol (−SH) groups of lipoic acid, making that compound unavailable to serve as a coenzyme. When it binds to lipoic acid in the PDHC, pyruvate (and, consequently, lactate)

accumulates. As with PDHC deficiency, this particularly affects the brain, causing neurologic disturbances and death.

B. Citrate synthesis

The irreversible condensation of acetyl CoA and OAA to form citrate (a TCA) is catalyzed by citrate synthase, the initiating enzyme of the TCA cycle (Fig. 9.4). This aldol condensation has a highly negative change in standard free energy ([ΔG^0]), which strongly favors citrate formation. The enzyme is inhibited by citrate (product inhibition). Substrate availability is another means of regulation for citrate synthase. The binding of OAA greatly increases the enzyme's affinity for acetyl CoA. (Note: Citrate, in addition to being an intermediate in the TCA cycle, is a source of acetyl CoA for the cytosolic synthesis of fatty acids and cholesterol. Citrate also inhibits phosphofructokinase-1 [PFK-1], the rate-limiting enzyme of glycolysis, and activates acetyl CoA carboxylase [the rate-limiting enzyme of fatty acid synthesis, see Chapter 16 Section III].)

C. Citrate isomerization

Citrate is isomerized to **isocitrate** through hydroxyl group migration catalyzed by **aconitase** (aconitate hydratase), an iron-sulfur protein (see Fig. 9.4). (Note: Aconitase is inhibited by fluoroacetate, a plant toxin that is used as a pesticide. Fluoroacetate is converted to fluoroacetyl CoA that condenses with OAA to form fluorocitrate, a potent inhibitor of aconitase.)

D. Oxidative decarboxylation of isocitrate

Isocitrate dehydrogenase catalyzes the irreversible oxidative decarboxylation of isocitrate to **α-ketoglutarate**, yielding the first of three NADH molecules produced by the cycle and the first release of CO_2 (see Fig. 9.4). This is one of the rate-limiting steps of the TCA cycle. The enzyme is allosterically activated by ADP (a low-energy signal) and Ca^{2+} and is inhibited by ATP and NADH, levels of which are elevated when the cell has abundant energy stores.

E. Oxidative decarboxylation of α-ketoglutarate

The irreversible conversion of **α-ketoglutarate** to **succinyl CoA** is catalyzed by the α-ketoglutarate dehydrogenase complex, a protein aggregate of multiple copies of three enzymes (Fig. 9.5). The mechanism of this oxidative decarboxylation is very similar to that used for the conversion of pyruvate to acetyl CoA by the PDHC. The reaction releases the second CO_2 and produces the second NADH of the cycle. The coenzymes required are TPP, lipoic acid, FAD, NAD^+, and CoA. Each functions as part of the catalytic mechanism in a way analogous to that described for the PDHC. The large negative ΔG^0 of the reaction favors formation of succinyl CoA, a high-energy thioester similar to acetyl CoA. The α-ketoglutarate dehydrogenase complex

is inhibited by its products, NADH and succinyl CoA, and activated by Ca^{2+}. However, it is not regulated by phosphorylation/dephosphorylation reactions as described for the PDHC. (Note: α-Ketoglutarate is also produced by the oxidative deamination and transamination of the amino acid glutamate.)

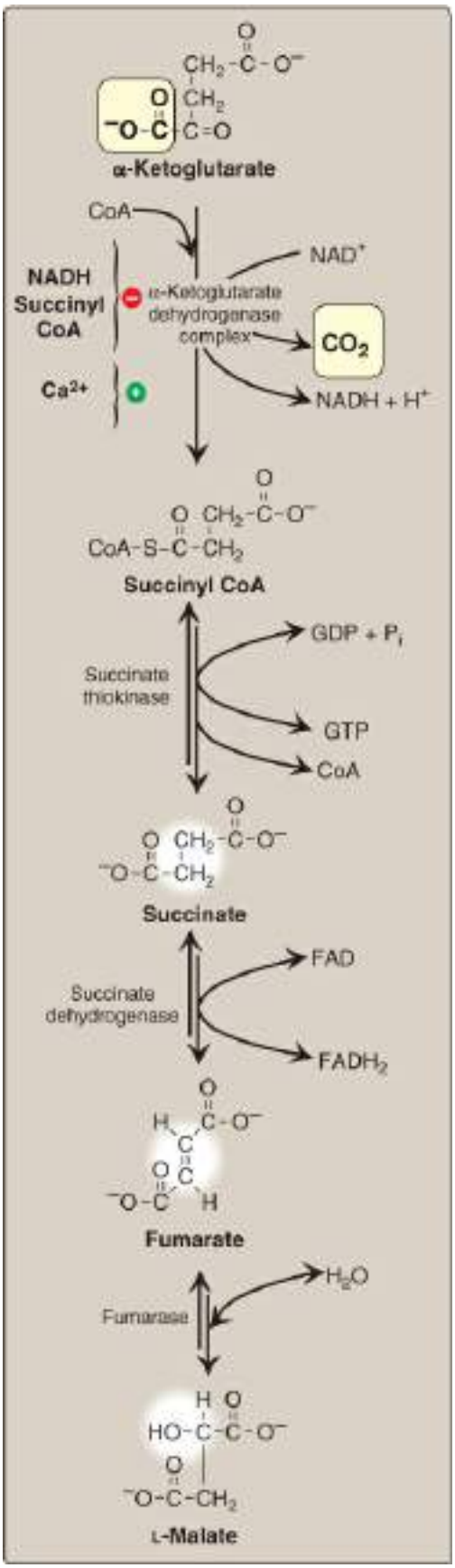


Figure 9.5
Formation of malate from α-ketoglutarate. FAD(H_2) and NAD(H) = flavin and nicotinamide adenine dinucleotides; GDP and GTP = guanosine di- and triphosphates; ~ = high-energy bond; CoA = coenzyme A.

F. Succinyl CoA cleavage

Succinate thiokinase (also called succinyl CoA synthetase, named for the reverse reaction) cleaves the high-energy thioester bond of succinyl CoA (see Fig. 9.5). This reaction is coupled to phosphorylation of guanosine diphosphate (GDP) to guanosine triphosphate (GTP). GTP and ATP are energetically interconvertible by the nucleoside diphosphate kinase reaction:

$$GTP + ADP \rightleftarrows GDP + ATP$$

The generation of GTP by succinate thiokinase is another example of **substrate-level** phosphorylation (see p. 112). (Note: Succinyl CoA is also produced from propionyl CoA derived from the metabolism of fatty acids with an odd number of carbon atoms and from the metabolism of several amino acids. It can be converted to pyruvate for gluconeogenesis [see Chapter 10] or used in heme synthesis [see Chapter 21].)

G. Succinate oxidation

Succinate is oxidized to **fumarate** by succinate dehydrogenase, as its coenzyme FAD is reduced to $FADH_2$ (see Fig. 9.5). Succinate dehydrogenase is the only enzyme of the TCA cycle that is embedded in the inner mitochondrial membrane. As such, it functions as Complex II of the ETC (see p. 83). (Note: FAD, rather than NAD^+, is the electron acceptor because the reducing power of succinate is not sufficient to reduce NAD^+.)

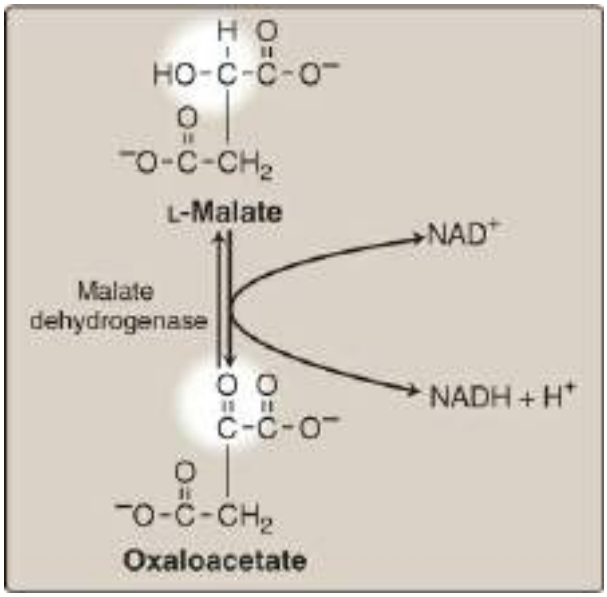


Figure 9.6
Formation (regeneration) of oxaloacetate from malate. NAD(H) = nicotinamide adenine dinucleotide.

H. Fumarate hydration

Fumarate is hydrated to **malate** in a freely reversible reaction catalyzed by **fumarase** (fumarate hydratase, see Fig. 9.5). (Note: Fumarate is also produced by the urea cycle, in purine synthesis [see Fig. 22.7], and during catabolism of the amino acids phenylalanine and tyrosine.)

I. Malate oxidation

Malate is oxidized to OAA by malate dehydrogenase (Fig. 9.6). This reaction produces the third and final **NADH** of the cycle. The ΔG^0 of the reaction is positive, but the reaction is driven in the direction of OAA by the highly exergonic citrate synthase reaction. (Note: OAA is also produced by the transamination of the amino acid aspartic acid.)

Energy-producing reaction	Number of ATP produced
3 NADH $\longrightarrow$ 3 NAD^+	9
$FADH_2 \longrightarrow FAD$	2
$GDP + P_i \longrightarrow GTP$	1
	12 ATP/acetyl CoA oxidized

Figure 9.7
Number of ATP molecules produced from the oxidation of one molecule of acetyl coenzyme A (CoA) using both substrate-level and oxidative phosphorylation. NAD(H) and FAD(H_2) = nicotinamide and flavin adenine dinucleotides; GDP and GTP = guanosine di- and triphosphates; P_i = inorganic phosphate.

III. ENERGY PRODUCED BY THE CYCLE

Four pairs of electrons are transferred during one turn of the TCA cycle: three pairs reducing three NAD^+ to NADH and one pair reducing FAD to $FADH_2$. Oxidation of one NADH by the ETC leads to formation of three ATP, whereas oxidation of $FADH_2$ produces two ATP. The total yield of ATP from the oxidation of one acetyl CoA is shown in Figure 9.7. Figure 9.8 summarizes the reactions of the TCA cycle. (Note: The cycle does not involve the net consumption or production of intermediates. Two carbons entering as acetyl CoA are balanced by two CO_2 exiting.)

IV. CYCLE REGULATION

In contrast to glycolysis, which is regulated primarily by PFK-1, the TCA cycle is controlled by the regulation of several enzymes (see Fig. 9.8). The most important of these regulated enzymes are those that catalyze reactions with highly negative ΔG^0: citrate synthase, isocitrate dehydrogenase, and the α-ketoglutarate dehydrogenase complex. Reducing equivalents needed for oxidative phosphorylation are generated by the PDHC and the TCA cycle, and both processes are upregulated in response to a decrease in the ATP/ADP ratio.

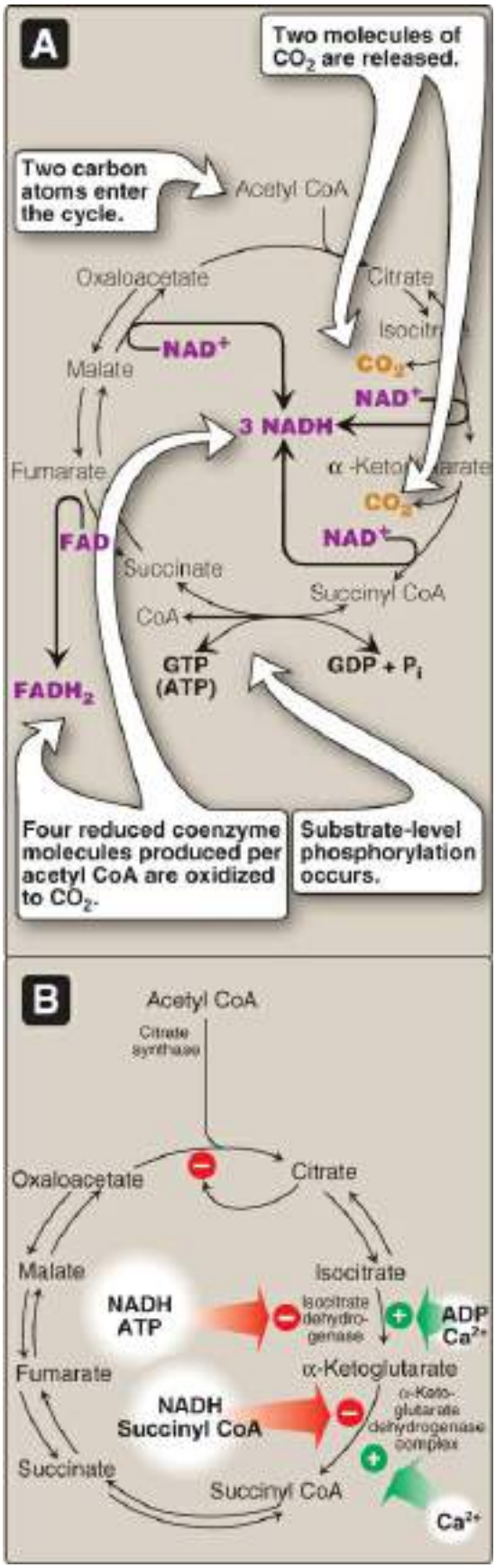


Figure 9.8
A: Production of reduced coenzymes, ATP, and carbon dioxide (CO_2) in the tricarboxylic acid cycle. (Note: Guanosine triphosphate [GTP] and ATP are interconverted by nucleoside diphosphate kinase.) **B:** Inhibitors and activators of the cycle.

V. Chapter Summary

- In the **TCA cycle**, also called the **Krebs cycle, pyruvate** is oxidatively decarboxylated by the **PDHC**, producing **acetyl CoA** (Fig. 9.9).
- The multienzyme PDHC requires five coenzymes: **TPP**, **lipoic acid**, **flavin adenine dinucleotide** (**FAD**), **nicotinamide adenine dinucleotide** (**NAD^+**), and **CoA**.
- PDHC is regulated by covalent modification of **E1**, by **PDH kinase** and **PDH phosphatase**: Phosphorylation inhibits E1.
- PDH kinase is allosterically activated by ATP, acetyl CoA, and NADH and inhibited by pyruvate. The phosphatase is activated by calcium (Ca^{2+}).
- **Pyruvate decarboxylase deficiency** is the most common biochemical cause of **congenital lactic acidosis**. The brain is particularly affected in this **X-linked dominant** disorder.
- **Arsenic poisoning** causes inactivation of the PDHC by binding to lipoic acid. In the TCA cycle, **citrate** is synthesized from **OAA** and **acetyl CoA** by **citrate synthase**, which is inhibited by product.
- **Citrate** is isomerized to **isocitrate** by **aconitase** (**aconitate hydratase**). Isocitrate is oxidatively decarboxylated by **isocitrate dehydrogenase** to **α-ketoglutarate**, producing **CO_2** and **NADH**. The enzyme is inhibited by ATP and NADH and activated by ADP and Ca^{2+}.
- **α-Ketoglutarate** is oxidatively decarboxylated to **succinyl CoA** by the **α-ketoglutarate dehydrogenase complex**, producing CO_2 and NADH. The enzyme is very similar to the PDHC and uses the same coenzymes.
- The α-ketoglutarate dehydrogenase complex is activated by Ca^{2+} and inhibited by NADH and succinyl CoA but is not covalently regulated. Succinyl CoA is cleaved by **succinate thiokinase** producing **succinate** and **GTP**. This is an example of **substrate-level phosphorylation**.
- Succinate is oxidized to **fumarate** by **succinate dehydrogenase**, producing **$FADH_2$**. Fumarate is hydrated to **malate** by **fumarase** (**fumarate hydratase**), and malate is oxidized to OAA by **malate dehydrogenase**, producing **NADH**.
- **Three NADH** and **one $FADH_2$** are produced by one round of the TCA cycle.
- The generation of acetyl CoA by the oxidation of pyruvate via the PDHC also produces an NADH. Oxidation of the NADH and $FADH_2$ by the ETC yields 14 ATP. The terminal phosphate of the GTP produced by substrate-level phosphorylation in the TCA cycle can be transferred to ADP by nucleoside diphosphate kinase, yielding another ATP.
- Therefore, a total of 15 ATP are produced from the complete mitochondrial oxidation of pyruvate to CO_2.

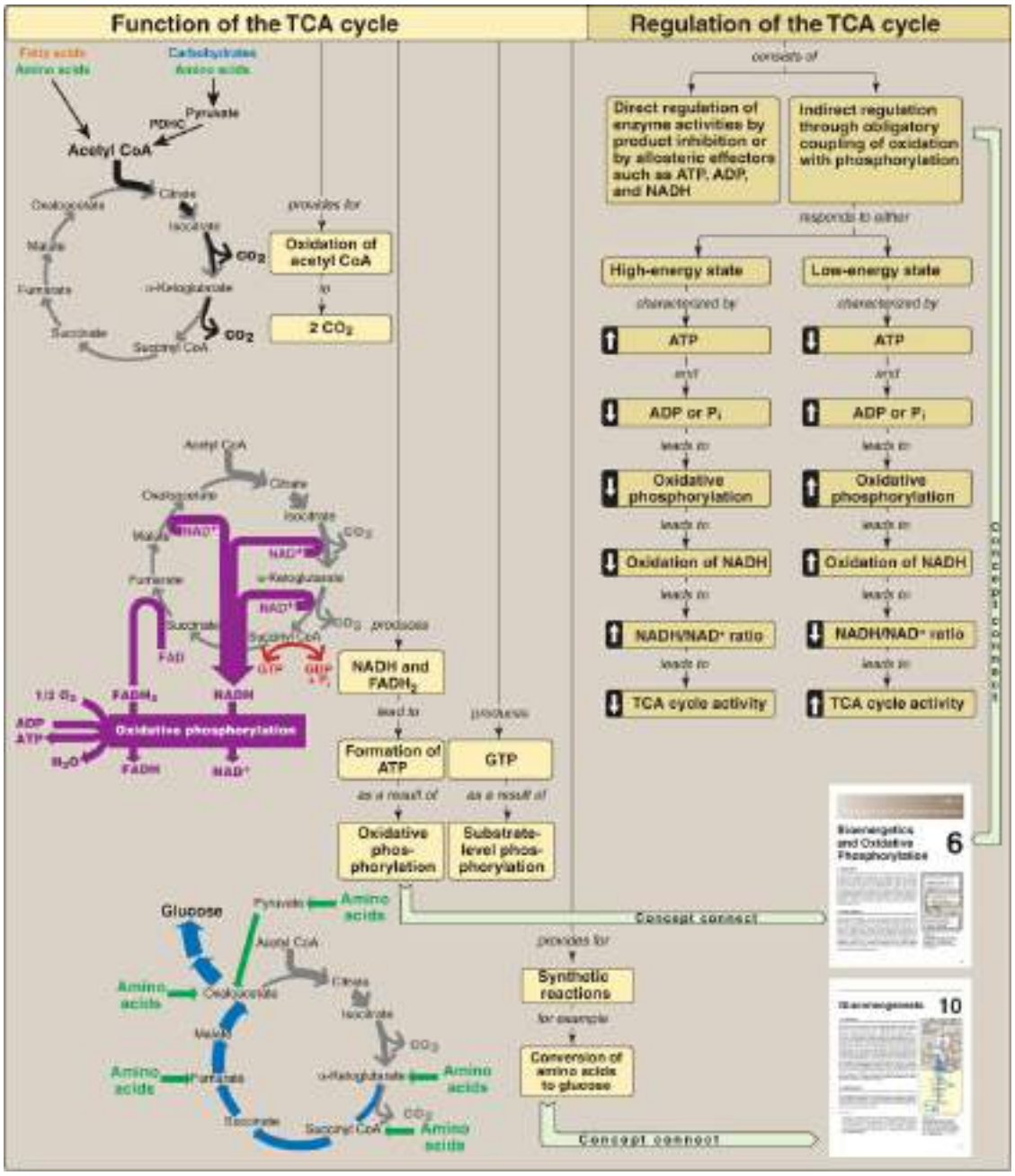


Figure 9.9
Key concept map for the tricarboxylic acid (TCA) cycle. PDHC = pyruvate dehydrogenase complex; CoA = coenzyme A; CO_2 = carbon dioxide; NAD(H) = nicotinamide adenine dinucleotide; FAD(H_2) = flavin adenine dinucleotide; GDP and GTP = guanosine di- and triphosphates; ADP = adenosine diphosphate; P_i = inorganic phosphate.

Study Questions

Choose the ONE best answer.

9.1 The conversion of pyruvate to acetyl coenzyme A and carbon dioxide:

A. involves the participation of lipoic acid.

B. is activated when pyruvate decarboxylase of the pyruvate dehydrogenase complex (PDHC) is phosphorylated by PDH kinase in the presence of ATP.
C. is reversible.
D. occurs in the cytosol.
E. requires the coenzyme biotin.

Correct answer = A. Lipoic acid is an intermediate acceptor of the acetyl group formed in the reaction. (Note: Lipoic acid linked to a lysine residue in E2 functions as a "swinging arm" that allows interaction with E1 and E3.) The PDHC catalyzes an irreversible reaction that is inhibited when the decarboxylase component (E1) is phosphorylated. The PDHC is located in the mitochondrial matrix. Biotin is utilized by carboxylases, not decarboxylases.

9.2 Which one of the following conditions decreases the oxidation of acetyl coenzyme A by the citric acid cycle?

A. A high availability of calcium
B. A high acetyl CoA/CoA ratio
C. A low ATP/ADP ratio
D. A low NAD^+/NADH ratio

Correct answer = D. A low NAD^+/NADH (oxidized to reduced nicotinamide adenine dinucleotide) ratio limits the rates of the NAD^+-requiring dehydrogenases. High availability of calcium and substrate (acetyl coenzyme A) and a low ATP/ADP (adenosine tri- to diphosphate) ratio stimulate the cycle.

9.3 The following is the sum of three steps in the citric acid cycle.

$$A + B + FAD + H_2O \rightarrow C + FADH_2 + NADH.$$

Choose the lettered answer that corresponds to the missing "A," "B," and "C" in the equation.

Reactant A	Reactant B	Product C
A. Succinyl CoA	GDP	Succinate
B. Succinate	NAD^+	Oxaloacetate
C. Fumarate	NAD^+	Oxaloacetate
D. Succinate	NAD^+	Malate
E. Fumarate	GTP	Malate

Correct answer = B. Succinate + NAD^+ + FAD + H_2O → oxaloacetate + NADH + $FADH_2$.

9.4 A 1-month-old male shows neurologic problems and lactic acidosis. An enzyme activity assay for pyruvate dehydrogenase complex (PDHC) performed on extracts of cultured skin fibroblasts showed 5% of normal activity with a low concentration of thiamine pyrophosphate (TPP) but 80% of normal activity when the assay contained a thousand-fold higher concentration of TPP. Which one of the following statements concerning this patient is correct?

A. Administration of thiamine is expected to reduce his serum lactate level and improve his clinical symptoms.
B. A high-carbohydrate diet would be expected to be beneficial for this patient.
C. Citrate production from aerobic glycolysis is expected to be increased.
D. PDH kinase, a regulatory enzyme of the PDHC, is expected to be active.

Correct answer = A. The patient appears to have a thiamine-responsive PDHC deficiency. The pyruvate decarboxylase (E1) component of the PDHC fails to bind thiamine pyrophosphate at low concentration but shows significant activity at a high concentration of the coenzyme. This mutation, which affects the K_m (Michaelis constant) of the enzyme for the coenzyme, is present in some, but not all, cases of PDHC deficiency. Because the PDHC is an integral part of carbohydrate metabolism, a diet low in carbohydrates would be expected to blunt the effects of the enzyme deficiency. Aerobic glycolysis generates pyruvate, the substrate of the PDHC. Decreased activity of the complex decreases production of acetyl coenzyme A, a substrate for citrate synthase. Because PDH kinase is allosterically inhibited by pyruvate, it is inactive.

9.5 Which coenzyme–cosubstrate is used by dehydrogenases in both glycolysis and the tricarboxylic acid cycle?

Oxidized nicotinamide adenine dinucleotide (NAD^+) is used by glyceraldehyde 3-phosphate dehydrogenase of glycolysis and by isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and malate dehydrogenase of the tricarboxylic acid cycle. (Note: E3 of the pyruvate dehydrogenase complex requires oxidized flavin adenine dinucleotide [FAD] and NAD^+.)

Gluconeogenesis 10

I. OVERVIEW

Some tissues, such as the brain, erythrocytes, kidney medulla, lens and cornea of the eye, testes, and exercising skeletal muscle, require a continuous supply of glucose as a metabolic fuel. Liver glycogen, an essential postprandial source of glucose, can meet these needs for <24 hours in the absence of dietary intake of carbohydrate (see p. 137). During a prolonged fast, however, hepatic glycogen stores are depleted, and glucose is made from noncarbohydrate precursors. The formation of glucose does not occur by a simple reversal of glycolysis, because the overall equilibrium of glycolysis strongly favors pyruvate formation. Instead, glucose is synthesized *de novo* by a special pathway, gluconeogenesis, which requires both mitochondrial and cytosolic enzymes. Deficiencies of gluconeogenic enzymes cause hypoglycemia. a an overnight fast, ~90% of gluconeogenesis occurs in the liver, with the remaining ~10% occurring in the kidneys. However, during prolonged fasting of 48 hours or longer, the kidneys become major glucose-producing organs, contributing ~40% of the total glucose production. The small intestine can also make glucose. Figure 10.1 shows the relationship of gluconeogenesis to other essential pathways of energy metabolism.

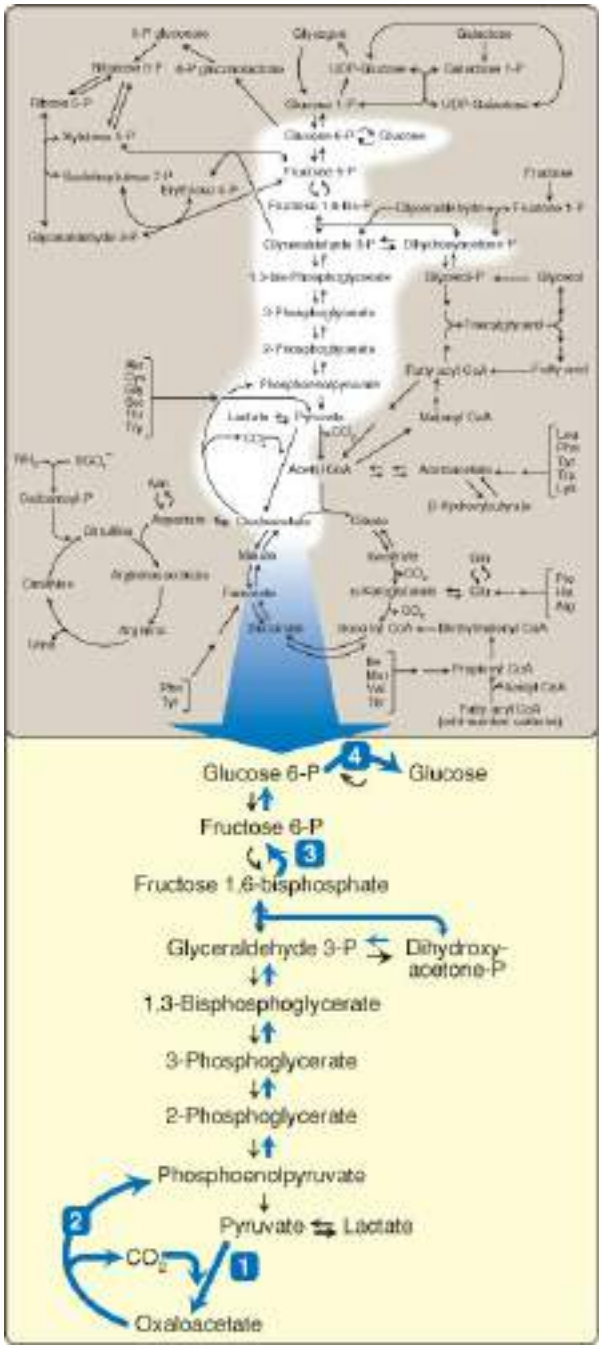


Figure 10.1
Gluconeogenesis shown as one of the essential pathways of energy metabolism. The numbered reactions are unique to gluconeogenesis. (Note: See Fig. 8.2, for a more detailed map of metabolism.) P = phosphate; CO_2 = carbon dioxide.

II. SUBSTRATES

Gluconeogenic precursors are molecules that can be used to produce a net synthesis of glucose. The most important gluconeogenic precursors are glycerol, lactate, and **α-keto acids** obtained from the metabolism of glucogenic amino acids. All but two amino

acids (leucine and lysine) are glucogenic.

A. Glycerol

Glycerol is released during the hydrolysis of triacylglycerols (TAGs) in adipose tissue and is delivered by the blood to the liver. Glycerol is phosphorylated by glycerol kinase to glycerol 3-phosphate, which is oxidized by glycerol 3-phosphate dehydrogenase to dihydroxyacetone phosphate, an intermediate of glycolysis and gluconeogenesis.

B. Lactate

Lactate from anaerobic glycolysis is released into the blood by exercising skeletal muscle and by erythrocytes, cells that lack mitochondria. In the Cori cycle, this lactate is taken up by the liver and oxidized to pyruvate that is converted to glucose, which is released back into the circulation (Fig. 10.2).

C. Amino acids

Amino acids produced by hydrolysis of tissue proteins are the major sources of glucose during a fast. Their metabolism generates α-keto acids, such as **pyruvate** that is converted to glucose, or **α-ketoglutarate** that can enter the tricarboxylic acid (TCA) cycle and form oxaloacetate (OAA), a direct precursor of **phosphoenolpyruvate (PEP)**. (Note: **Acetyl coenzyme A [CoA]** and compounds that give rise only to acetyl CoA [e.g., acetoacetate, lysine, and leucine] cannot give rise to a net synthesis of **glucose**. This is because of the irreversible nature of the pyruvate dehydrogenase complex [PDHC], which converts pyruvate to acetyl CoA. These compounds give rise instead to ketone bodies and are termed ketogenic.)

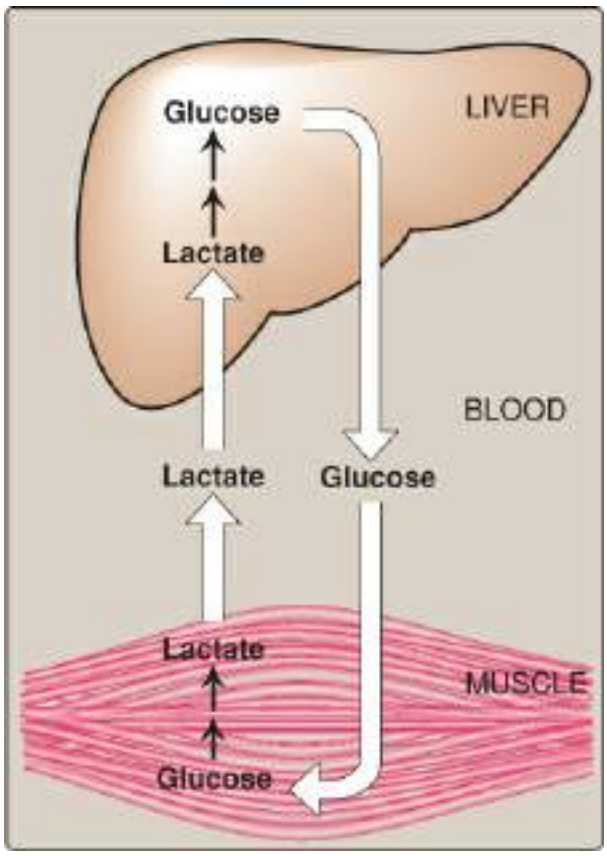


Figure 10.2
The intertissue Cori cycle links gluconeogenesis with glycolysis. (Note: Diffusion of lactate and glucose across membranes is facilitated by transport proteins.)

III. REACTIONS

Seven glycolytic reactions are reversible and are used in the synthesis of glucose from lactate or pyruvate. However, three glycolytic reactions are irreversible and must be circumvented by four alternate reactions that energetically favor the synthesis of glucose. These irreversible reactions, which together are unique to gluconeogenesis, are described below.

A. Pyruvate carboxylation

The first roadblock to overcome in the synthesis of glucose from pyruvate is the irreversible conversion in glycolysis of PEP to pyruvate by **pyruvate kinase (PK)**. In gluconeogenesis, pyruvate is carboxylated by pyruvate carboxylase (PC) to OAA, which is converted to PEP by PEP-carboxykinase (PEPCK) (Fig. 10.3).

1. Biotin: PC requires the coenzyme biotin (see p. 431) covalently bound to the ε-amino group of a lysine residue in the enzyme (see Fig. 10.3). ATP hydrolysis drives formation of an enzyme–biotin–carbon dioxide (CO_2) intermediate, which then carboxylates pyruvate to form OAA. (Note: HCO_3^- provides the CO_2.) The

PC reaction occurs in the mitochondria of liver and kidney cells and has two purposes: to allow production of PEP, an important substrate for gluconeogenesis, and to provide OAA that can replenish the TCA cycle intermediates that may become depleted. Muscle cells also contain PC but use the OAA product only for the replenishment (anaplerotic) purpose and do not synthesize glucose. (Note: Pyruvate carrier protein moves pyruvate from the cytosol into mitochondria.)

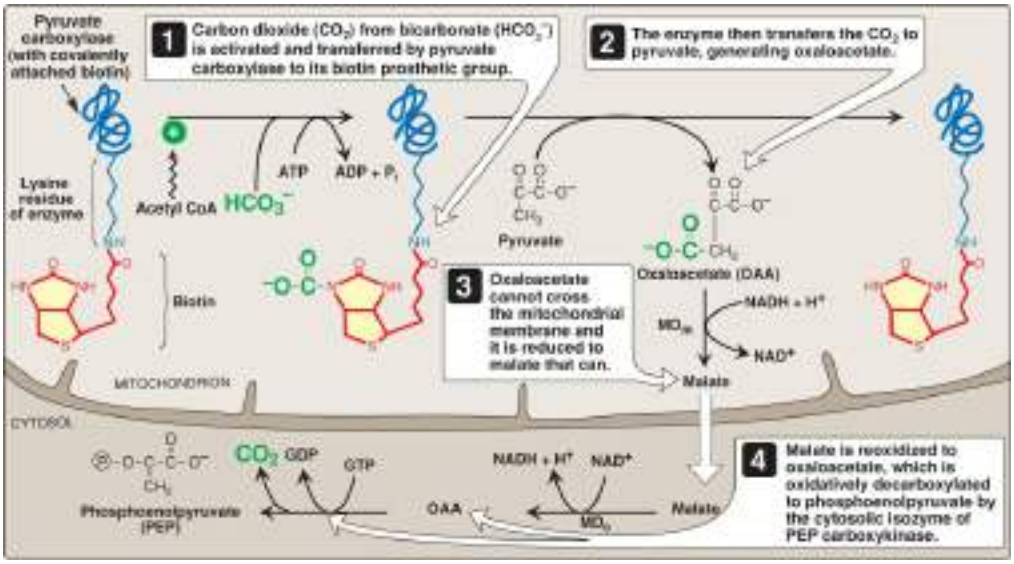


Figure 10.3
PEP synthesis in the cytosol. (Note: The process moves nicotinamide adenine dinucleotide [NADH]-reducing equivalents required for gluconeogenesis out of mitochondria into the cytosol.) MD_m and MD_c = mitochondrial and cytosolic isozymes of malate dehydrogenase; GTP and GDP = guanosine tri- and diphosphates; ADP = adenosine diphosphate.

PC is one of several carboxylases that require biotin. Others include acetyl CoA carboxylase (p. 203), propionyl CoA carboxylase (p. 215), and methylcrotonyl CoA carboxylase (p. 295).

2. Allosteric regulation: PC is allosterically activated by acetyl CoA. Elevated levels of acetyl CoA in mitochondria signal a metabolic state in which increased synthesis of OAA is required. For example, this occurs during fasting, when OAA is used for gluconeogenesis in the liver and kidneys. Conversely, at low levels of acetyl CoA, PC is largely inactive, and pyruvate is primarily oxidized by the PDHC to acetyl CoA that can be further oxidized by the TCA cycle.

B. Oxaloacetate transport to the cytosol

For gluconeogenesis to continue, OAA must be converted to PEP by PEPCK. PEP production in the cytosol requires transport of OAA out of mitochondria. However, there is no OAA transporter in the inner mitochondrial membrane, and OAA is first reduced to malate by mitochondrial malate dehydrogenase (MD). Malate is

transported into the cytosol and reoxidized to OAA by cytosolic MD as nicotinamide adenine dinucleotide (NAD^+) is reduced to NADH (see Fig. 10.3). The NADH is used in the reduction of 1,3-bisphosphoglycerate to glyceraldehyde 3-phosphate by glyceraldehyde 3-phosphate dehydrogenase, a reaction common to glycolysis and gluconeogenesis. (Note: When abundant, lactate is oxidized to pyruvate as NAD^+ is reduced. The pyruvate is transported into mitochondria and carboxylated by PC to OAA, which can be converted to PEP by the mitochondrial isozyme of PEPCK. PEP is transported to the cytosol. OAA can also be converted to aspartate that is transported into the cytosol.)

C. Cytosolic oxaloacetate decarboxylation

OAA is decarboxylated and phosphorylated to PEP in the cytosol by PEPCK. The reaction is driven by hydrolysis of guanosine triphosphate (GTP) (see Fig. 10.3). The combined actions of PC and PEPCK provide an energetically favorable pathway from pyruvate to PEP. PEP is then acted on by the reactions of glycolysis running in the reverse direction until it becomes fructose 1,6-bisphosphate.

> The pairing of carboxylation with decarboxylation drives reactions that would otherwise be energetically unfavorable. This strategy is also used in fatty acid (FA) synthesis.

D. Fructose 1,6-bisphosphate dephosphorylation

Hydrolysis of fructose 1,6-bisphosphate by fructose 1,6-bisphosphatase, found in the liver and kidneys, bypasses the irreversible **phosphofructokinase-1 (PFK-1)** reaction of glycolysis and provides an energetically favorable pathway for the formation of fructose 6-phosphate (Fig. 10.4). This reaction is an important regulatory site of gluconeogenesis.

1. Regulation by intracellular energy levels: Fructose 1,6-bisphosphatase is inhibited by a rise in the ratio of adenosine monophosphate (AMP) to ATP, called the AMP to ATP ratio, which signals a low-energy state in the cell. Conversely, low AMP and high ATP levels stimulate gluconeogenesis, an energy-requiring pathway.

2. Regulation by fructose 2,6-bisphosphate: Fructose 1,6- bisphosphatase is inhibited by fructose 2,6-bisphosphate, an allosteric effector whose concentration is influenced by the insulin/glucagon ratio. When glucagon is high, the effector is not made by hepatic PFK-2, and thus, the phosphatase is active (Fig. 10.5). (Note: The signals that inhibit [low-energy, high fructose 2,6-bisphosphate] or activate [high-energy, low fructose 2,6-bisphosphate] gluconeogenesis have the opposite effect on glycolysis, providing reciprocal control of the pathways that synthesize and oxidize glucose.)

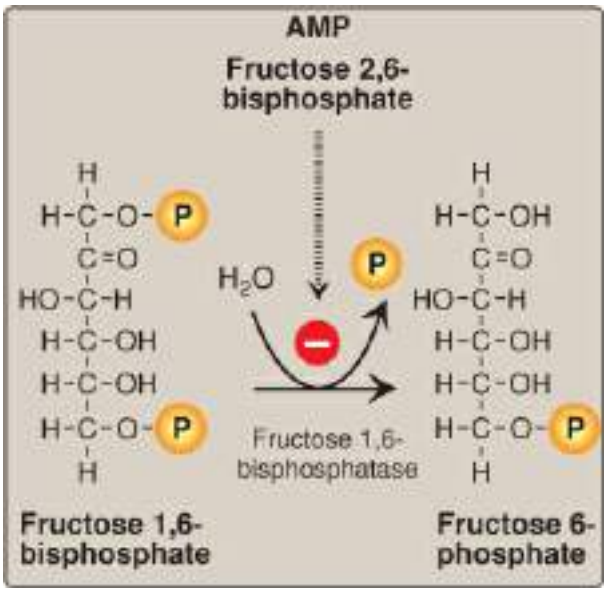


Figure 10.4
Dephosphorylation of fructose 1,6-bisphosphate. AMP = adenosine monophosphate; P = phosphate.

E. Glucose 6-phosphate dephosphorylation

Glucose 6-phosphate hydrolysis by **glucose 6-phosphatase** bypasses the irreversible hexokinase/glucokinase reaction and provides an energetically favorable pathway for the formation of free glucose (Fig. 10.6). The liver is the primary organ that produces free glucose from glucose 6-phosphate. This process requires a complex of two proteins found only in gluconeogenic tissue: glucose 6-phosphate translocase, which transports glucose 6-phosphate across the endoplasmic reticular (ER) membrane, and glucose 6-phosphatase, which removes the phosphate, producing free glucose (see Fig. 10.6). These ER membrane proteins are also required for the final step of glycogen degradation.

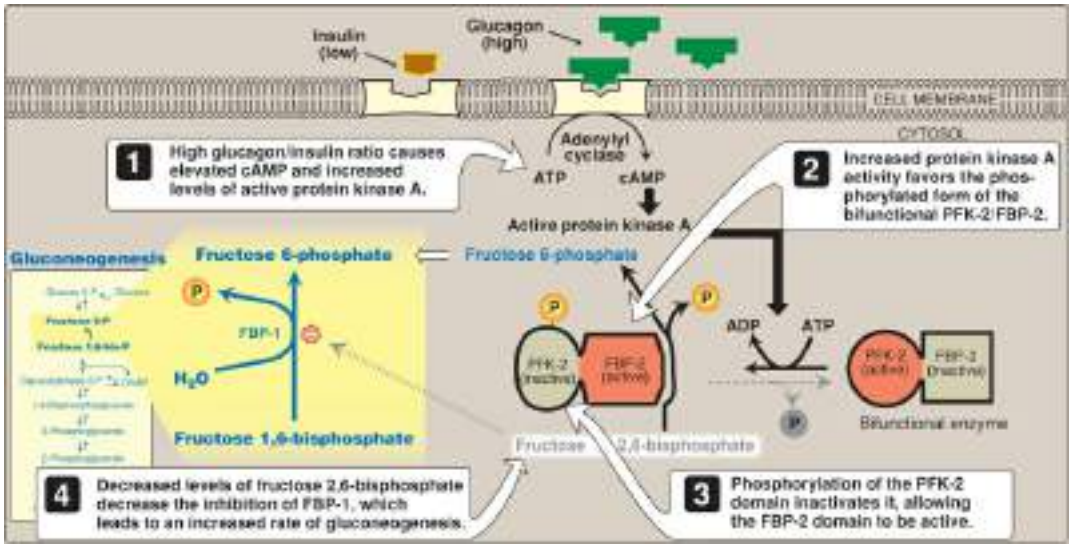


Figure 10.5
Effect of elevated glucagon on the intracellular concentration of fructose 2,6-bisphosphate in the liver. AMP and ADP = adenosine mono- and diphosphates; cAMP = cyclic AMP; PFK-2 = phosphofructokinase-2; FBP-2 = fructose 2,6-bisphosphatase; FBP-1 = fructose 1,6-bisphosphatase; P and P = phosphate.

Glycogen storage diseases Ia and Ib, caused by deficiencies in the phosphatase and the translocase, respectively, are characterized by severe fasting hypoglycemia, because free glucose is unable to be produced from either gluconeogenesis or glycogenolysis. Specific transporters are responsible for moving the free glucose into the cytosol and then into blood.

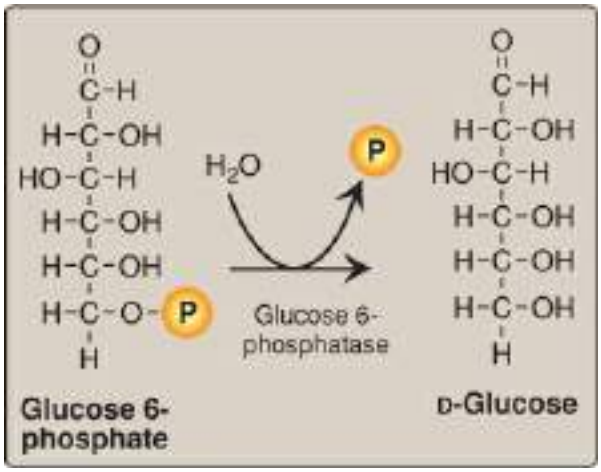


Figure 10.6
Dephosphorylation of glucose 6-phosphate allows release of free glucose from gluconeogenic tissues (primarily the liver) into blood. P = phosphate.

F. Summary of the reactions of glycolysis and gluconeogenesis

Of the 11 reactions required to convert pyruvate to free glucose, 7 are catalyzed by reversible glycolytic enzymes (Fig. 10.7). The 3 irreversible reactions, catalyzed by hexokinase/glucokinase, PFK-1, and PK are circumvented by reactions catalyzed by glucose 6-phosphatase, fructose 1,6-bisphosphatase, PC, and PEPCK. In gluconeogenesis, the equilibria of the reversible glycolytic reactions are pushed toward glucose synthesis as a result of the essentially irreversible formation of PEP, fructose 6-phosphate, and glucose by the gluconeogenic enzymes. (Note: The stoichiometry of gluconeogenesis from two pyruvate molecules couples the cleavage of six high-energy phosphate bonds and the oxidation of two NADH with the formation of one glucose molecule [see Fig. 10.7].)

IV. REGULATION

The moment-to-moment regulation of gluconeogenesis is determined primarily by the circulating level of glucagon and by the availability of gluconeogenic substrates. In

addition, slow adaptive changes in enzyme amount result from an alteration in the rate of enzyme synthesis or degradation or both. (Note: Hormonal control of the glucoregulatory system is presented in Chapter 23.)

A. Glucagon

This peptide hormone from pancreatic islet α-cells (see p. 347) stimulates gluconeogenesis by three mechanisms.

1. Changes in allosteric effectors: Glucagon lowers hepatic fructose 2,6-bisphosphate, resulting in fructose 1,6-bisphosphatase activation and PFK-1 inhibition, thereby favoring gluconeogenesis over glycolysis (see Fig. 10.5).
2. Covalent modification of enzyme activity: Glucagon binds its G protein–coupled receptor and, via an elevation in cyclic AMP (cAMP) level and cAMP-dependent protein kinase A activity, stimulates the conversion of hepatic PK to its inactive (phosphorylated) form. This decreases PEP conversion to pyruvate, which has the effect of diverting PEP to gluconeogenesis (Fig. 10.8).
3. Induction of enzyme synthesis: Glucagon increases transcription of the gene for PEPCK via the transcription factor cAMP response element–binding (CREB) protein, thereby increasing the availability of this enzyme as levels of its substrate rise during fasting. Cortisol, a glucocorticoid, also increases expression of the gene, whereas insulin decreases expression.

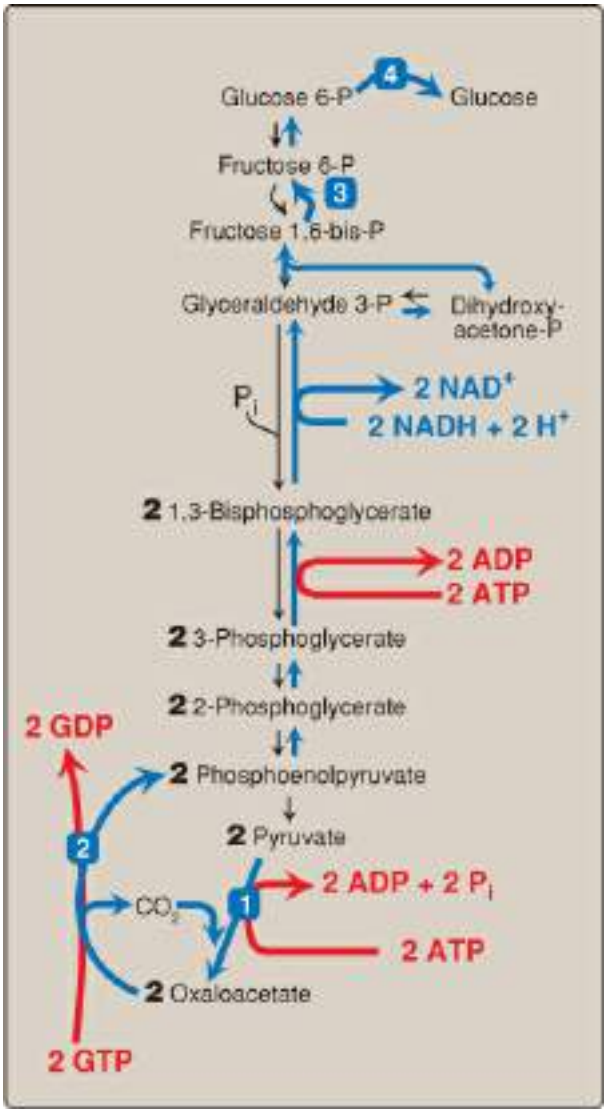


Figure 10.7
Summary of the reactions of glycolysis and gluconeogenesis, showing the energy requirements of gluconeogenesis. The numbered reactions are unique to gluconeogenesis. P = phosphate; GDP and GTP = guanosine di- and triphosphates; NAD(H) = nicotinamide adenine dinucleotide; ADP = adenosine diphosphate.

B. Substrate availability

The availability of gluconeogenic precursors, particularly glucogenic amino acids, significantly influences the rate of glucose synthesis. Decreased insulin levels favor mobilization of amino acids from muscle protein to provide the carbon skeletons for gluconeogenesis. The ATP and NADH coenzymes required for gluconeogenesis are primarily provided by FA oxidation.

C. Allosteric activation by acetyl CoA

Allosteric activation of hepatic PC by acetyl CoA occurs during fasting. As a result of

increased TAG hydrolysis in adipose tissue, the liver is flooded with FA. The rate of formation of acetyl CoA by β-oxidation of these FA exceeds the capacity of the liver to oxidize it to CO_2 and water. As a result, acetyl CoA accumulates and activates PC. (Note: Acetyl CoA inhibits the PDHC [by activating PDH kinase]. Thus, this single compound can divert pyruvate toward gluconeogenesis and away from the TCA cycle [Fig. 10.9].)

D. Allosteric inhibition by AMP

Fructose 1,6-bisphosphatase is inhibited by AMP, a compound that activates PFK-1. This results in reciprocal regulation of glycolysis and gluconeogenesis seen previously with **fructose 2,6-bisphosphate** (see p. 122). Thus, elevated AMP stimulates energy-producing pathways and inhibits energy-requiring ones.

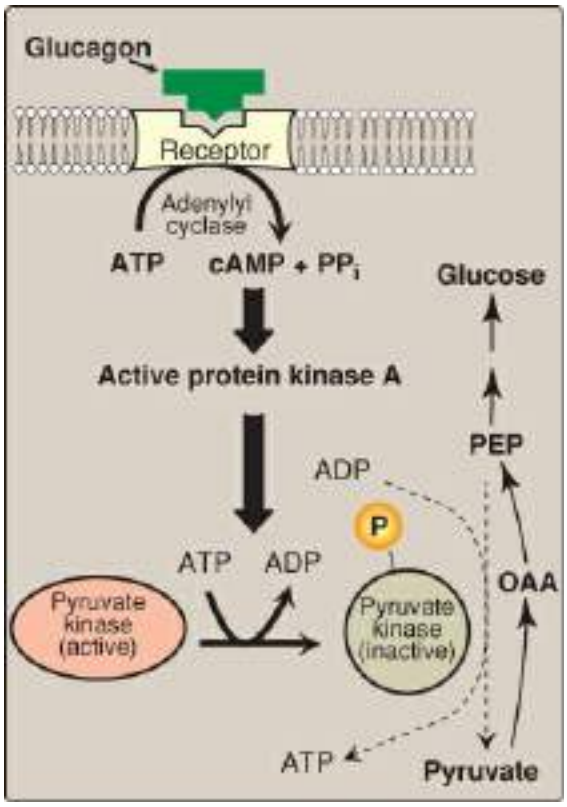


Figure 10.8
Covalent modification of pyruvate kinase results in inactivation of the enzyme. (Note: Only the hepatic isozyme is subject to covalent regulation.) OAA = oxaloacetate; PEP = phosphoenolpyruvate; PP_i = pyrophosphate; P = phosphate; AMP and ADP = adenosine mono- and diphosphates; cAMP = cyclic AMP.

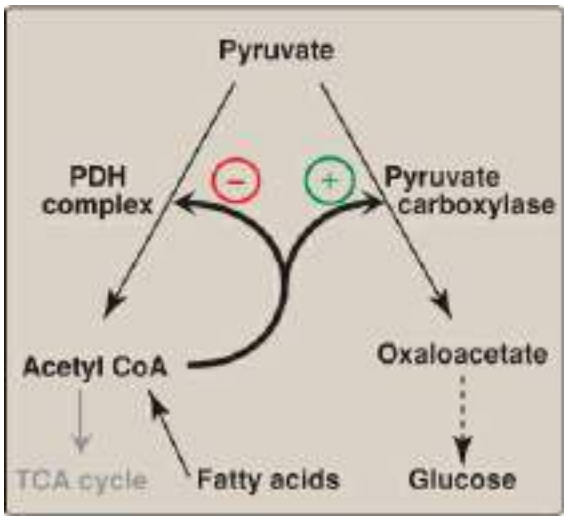


Figure 10.9
Acetyl coenzyme A (CoA) diverts pyruvate away from oxidation and toward gluconeogenesis. PDH = pyruvate dehydrogenase; TCA = tricarboxylic acid.

V. Chapter Summary

- **Gluconeogenic precursors** include **glycerol** released during TAG hydrolysis in adipose tissue, **lactate** released by cells that lack mitochondria and by exercising skeletal muscle, and **α-keto acids** (e.g., **α-ketoglutarate** and **pyruvate**) derived from glucogenic amino acid metabolism (Fig. 10.10).
- Seven of the reactions of glycolysis are reversible and are used for gluconeogenesis in the liver and kidneys.
- Three reactions, catalyzed by **PK**, **PFK-1**, and glucokinase/hexokinase, are physiologically irreversible and must be circumvented.
- **Pyruvate** is converted to **OAA** and then to **PEP** by **PC** and **PEPCK**.
- PC requires **biotin** and **ATP** and is allosterically activated by **acetyl CoA** and PEPCK requires **GTP**.
- Fructose 1,6-bisphosphate is converted to fructose 6-phosphate by **fructose 1,6-bisphosphatase**. This enzyme is inhibited by a high AMP/ATP ratio and by **fructose 2,6-bisphosphate**, the primary allosteric activator of glycolysis.
- **Glucose 6-phosphate** is dephosphorylated to **glucose** by **glucose 6-phosphatase**. This enzyme of the ER membrane catalyzes the final step in gluconeogenesis and in glycogen degradation. Its deficiency results in severe, fasting hypoglycemia.

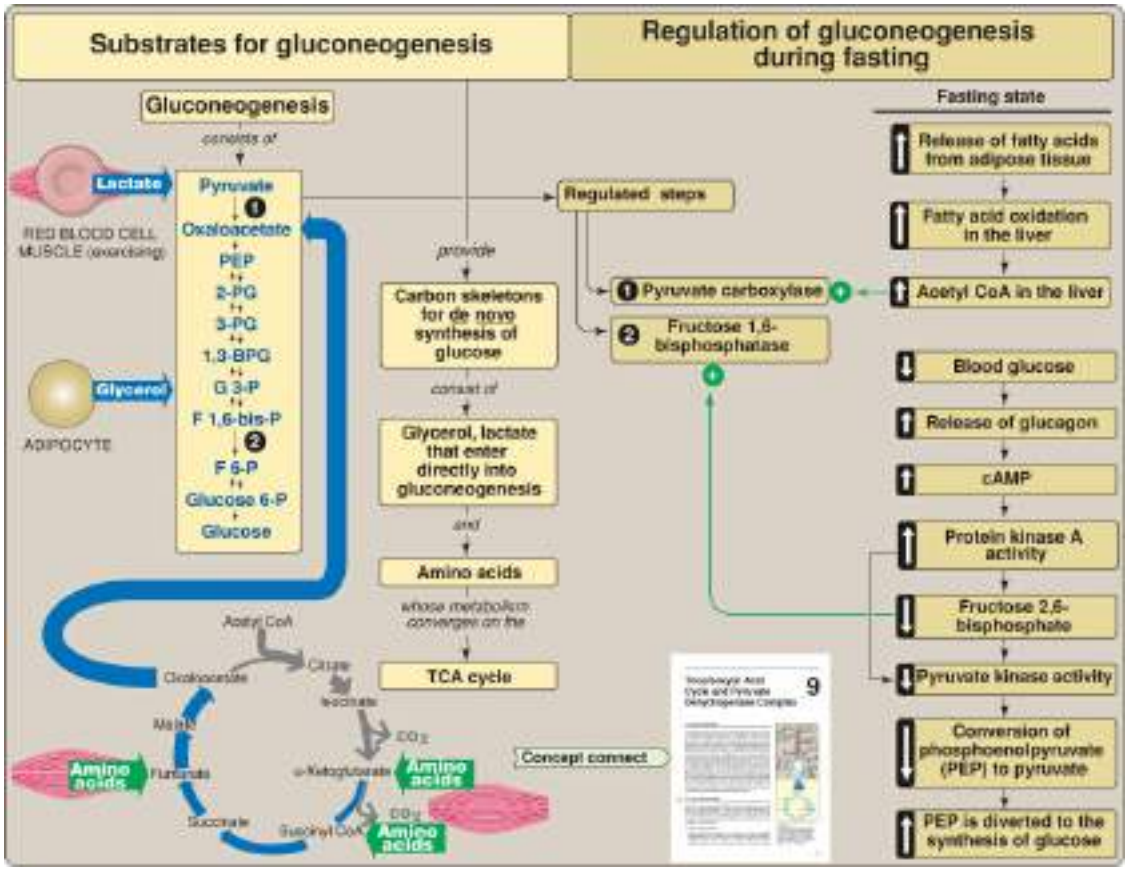


Figure 10.10
Key concept map for gluconeogenesis. TCA = tricarboxylic acid. CoA = coenzyme A; cAMP = cyclic adenosine monophosphate; P = phosphate; (B)PG = (bis)phosphoglycerate; G = glyceraldehyde; F = fructose; CO_2 = carbon dioxide.

Study Questions

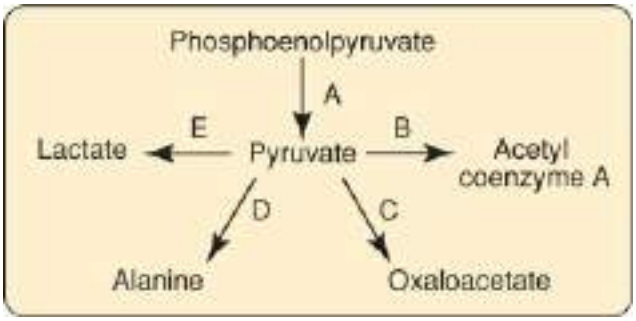
Choose the ONE best answer.

10.1 Which one of the following statements concerning gluconeogenesis is correct?

A. It is an energy-producing (exergonic) process.
B. It is important in maintaining blood glucose during a 2-day fast.
C. It is inhibited by a fall in the insulin/glucagon ratio.
D. It occurs in the cytosol of muscle cells.
E. It uses carbon skeletons provided by fatty acid degradation.

Correct answer = B. During a 2-day fast, glycogen stores are depleted, and gluconeogenesis maintains blood glucose. This is an energy-requiring (endergonic) pathway (both ATP and GTP get hydrolyzed) that occurs primarily in the liver, with the kidneys becoming major glucose producers in prolonged fasting. Gluconeogenesis uses both mitochondrial and cytosolic enzymes and is stimulated by a fall in the insulin/glucagon ratio. Fatty acid degradation yields acetyl coenzyme A (CoA), which cannot be converted to glucose. This is because there is no net gain of carbons from acetyl CoA in the tricarboxylic acid cycle, and the pyruvate dehydrogenase complex is physiologically irreversible. It is the carbon skeletons of most amino acids that are glucogenic.

10.2 Which reaction in the diagram below would be inhibited in the presence of large amounts of avidin, an egg white protein that binds and sequesters biotin?



Correct answer = C. Pyruvate is carboxylated to oxaloacetate by pyruvate carboxylase, a biotin-requiring enzyme. B (pyruvate dehydrogenase complex) requires thiamine pyrophosphate, lipoic acid, flavin and nicotinamide adenine dinucleotides (FAD and NAD^+, respectively), and coenzyme A; D (transaminase) requires pyridoxal phosphate; E (lactate dehydrogenase) requires NADH.

10.3 Which one of the following reactions is unique to gluconeogenesis?

A. 1,3-Bisphosphoglycerate → 3-phosphoglycerate
B. Lactate → pyruvate
C. Oxaloacetate → phosphoenolpyruvate
D. Phosphoenolpyruvate → pyruvate

Correct answer = C. The other reactions are common to both gluconeogenesis and glycolysis.

10.4 Use the chart below to show the effect of adenosine monophosphate (AMP) and fructose 2,6-bisphosphate on the listed enzymes of gluconeogenesis and glycolysis.

Enzyme	Fructose 2,6-bisphosphate	AMP
Fructose 1,6-bisphosphatase		

Phosphofructokinase-1		

Both fructose 2,6-bisphosphate and adenosine monophosphate inhibit fructose 1,6-bisphosphatase of gluconeogenesis and activate phosphofructokinase-1 of glycolysis. This results in reciprocal regulation of the two pathways.

10.5 The metabolism of ethanol by alcohol dehydrogenase produces reduced nicotinamide adenine dinucleotide (NADH) from the oxidized (NAD^+) form. What effect is the fall in the NAD^+/NADH ratio expected to have on gluconeogenesis? Explain.

The increase in NADH as ethanol is oxidized decreases the availability of oxaloacetate (OAA) because the reversible oxidation of malate to OAA by malate dehydrogenase of the tricarboxylic acid cycle is driven in the reverse direction by NADH. Additionally, the reversible reduction of pyruvate to lactate by lactate dehydrogenase is driven to lactate by NADH. Thus, two important gluconeogenic substrates, OAA and pyruvate, decrease as a result of the increase in NADH during ethanol metabolism. Consequently, gluconeogenesis decreases.

10.6 Given that acetyl coenzyme A cannot be a substrate for gluconeogenesis, why is its production in fatty acid oxidation essential for gluconeogenesis?

Acetyl coenzyme A inhibits the pyruvate dehydrogenase complex and activates pyruvate carboxylase, pushing pyruvate to gluconeogenesis and away from oxidation.

Glycogen Metabolism 11

I. OVERVIEW

A constant source of blood glucose is an absolute requirement for human life. Glucose is the greatly preferred energy source for the brain and the required energy source for cells with few or no mitochondria such as mature red blood cells. Glucose is also essential as an energy source for exercising muscle, where it is the substrate for anaerobic glycolysis. Blood glucose can be obtained from three primary sources: the diet, glycogen degradation, and gluconeogenesis. Dietary intake of glucose and glucose precursors, such as starch (a polysaccharide), disaccharides, and monosaccharides, is sporadic and, depending on the diet, is not always a reliable source of blood glucose. In contrast, gluconeogenesis can provide sustained synthesis of glucose, but it is somewhat slow in responding to a falling blood glucose level. Therefore, the body has developed mechanisms for storing a supply of glucose in a rapidly mobilized form, namely, glycogen. In the absence of a dietary source of glucose, this sugar is rapidly released into the blood from liver glycogen. Similarly, muscle glycogen is extensively degraded in exercising muscle to provide that tissue with an important energy source. When glycogen stores are depleted, specific tissues synthesize glucose *de novo*, using glycerol, lactate, pyruvate, and amino acids as carbon sources for gluconeogenesis (see Chapter 10). Figure 11.1 shows the reactions of glycogen synthesis and degradation as part of the essential pathways of energy metabolism.

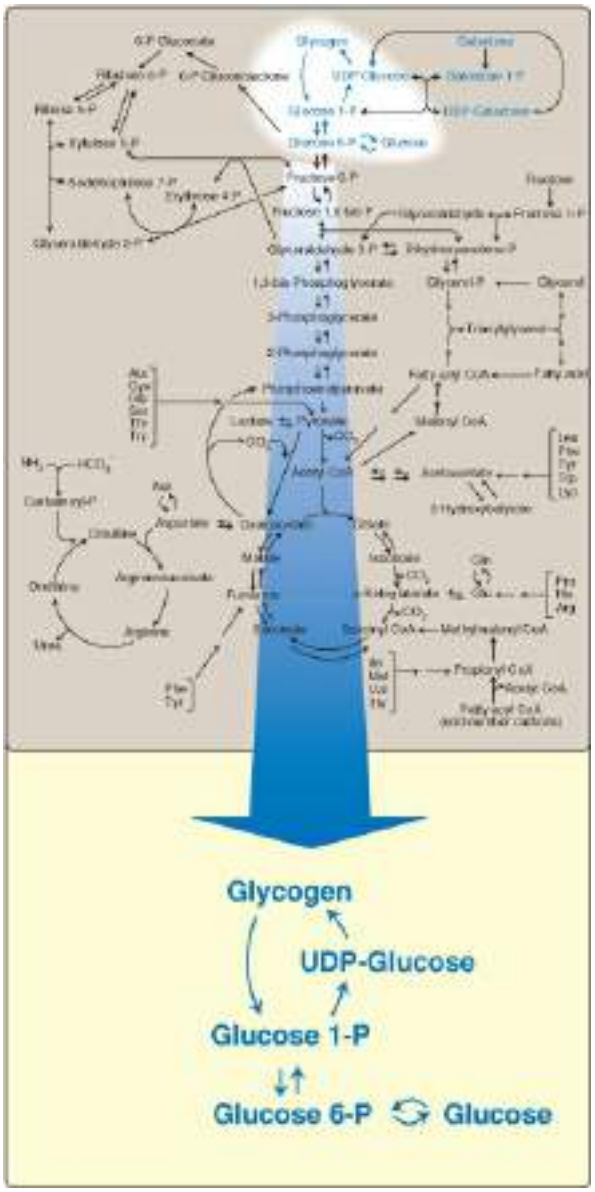


Figure 11.1
Glycogen synthesis and degradation shown as a part of the essential pathways of energy metabolism. (Note: See Fig. 8.2, for a more detailed map of metabolism.) P = phosphate; UDP = uridine diphosphate.

II. STRUCTURE AND FUNCTION

The main stores of glycogen are found in skeletal muscle and liver, although most other cells store small amounts of glycogen for their own use. The function of muscle glycogen is to serve as a fuel reserve for the synthesis of ATP during muscle contraction, while the purpose of liver glycogen is to maintain the blood glucose concentration, particularly during the early stages of a fast (Fig. 11.2). (Note: Liver glycogen can maintain blood glucose for <24 hours.)

A. Amounts in liver and muscle

Approximately 400 g of glycogen make up 1% to 2% of the fresh weight of resting muscle, and ~100 g of glycogen make up to 10% of the fresh weight of a well-fed adult liver. What limits the production of glycogen at these levels is not clear. However, in some glycogen storage diseases (GSDs) (see Fig. 11.8), the amount of glycogen in the liver and/or muscle can be significantly higher. (Note: In the body, muscle mass is greater than liver mass. Consequently, most of the body's glycogen is found in skeletal muscle.)

B. Structure

Glycogen is a branched-chain polysaccharide made exclusively from α-D-glucose. The primary glycosidic bond is an α(1→4) linkage. After an average of 8 to 14 glucosyl residues, there is a branch containing an α(1→6) linkage (Fig. 11.3). A single glycogen molecule can contain up to 55,000 glucosyl residues. These polymers of glucose exist as large, spherical, cytoplasmic granules (particles) that also contain most of the enzymes necessary for glycogen synthesis and degradation.

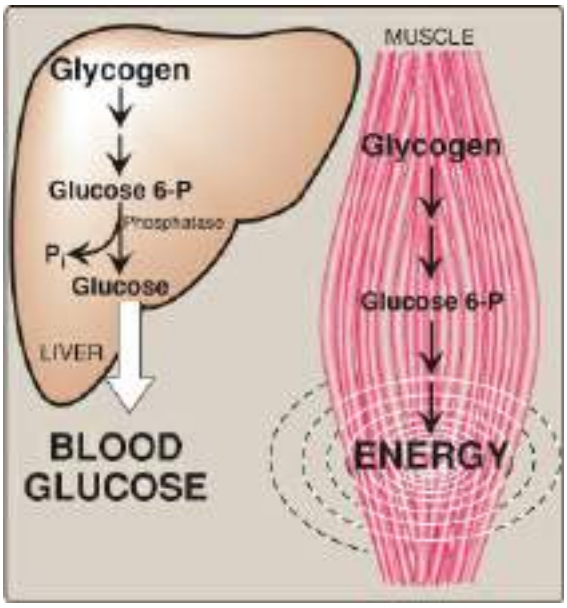


Figure 11.2
Functions of muscle and liver glycogen. (Note: The presence of glucose 6-phosphatase in liver allows release of glucose into blood.) P = phosphate; P_i = inorganic phosphate.

C. Glycogen store fluctuation

Liver glycogen stores increase during the well-fed state and are depleted during a fast. Muscle glycogen is not affected by short periods of fasting (a few days) and is only moderately decreased in prolonged fasting (weeks). Muscle glycogen is synthesized to replenish muscle stores after they have been depleted following strenuous exercise. (Note: Glycogen synthesis and degradation go on continuously. The difference between the rates of these two processes determines the levels of

stored glycogen during specific physiologic states.)

III. SYNTHESIS (GLYCOGENESIS)

Glycogen is synthesized from molecules of α-D-glucose. The process occurs in the cytosol and requires energy supplied by ATP (for the phosphorylation of glucose) and uridine triphosphate (UTP).

A. Uridine diphosphate glucose synthesis

α-D-Glucose attached to uridine diphosphate (UDP) is the source of all the glucosyl residues that are added to the growing glycogen molecule. UDP-glucose (Fig. 11.4) is synthesized from glucose 1-phosphate and UTP by UDP–glucose pyrophosphorylase (Fig. 11.5). Pyrophosphate (PP_i), the second product of the reaction, is hydrolyzed to two inorganic phosphates (P_i) by pyrophosphatase. The hydrolysis is exergonic, which ensures that the UDP–glucose pyrophosphorylase reaction proceeds in the direction of UDP-glucose production. (Note: Glucose 1-phosphate is generated from glucose 6-phosphate by phosphoglucomutase. Glucose 1,6-bisphosphate is an obligatory intermediate in this reversible reaction [Fig. 11.6].)

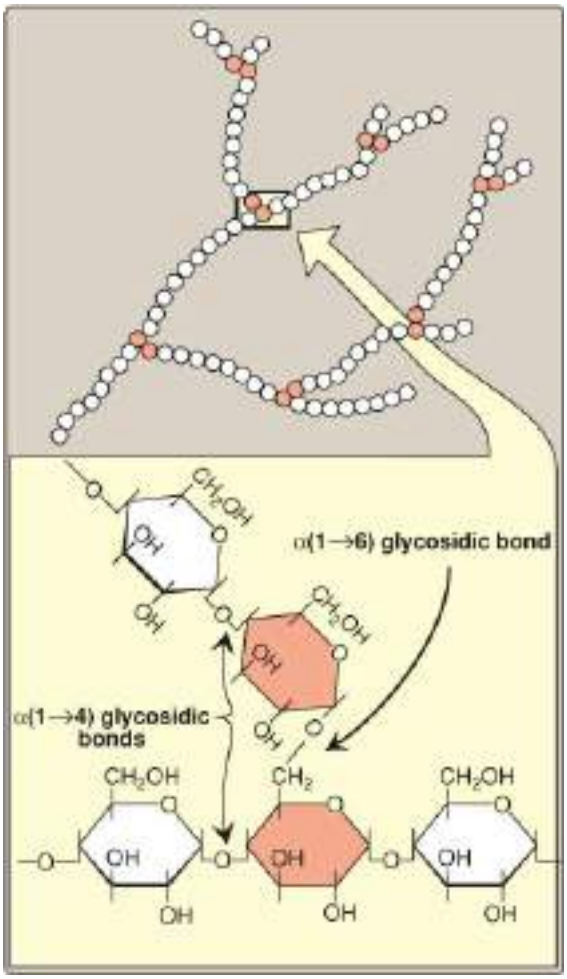


Figure 11.3
Branched structure of glycogen, showing α(1→4) and α(1→6) glycosidic bonds.

B. Primer requirement and synthesis

Glycogen synthase catalyzes the α(1→4) linkages in glycogen. This enzyme cannot initiate chain synthesis using free glucose as an acceptor of a molecule of glucose from UDP-glucose. Instead, it can only elongate already existing chains of glucose and, therefore, requires a primer. A fragment of glycogen can serve as a primer. In the absence of a fragment, the homodimeric protein glycogenin can serve as an acceptor of glucose from UDP-glucose (see Fig. 11.5). The side-chain hydroxyl group of tyrosine-194 in the protein is the site at which the initial glucosyl unit is attached. Because the reaction is catalyzed by glycogenin itself via autoglucosylation, glycogenin is an enzyme. Glycogenin then catalyzes the transfer of at least four molecules of glucose from UDP-glucose, producing a short, α(1→4)-linked glucosyl chain. This short chain serves as a primer that is able to be elongated by glycogen synthase, which is recruited by glycogenin, as described in C. below. (Note: Glycogenin stays associated with and forms the core of a glycogen granule.)

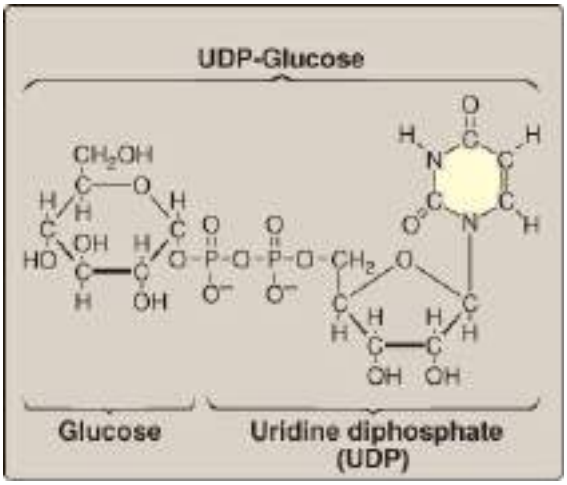


Figure 11.4
The structure of UDP-glucose, a nucleotide sugar.

C. Elongation by glycogen synthase

Elongation of a glycogen chain involves the transfer of glucose from UDP-glucose to the nonreducing end of the growing chain, forming a new glycosidic bond between the anomeric hydroxyl group of carbon 1 of the activated glucose and carbon 4 of the accepting glucosyl residue (see Fig. 11.5). (Note: The nonreducing end of a carbohydrate chain is one in which the anomeric carbon of the terminal sugar is linked by a glycosidic bond to another molecule, making the terminal sugar nonreducing.) The enzyme responsible for making the α(1→4) linkages in glycogen is glycogen synthase. (Note: The UDP released when the new α[1→4] glycosidic bond is made can be phosphorylated to UTP by nucleoside diphosphate kinase [UDP + ATP ⇄ UTP + ADP].)

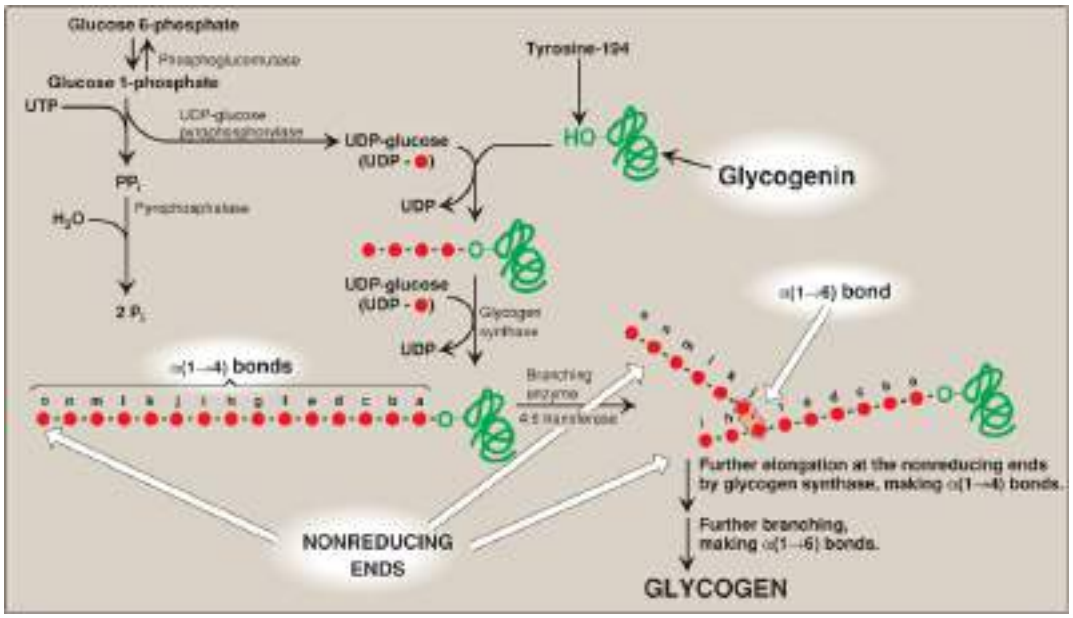


Figure 11.5
Glycogen synthesis. UDP and UTP = uridine di- and triphosphates; PP_i = pyrophosphate; P_i = inorganic phosphate.

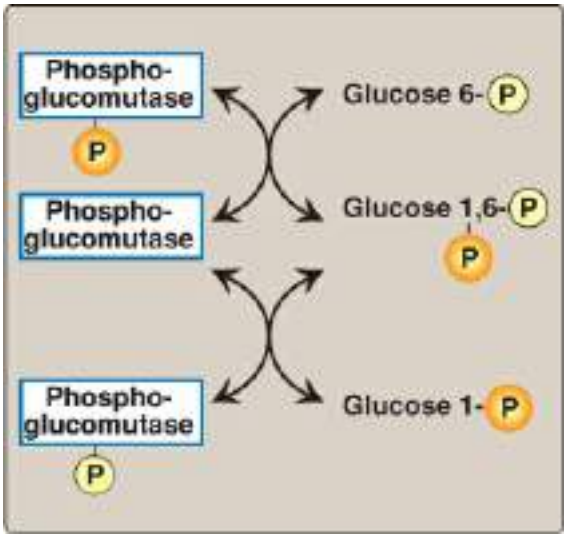


Figure 11.6
Interconversion of glucose 6-phosphate and glucose 1-phosphate by phosphoglucomutase. P and P = phosphate.

D. Branch formation

If no other synthetic enzyme acted on the chain, the resulting structure would be a linear (unbranched) chain of glucosyl residues attached by α(1→4) linkages. Such a compound is found in plant tissues and is called amylose. In contrast, glycogen has branches located, on average, eight glucosyl residues apart, resulting in a highly branched, tree-like structure (see Fig. 11.3) that is far more soluble than the unbranched amylose. Branching also increases the number of nonreducing ends to which new glucosyl residues can be added (and also, as described in IV. below, from which these residues can be removed), thereby greatly accelerating the rate at which glycogen synthesis can occur and dramatically increasing the size of the glycogen molecule.

1. Branch synthesis: Branches are made by the action of the branching enzyme, amylo-α(1→4)→α(1→6)-transglycosylase. This enzyme removes a set of six to eight glucosyl residues from the nonreducing end of the glycogen chain, breaking an α(1→4) bond to another residue on the chain, and attaches it to a nonterminal glucosyl residue by an α(1→6) linkage, thus functioning as a 4:6 transferase. The resulting new, nonreducing end (see "i" in Fig. 11.5), as well as the old nonreducing end from which the six to eight residues were removed (see "o" in Fig. 11.5), can now be further elongated by glycogen synthase.

2. Additional branch synthesis: After elongation of these two ends has been accomplished, their terminal six to eight glucosyl residues can be removed and

used to make additional branches.

IV. DEGRADATION (GLYCOGENOLYSIS)

The degradative pathway that mobilizes stored glycogen in liver and skeletal muscle is not a reversal of the synthetic reactions. Instead, a separate set of cytosolic enzymes is required. When glycogen is degraded, the primary product is glucose 1-phosphate, obtained by breaking α(1→4) glycosidic bonds. In addition, free glucose is released from each α(1→6)–linked glucosyl residue (branch point).

A. Chain shortening

Glycogen phosphorylase sequentially cleaves the α(1→4) glycosidic bonds between the glucosyl residues at the nonreducing ends of the glycogen chains by simple phosphorolysis (producing glucose 1-phosphate) until four glucosyl units remain on each chain at a branch point (Fig. 11.7). The resulting structure is called a limit dextrin, and phosphorylase cannot degrade it any further (Fig. 11.8). (Note: Phosphorylase requires pyridoxal phosphate [a derivative of vitamin B_6] as a coenzyme.)

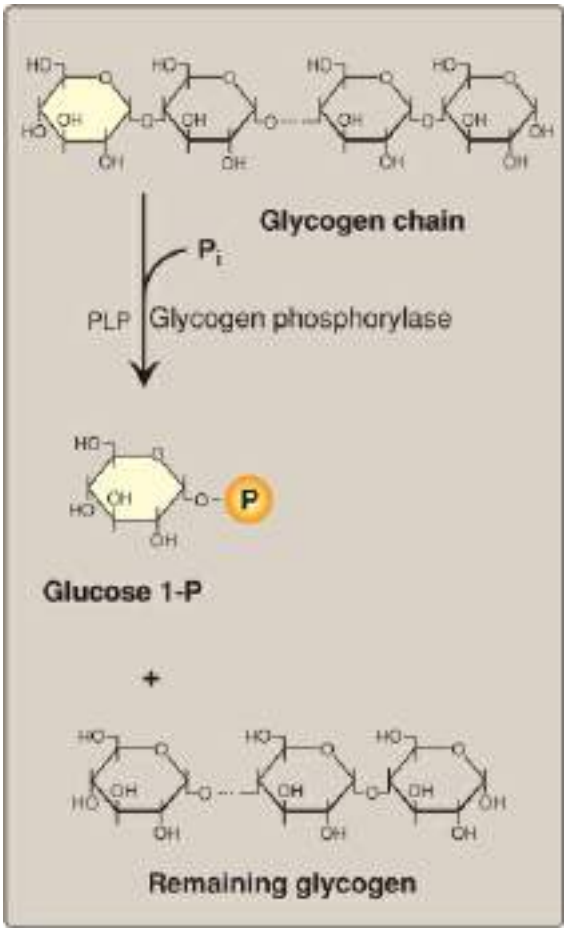
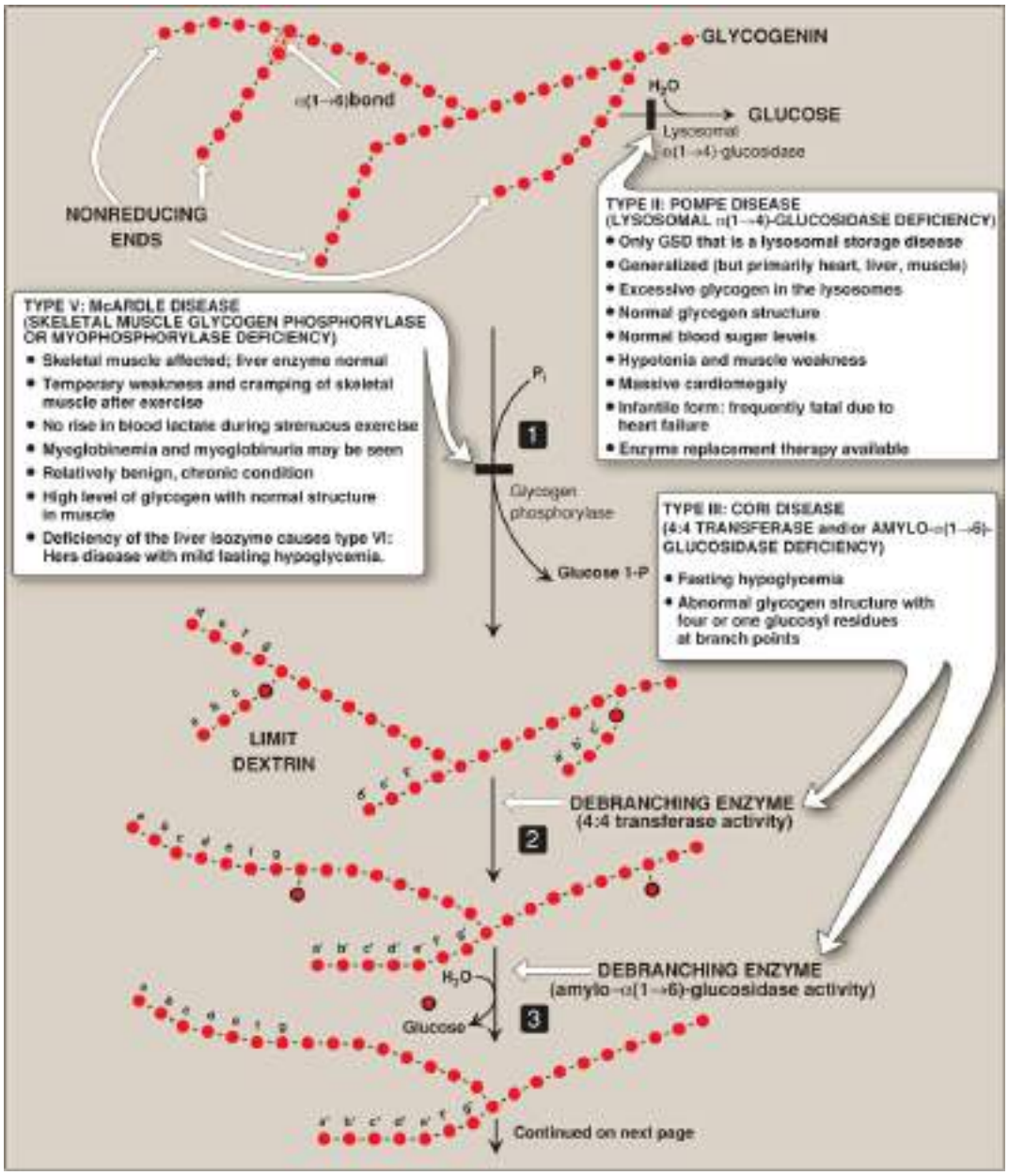


Figure 11.7
Cleavage of an α(1→4)-glycosidic bond. PLP = pyridoxal phosphate; P_i = inorganic phosphate; Ⓟ = phosphate.



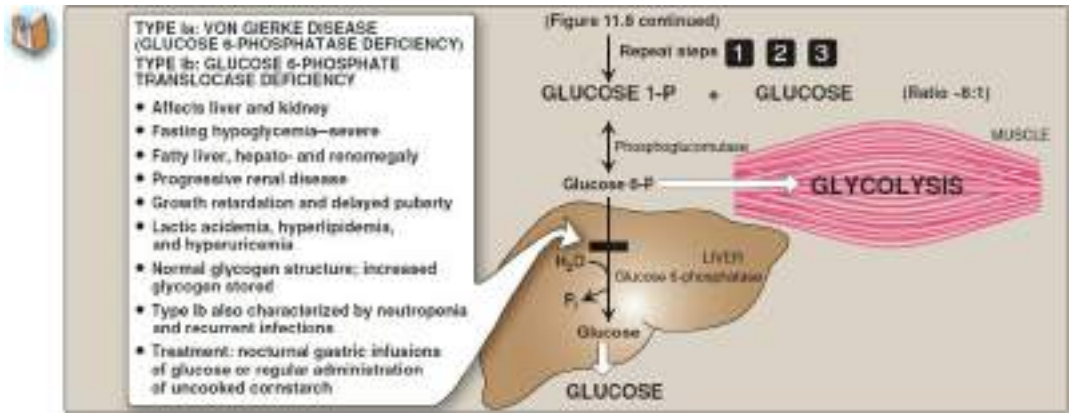


Figure 11.8
Glycogen degradation, showing some of the glycogen storage diseases (GSD). (Note: GSD type IV: Andersen disease is caused by defects in branching enzyme, an enzyme of synthesis, resulting in liver cirrhosis that can be fatal in early childhood.) P_i = inorganic phosphate; P = phosphate.

B. Branch removal

Branches are removed by the two enzymic activities of a single bifunctional protein, the debranching enzyme (see Fig. 11.8). First, oligo-α(1→4)→α(1→4)-glucantransferase activity removes the outer three of the four glucosyl residues remaining at a branch. It next transfers them to the nonreducing end of another chain, lengthening it accordingly. Thus, an α(1→4) bond is broken and an α(1→4) bond is made, and the enzyme functions as a 4:4 transferase. Next, the remaining glucose residue attached in an α(1→6) linkage is removed hydrolytically by amylo-α(1→6)-glucosidase activity, releasing free (nonphosphorylated) glucose. The glucosyl chain is now available again for degradation by glycogen phosphorylase until four glucosyl units in the next branch are reached.

C. Glucose 1-phosphate isomerization to glucose 6-phosphate

Glucose 1-phosphate, produced by glycogen phosphorylase, is isomerized in the cytosol to glucose 6-phosphate by phosphoglucomutase (see Fig. 11.6). In the liver, glucose 6-phosphate is transported into the endoplasmic reticulum (ER) by glucose 6-phosphate translocase. There, it is dephosphorylated to glucose by glucose 6-phosphatase (the same enzyme used in the last step of gluconeogenesis; see p. 131). The glucose is then transported from the ER to the cytosol. Hepatocytes release glycogen-derived glucose into the blood to help maintain blood glucose levels until the gluconeogenic pathway is actively producing glucose. (Note: Muscle lacks glucose 6-phosphatase. Consequently, glucose 6-phosphate cannot be dephosphorylated and sent into the blood. Instead, it enters glycolysis, providing energy needed for muscle contraction.)

D. Lysosomal degradation

A small amount (1% to 3%) of glycogen is degraded by the lysosomal enzyme, acid

α(1→4)-glucosidase (acid maltase). The purpose of this autophagic pathway is unknown. However, a deficiency of this enzyme causes accumulation of glycogen in vacuoles in the lysosomes, resulting in the serious GSD type II: Pompe disease (see Table 11.1 and Fig. 11.8). (Note: Pompe disease, caused by acid maltase deficiency, is the only GSD that is a lysosomal storage disease.)

Table 11.1 Descriptions of Glycogen Storage Diseases

Type[a]	Deficient Enzyme	Main Signs/Symptoms
I – Von Gierke disease	Glucose-6-phosphatase	Lactic acidosis, hypoglycemia, hyperuricemia, Impaired growth, bone thinning
II – Pompe disease[a]	Acid α-glucosidase (acid maltase)	Excess glycogen in lysosomes. Normal blood sugar. Enlarged liver and heart; muscle weakness and heart problems in severe forms
III – Cori disease[a]	Glycogen debranching enzyme (4:4 transferase)	Enlarged liver, growth delay, fasting hypoglycemia, abnormal glycogen structure, elevated fat in blood, possible muscle weakness
IV – Andersen disease	Glycogen branching enzyme (4:6 transferase)	Growth delay, enlarged liver, myopathy; death by age 5 usually
V – McArdle disease[a]	Muscle glycogen phosphorylase (myophosphorylase)	Muscle weakness and cramping after exercise; usually a relatively benign, chronic condition
VI – Hers disease	Liver glycogen phosphorylase	Liver enlargement; hypoglycemia; developmental delay
VII – Tarui disease	Muscle phosphofructokinase	Exercise-induced muscle cramps, developmental delay, hemolytic anemia in some

[a]This describes 7 of the 15 types of GSDs. See also Figure 11.3.

> Lysosomal storage diseases are genetic disorders characterized by the accumulation of abnormal amounts of carbohydrates or lipids primarily due to their decreased lysosomal degradation resulting from absence, or decreased activity or amount of the specific lysosomal acid hydrolase that is normally responsible for its degradation.

V. GLYCOGENESIS AND GLYCOGENOLYSIS REGULATION

Because of the importance of maintaining blood glucose levels, the synthesis and degradation of its glycogen storage form are tightly regulated. In the liver, glycogenesis accelerates during periods when the body has been well fed, whereas glycogenolysis accelerates during periods of fasting. In skeletal muscle, glycogenolysis occurs during active exercise, and glycogenesis begins as soon as the muscle is again at rest.

Regulation of synthesis and degradation is accomplished on two levels. First, glycogen synthase and glycogen phosphorylase are hormonally regulated (by covalent phosphorylation/dephosphorylation) to meet the needs of the body as a whole. Second, these same enzymes are allosterically regulated (by effector molecules) to meet the needs of a particular tissue.

A. Covalent activation of glycogenolysis

The binding of hormones, such as glucagon or epinephrine, to plasma membrane G protein–coupled receptors signals the need for glycogen to be degraded, either to elevate blood glucose levels or to provide energy for exercising muscle.

1. Protein kinase A activation: Binding of glucagon or epinephrine to their specific hepatocyte GPCR, or of epinephrine to a specific myocyte GPCR, results in the G protein–mediated activation of adenylyl cyclase. This enzyme catalyzes the synthesis of cyclic adenosine monophosphate (cAMP), which activates cAMP-dependent protein kinase A (PKA). cAMP binds the two regulatory subunits of tetrameric PKA, releasing two individual catalytic subunits that are active (Fig. 11.9). PKA then phosphorylates several enzymes of glycogen metabolism, affecting their activity. (Note: When cAMP is removed, the inactive PKA tetramer reforms.)

2. Phosphorylase kinase activation: Phosphorylase kinase exists in two forms: an inactive “b” form and an active “a” form. Active PKA phosphorylates the inactive “b” form of phosphorylase kinase, producing the active “a” form (see Fig. 11.9).

3. Glycogen phosphorylase activation: Glycogen phosphorylase also exists in a dephosphorylated, inactive “b” form and a phosphorylated, active “a” form. Phosphorylase kinase a is the only enzyme that phosphorylates glycogen phosphorylase b to its active “a” form, which then begins glycogenolysis (see Fig. 11.9).

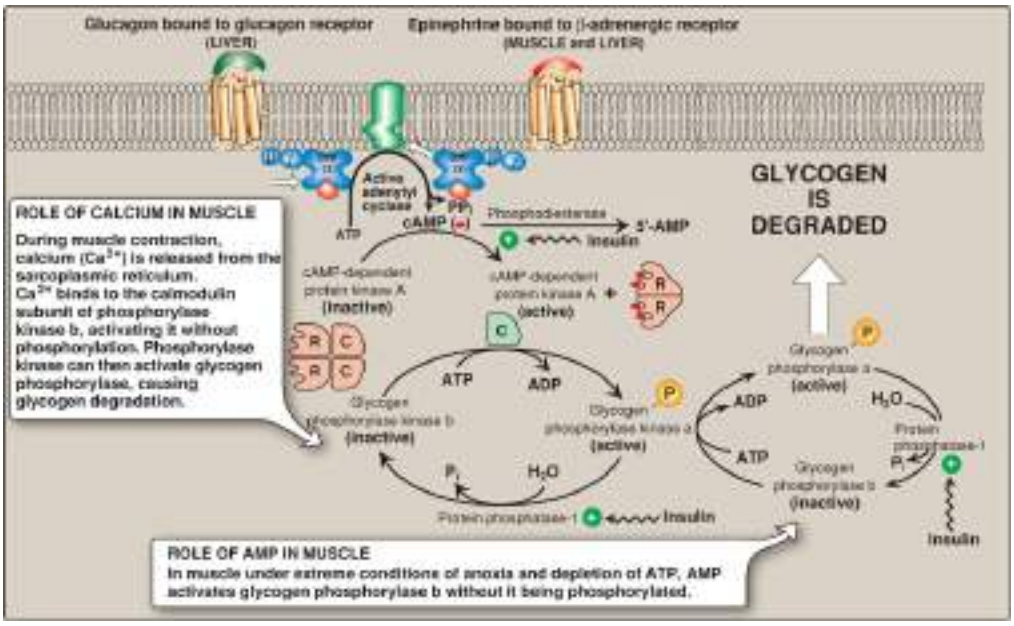


Figure 11.9
Stimulation and inhibition of glycogen degradation. AMP = adenosine monophosphate; cAMP = cyclic AMP; GTP = guanosine triphosphate; P = phosphate; PP_i = pyrophosphate; R = regulatory subunit; C = catalytic subunit.

4. Signal amplification: The cascade of reactions described above activates glycogenolysis. The large number of sequential steps serves to amplify the effect of the hormonal signal, that is, a few hormone molecules binding to their GPCR result in a number of PKA molecules being activated that can each activate many phosphorylase kinase molecules. This causes the production of many molecules of active glycogen phosphorylase a that can degrade glycogen.

5. Phosphorylated state maintenance: The phosphate groups added to phosphorylase kinase and phosphorylase in response to cAMP are maintained because the enzyme that hydrolytically removes the phosphate, protein phosphatase-1 (PP1), is inactivated by inhibitor proteins that are also phosphorylated and activated in response to cAMP (see Fig. 11.9). Because insulin also activates the phosphodiesterase that degrades cAMP, it opposes the effects of glucagon and epinephrine.

B. Covalent inhibition of glycogenesis

The regulated enzyme in glycogenesis, glycogen synthase, also exists in two forms, the active “a” form and the inactive “b” form. However, in contrast to phosphorylase kinase and phosphorylase, the active form of glycogen synthase is dephosphorylated, whereas the inactive form is phosphorylated at several sites on the enzyme, with the level of inactivation proportional to the degree of phosphorylation (Fig. 11.10). Phosphorylation is catalyzed by several different protein kinases in response to cAMP (for example, PKA and phosphorylase kinase) or other signaling mechanisms (see C. below). Glycogen synthase b can be reconverted to the “a” form by PP1. Figure 11.11 summarizes the covalent regulation of glycogen metabolism.

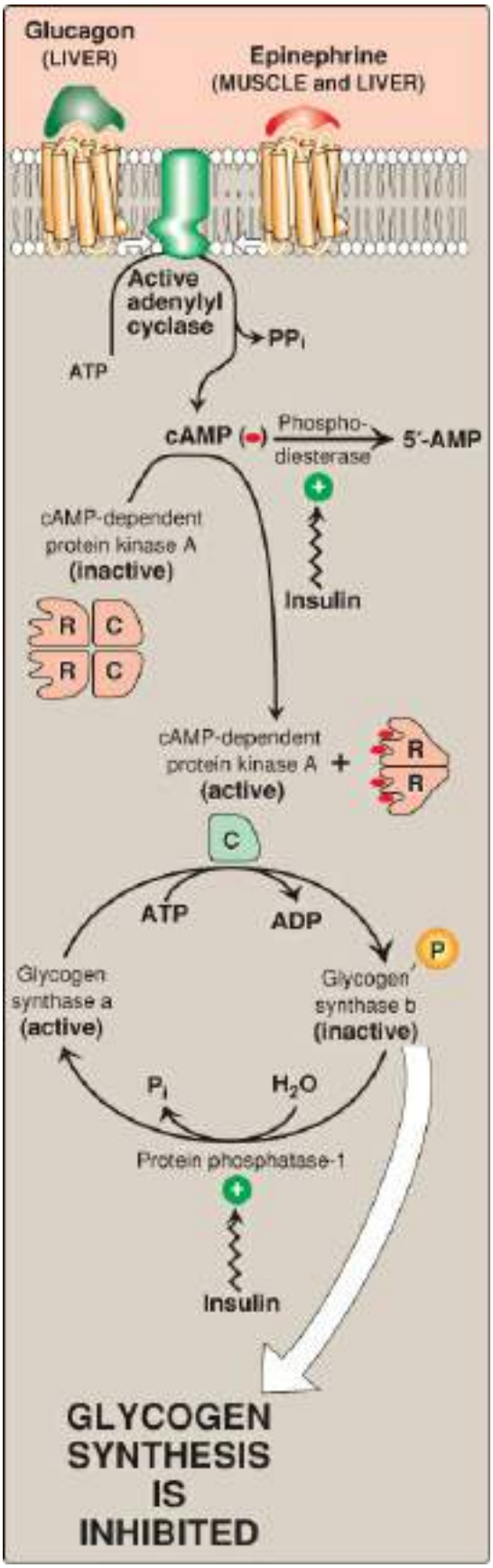


Figure 11.10
Hormonal regulation of glycogen synthesis. (Note: In contrast to glycogen phosphorylase, glycogen synthase is inactivated by phosphorylation.) cAMP = cyclic adenosine monophosphate;  = phosphate; PP_i = pyrophosphate; R = regulatory subunit; C = catalytic subunit; ADP = adenosine diphosphate.

C. Allosteric regulation of glycogenesis and glycogenolysis

In addition to hormonal signals, glycogen synthase and glycogen phosphorylase respond to the levels of metabolites and energy needs of the cell. Glycogenesis is stimulated when glucose and energy levels are high, whereas glycogenolysis is increased when glucose and energy levels are low. This allosteric regulation allows a rapid response to the needs of a cell and can override the effects of hormone-mediated covalent regulation. (Note: The “a” and “b” forms of the allosteric enzymes of glycogen metabolism are each in an equilibrium between the R [relaxed, more active] and T [tense, less active] conformations [see p. 29]. The binding of effectors shifts the equilibrium and alters enzymic activity without directly altering the covalent modification.)

1. Regulation in the well-fed state: In the well-fed state, glycogen synthase b in both liver and muscle is allosterically activated by glucose 6-phosphate, which is present in elevated concentrations (Fig. 11.12). In contrast, glycogen phosphorylase a is allosterically inhibited by glucose 6-phosphate, as well as by ATP, a high-energy signal. Note that in liver, but not muscle, free glucose is also an allosteric inhibitor of glycogen phosphorylase a.

2. Glycogenolysis activation by AMP: Muscle glycogen phosphorylase (myophosphorylase), but not the liver isozyme, is active in the presence of the high AMP concentrations that occur under extreme conditions of anoxia and ATP depletion. AMP binds to glycogen phosphorylase b, causing its activation without phosphorylation (see Fig. 11.9). Recall that AMP also activates phosphofructokinase-1 of glycolysis, allowing glucose from glycogenolysis to be oxidized.

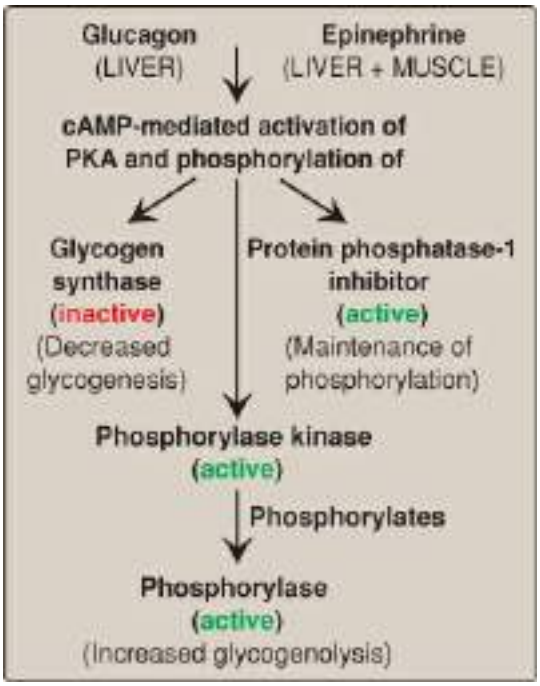


Figure 11.11
Summary of the hormone-mediated covalent regulation of glycogen metabolism. cAMP = cyclic adenosine monophosphate; PKA = protein kinase A.

3. Glycogenolysis activation by calcium: Calcium (Ca^{2+}) is released into the sarcoplasm in muscle cells (myocytes) in response to neural stimulation and in the liver in response to epinephrine binding to α_1-adrenergic receptors. The Ca^{2+} binds to calmodulin (CaM), the most widely distributed member of a family of small, Ca^{2+}-binding proteins. The binding of four molecules of Ca^{2+} to CaM triggers a conformational change such that the activated Ca^{2+}–CaM complex binds to and activates protein molecules, often enzymes that are inactive in the absence of this complex (Fig. 11.13). Thus, CaM functions as an essential subunit of many complex proteins. One such protein is the tetrameric phosphorylase kinase, whose "b" form is activated by the binding of Ca^{2+} to its δ subunit (CaM) without the need for the kinase to be phosphorylated by PKA. (Note: Epinephrine at β-adrenergic receptors signals through a rise in cAMP, not via a rise in Ca^{2+}.)

a. Muscle phosphorylase kinase activation: During muscle contraction, there is a rapid and urgent need for ATP. It is supplied by the degradation of muscle glycogen to glucose 6-phosphate, which enters glycolysis. Nerve impulses cause membrane depolarization, which promotes Ca^{2+} release from the sarcoplasmic reticulum into the sarcoplasm of myocytes. The Ca^{2+} binds the CaM subunit, and the complex activates muscle phosphorylase kinase b (see Fig. 11.9).

b. Liver phosphorylase kinase activation: During physiologic stress,

epinephrine is released from the adrenal medulla and signals the need for blood glucose. This glucose initially comes from hepatic glycogenolysis. Binding of epinephrine to hepatocyte α_1-adrenergic GPCR activates a phospholipid-dependent cascade that results in movement of Ca^{2+} from the ER into the cytoplasm. A Ca^{2+}–CaM complex forms and activates hepatic phosphorylase kinase b. Note that the released Ca^{2+} also helps to activate protein kinase C that can phosphorylate and inactivate glycogen synthase a.

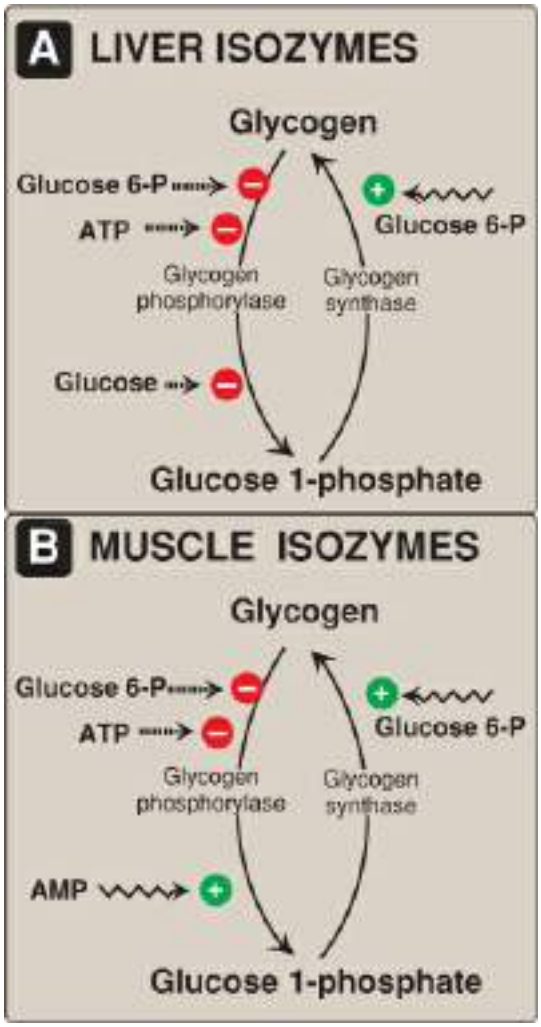


Figure 11.12
Allosteric regulation of glycogenesis and glycogenolysis in liver, (A) and muscle, (B). P = phosphate; AMP = adenosine monophosphate.

VI. GLYCOGEN STORAGE DISEASES

GSDs are a group of genetic diseases caused by defects in enzymes required for glycogen degradation or, more rarely, glycogen synthesis. The most common symptoms are hypoglycemia (low blood glucose), enlarged liver, slow growth, and muscle

weakness or cramping.

These disorders result either in formation of glycogen that has an abnormal structure or in the accumulation of excessive amounts of normal glycogen in specific tissues as a result of impaired degradation. A particular enzyme may be defective in a single tissue, such as the liver (resulting in hypoglycemia) or muscle (causing muscle weakness), or the defect may be more generalized, affecting a variety of tissues, such as the heart and kidneys. Severity ranges from fatal in early childhood to mild disorders that are not life threatening. Overall there are 15 recognized types of GSD; some are quite rare. The more prevalent types of GSD are described in Table 11.1 and three of the most common GSD are illustrated in Figure 11.8.

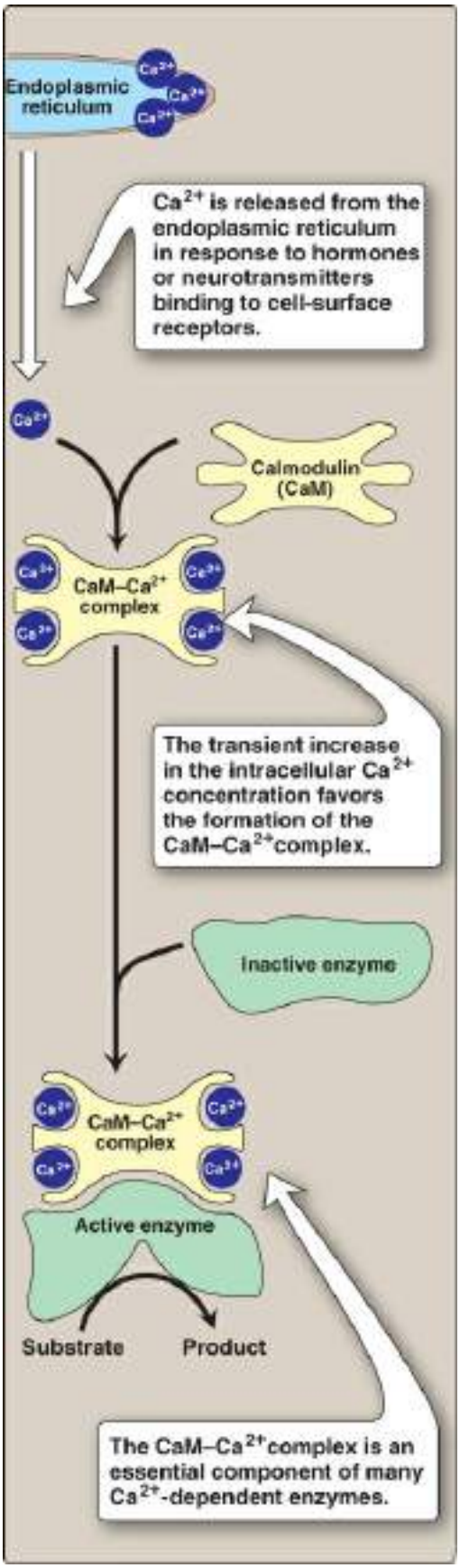


Figure 11.13
Calmodulin mediates many effects of intracellular calcium (Ca^{2+}). (Note: Ca^{2+} activates phosphorylase kinase in liver and muscles.)

VII. Chapter Summary

- The main stores of **glycogen** in the body are found in **skeletal muscle**, where they serve as a fuel reserve for the synthesis of **ATP** during muscle **contraction**, and in the **liver**, where they are used to maintain the **blood glucose** concentration, particularly during the early stages of a **fast**.
- Glycogen is a highly **branched polymer** of **α-D-glucose**.
- **UDP-glucose**, the building block of glycogen, is synthesized from **glucose 1-phosphate** and **UTP** by **UDP–glucose pyrophosphorylase** (Fig. 11.14).
- **Glucose** from UDP-glucose is transferred to the **nonreducing ends** of glycogen chains by primer-requiring **glycogen synthase**, which makes the α(1→4) linkages. The **primer** is made by **glycogenin**. Branches are formed by **amylo-α(1→4)→α(1→6)-transglycosylase** (a **4:6 transferase**), which transfers a set of six to eight glucosyl residues from the nonreducing end of the glycogen chain (breaking an α[1→4] linkage), and making an α(1→6) linkage to another residue in the chain.
- G**lycogen phosphorylase** cleaves the α(1→4) bonds between glucosyl residues at the nonreducing ends of the glycogen chains, producing **glucose 1-phosphate**.
- Glucose 1-phosphate is converted to **glucose 6-phosphate** by **phosphoglucomutase**.
- In **muscle**, glucose 6-phosphate enters glycolysis. In **liver**, the phosphate is removed by **glucose 6-phosphatase**, releasing free glucose that can be used to maintain blood glucose levels at the beginning of a fast.
- A deficiency of the phosphatase causes **von Gierke disease** and results in an inability of the liver to provide free glucose to the body during a fast. It affects both glycogen degradation and gluconeogenesis.
- Glycogen synthesis and degradation are **reciprocally regulated** to meet whole-body needs by the same hormonal signals, namely, an **elevated insulin** level results in overall **increased glycogenesis** and **decreased glycogenolysis**, whereas an **elevated glucagon**, or **epinephrine**, level causes the opposite effects.
- Key enzymes are phosphorylated by **protein kinases**, some of which are dependent on **cAMP**, a compound increased by glucagon and epinephrine. Phosphate groups are removed by **PP-1**.
- In addition to this **covalent regulation**, **glycogen synthase**, **phosphorylase kinase**, and **phosphorylase** are **allosterically regulated** to meet tissues' needs.
- In the well-fed state, glycogen synthase is activated by glucose 6-phosphate, but glycogen phosphorylase is inhibited by glucose 6-phosphate as well as by ATP.
- In the liver, free glucose also serves as an allosteric inhibitor of glycogen phosphorylase.
- The rise in **calcium** in muscle during exercise and in liver in response to epinephrine activates phosphorylase kinase by binding to the enzyme's C**aM** subunit. This allows the enzyme to activate glycogen phosphorylase, thereby causing glycogen degradation.

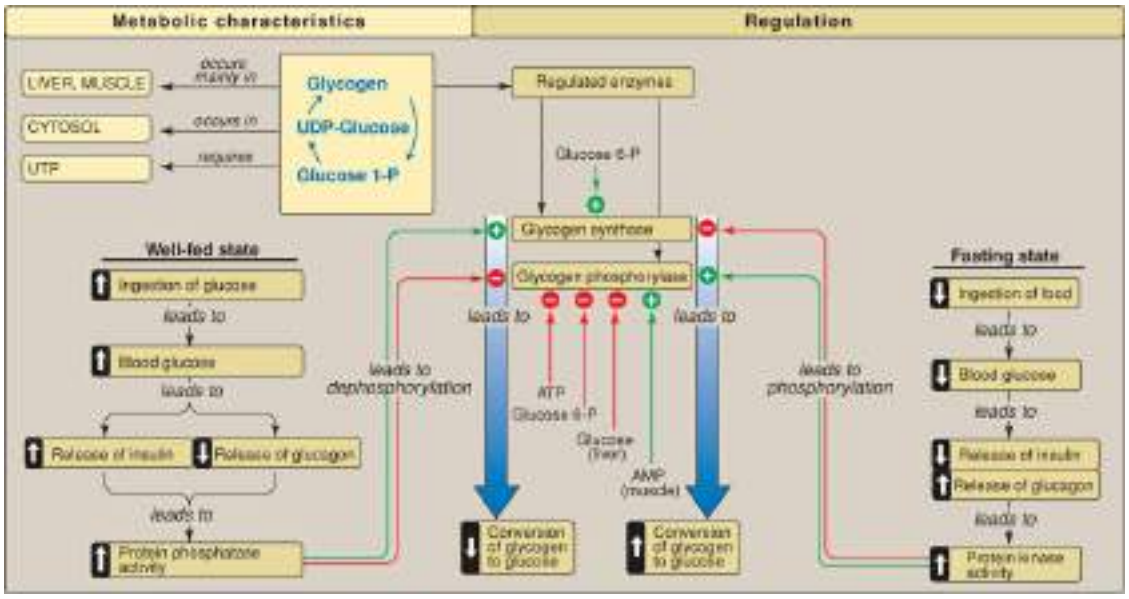


Figure 11.14
Key concept map for glycogen metabolism in the liver. (Note: Glycogen phosphorylase is phosphorylated by phosphorylase kinase, the “b” form of which can be activated by calcium.) UDP and UTP = uridine di- and triphosphates; P = phosphate; AMP = adenosine monophosphate.

Study Questions

Choose the ONE best answer.

For Questions 11.1 to 11.4, match the deficient enzyme to the clinical finding in selected glycogen storage diseases (GSDs).

Choice	GSD	Deficient Enzyme
A	Von Gierke disease Type Ia	Glucose 6-phosphatase
B	Pompe disease Type II	Acid maltase
C	Cori disease Type III	4:4 Transferase
D	Andersen disease Type IV	4:6 Transferase
E	McArdle disease Type V	Myophosphorylase
F	Hers disease Type VI	Liver phosphorylase

11.1 Exercise intolerance, with no rise in blood lactate during exercise

Correct answer = E. Myophosphorylase (the muscle isozyme of glycogen phosphorylase) deficiency (or, McArdle disease) prevents glycogen degradation in muscle, depriving muscle of glycogen-derived glucose, resulting in decreased glycolysis and its anaerobic product, lactate.

11.2 Fatal, progressive cirrhosis and glycogen with longer-than-normal outer chains

Correct answer = D. 4:6 Transferase (branching enzyme) deficiency (Andersen disease), a defect in glycogen

synthesis, results in glycogen with fewer branches and decreased solubility.

11.3 Generalized accumulation of glycogen, severe hypotonia, and death from heart failure

Correct answer = B. Acid maltase (acid α[1→4]-glucosidase) deficiency (or, Pompe disease) prevents degradation of any glycogen brought into lysosomes. A variety of tissues are affected, with the most severe pathology resulting from heart damage.

11.4 Severe fasting hypoglycemia, lactic acidemia, hyperuricemia, and hyperlipidemia

Correct answer = A. Glucose 6-phosphatase deficiency (von Gierke disease) prevents the liver from releasing free glucose into the blood, causing severe fasting hypoglycemia, lactic acidemia, hyperuricemia, and hyperlipidemia.

11.5 Both epinephrine and glucagon have which effect on hepatic glycogen metabolism?

A. Both phosphorylate and activate glycogen phosphorylase and glycogen synthase.
B. Both phosphorylate and inactivate glycogen phosphorylase and glycogen synthase.
C. Both cause increased glycogen degradation and decreased synthesis in the liver.
D. Both cause the synthesis of glycogen to have a net increase.

Correct answer = C. Epinephrine and glucagon both cause increased glycogen degradation and decreased synthesis in the liver through covalent modification (phosphorylation) of key enzymes of glycogen metabolism. Glycogen phosphorylase is phosphorylated and active (“a” form), whereas glycogen synthase is phosphorylated and inactive (“b” form). Glucagon does not cause a rise in calcium.

11.6 In contracting skeletal muscle, a sudden elevation of the sarcoplasmic calcium concentration will result in:

A. activation of cyclic adenosine monophosphate (cAMP)-dependent protein kinase A.
B. conversion of cAMP to AMP by phosphodiesterase.
C. direct activation of glycogen synthase b.
D. direct activation of phosphorylase kinase b.
E. inactivation of phosphorylase kinase a by the action of protein phosphatase-1.

Correct answer = D. Calcium (Ca^{2+}) released from the sarcoplasmic reticulum during exercise binds to the calmodulin subunit of phosphorylase kinase, thereby allosterically activating the dephosphorylated “b” form of this enzyme. The other choices are not caused by an elevation of cytosolic Ca^{2+}. (Note: Ca^{2+} also activates hepatic phosphorylase kinase b.)

11.7 Explain why the hypoglycemia seen with type 1 von Gierke disease (glucose 6-phosphatase deficiency) is severe, whereas that seen with type VI Hers disease (liver phosphorylase deficiency) is mild.

With von Gierke disease, the liver is unable to generate free glucose either from glycogenolysis or gluconeogenesis because both processes produce glucose 6-phosphate. With Hers disease, the liver is still able to produce free glucose from gluconeogenesis, but glycogenolysis is inhibited.

Monosaccharide and Disaccharide Metabolism 12

I. OVERVIEW

Glucose is the most common monosaccharide consumed by humans; its metabolism has already been discussed. Two other monosaccharides, fructose and galactose, also occur in significant amounts in the diet, primarily in disaccharides, and make important contributions to energy metabolism. In addition, galactose is an important component of glycosylated proteins. Figure 12.1 shows the metabolism of fructose and galactose as part of the essential pathways of energy metabolism.

II. FRUCTOSE METABOLISM

About 10% of the calories in the typical Western diet are supplied by fructose (~55 g/day). The major source of fructose is the disaccharide sucrose, which, when cleaved in the intestine, releases equimolar amounts of fructose and glucose. Fructose is also found as a free monosaccharide in many fruits, in honey, and in high-fructose corn syrup (typically, 55% fructose and 45% glucose), which is used to sweeten soft drinks and many foods. Fructose transport into cells is not insulin dependent (unlike that of glucose into certain tissues), and, in contrast to glucose, fructose does not promote the secretion of insulin.

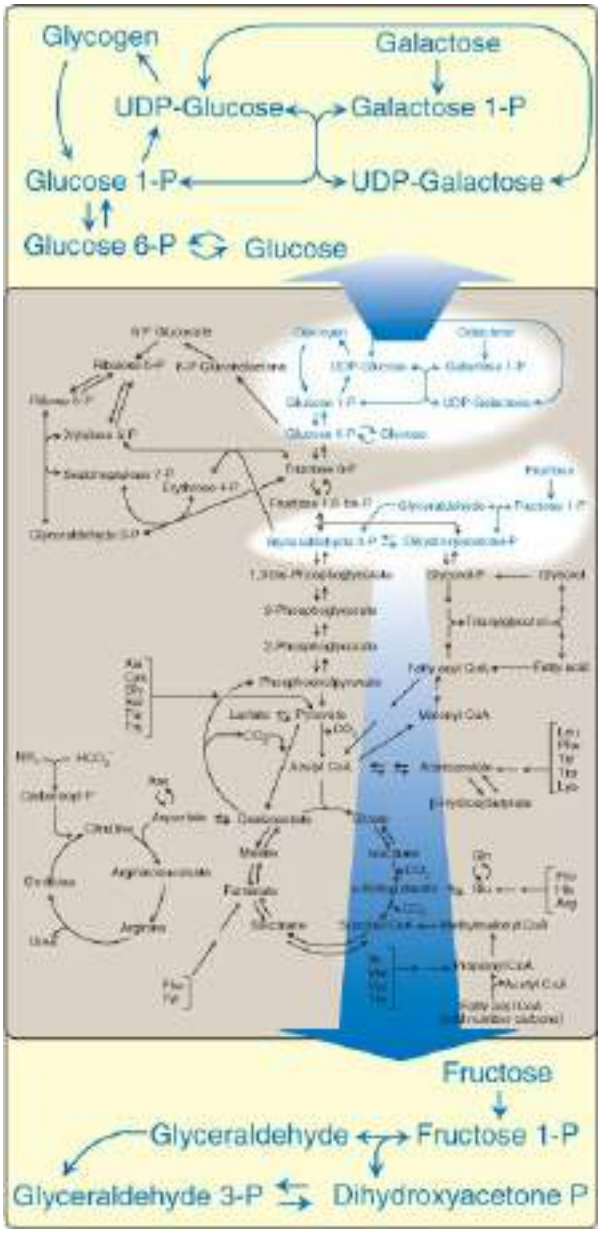


Figure 12.1
Galactose and fructose metabolism as part of the essential pathways of energy metabolism. (Note: See Fig. 8.2, for a more detailed map of metabolism.) UDP = uridine diphosphate; P = phosphate.

A. Phosphorylation

For fructose to enter the pathways of intermediary metabolism, it must first be phosphorylated (Fig. 12.2). This can be accomplished by actions of either hexokinase or fructokinase. Hexokinase phosphorylates glucose in most cells of the body, and several additional hexoses can serve as substrates for this enzyme. However, it has a low affinity (a high K_m) for fructose.

Therefore, unless the intracellular concentration of fructose becomes unusually

high, the normal presence of saturating concentrations of glucose means that little fructose is phosphorylated by hexokinase. Fructokinase provides the primary mechanism for fructose phosphorylation (see Fig. 12.2). The enzyme has a low K_m for fructose and a high V_{max} (maximal velocity). It is found in the liver (which processes most of the dietary fructose), kidneys, and the small intestine and converts fructose to fructose 1-phosphate, using ATP as the phosphate donor. (Note: These three tissues also contain aldolase B, discussed in section B.)

B. Fructose 1-phosphate cleavage

Fructose 1-phosphate is not phosphorylated to fructose 1,6- bisphosphate as is fructose 6-phosphate (see p. 109) but is cleaved by aldolase B (also called fructose 1-phosphate aldolase) to two trioses, dihydroxyacetone phosphate (DHAP) and glyceraldehyde. (Note: Humans express three distinct aldolase isoenzymes, the products of three different genes: aldolase A in most tissues; aldolase B in the liver, kidneys, and small intestine; and aldolase C in the brain. All cleave fructose 1,6-bisphosphate produced during glycolysis to DHAP and glyceraldehyde 3-phosphate, but only aldolase B cleaves fructose 1-phosphate.) DHAP can be used in glycolysis or gluconeogenesis, whereas glyceraldehyde can be metabolized by a number of pathways, as illustrated in Figure 12.3.

C. Kinetics

The rate of fructose metabolism is more rapid than that of glucose because triose production from fructose 1-phosphate bypasses phosphofructokinase-1, the major rate-limiting step in glycolysis.

D. Disorders

A deficiency of one of the key enzymes required for the entry of fructose into metabolic pathways can result in either a benign condition as a result of fructokinase deficiency (essential fructosuria) or a severe disturbance of liver and kidney metabolism as a result of aldolase B deficiency, or hereditary fructose intolerance (HFI), which occurs in ~1:20,000 live births (see Fig. 12.3).

The first symptoms of HFI appear when a baby is weaned from lactose-containing milk and begins ingesting food containing sucrose or fructose. Fructose 1-phosphate accumulates, resulting in a drop in the level of inorganic phosphate (P_i) and, therefore, of ATP production. As ATP falls, adenosine monophosphate (AMP) rises. The AMP is degraded, causing hyperuricemia and lactic acidemia. The decreased availability of hepatic ATP decreases gluconeogenesis (causing hypoglycemia with vomiting) and protein synthesis (causing a decrease in blood-clotting factors and other essential proteins). Renal reabsorption of P_i is also decreased. (Note: The drop in P_i also inhibits glycogenolysis.)

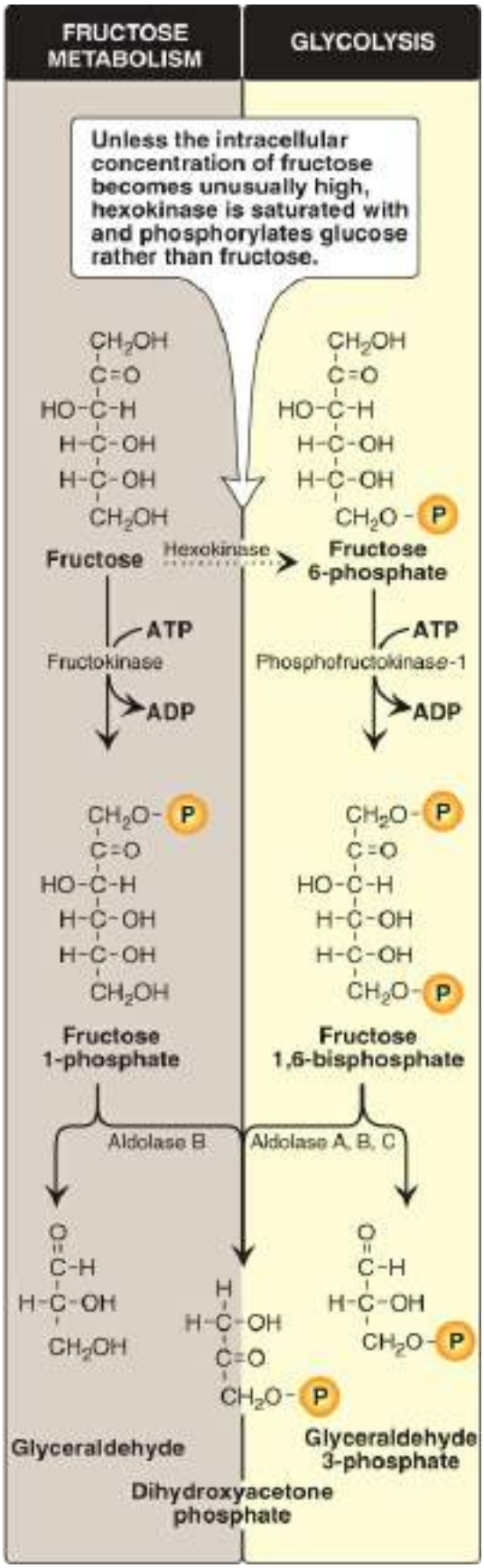


Figure 12.2
Fructose phosphorylation products and their cleavage. P = phosphate; ADP = adenosine diphosphate.

Diagnosis of HFI can be made on the basis of fructose in the urine, enzyme assay using liver cells, or by DNA-based testing (see Chapter 34). With HFI, sucrose, as well as fructose, must be removed from the diet to prevent liver failure and possible death. Note that individuals with HFI tend to display a life-long aversion to sweets.

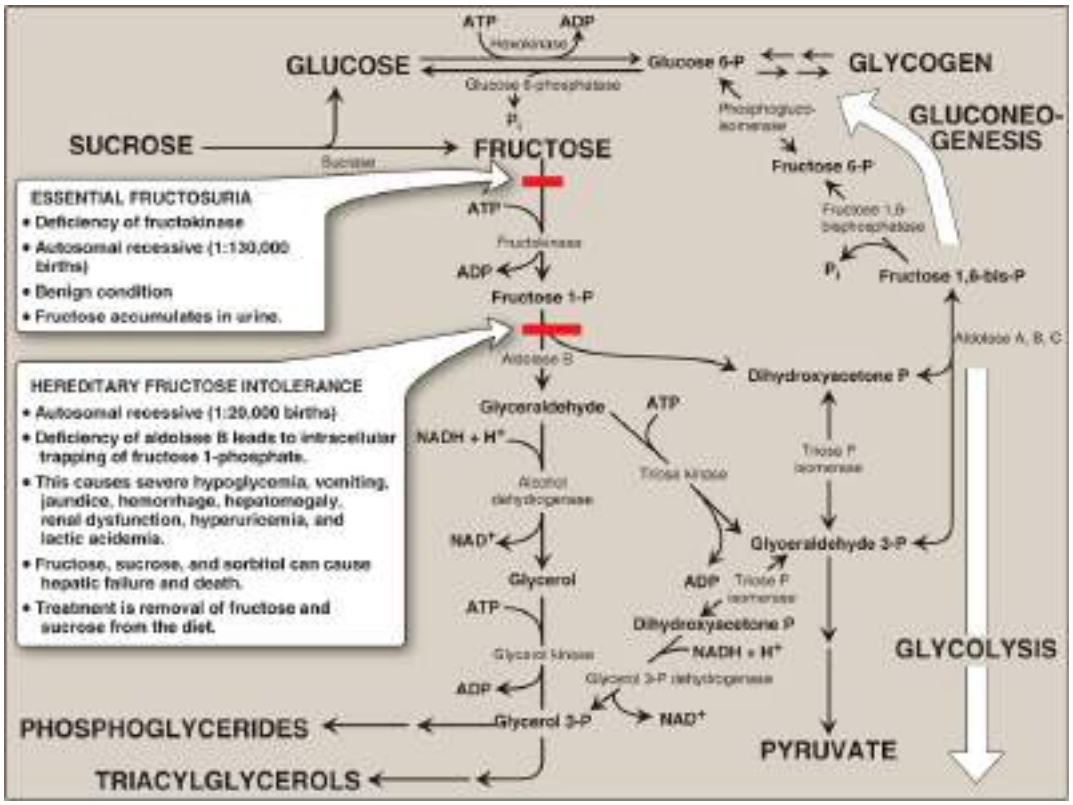


Figure 12.3
Summary of fructose metabolism. P = phosphate; P_i = inorganic phosphate; NAD(H) = nicotinamide adenine dinucleotide; ADP = adenosine diphosphate.

E. Mannose conversion to fructose 6-phosphate

Mannose, the C-2 epimer of glucose, is an important component of glycoproteins. Hexokinase phosphorylates mannose, producing mannose 6-phosphate, which, in turn, is reversibly isomerized to fructose 6-phosphate by phosphomannose isomerase. (Note: Most intracellular mannose is synthesized from fructose or is pre-existing mannose produced by the degradation of glycoproteins and salvaged by hexokinase. Dietary carbohydrates contain little mannose.)

F. Glucose conversion to fructose via sorbitol

Most sugars are rapidly phosphorylated following their entry into cells. Therefore,

they are trapped within the cells, because organic phosphates cannot freely cross membranes without specific transporters. An alternate mechanism for metabolizing a monosaccharide is to convert it to a polyol (sugar alcohol) by the reduction of an aldehyde group, thereby producing an additional hydroxyl group.

1. Sorbitol synthesis: Aldose reductase reduces glucose, producing sorbitol (or, glucitol; Fig. 12.4), but the K_m is high. This enzyme is found in many tissues, including the retina, lens, kidneys, peripheral nerves, ovaries, and seminal vesicles. A second enzyme, sorbitol dehydrogenase, can oxidize sorbitol to fructose in cells of the liver, ovaries, and seminal vesicles (see Fig. 12.4). The two-reaction pathway from glucose to fructose in the seminal vesicles benefits sperm cells, which use fructose as a major carbohydrate energy source. The pathway from sorbitol to fructose in the liver provides a mechanism by which any available sorbitol is converted into a substrate that can enter glycolysis.

2. Hyperglycemia and sorbitol metabolism: Because insulin is not required for the entry of glucose into cells of the retina, lens, kidneys, and peripheral nerves, large amounts of glucose may enter these cells during times of hyperglycemia (e.g., in poorly controlled diabetes mellitus). An elevated intracellular glucose concentration and an adequate supply of reduced nicotinamide adenine dinucleotide phosphate (NADPH) cause aldose reductase to produce a significant increase in sorbitol within the cell, which cannot pass efficiently through cell membranes and, therefore, remains trapped inside the cell (see Fig. 12.4). This is exacerbated when sorbitol dehydrogenase production is low or absent. As a result, sorbitol accumulates in these cells, causing strong osmotic effects and cell swelling due to water influx and retention.

   Some of the pathologic alterations associated with diabetes mellitus can be partly attributed to this osmotic stress, including cataract formation, peripheral neuropathy, and microvascular problems leading to nephropathy and retinopathy. Use of NADPH in the aldose reductase reaction decreases the generation of reduced glutathione, an important antioxidant, and may also be related to complications of diabetes.

III. GALACTOSE METABOLISM

The major dietary source of galactose is lactose (galactosyl β-1,4-glucose) obtained from milk and milk products. (Note: The digestion of lactose by β-galactosidase, also called lactase, was discussed on p. 97.) Some galactose can also be obtained by lysosomal degradation of glycoproteins and glycolipids. Like fructose (and mannose), the transport of galactose into cells is not insulin dependent.

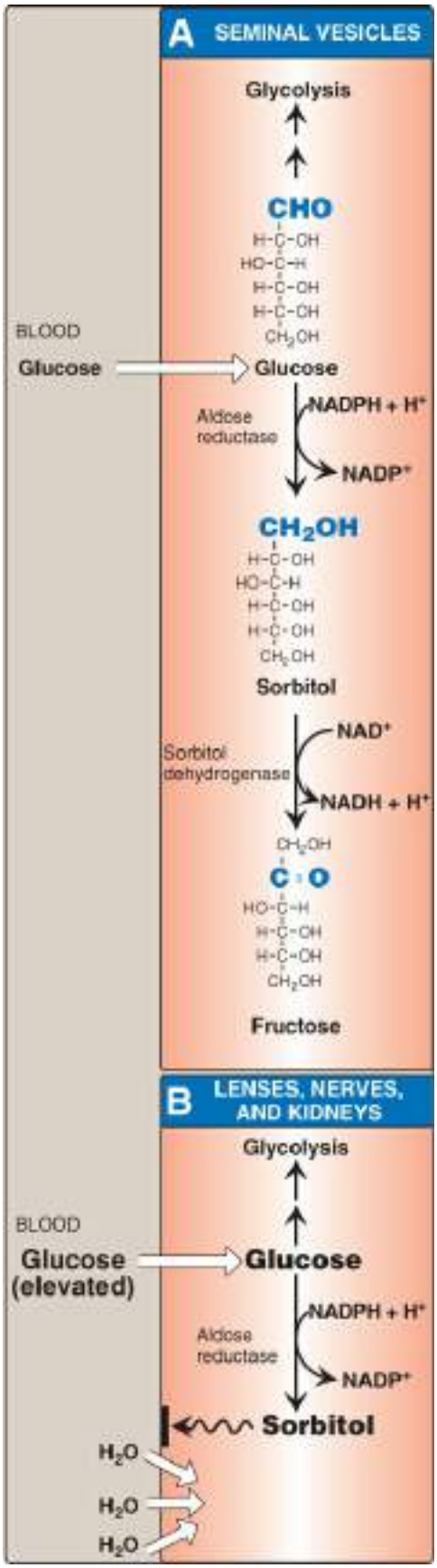


Figure 12.4
Sorbitol metabolism. NAD(H) = nicotinamide adenine dinucleotide; NADP(H) = nicotinamide adenine dinucleotide phosphate.

A. Phosphorylation

Like fructose, galactose must be phosphorylated before it can be further metabolized. Most tissues have a specific enzyme for this purpose, galactokinase, which produces galactose 1-phosphate (Fig. 12.5). As with other kinases, ATP is the phosphate donor.

B. Uridine diphosphate–galactose formation

Galactose 1-phosphate cannot enter the glycolytic pathway unless it is first converted to uridine diphosphate (UDP)-galactose (Fig. 12.6). This occurs in an exchange reaction, in which UDP-glucose reacts with galactose 1-phosphate, producing UDP-galactose and glucose 1-phosphate (see Fig. 12.5). The reaction is catalyzed by galactose 1-phosphate uridylyltransferase (GALT). (Note: The glucose 1-phosphate product can be isomerized to glucose 6-phosphate, which can enter glycolysis or be dephosphorylated.)

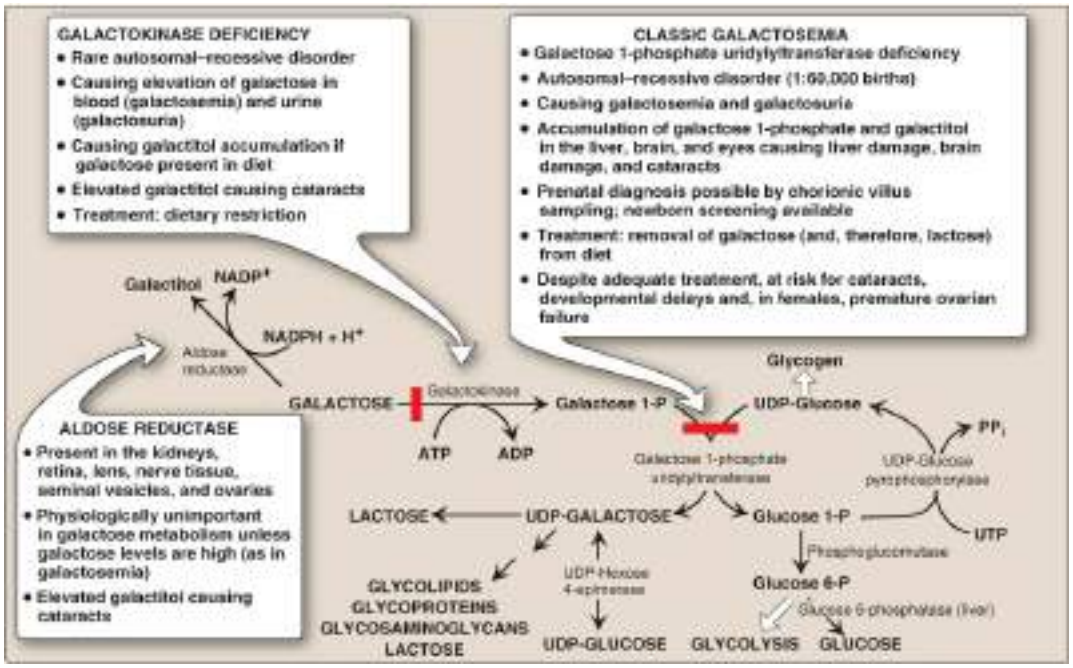


Figure 12.5
Metabolism of galactose. UDP and UTP = uridine di- and triphosphates; P = phosphate; PP_i = pyrophosphate; NADP(H) = nicotinamide adenine dinucleotide phosphate; ADP = adenosine diphosphate.

C. UDP-galactose conversion to UDP-glucose

For UDP-galactose to enter the mainstream of glucose metabolism, it must first be

isomerized to its C-4 epimer, UDP-glucose, by UDP-hexose 4-epimerase. This "new" UDP-glucose (produced from the original UDP-galactose) can participate in biosynthetic reactions (e.g., glycogenesis) as well as in the GALT reaction. (Note: See Fig. 12.5 for a summary of the interconversions.)

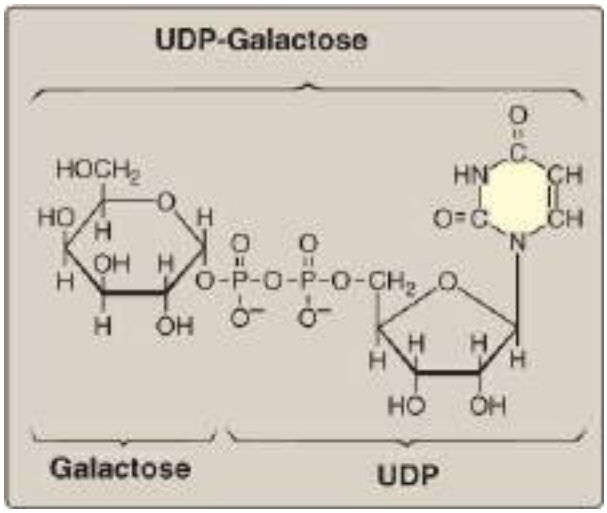


Figure 12.6
Structure of UDP-galactose. UDP = uridine diphosphate.

D. UDP-galactose in biosynthetic reactions

UDP-galactose can serve as the donor of galactose units in a number of synthetic pathways, including synthesis of lactose (see IV. below), glycoproteins, glycolipids, and glycosaminoglycans. (Note: If galactose is not provided by the diet [e.g., when it cannot be released from lactose owing to a lack of β-galactosidase in people who are lactose intolerant], all tissue requirements for UDP-galactose can be met by the action of UDP-hexose 4-epimerase on UDP-glucose, which is efficiently produced from glucose 1-phosphate and uridine triphosphate [see Fig. 12.5].)

E. Disorders

GALT is severely deficient in individuals with classic galactosemia (see Fig. 12.5). In this disorder, galactose 1-phosphate and, therefore, galactose accumulate. Physiologic consequences are similar to those found in HFI, but a broader spectrum of tissues is affected. The accumulated galactose is shunted into side pathways such as that of galactitol production. This reaction is catalyzed by aldose reductase, the same enzyme that reduces glucose to sorbitol. GALT deficiency is part of the newborn screening panel. Treatment of galactosemia requires removal of galactose and lactose from the diet. Deficiencies in galactokinase and the epimerase result in less severe disorders of galactose metabolism, although cataracts are common (see Fig. 12.5).

IV. LACTOSE SYNTHESIS

Lactose is a disaccharide that consists of a molecule of β-galactose attached by a β(1→4) linkage to glucose. Therefore, lactose is galactosyl β(1→4)-glucose. Because lactose, the sugar in milk, is made by lactating (milk-producing) mammary glands, milk and other dairy products are the dietary sources of lactose.

Lactose synthase (UDP-galactose:glucose galactosyltransferase) catalyzes lactose synthesis in the Golgi. This enzyme, composed of A and B proteins, transfers galactose from UDP-galactose to glucose, releasing UDP (Fig. 12.7). Protein A is a β-D-galactosyltransferase and is found in a number of body tissues. In tissues other than the lactating mammary gland, this enzyme transfers galactose from UDP-galactose to N-acetyl-D-glucosamine, forming the same β(1→4) linkage found in lactose, and producing N-acetyllactosamine, a component of the structurally important N-linked glycoproteins (see p. 185). In contrast, protein B is found only in lactating mammary glands. It is α-lactalbumin, and its synthesis is stimulated by the peptide hormone prolactin. Protein B forms a complex with the enzyme, protein A, changing the specificity of that transferase (by decreasing the K_m for glucose) so that lactose, rather than N-acetyllactosamine, is produced (see Fig. 12.7).

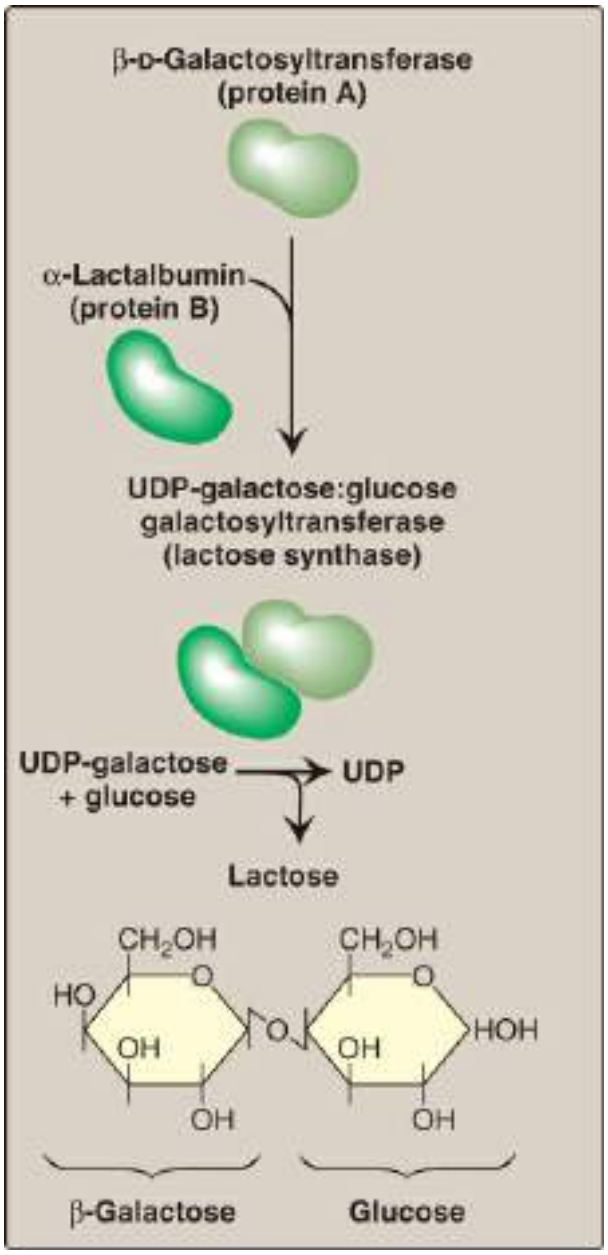


Figure 12.7
Lactose synthesis. UDP = uridine diphosphate.

Clinical Application 12.1: Lactose Intolerance

Lactose intolerance, also called lactose malabsorption, affects up to 60% of adults with ancestries other than Northern European. It results from deficiency of β-galactosidase or lactase in the small intestine. Recall (see p. 97 and Fig. 7.11) that with insufficient lactase there is an inability to fully digest dairy products. After consuming dairy, lactose intolerant individuals can experience cramping, diarrhea, and bloating. Lactase supplements and avoidance of dairy products can be effective in treating the condition.

V. Chapter Summary

- The major source of fructose is the disaccharide **sucrose**, which, when cleaved, releases equimolar amounts of **fructose** and **glucose** (Fig. 12.8).
- Transport of fructose into cells is **insulin independent**.
- Fructose is first phosphorylated to **fructose 1-phosphate** by **fructokinase** and then cleaved by **aldolase B** to **DHAP** and **glyceraldehyde**. These enzymes are found in the **liver**, **kidneys**, and **small intestine**.
- A deficiency of fructokinase causes a benign condition**, essential fructosuria**, whereas a deficiency of aldolase B causes **HFI**, in which **severe hypoglycemia** and **liver failure** lead to **death** if fructose (and sucrose) is not removed from the diet.
- **Mannose**, an important component of **glycoproteins**, is phosphorylated by **hexokinase** to **mannose 6-phosphate**, which is reversibly isomerized to **fructose 6-phosphate** by **phosphomannose isomerase**.
- Glucose can be reduced to **sorbitol** (**glucitol**) by **aldose reductase** in many tissues, including the **lens**, **retina**, **peripheral nerves**, **kidneys**, **ovaries**, and **seminal vesicles**. In the liver, ovaries, and seminal vesicles, a second enzyme, **sorbitol dehydrogenase**, can oxidize sorbitol to produce **fructose**.
- **Hyperglycemia** results in the accumulation of sorbitol in those cells lacking sorbitol dehydrogenase. The resulting **osmotic events** cause cell swelling and may contribute to the **cataract formation**, **peripheral neuropathy**, **nephropathy**, and **retinopathy** seen in **diabetes**.
- The major dietary source of **galactose** is **lactose**. The transport of galactose into cells is insulin independent. Galactose is first phosphorylated to galactose 1-phosphate by **galactokinase**, a deficiency of which results in cataracts.
- Galactose 1-phosphate is converted to **UDP-galactose** by **GALT**, with the nucleotide supplied by UDP-glucose. A deficiency of this enzyme causes **classic galactosemia**. Galactose 1-phosphate accumulates, and excess galactose is converted to **galactitol** by **aldose reductase**. This causes **liver damage**, **brain damage**, and **cataracts**. Treatment requires removal of galactose (and lactose) from the diet.
- For UDP-galactose to enter the mainstream of glucose metabolism, it must first be isomerized to UDP-glucose by **UDP-hexose 4-epimerase**. This enzyme can also be used to produce UDP-galactose from UDP-glucose when the former is required for glycoprotein and glycolipid synthesis.
- **Lactose** is a disaccharide of **galactose** and **glucose**. D**airy products** are the dietary sources of lactose. Lactose is synthesized by **lactose synthase** from **UDP-galactose** and **glucose** in the **lactating mammary gland**. The enzyme has two subunits, **protein A** (which is a **galactosyltransferase** found in most cells where it synthesizes **N-acetyllactosamine**) and **protein B** (**α-lactalbumin**, which is found only in lactating mammary glands, and whose synthesis is stimulated by the peptide hormone **prolactin**). When both subunits are present, the transferase produces lactose.

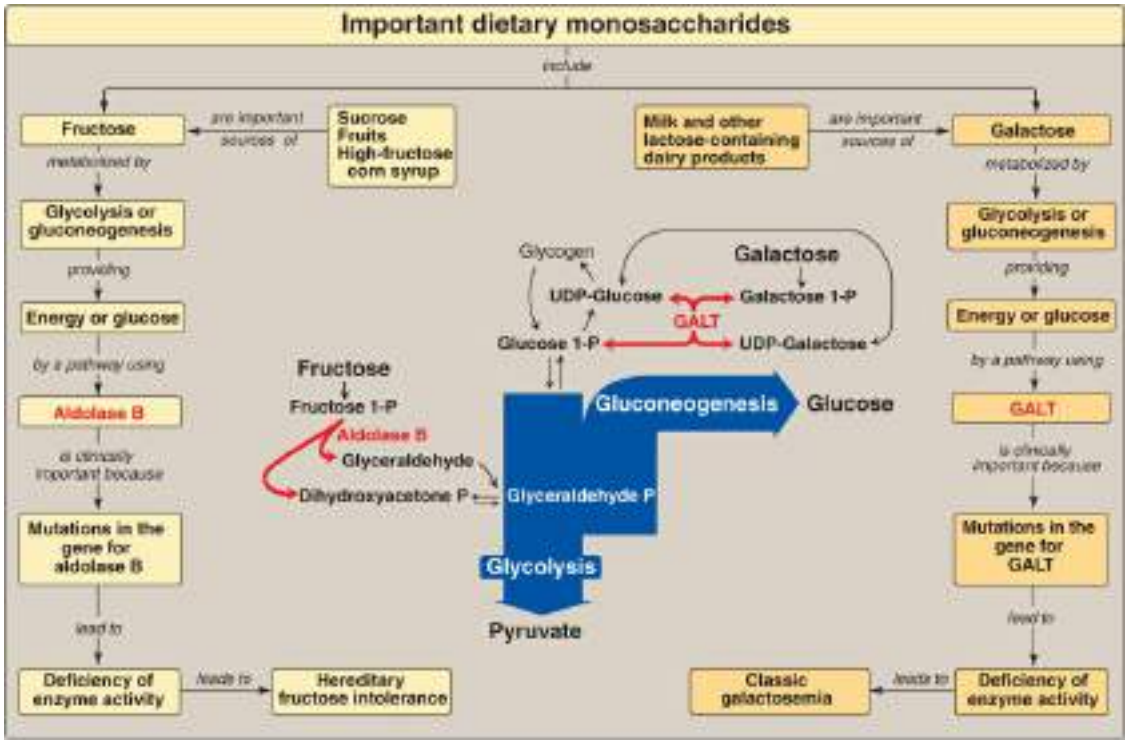


Figure 12.8
Key concept map for metabolism of fructose and galactose. GALT = galactose 1-phosphate uridylyltransferase; UDP = uridine diphosphate; P = phosphate.

Study Questions

Choose the ONE best answer.

12.1 A female with classic galactosemia who is on a galactose-free diet delivers a full-term infant. She is able to produce lactose in her breast milk because:

A. galactose can be produced from fructose by isomerization.
B. galactose can be produced from a glucose metabolite by epimerization.
C. hexokinase can efficiently phosphorylate galactose to galactose 1-phosphate.
D. the enzyme affected in galactosemia is activated by a mammary gland hormone.

Correct answer = B. Uridine diphosphate (UDP)-glucose is converted to UDP-galactose by UDP-hexose 4-epimerase, thereby providing the appropriate form of galactose for lactose synthesis. Isomerization of fructose to galactose does not occur in the human body. Galactose is not converted to galactose 1-phosphate by hexokinase. A galactose-free diet provides no galactose. Galactosemia is the result of an enzyme (galactose 1-phosphate uridylyltransferase) deficiency.

12.2 A 6-month-old male child is brought to his pediatrician because of vomiting, night sweats, and tremors. History reveals that these symptoms began after fruit juices were introduced to his diet as he was being weaned off breast milk. The physical examination was remarkable for hepatomegaly. Tests on his urine were positive for reducing sugar but negative for glucose. The infant most likely has a deficiency of:

A. aldolase B.
B. fructokinase.
C. galactokinase.
D. β-galactosidase.

Correct answer = A. The symptoms suggest hereditary fructose intolerance, a deficiency in aldolase B. Deficiencies in fructokinase or galactokinase result in relatively benign conditions characterized by elevated levels of fructose or galactose in the blood and urine. Deficiency in β-galactosidase (lactase) results in a decreased ability to degrade lactose (milk sugar). Congenital lactase deficiency is quite rare and would have presented much earlier in this baby (and with different symptoms). Typical lactase deficiency (adult lactose intolerance) presents at a later age.

12.3 In lactose synthesis:

A. α-lactalbumin expression is decreased by the hormone prolactin.
B. galactosyltransferase catalyzes transfer of galactose from galactose 1-phosphate to glucose.
C. protein A is used exclusively.
D. α-lactalbumin decreases the affinity of protein A for glucose.
E. protein B expression is stimulated by prolactin.

Correct answer = D. α-Lactalbumin (protein B) expression is increased by the hormone prolactin. Uridine diphosphate–galactose is the form used by the galactosyltransferase (protein A). Protein A is also involved in the synthesis of the amino sugar N-acetyllactosamine. Protein B decreases the Michaelis constant (K_m) and, so, increases the affinity of protein A for glucose.

12.4 A 3-month-old child is evaluated for cloudiness of her eyes. Her physical examination reveals cataracts. Other than not having a social smile or being able to track objects visually, all other aspects of her examination are normal. Tests on her urine are positive for reducing sugar but negative for glucose. Which enzyme is most likely deficient in this child?

A. Aldolase B
B. Fructokinase
C. Galactokinase
D. Galactose 1-phosphate uridylyltransferase

Correct answer = C. The child is deficient in galactokinase and is unable to appropriately phosphorylate galactose. Galactose accumulates in the blood (and urine). In the lens of the eye, galactose is reduced by aldose reductase to galactitol, a sugar alcohol, which causes osmotic effects that result in cataract formation. Deficiency of galactose 1-phosphate uridylyltransferase also results in cataracts but is characterized by liver damage and neurologic effects. Fructokinase deficiency is a benign condition. Aldolase B deficiency is severe, with effects on several tissues but cataracts are not typically seen.

12.5 In a person with elevated blood glucose and an adequate supply of NADPH, which of the following will be produced in high concentration and then remain trapped in the cell?

A. fructose
B. galactose
C. lactose
D. sorbitol
E. sucrose

Correct answer = D. Sorbitol will be elevated in this situation. An elevated intracellular glucose concentration and an adequate supply of reduced NADPH cause aldose reductase to produce a significant increase in sorbitol, which cannot pass efficiently through cell membranes and, therefore, remains trapped inside the cell. Sorbitol trapped in the cells then contributes to complications of diabetes mellitus including cataract formation, peripheral neuropathy, and microvascular problems.

Pentose Phosphate Pathway and Nicotinamide Adenine Dinucleotide Phosphate 13

I. OVERVIEW

The pentose phosphate pathway, also known as the hexose monophosphate shunt, provides ribose 5-phosphate for the biosynthesis of nucleotides and is important as the body's main source of nicotinamide adenine dinucleotide phosphate (NADPH), a biochemical reductant. NADPH is the cellular source of reducing equivalents for biosynthesis of fatty acids and cholesterol and for the reduction of hydrogen peroxide (H_2O_2) formed in response to oxidative stress and as a byproduct of aerobic metabolism. Glucose-6 phosphate dehydrogenase (G6PD) catalyzes the first, rate-limiting step of the pathway; X-linked inheritance of G6PD deficiency results in insufficient NADPH, particularly in red blood cells, making them susceptible to lysis in response to oxidant stress. The pathway does not produce or consume ATP.

Reactions of this pathway occur in the cytosol and include an irreversible oxidative phase, followed by a series of reversible sugar–phosphate interconversions (Fig. 13.1). In the oxidative phase, carbon 1 of a glucose 6-phosphate molecule is released as carbon dioxide (CO_2), and one pentose sugar–phosphate plus two reduced NADPHs are produced. The rate and direction of the reversible reactions are determined by the supply of and demand for intermediates of the pathway. The pentose phosphate pathway also produces ribose 5-phosphate, required for nucleotide biosynthesis (see also Chapter 22 III.), and provides a mechanism for the conversion of pentose sugars to triose and hexose intermediates of glycolysis.

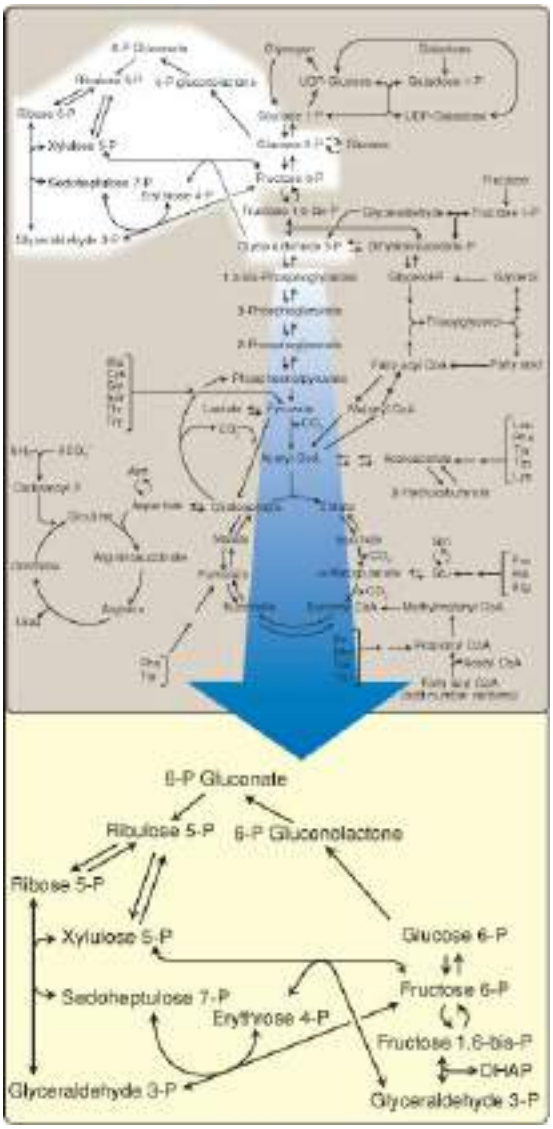


Figure 13.1
Pentose phosphate pathway shown as a component of the metabolic map. (Note: See Fig. 8.2, for a more detailed map of metabolism.) P = phosphate; DHAP = dihydroxyacetone phosphate.

II. IRREVERSIBLE OXIDATIVE REACTIONS

The oxidative portion of the pentose phosphate pathway consists of three irreversible reactions that lead to the formation of ribulose 5-phosphate, CO_2, and two molecules of NADPH for each molecule of glucose 6-phosphate oxidized (Fig. 13.2). This portion of the pathway is particularly important in the liver, lactating mammary glands, and adipose tissue for the NADPH-dependent biosynthesis of fatty acids (see also Chapter 15 III.); in the testes, ovaries, placenta, and adrenal cortex for the NADPH-dependent biosynthesis of steroid hormones (see also Chapter 18); and in red blood cells for the NADPH-dependent reduction of glutathione.

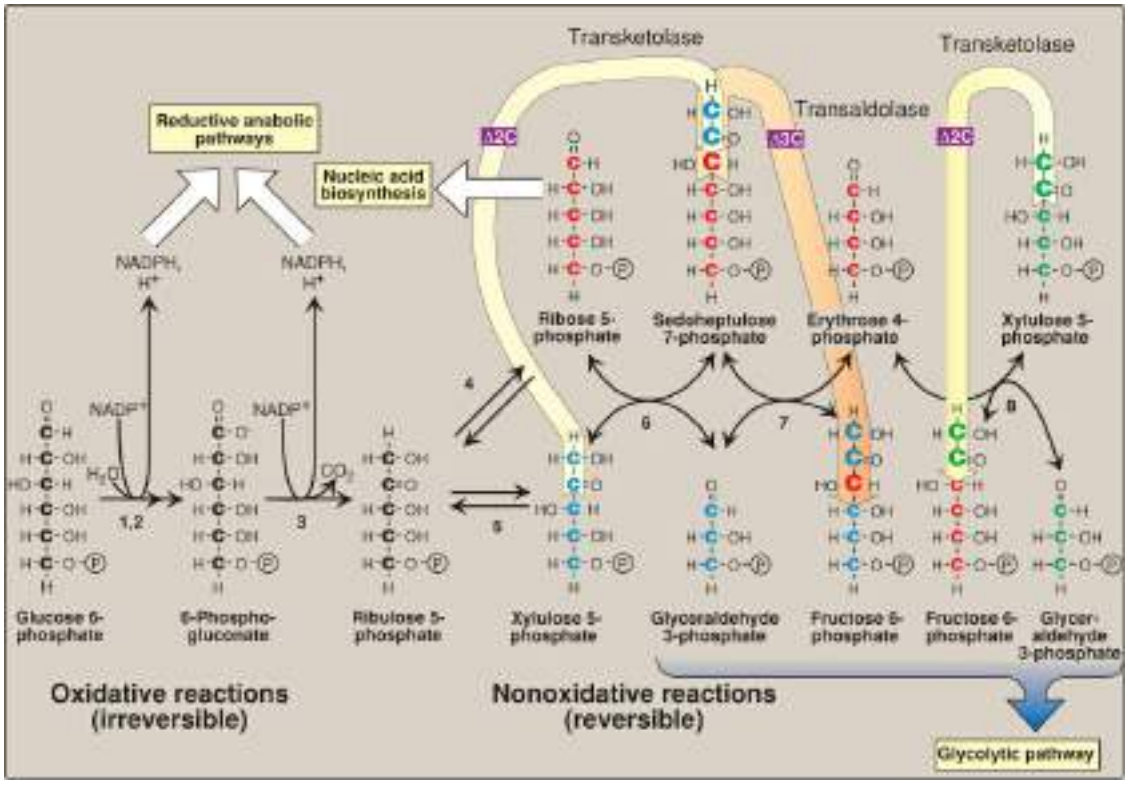


Figure 13.2
Reactions of the pentose phosphate pathway. Enzymes numbered above are: (1, 2) glucose 6-phosphate dehydrogenase and 6-phosphogluconolactone hydrolase, (3) 6-phosphogluconate dehydrogenase, (4) ribose 5-phosphate isomerase, (5) phosphopentose epimerase, (6, 8) transketolase (coenzyme: thiamine pyrophosphate), and (7) transaldolase. Δ2C, two carbons are transferred from a ketose donor to an aldose acceptor in transketolase reactions; Δ3C, three carbons are transferred in the transaldolase reaction. This can be represented as: 5C sugar + 5C sugar → 7C sugar + 3C sugar → 4C sugar + 6C sugar. NADP(H) = nicotinamide adenine dinucleotide phosphate; Ⓟ, phosphate; CO_2, carbon dioxide.

A. Glucose 6-phosphate dehydrogenation

Glucose 6-phosphate dehydrogenase (G6PD) catalyzes the oxidation of glucose 6-phosphate to 6-phosphogluconolactone as the coenzyme $NADP^+$ is reduced to NADPH. This initial reaction is the committed, rate-limiting, and regulated step of the pathway. NADPH is a potent competitive inhibitor of G6PD, and the ratio of $NADPH/NADP^+$ is sufficiently high to substantially inhibit the enzyme under most metabolic conditions. However, with increased demand for NADPH, the ratio of $NADPH/NADP^+$ decreases, and flux through the pathway increases in response to the enhanced activity of G6PD. It should be noted that insulin upregulates expression of the gene for G6PD, and flux through the pathway increases in the absorptive state (see also Chapter 24 III.).

B. Ribulose 5-phosphate formation

6-Phosphogluconolactone is hydrolyzed by 6-phosphogluconolactone hydrolase in the second step. The oxidative decarboxylation of the product, 6-phosphogluconate,

is catalyzed by 6-phosphogluconate dehydrogenase. This third irreversible step produces ribulose 5-phosphate, a pentose sugar–phosphate, CO_2 (from carbon 1 of glucose), and a second molecule of NADPH (see Fig. 13.2).

III. REVERSIBLE NONOXIDATIVE REACTIONS

The nonoxidative reactions of the pentose phosphate pathway occur in all cell types synthesizing nucleotides and nucleic acids. These reactions catalyze the interconversion of sugars containing three to seven carbons (see Fig. 13.2). These reversible reactions permit ribulose 5-phosphate produced by the oxidative portion of the pathway to be converted either to ribose 5-phosphate needed for nucleotide synthesis (see also Chapter 22 III.) or to intermediates of glycolysis, fructose 6-phosphate and glyceraldehyde 3-phosphate.

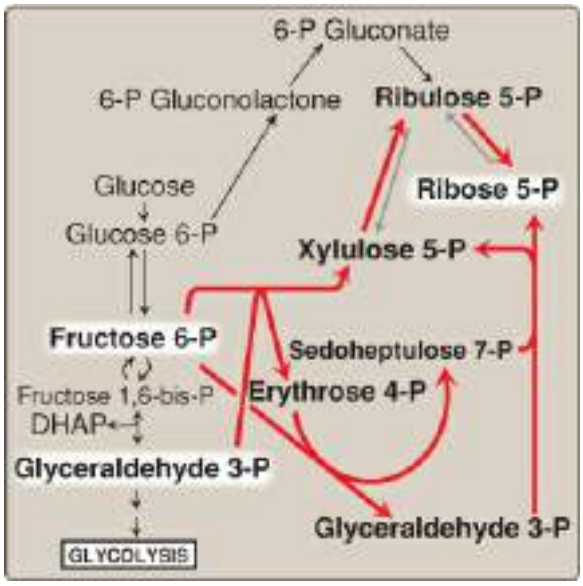


Figure 13.3
Formation of ribose 5-phosphate from intermediates of glycolysis. P = phosphate; DHAP = dihydroxyacetone phosphate.

Many cells that carry out reductive biosynthetic reactions have a greater need for NADPH than for ribose 5-phosphate. In this case, transketolase, which transfers two-carbon units in a thiamine pyrophosphate (TPP)-requiring reaction, and transaldolase, which transfers three-carbon units, convert the ribulose 5-phosphate produced as an end product of the oxidative phase to glyceraldehyde 3-phosphate and fructose 6-phosphate. In contrast, when the demand for ribose for nucleotides and nucleic acids is greater than the need for NADPH, the nonoxidative reactions can provide the ribose 5-phosphate from glyceraldehyde 3-phosphate and fructose 6-phosphate in the absence of the oxidative steps (Fig. 13.3).

In addition to transketolase, TPP is required by the multienzyme complexes pyruvate dehydrogenase (see also Chapter 9 II.), α-ketoglutarate dehydrogenase of the tricarboxylic acid cycle (see also Chapter 9 II.), and branched-chain α-keto acid dehydrogenase of branched-chain amino acid catabolism (see also Chapter 20 III.).

IV. USES OF NADPH

The coenzyme NADPH differs from nicotinamide adenine dinucleotide (NADH) only by the presence of a phosphate group on one of the ribose units (Fig. 13.4). This seemingly small change in structure allows NADPH to interact with NADPH-specific enzymes that have unique roles in the cell. For example, in the cytosol of hepatocytes, the steady-state $NADP^+$/NADPH ratio is ~0.1, which favors the use of NADPH in reductive biosynthetic reactions. This contrasts with the high NAD^+/NADH ratio (~1,000), which favors an oxidative role for NAD^+. This summarizes some important NADPH-specific functions in reductive biosynthesis and detoxification reactions.

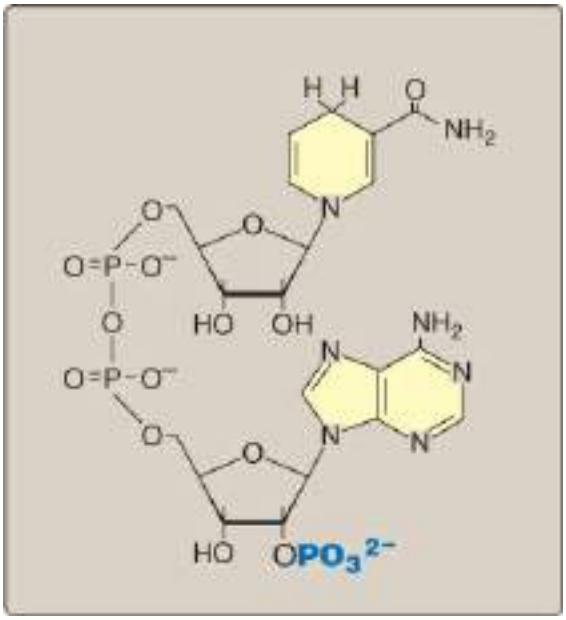


Figure 13.4
Structure of reduced nicotinamide adenine dinucleotide phosphate (NADPH).

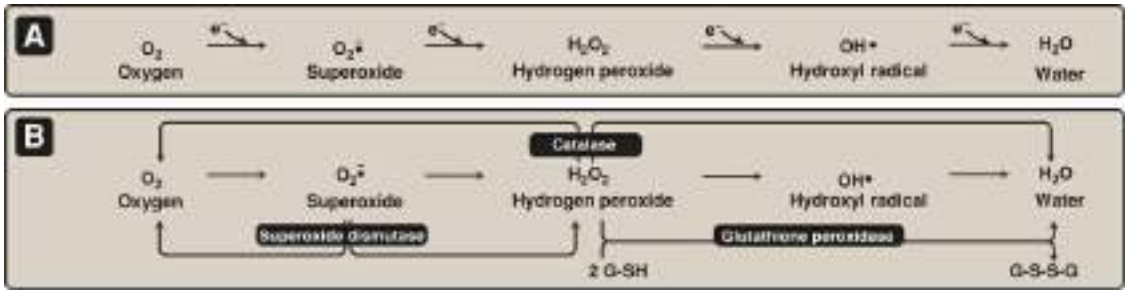


Figure 13.5
A: Formation of reactive intermediates from oxygen. e^- = electrons. **B:** Actions of antioxidant enzymes. G-SH = reduced glutathione; G-S-S-G = oxidized glutathione. (Note: See Fig. 13.6B for the regeneration of G-SH.)

A. Reductive biosynthesis

Like NADH, NADPH can be thought of as a high-energy molecule. However, the electrons of NADPH are used for reductive biosynthesis, rather than for transfer to the electron transport chain as is seen with NADH (see Chapter 6 V.). In the metabolic transformations of the pentose phosphate pathway, part of the energy of glucose 6-phosphate is conserved in NADPH, a molecule with a negative reduction potential (see Chapter 6), which, therefore, can be used in reactions requiring an electron donor, such as fatty acid (see Chapter 16 III.), cholesterol, and steroid hormone synthesis (see also Chapter 18 III. and VII.).

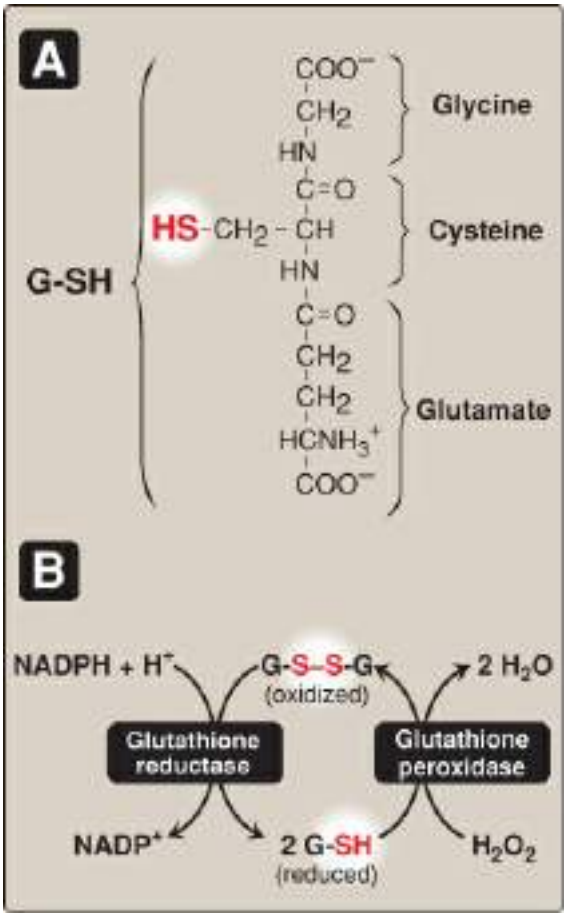


Figure 13.6
A: Structure of reduced glutathione (G-SH). (Note: Glutamate is linked to cysteine through a γ-carboxyl, rather than an α-carboxyl.) **B:** The roles of G-SH and reduced nicotinamide adenine dinucleotide phosphate (NADPH) in the reduction of hydrogen peroxide (H_2O_2) to water. G-S-S-G = oxidized glutathione.

B. Reduction of H_2O_2

H_2O_2 is one of a family of reactive oxygen species (ROS) that are formed from the partial reduction of molecular oxygen, O_2 (Fig. 13.5A). These compounds are generated continuously as byproducts of aerobic metabolism, through reactions with drugs and environmental toxins, or when the level of antioxidants is diminished, all creating the condition of oxidative stress. These highly reactive oxygen intermediates can cause serious chemical damage to DNA, proteins, and unsaturated lipids and can lead to cell death. ROS have been implicated in a number of pathologic processes, including reperfusion injury, cancer, inflammatory disease, and aging. The cell has several protective mechanisms that minimize the toxic potential of these compounds. ROS can also be generated in the killing of microbes by white blood cells (see Section D., on page 165).

1. Enzymes that catalyze antioxidant reactions: Reduced glutathione (G-SH), a tripeptide-thiol (γ-glutamylcysteinylglycine) present in most cells, can chemically detoxify H_2O_2 (Fig. 13.5B). This reaction, catalyzed by glutathione peroxidase, forms oxidized glutathione (G-S-S-G), which no longer has protective properties. The cell regenerates G-SH in a reaction catalyzed by glutathione reductase, using NADPH as a source of reducing equivalents. Thus, NADPH indirectly provides electrons for the reduction of H_2O_2 (Fig. 13.6). Additional enzymes, such as superoxide dismutase and catalase, catalyze the conversion of other ROS to harmless products (see Fig. 13.5B). As a group, these enzymes serve as a defense system to guard against the toxic effects of ROS.

2. Antioxidant chemicals: A number of intracellular reducing agents, such as ascorbate or vitamin C, vitamin E, and β-carotene, are able to reduce and, thereby, detoxify ROS in the laboratory. Consumption of foods rich in these antioxidant compounds has been correlated with a reduced risk for certain types of cancers as well as decreased frequency of certain other chronic health problems. Therefore, it is tempting to speculate that the effects of these compounds are, in part, an expression of their ability to quench the toxic effect of ROS. However, clinical trials with antioxidants as dietary supplements have failed to show clear beneficial effects. In the case of dietary supplementation with β-carotene, the rate of lung cancer in smokers increased rather than decreased. Thus, the health-promoting effects of dietary fruits and vegetables likely reflect a complex interaction among many naturally occurring compounds, which has not been duplicated by consumption of isolated antioxidant compounds (see also Chapter 28).

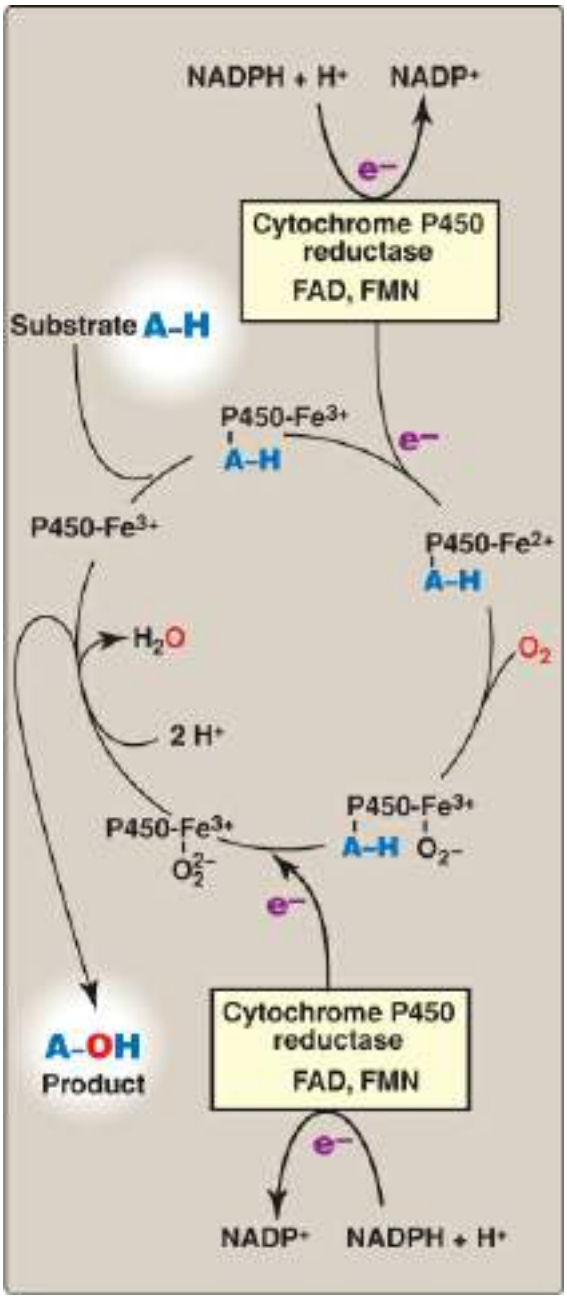


Figure 13.7

Cytochrome P450 (CYP) monooxygenase catalytic cycle (simplified). Electrons (e^-) move from nicotinamide adenine dinucleotide phosphate (NADPH) to flavin adenine dinucleotide (FAD) to flavin adenine mononucleotide (FMN) of the reductase and then to the heme iron (Fe) of the microsomal CYP enzyme. (Note: In the mitochondrial system, e^- move from FAD to an iron–sulfur protein and then to the CYP enzyme.)

C. Cytochrome P450 monooxygenase system

Monooxygenases (mixed-function oxidases) incorporate one atom from O_2 into a substrate (creating a hydroxyl group), with the other atom being reduced to water (H_2O). In the cytochrome P450 (CYP) monooxygenase system, NADPH provides

the reducing equivalents required by this series of reactions (Fig. 13.7). This system performs different functions in two separate locations in cells. The overall reaction catalyzed by a CYP enzyme is

$$R{-}H + O_2 + NADPH + H^+ \rightarrow R{-}OH + H_2O + NADP^+$$

where R may be a steroid, drug, or other chemical. CYP enzymes are actually a superfamily of related, heme-containing monooxygenases that participate in a broad variety of reactions. The P450 in the name reflects the absorbance at 450 nm by the protein.

1. Mitochondrial system: An important function of the CYP monooxygenase system found associated with the inner mitochondrial membrane is the biosynthesis of steroid hormones. In steroidogenic tissues, such as the placenta, ovaries, testes, and adrenal cortex, it is used to hydroxylate intermediates in the conversion of cholesterol to steroid hormones, a process that makes these hydrophobic compounds more water soluble (see Chapter 18 VII.). The liver uses this same system in bile acid synthesis (see Chapter 18 V.) and the hydroxylation of cholecalciferol to 25-hydroxycholecalciferol (vitamin D_3; see Chapter 28 XII.), and the kidney uses it to hydroxylate vitamin D_3 to its biologically active 1,25-dihydroxylated form.

2. Microsomal system: The microsomal CYP monooxygenase system found associated with the membrane of the smooth endoplasmic reticulum, particularly in the liver, functions primarily in the detoxification of foreign compounds or xenobiotics. These include numerous drugs and such varied pollutants as petroleum products and pesticides. CYP enzymes of the microsomal system, for example, CYP3A4, can be used to hydroxylate these toxins (phase I). The purpose of these modifications is twofold. First, it may itself activate or inactivate a drug and second, make a toxic compound more soluble, thereby facilitating its excretion in the urine or feces. Frequently, however, the new hydroxyl group will serve as a site for conjugation with a polar molecule, such as glucuronic acid (see Chapter 14 III.), which will significantly increase the compound's solubility (phase II). It should be noted that polymorphisms (see Chapter 34) in the genes for CYP enzymes can lead to differences in drug metabolism.

D. White blood cell phagocytosis and microbe killing

Phagocytosis is the ingestion by receptor-mediated endocytosis of microorganisms, foreign particles, and cellular debris by leukocytes such as neutrophils and macrophages (monocytes). It is an important defense mechanism, particularly in bacterial infections. Neutrophils and monocytes are armed with both oxygen-independent and oxygen-dependent mechanisms for killing bacteria.

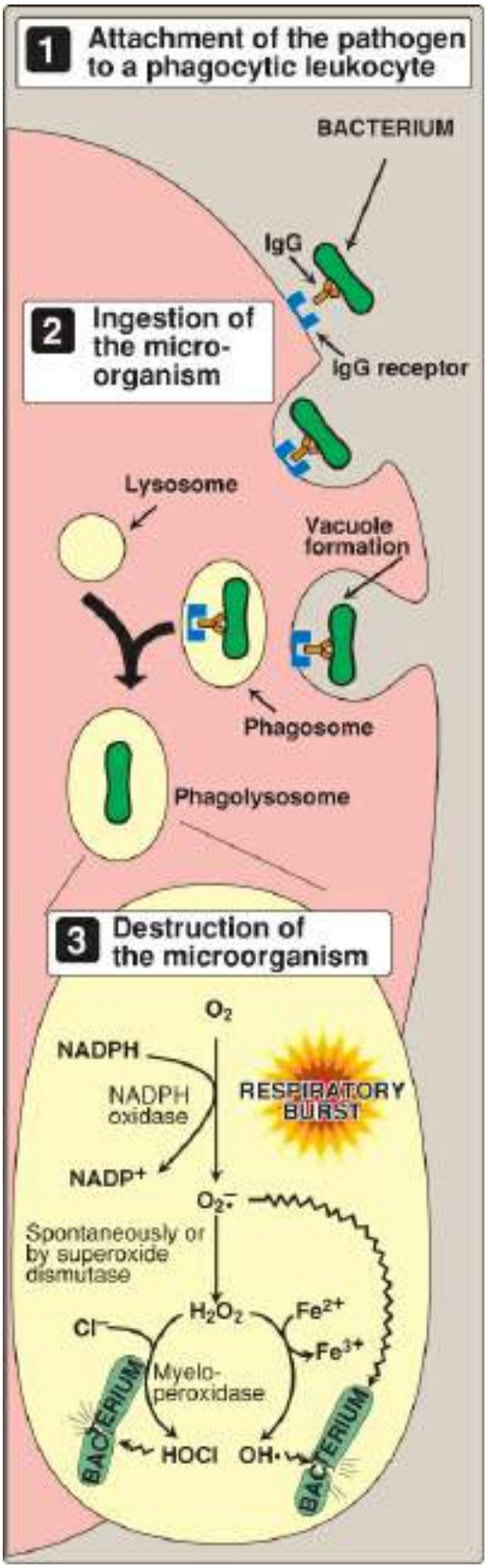


Figure 13.8
Phagocytosis and the oxygen (O_2)-dependent pathway of microbial killing. IgG = immunoglobulin G; NADP(H) = nicotinamide adenine dinucleotide phosphate; O_2^- = superoxide; H_2O_2 = hydrogen peroxide; HOCl = hypochlorous acid; OH• = hydroxyl radical

1. Oxygen independent: Oxygen-independent mechanisms use pH changes in phagolysosomes and lysosomal enzymes to destroy pathogens.

2. Oxygen dependent: Oxygen-dependent mechanisms include the enzymes NADPH oxidase and myeloperoxidase (MPO) that work together in killing bacteria (Fig. 13.8). Overall, the MPO system is the most potent of the bactericidal mechanisms. An invading bacterium is recognized by the immune system and attacked by antibodies that bind it to a receptor on a phagocytic cell. After internalization of the microorganism has occurred, NADPH oxidase, located in the leukocyte cell membrane, is activated and reduces O_2 from the surrounding tissue to superoxide (O_2^-), a free radical ROS, as NADPH is oxidized. The rapid consumption of O_2 that accompanies its formation is referred to as the respiratory burst. (Note: Active NADPH oxidase is a membrane-associated complex containing a flavocytochrome plus additional peptides that translocate from the cytoplasm upon activation of the leukocyte. Electrons move from NADPH to O_2 via flavin adenine nucleotide [FAD] and heme, generating O_2^-.])

   Rare genetic deficiencies in NADPH oxidase cause chronic granulomatous disease (CGD) characterized by severe, persistent infections and the formation of granulomas (nodular areas of inflammation) that sequester the bacteria that were not destroyed. Next, O_2^- is converted to H_2O_2 (also a ROS), either spontaneously or catalyzed by superoxide dismutase. In the presence of MPO, a heme-containing lysosomal enzyme present within the phagolysosome, peroxide plus chloride ions are converted to hypochlorous acid, HOCl, the major component of household bleach, which kills the bacteria. The peroxide can also be partially reduced to the hydroxyl radical (OH•), an ROS, or be fully reduced to H_2O by catalase or glutathione peroxidase. Deficiencies in MPO do not confer increased susceptibility to infection because peroxide from NADPH oxidase is bactericidal.

E. Nitric oxide synthesis

Nitric oxide (NO) is recognized as a mediator in a broad array of biologic systems. NO is the endothelium-derived relaxing factor that causes vasodilation by relaxing vascular smooth muscle. It also acts as a neurotransmitter, prevents platelet aggregation, and plays an essential role in macrophage function. It has a very short half-life in tissues (3 to 10 seconds) because it reacts with O_2 and is converted into nitrates and nitrites including peroxynitrite ($O = NOO^-$), a reactive nitrogen species

(RNS). Note that NO is a free radical gas that is often confused with nitrous oxide (N_2O), the "laughing gas" that is used as an anesthetic and is chemically stable.

1. Nitric oxide synthase: Arginine, O_2, and NADPH are substrates for cytosolic NO synthase ([NOS], Fig. 13.9). Flavin mononucleotide (FMN), FAD, heme, and tetrahydrobiopterin (see Chapter 20 V.) are coenzymes, and NO and citrulline are products of the reaction. Three NOS isozymes, each the product of a different gene, have been identified. Two are constitutive (synthesized at a constant rate), calcium (Ca^{2+})–calmodulin (CaM)-dependent enzymes (see Chapter 11 V.). They are found primarily in endothelium (eNOS) and neural tissue (nNOS) and constantly produce very low levels of NO for vasodilation and neurotransmission. An inducible, Ca^{2+}-independent enzyme (iNOS) can be expressed in many cells, including macrophages and neutrophils, as an early defense against pathogens. The specific inducers for *iNOS* vary with cell type and include proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ), and bacterial endotoxins such as lipopolysaccharide (LPS). These compounds promote synthesis of iNOS, which can result in large amounts of NO being produced over hours or even days.

2. Nitric oxide and vascular endothelium: NO is an important mediator in the control of vascular smooth muscle tone. NO is synthesized by eNOS in endothelial cells and diffuses to vascular smooth muscle, where it activates the cytosolic form of guanylyl cyclase (or, guanylate cyclase) to form cyclic guanosine monophosphate (cGMP). This reaction is analogous to the formation of cyclic adenosine monophosphate (cAMP) by adenylyl cyclase (see Chapter 8 II. D.). The resultant rise in cGMP causes activation of protein kinase G, which phosphorylates Ca^{2+} channels, causing decreased entry of Ca^{2+} into smooth muscle cells. This decreases the Ca^{2+}–CaM activation of myosin light-chain kinase, thereby decreasing smooth muscle contraction and favoring relaxation.

   Vasodilator nitrates, such as nitroglycerin, are metabolized to NO, which causes relaxation of vascular smooth muscle and, therefore, lowers blood pressure. Thus, NO can be envisioned as an endogenous nitrovasodilator. Note that under hypoxic conditions, nitrite (NO_2^-) can be reduced to NO, which binds to deoxyhemoglobin. The NO is released into the blood, causing vasodilation and increasing blood flow.

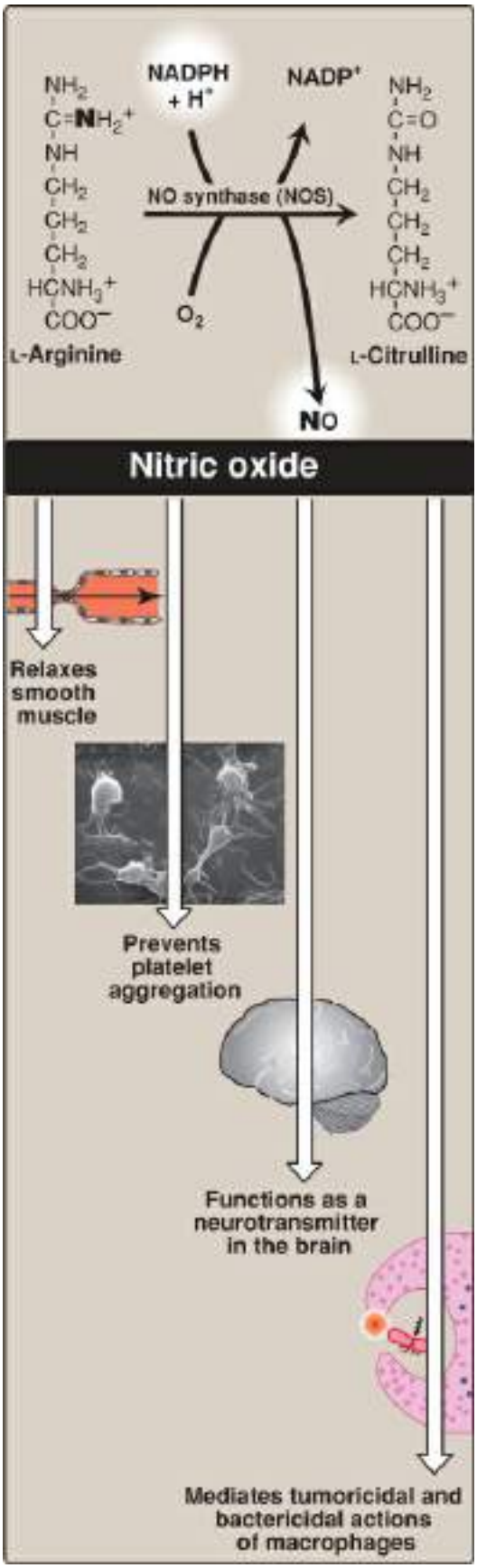


Figure 13.9
Synthesis and some actions of nitric oxide (NO). (Note: Flavin mononucleotide, flavin adenine dinucleotide, heme, and tetrahydrobiopterin are additional coenzymes required by *NOS*.) NADP(H) = nicotinamideadenine dinucleotide phosphate.

3. Nitric oxide and macrophage bactericidal activity: In macrophages, iNOS activity is normally low, but synthesis of the enzyme is significantly stimulated by bacterial LPS and by release of IFN-γ and TNF-α in response to infection. Activated macrophages form radicals that combine with NO to form intermediates that decompose, producing the highly bactericidal OH• radical.

4. Additional functions: NO is a potent inhibitor of platelet adhesion and aggregation (by activating the cGMP pathway). It is also characterized as a neurotransmitter in the central and peripheral nervous systems.

V. G6PD DEFICIENCY

Deficiency of G6PD, a hereditary condition that affects mostly males, is characterized by hemolytic anemia when the affected individual is exposed to an oxidant stress. The anemia is caused by the inability of red blood cells (erythrocytes) to detoxify oxidizing agents. With G6PD deficiency, less NADPH is available to maintain a pool of reduced glutathione to detoxify H_2O_2 generated in response to oxidant stress.

A. G6PD role in erythrocytes

Adequate G6PD activity is required for cells to form NADPH essential for the maintenance of the G-SH pool. Although G6PD deficiency occurs in all cells of the affected individual, it is most severe in erythrocytes, where the pentose phosphate pathway provides the only means of generating NADPH. Additionally, since red blood cells have no nucleus or ribosomes they cannot renew their supply of the enzyme, leaving erythrocytes particularly vulnerable to enzyme variants with diminished stability. Other tissues have an alternative pathway to produce NADPH (via $NADP^+$-dependent malate dehydrogenase [malic enzyme]; see Chapter 16 III.).

Clinical Application 13.1: Characteristics of G6PD Deficiency

Inherited as an X-linked trait, G6PD deficiency affects mostly males and is the most common disease-producing enzyme abnormality in humans. More than 400 million individuals are affected worldwide. This enzyme deficiency has the highest prevalence in persons whose ancestries come from the Middle East, tropical Africa and Asia, and parts of the Mediterranean. G6PD deficiency is actually a family of deficiencies caused by a number of different mutations in the G6PD gene. Only some of the resulting protein variants cause clinical symptoms.

In addition to periodic bouts of hemolytic anemia in response to oxidant stress, a common clinical manifestation of G6PD deficiency is neonatal jaundice appearing 1 to 4 days after birth. The jaundice, which may be severe, typically results from increased production of unconjugated bilirubin (see Chapter 21 II.). The lifespan of individuals with a severe form of G6PD deficiency may be somewhat shortened as a result of complications arising from chronic hemolysis. This negative effect of G6PD deficiency has been

balanced in evolution by an increased resistance to malaria caused by *Plasmodium falciparum*. Infection of red blood cells by the parasite induces oxidant stress, resulting of lysis of the red blood cells, and protecting the host from developing malaria.

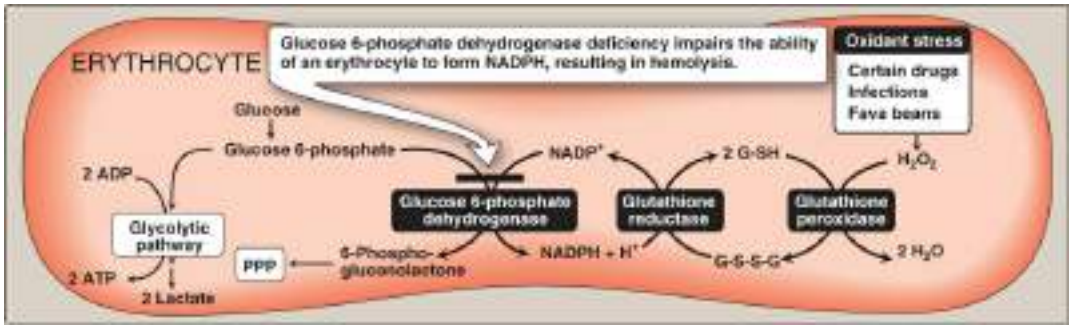


Figure 13.10
Pathways of glucose 6-phosphate metabolism in the erythrocyte. NADP(H) = nicotinamide adenine dinucleotide phosphate; G-SH = reduced glutathione; G-S-S-G = oxidized glutathione; H_2O_2 = hydrogen peroxide; PPP = pentose phosphate pathway.

Deficiency of G6PD impairs the process of detoxification of free radicals and peroxides formed within the cell (Fig. 13.10). G-SH also helps maintain the reduced states of sulfhydryl groups in proteins, including hemoglobin. Oxidation of those sulfhydryl groups leads to the formation of denatured proteins that form insoluble masses called Heinz bodies that attach to red cell membranes (Fig. 13.11). Additional oxidation of membrane proteins causes erythrocyte membranes to be rigid (less deformable), and they are removed from the circulation by macrophages in the spleen and liver.

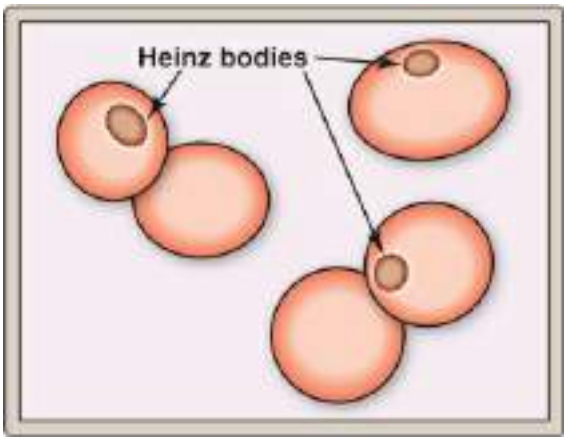


Figure 13.11
A drawing depicting the appearance of Heinz bodies in the erythrocytes of a patient with glucose 6-phosphate dehydrogenase deficiency.

B. Precipitating factors in G6PD deficiency

Male individuals who inherit a *G6PD* mutation on their lone X chromosome are considered to be hemizygous for the G6PD deficiency trait since they have only one

X chromosome. Affected individuals will normally remain asymptomatic unless or until they experience a strong oxidant stress, which may be from treatment with an oxidant drug, ingestion of fava beans, or a severe infection. Lysis of red blood cells and hemolytic anemia results in G6PD-deficient individuals in response to oxidant stress-inducing agents.

1. Oxidant drugs: Drugs that can cause oxidant stress and produce hemolytic anemia in patients with G6PD deficiency are often in categories that begin with the letter A: some *antibiotics* (particularly sulfa drugs), some *antimalarials*, some *analgesics*, and some *antipyretics*. Only certain drugs in each category are implicated. Drug lists are available for prescribers that include usually safe agents and those best avoided by G6PD-deficient individuals.

2. Favism: Persons with some forms of G6PD deficiency, especially the Mediterranean variant, are particularly susceptible to the hemolytic effect of the fava or broad bean, a dietary staple in the Mediterranean region. Favism, the hemolytic effect of ingesting fava beans, is not observed in all individuals with G6PD deficiency, but all patients with favism do have G6PD deficiency.

3. Infection: Infection is a common precipitating factor of hemolysis in persons with G6PD deficiency. The inflammatory response to infection results in the generation of free radicals in macrophages. The radicals can diffuse into red blood cells and cause oxidative damage.

C. *G6PD* gene variants

The cloning and sequencing of the *G6PD* gene (see Chapter 34) have led to identification of more than 400 *G6PD* variants that result in G6PD enzyme deficiency. Some mutations do not affect enzymatic activity. Most mutations that do result in low G6PD enzyme function are missense point mutations (see Chapter 32 II.); some cause decreased catalytic activity, others decreased stability while other *G6PD* mutations alter the binding affinity for $NADP^+$ or glucose 6-phosphate. Active G6PD enzyme exists as a homodimer or tetramer. Mutations at the interface between subunits can affect enzyme stability.

Class	Clinical symptoms	Residual enzyme activity
I	Very severe (chronic, nonspher-ocytic hemolytic anemia)	<10%
*II	Severe (acute hemolytic anemia)	<10%
*III	Moderate	10%–60%
IV	None	>60%

Figure 13.12
Classification of glucose 6-phosphate dehydrogenase (G6PD) deficiency variants. (Note: Class V variants (not shown) result in overproduction of G6PD.) * = most common.

The severity of hemolytic anemia in those with G6PD deficiency usually correlates with the amount of residual enzyme activity in the patient's red blood cells. G6PD variants can be classified as shown in Figure 13.12. G6PD A^- is the prototype of the moderate (class III) form of the disease. Red blood cells contain an unstable but kinetically normal G6PD, with most of the enzyme activity present in the reticulocytes and younger red cells (Fig. 13.13). The oldest red blood cells have the lowest level of G6PD activity and are preferentially removed in a hemolytic episode. Because hemolysis does not affect younger cells, the episodes are self-limiting. G6PD Mediterranean is the prototype of a more severe (class II) deficiency. Class I mutations (rare) are the most severe and are associated with chronic nonspherocytic hemolytic anemia, even in the absence of oxidative stress.

Both G6PD A^- and G6PD Mediterranean proteins represent mutant enzymes that differ from the respective normal variants by a single amino acid. Large deletions or frameshift mutations have not been identified, suggesting that complete absence of G6PD enzyme activity is most likely lethal.

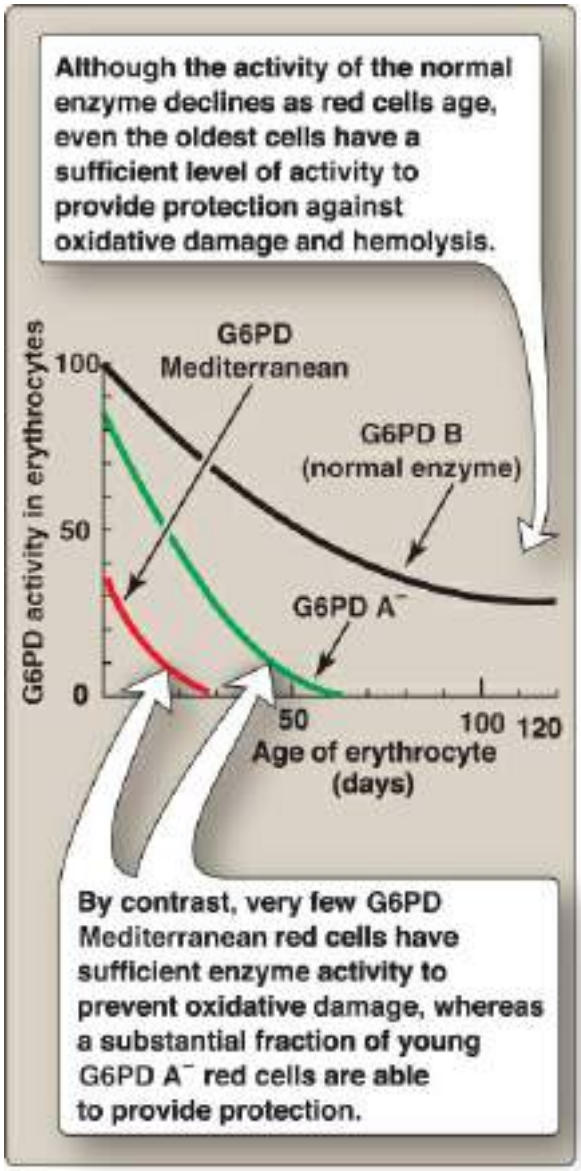


Figure 13.13
Decline of erythrocyte glucose 6-phosphate dehydrogenase (G6PD) activity with cell age for the three most commonly encountered forms of the enzyme.

VI. Chapter Summary

- The **pentose phosphate pathway** is the main producer of **NADPH** in the body (Fig. 13.14).
- **No ATP** is used or consumed in the pathway.
- The pathway includes an irreversible oxidative phase followed by a series of reversible sugar–phosphate interconversions.
- **Reversible nonoxidative reactions** interconvert sugars. Ribulose 5-phosphate is converted to **ribose 5-phosphate**, required for nucleotide and nucleic acid synthesis, or to **fructose 6-phosphate** and **glyceraldehyde 3-phosphate** (glycolytic intermediates).
- The NADPH-producing **oxidative portion** of the pathway provides reducing equivalents for reductive biosynthesis and detoxification reactions.
- In this part of the pathway, **glucose 6-phosphate** is irreversibly converted to **ribulose 5-phosphate**, and **two NADPHs** are produced. The regulated step is catalyzed by **G6PD**, which is strongly inhibited by a rise in the **NADPH/$NADP^+$ ratio**.
- NADPH is a source of **reducing equivalents** in **reductive biosynthesis**, such as the production of fatty acids in liver, adipose tissue, and the mammary gland; cholesterol in the liver; and steroid hormones in the placenta, ovaries, testes, and adrenal cortex.
- NADPH is also required by erythrocytes for reduction of H_2O_2 produced as a consequence of aerobic metabolism.
- **G-SH** is used by **glutathione peroxidase** to reduce the peroxide to water. The **oxidized glutathione** (**G-S-S-G**) produced is reduced by **glutathione reductase**, using NADPH as the source of electrons.
- NADPH provides reducing equivalents for the **mitochondrial cytochrome P450 monooxygenase system**, which is used in **steroid hormone synthesis** in steroidogenic tissue, **bile acid synthesis** in the liver, and **vitamin D activation** in the liver and kidneys.
- The **microsomal system** uses NADPH to **detoxify** xenobiotics, such as drugs and a variety of pollutants. NADPH provides the reducing equivalents for phagocytes involved in eliminating invading microorganisms. **NADPH oxidase** uses molecular oxygen (O_2) and electrons from NADPH to produce **superoxide radicals**, which, in turn, can be converted to peroxide by **superoxide dismutase**.
- **G6PD deficiency, an X-linked disease that affects mostly males,** impairs **erythrocyte** ability to form NADPH essential for maintaining the G-SH pool. Erythrocytes are most affected because they do not have additional sources of NADPH. It is characterized by **hemolytic anemia** caused by the production of free radicals and peroxides after exposure to oxidant stress, including severe infection, **oxidant drugs, or fava beans**. The extent of the anemia depends on the amount of residual enzyme. Neonates with G6PD deficiency may experience prolonged **neonatal jaundice**.

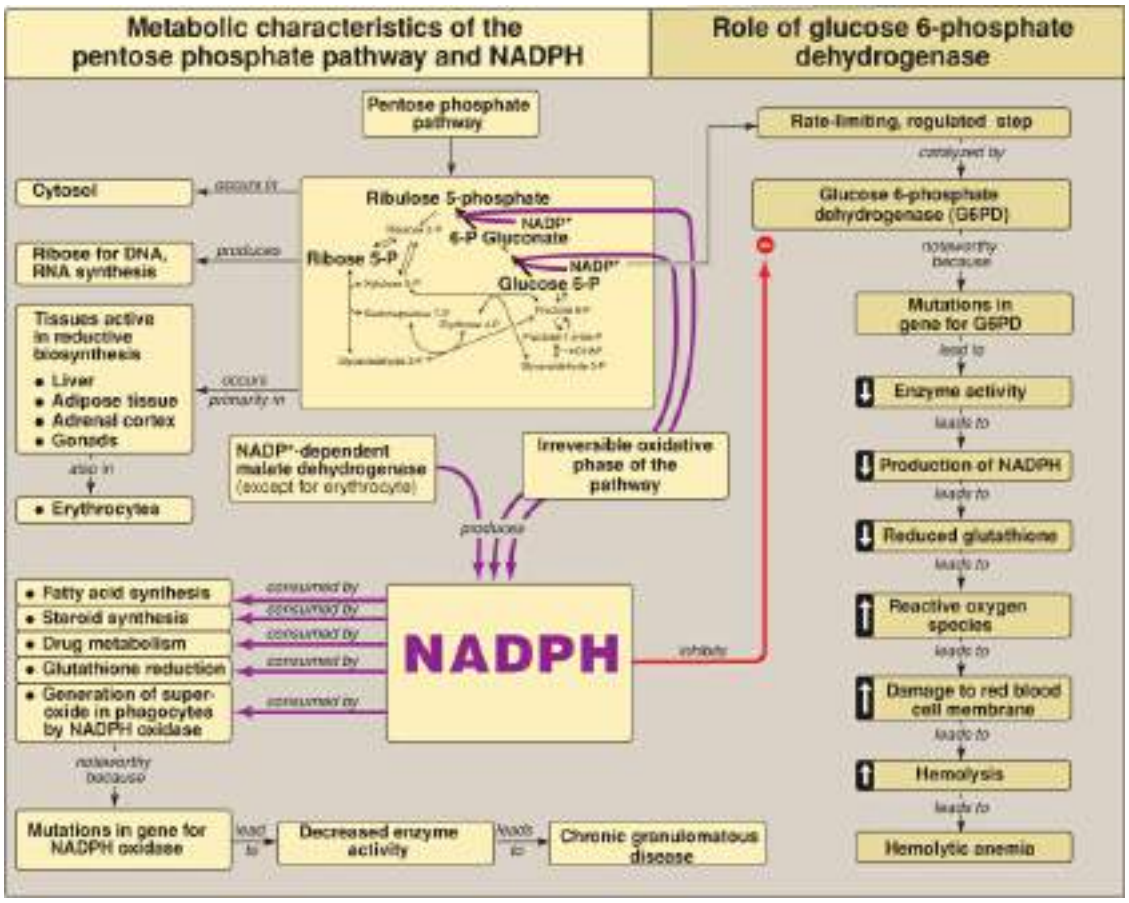


Figure 13.14
Key concept map for the pentose phosphate pathway and nicotinamide adenine dinucleotide phosphate (NADPH).

Study Questions

Choose the ONE best answer.

13.1 In preparation for a trip to an area of India, a young male is given an antimalarial drug prophylactically. Several days after initiation of this therapy he develops jaundice and is diagnosed with anemia. A low level of which of the following is a consequence of the most likely enzyme deficiency and the underlying cause of the patient's presentation?

A. Glucose 6-phosphate
B. Oxidized form of nicotinamide adenine dinucleotide
C. Reduced form of glutathione
D. Ribose 5-phosphate

Correct answer = C. Glutathione (G-SH) is essential for red cell integrity and is maintained in this reduced (functional) form by nicotinamide adenine dinucleotide phosphate (NADPH)-dependent glutathione reductase. The NADPH is from the oxidative portion of the pentose phosphate pathway. Individuals with a deficiency of the regulated enzyme of this pathway, glucose 6-phosphate dehydrogenase (G6PD), have a decreased ability to generate NADPH and, therefore, a decreased ability to keep G-SH reduced. When treated with some antimalarials that induce an oxidant stress, some patients with G6PD deficiency develop a hemolytic anemia. Levels of glucose 6-phosphate are not altered. Nicotinamide adenine dinucleotide (NAD[H]) is neither produced by the pathway nor used as a coenzyme by G-SH reductase. A decrease in ribose 5-phosphate does not cause

hemolysis.

13.2 Low blood pressure (hypotension), is a sign of septic shock, resulting from a severe inflammatory response to a bacterial infection. Based on this information, a likely cause of this hypotension is:

A. Activation of endothelial nitric oxide synthase causing a decrease in nitric oxide.
B. The long half-life of nitric oxide promotes long-term, excess vasoconstriction.
C. Lysine, the nitrogen source for nitric oxide synthesis, is deaminated by bacteria.
D. Bacterial endotoxin promoting iNOS synthesis causing increased NO production.

Correct answer = D. Overproduction of short-lived nitric oxide (NO) by calcium-independent, inducible nitric oxide synthase (iNOS) results in excessive vasodilation, leading to hypotension. The endothelial enzyme (eNOS) is constitutive and produces low levels of NO at a consistent rate. NOS uses arginine, not lysine, as the source of the nitrogen.

13.3 An individual who has recently been prescribed a drug (atorvastatin) to lower cholesterol levels is advised to limit consumption of grapefruit juice, because high intake of the juice reportedly results in an increased level of the drug in the blood, increasing the risk of side effects. Atorvastatin is a substrate for the cytochrome P450 enzyme CYP3A4, and grapefruit juice inhibits the enzyme. Based on this information, CYP enzymes most likely:

A. Accept electrons from reduced nicotinamide adenine dinucleotide.
B. Catalyze the hydroxylation of hydrophobic molecules.
C. Differ from nitric oxide synthase in that they contain heme.
D. Function in association with an oxidase.

Correct answer = B. The CYP enzymes hydroxylate hydrophobic compounds, making them more water soluble. Reduced nicotinamide adenine dinucleotide phosphate (NADPH) from the pentose phosphate pathway is the electron donor. Both the CYP enzymes and the nitric oxide synthase isozymes contain heme.

13.4 In males who are hemizygous for glucose 6-phosphate dehydrogenase deficiency, pathophysiologic consequences are more apparent in red blood cells than in other cells such as in the liver. The best explanation for these findings is that:

A. Excess glucose 6-phosphate in the liver, but not in red blood cells, can be channeled to glycogen, thereby averting cellular damage.
B. Liver cells, in contrast to red blood cells, have alternative mechanisms for supplying the reduced nicotinamide adenine dinucleotide phosphate required for maintaining cell integrity.
C. Red blood cell production of ATP required to maintain cell integrity depends exclusively on the shunting of glucose 6-phosphate to the pentose phosphate pathway.
D. In contrast to liver cells, red cell glucose 6-phosphatase activity decreases the level of glucose 6-phosphate, resulting in cell damage.

Correct answer = B. Cellular damage is directly related to decreased ability of the cell to regenerate reduced glutathione, for which large amounts of reduced nicotinamide adenine dinucleotide phosphate (NADPH) are needed, and red blood cells have no means other than the pentose phosphate pathway of generating NADPH. It is decreased product (NADPH), not increased substrate (glucose 6-phosphate), that is the problem. Red blood cells do not have glucose 6-phosphatase. The pentose phosphate pathway does not generate ATP.

13.5 An essential coenzyme for several enzymes of metabolism is derived from the vitamin thiamine. The thiamine status in the body can be determined using a measurement of the activity of which enzyme?

A. Transketolase
B. Glucose-6-phosphate dehydrogenase
C. Pyruvate dehydrogenase
D. Glutathione peroxidase

Correct answer = B. Red blood cells do not have mitochondria and, so, do not contain mitochondrial enzymes

such as pyruvate dehydrogenase that require the thiamine-derived coenzyme thiamine pyrophosphate (TPP). However, they do contain the cytosolic TPP-requiring transketolase, whose activity is used clinically to assess thiamine status.

Glycosaminoglycans, Proteoglycans, and Glycoproteins 14

I. GLYCOSAMINOGLYCAN OVERVIEW

Glycosaminoglycans (GAGs) are large complexes of negatively charged heteropolysaccharide chains. They are generally associated with a small amount of protein-forming structures known as *proteoglycans*, which typically consist of up to 95% carbohydrate. GAGs have the special ability to bind large amounts of water, producing the gel-like matrix that forms the basis of the body's ground substance, which, along with fibrous structural proteins such as collagen, elastin, and fibrillin-1, and adhesive proteins such as fibronectin, makes up the extracellular matrix (ECM). [a]Hydrated GAGs serve as a flexible support for the ECM, interacting with the structural and adhesive proteins, and as a molecular sieve, influencing movement of materials through the ECM. The viscous, lubricating properties of mucous secretions also result from the presence of GAGs, which led to the original naming of these compounds as mucopolysaccharides.

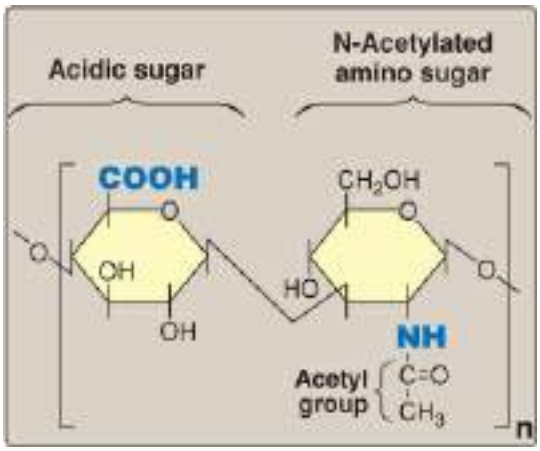


Figure 14.1
Repeating disaccharide unit of glycosaminoglycans.

II. STRUCTURE

GAGs are long, unbranched, heteropolysaccharide composed of repeating disaccharide chains where one of the sugars is an *N*-acetylated amino sugar, either *N*-acetylglucosamine (GlcNAc) or *N*-acetylgalactosamine (GalNAc) (Fig. 14.1), and the other is an acidic sugar. A single exception is keratan sulfate, which contains galactose rather than an acidic sugar. The amino sugar is either D-glucosamine or D-

galactosamine, in which the amino group is usually acetylated, eliminating its positive charge. The amino sugar may also be sulfated on carbon 4 or 6 or on a nonacetylated nitrogen. The acidic sugar is either D-glucuronic acid or its C-5 epimer L-iduronic acid (Fig. 14.2). These uronic sugars contain carboxyl groups that are negatively charged at physiologic pH and, together with the sulfate groups ($-SO_4^{2-}$), give GAGs their strongly negative nature.

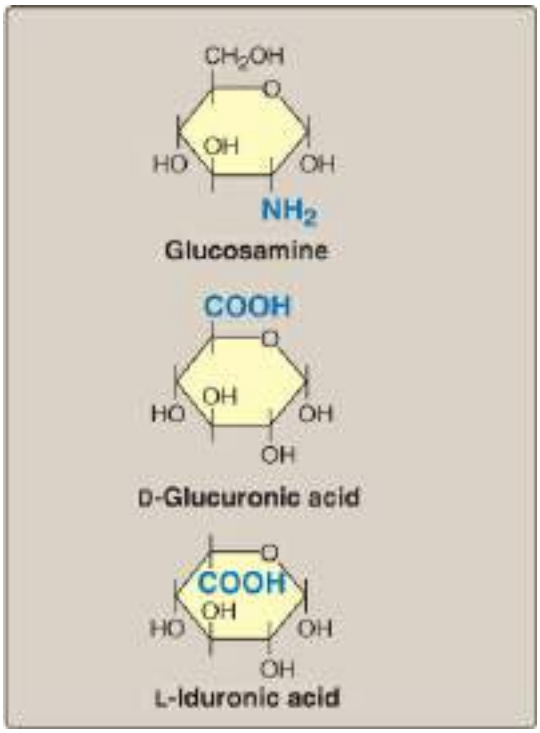


Figure 14.2
Some monosaccharide units found in glycosaminoglycans.

A. Structure–function relationship

Because of the high concentration of negative charges, these repeating disaccharide chains tend to be extended in solution. They repel each other and are surrounded by a shell of water molecules. When brought together, they slide past each other, much as two magnets with the same polarity seem to slide past each other. This produces the slippery consistency of mucous secretions and synovial fluid. When a solution containing GAGs is compressed, the water is squeezed out, and the GAGs are forced to occupy a smaller volume. When the compression is released, the GAGs spring back to their original, hydrated volume because of the repulsion of their negative charges. This property contributes to the resilience of cartilage, synovial fluid, and the vitreous humor of the eye (Fig. 14.3).

B. Classification

The six major types of GAGs are divided according to monomeric composition, type

of glycosidic linkages, and degree and location of sulfate units. The structure of the GAGs and their distribution in the body is illustrated in Figure 14.4. All GAGs, except for hyaluronic acid, are sulfated and are found covalently attached to protein, forming proteoglycan monomers.

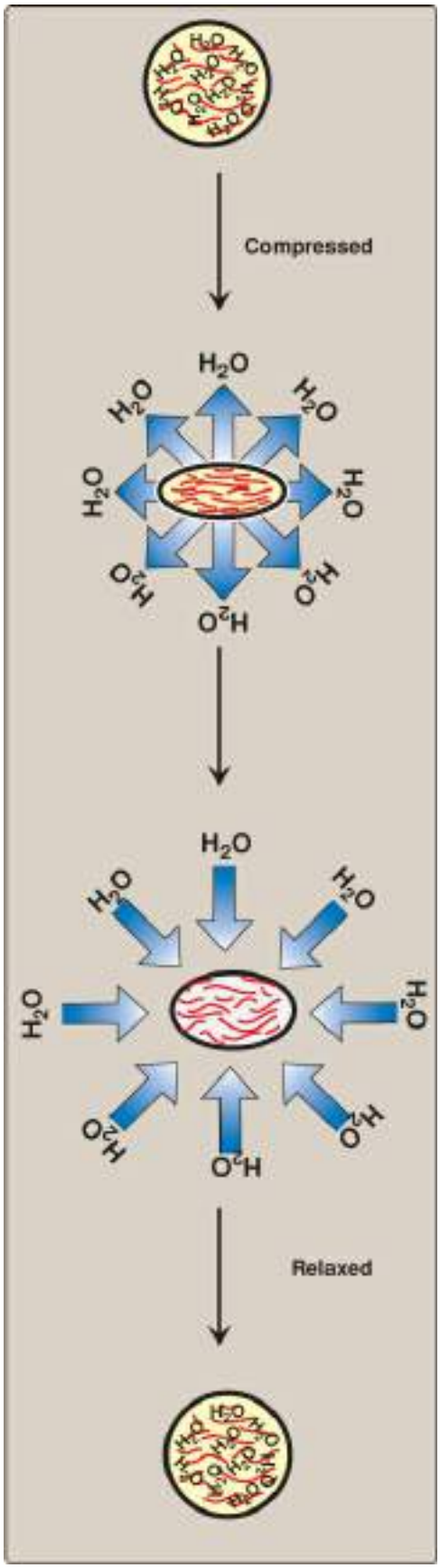


Figure 14.3
Resilience of glycosaminoglycans.

C. Proteoglycans

Proteoglycans are found in the ECM and on the outer surface of cells.

1. Monomer structure: A proteoglycan monomer found in cartilage consists of a core protein to which up to 100 linear chains of GAGs are covalently attached. These chains, which may each be composed of up to 200 disaccharide units, extend out from the core protein and remain separated from each other because of charge repulsion. The resulting structure resembles a bottle brush (Fig. 14.5). In cartilage proteoglycans, chondroitin sulfate and keratan sulfate are the main types of GAGs. Note that proteoglycans are grouped into gene families that encode core proteins with common structural features. The aggrecan family (aggrecan, versican, neurocan, and brevican), abundant in cartilage, is an example.

2. GAGs–protein linkage: GAGs attached to core protein via covalent linkage are most commonly through a trihexoside (galactose–galactose–xylose) and a serine residue in the protein. An O-glycosidic bond is formed between the xylose and the hydroxyl group of the serine (Fig. 14.6).

Clinical Application 14.1: Proteoglycans, Cartilage, and Osteoarthritis

Osteoarthritis affects millions of individuals worldwide. In this disease, joint cartilage is degraded and proteoglycans that normally help provide a cushion for the joint are lost. Without the resilience of the cartilage protecting the joint, there is pain, stiffness, and swelling, with progressive worsening of signs and symptoms. Glucosamine and chondroitin have been reported both to relieve pain and to stop progression of osteoarthritis. These compounds are readily available as over-the-counter dietary supplements in the United States. Based on several well-controlled clinical studies, it appears that glucosamine sulfate (but not glucosamine hydrochloride) and chondroitin sulfate may have a small to moderate effect in relieving symptoms of osteoarthritis.

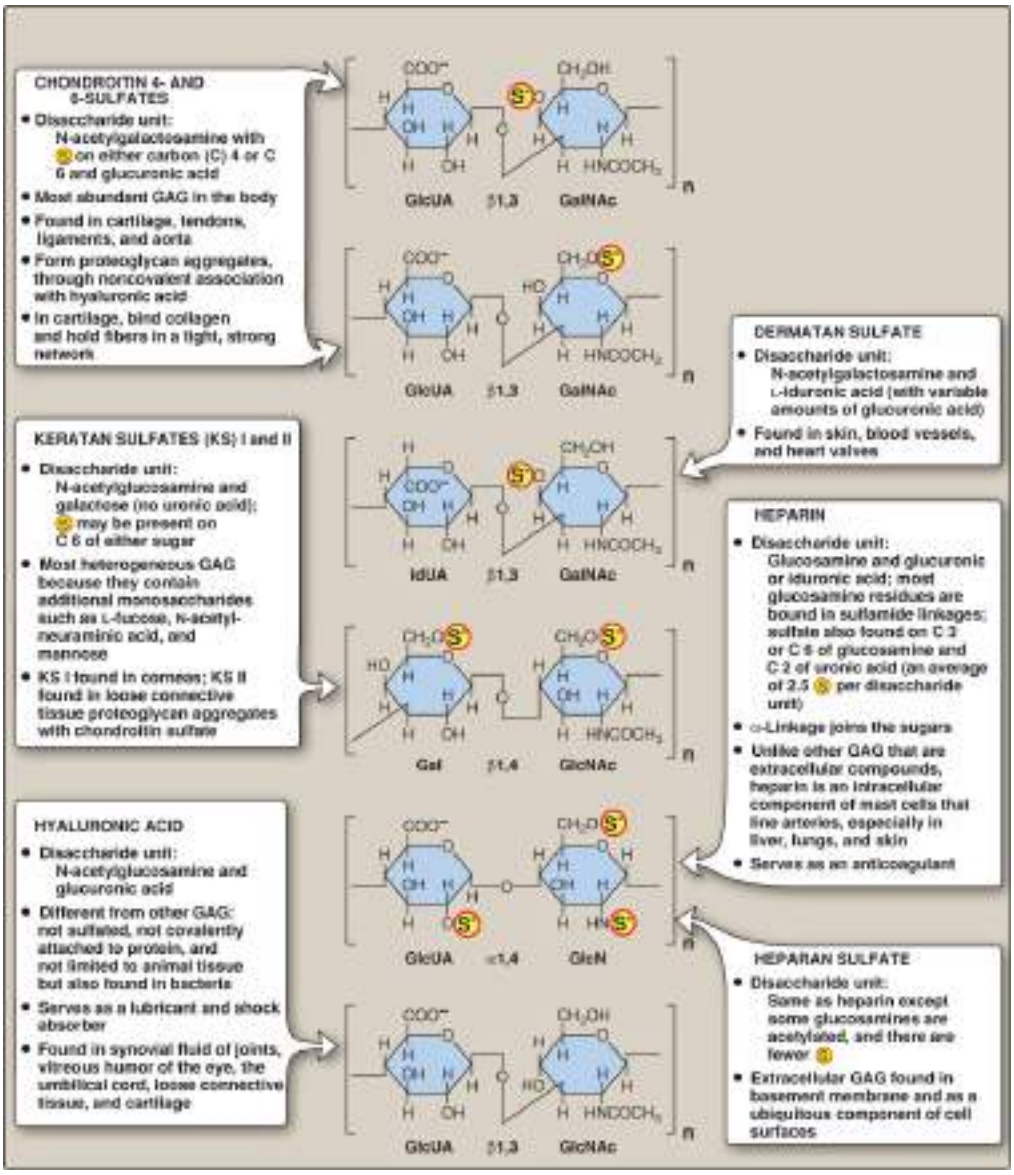


Figure 14.4
Structure of repeating units in and distribution of glycosaminoglycans (GAGs). Sulfate groups (S) are shown in all possible positions. GlcUA and IdUA = glucuronic and iduronic acids; GalNAc = N-acetylgalactosamine; GlcNAc = N-acetylglucosamine; GlcN = glucosamine; Gal = galactose.

3. Aggregate formation: Many proteoglycan monomers can associate with one molecule of hyaluronic acid to form proteoglycan aggregates. The association is not covalent and occurs primarily through ionic interactions between the core protein and the hyaluronic acid. The association is stabilized by additional small proteins called link proteins (Fig. 14.7).

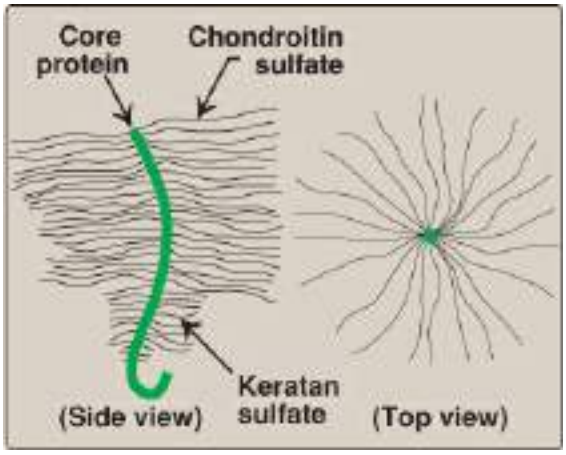


Figure 14.5
Bottle brush model of a cartilage proteoglycan monomer.

III. SYNTHESIS

The heteropolysaccharide chains are elongated by the sequential addition of alternating acidic and amino sugars donated primarily by their uridine diphosphate (UDP) derivatives. The reactions are catalyzed by a family of specific glycosyltransferases. Because GAGs are produced for export from the cell, their synthesis occurs primarily in the Golgi.

A. Amino sugar synthesis

Amino sugars are essential components of glycoconjugates such as proteoglycans, glycoproteins, and glycolipids. The synthetic pathway of amino sugars (hexosamines) is very active in connective tissues, where as much as 20% of glucose flows through this pathway.

1. N-Acetylglucosamine and N-acetylgalactosamine: The monosaccharide fructose 6-phosphate is the precursor of GlcNAc and GalNAc. A hydroxyl group on the fructose is replaced by the amide nitrogen of a glutamine, and the glucosamine 6-phosphate product gets acetylated, isomerized, and activated, producing the nucleotide sugar UDP-GlcNAc (Fig. 14.8). UDP-GalNAc is generated by the epimerization of UDP-GlcNAc. It is these nucleotide sugar forms of the amino sugars that are used to elongate the carbohydrate chains.

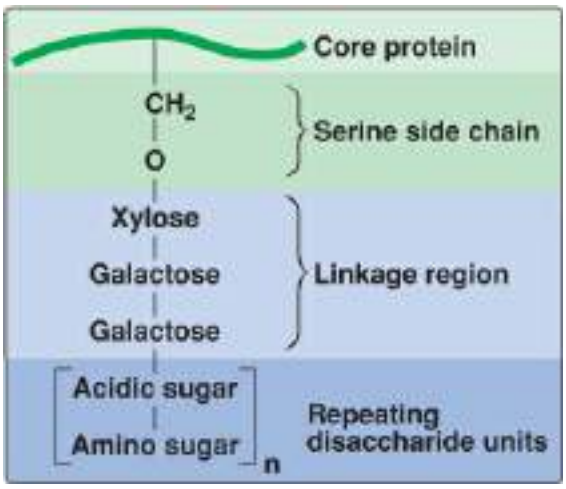


Figure 14.6
Glycosaminoglycan linkage regions.

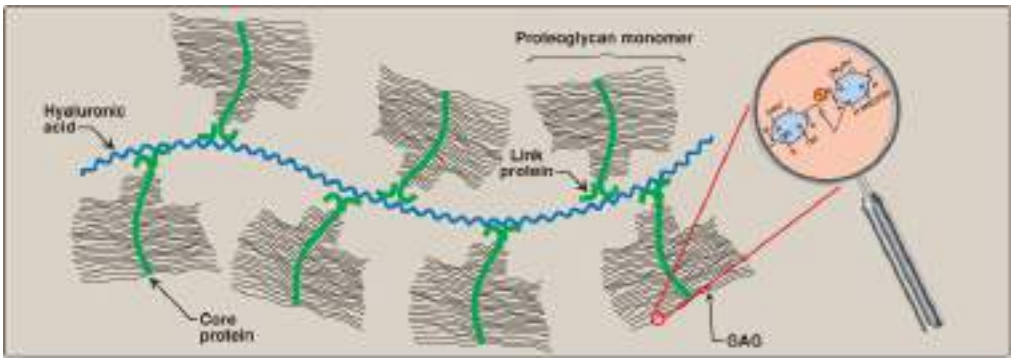


Figure 14.7
Proteoglycan aggregate. GAGs = glycosaminoglycan.

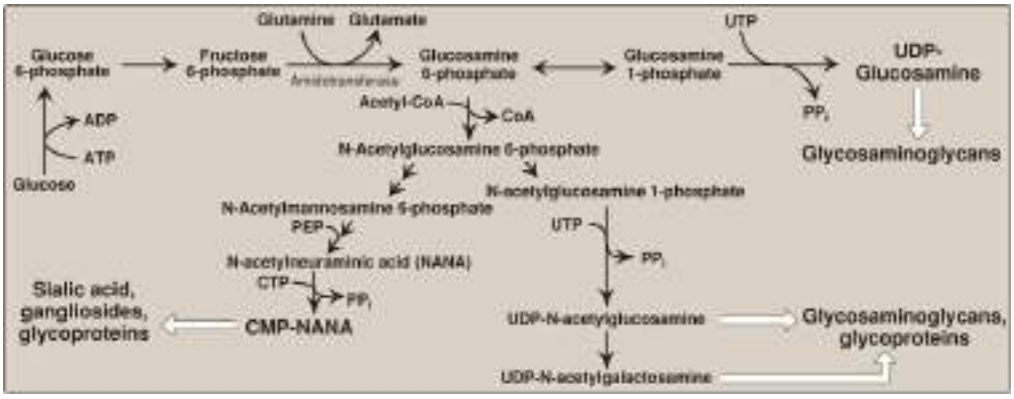


Figure 14.8
Synthesis of the amino sugars. ADP = adenosine diphosphate; UTP and UDP = uridine tri- and diphosphates; CoA = coenzyme A; PEP = phosphoenolpyruvate; CTP and CMP = cytidine tri- and monophosphates; PP_i = pyrophosphate.

2. N-Acetylneuraminic acid: NANA, a nine-carbon, acidic monosaccharide (see Fig. 17.15), is a member of the family of sialic acids, each of which is acylated at

a different site. These compounds are usually found as terminal carbohydrate residues of oligosaccharide side chains of glycoproteins, of glycolipids, or, less frequently, of GAGs. N-Acetylmannosamine 6-phosphate (derived from fructose 6-phosphate) and phosphoenolpyruvate (an intermediate in glycolysis) are the immediate sources of the carbons and nitrogens for NANA synthesis (see Fig. 14.8). Before NANA can be added to a growing oligosaccharide, it must be activated to cytidine monophosphate (CMP)-NANA by reacting with cytidine triphosphate (CTP). CMP-NANA synthetase catalyzes the reaction. CMP-NANA is the only nucleotide sugar in human metabolism in which the carrier nucleotide is a monophosphate rather than a diphosphate.

B. Acidic sugar synthesis

D-Glucuronic acid, whose structure is that of glucose with an oxidized carbon 6 ($-CH_2OH \rightarrow -COOH$), and its C-5 epimer, L-iduronic acid, are essential components of GAGs. Glucuronic acid is also required for the detoxification of lipophilic compounds, such as bilirubin, steroids, and many drugs, including the statins, because conjugation with glucuronate (glucuronidation) increases water solubility. In plants and mammals (other than guinea pigs and primates, including humans), glucuronic acid is a precursor of ascorbic acid (vitamin C) as shown in Figure 14.9. This uronic acid pathway also provides a mechanism by which dietary D-xylulose can enter the central metabolic pathways.

1. Glucuronic acid: Glucuronic acid can be obtained in small amounts from the diet and from the lysosomal degradation of GAGs. It also can be synthesized by the uronic acid pathway, in which glucose 1-phosphate reacts with uridine triphosphate (UTP) and is converted to UDP-glucose. Oxidation of UDP-glucose produces UDP-glucuronic acid, the form that supplies glucuronic acid for GAGs synthesis and glucuronidation (Fig. 14.10). The end product of glucuronic acid metabolism in humans is D-xylulose 5-phosphate, which can enter the pentose phosphate pathway and produce the glycolytic intermediates glyceraldehyde 3-phosphate and fructose 6-phosphate (see Fig. 14.9; see also Fig. 13.2).

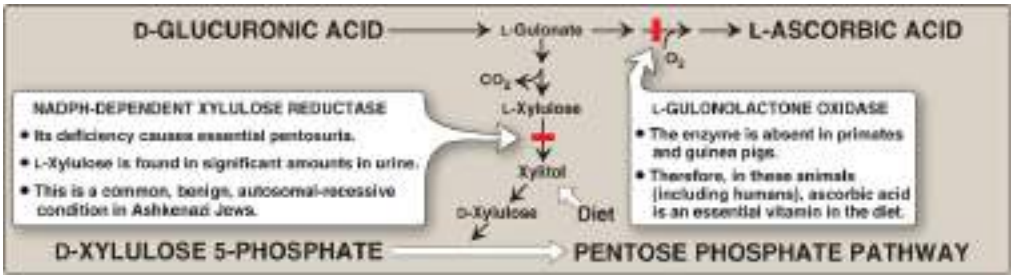


Figure 14.9
Metabolism of glucuronic acid. NADPH = reduced nicotinamide adenine dinucleotide phosphate; CO_2 = carbon dioxide.

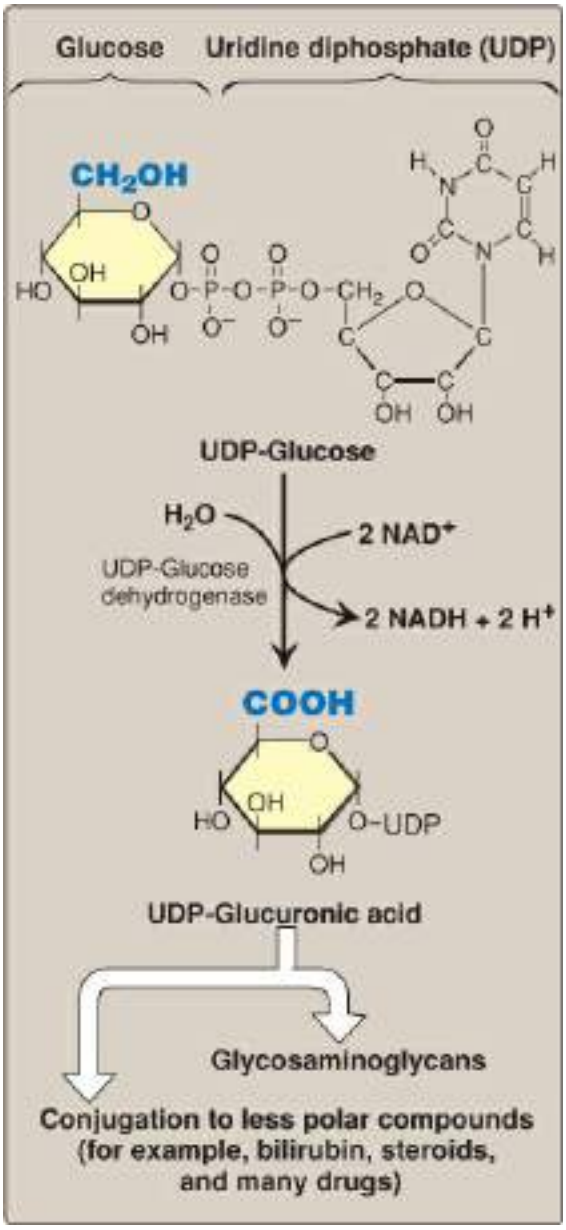


Figure 14.10
Oxidation of UDP-glucose to UDP-glucuronic acid. NAD(H) = nicotinamide adenine dinucleotide.

2. L-Iduronic acid: Synthesis of L-iduronic acid occurs after D-glucuronic acid has been incorporated into the carbohydrate chain. Uronosyl 5-epimerase causes epimerization of the D- to the L-sugar.

C. Core protein synthesis

The core protein is made by ribosomes on the rough endoplasmic reticulum (RER), enters the RER lumen, and then moves to the Golgi, where it is glycosylated by membrane-bound glycosyltransferases.

D. Carbohydrate chain synthesis

Carbohydrate chain formation is initiated by synthesis of a short linker on the core protein on which carbohydrate chain synthesis will occur. The most common linker is a trihexoside formed by the transfer of a xylose from UDP-xylose to the hydroxyl group of a serine (or threonine) catalyzed by xylosyltransferase. Two galactose molecules are then added, completing the trihexoside. This is followed by sequential addition of alternating acidic and amino sugars (Fig. 14.11) and epimerization of some D-glucuronyl to L-iduronyl residues.

E. Sulfate group addition

Sulfation of a GAG occurs after the monosaccharide to be sulfated has been incorporated into the growing carbohydrate chain. The source of the sulfate is 3′-phosphoadenosyl-5′-phosphosulfate (PAPS) a molecule of adenosine monophosphate with a sulfate group attached to the 5′-phosphate; see also Fig. 17.16. The sulfation reaction is catalyzed by sulfotransferases. Synthesis of the sulfated GAG chondroitin sulfate is shown in Figure 14.11. Note that PAPS is also the sulfur donor in glycosphingolipid synthesis.

IV. DEGRADATION

GAGs are degraded in lysosomes, which contain hydrolytic enzymes that are most active at a pH of ~5. Therefore, as a group, these enzymes are called acid hydrolases. The low pH optimum within lysosomes is a protective mechanism that prevents the enzymes from destroying the cell should leakage occur into the cytosol where the pH is neutral. [b]The half-lives of GAGs vary from minutes to months and are influenced by the type of GAG and its location in the body.

A. GAGs and phagocytosis

Because GAGs are extracellular or cell-surface compounds, they must first be engulfed by invagination of the cell membrane (phagocytosis), forming a vesicle inside of which are the GAGs to be degraded. This vesicle then fuses with a lysosome, forming a single digestive vesicle in which the GAGs are efficiently degraded.

B. Lysosomal degradation

The lysosomal degradation of GAGs requires a large number of acid hydrolases for complete digestion. First, the polysaccharide chains are cleaved by endoglycosidases, producing oligosaccharides. Further degradation of the oligosaccharides occurs sequentially from the nonreducing end of each chain, the last group (sulfate or sugar) added during synthesis being the first group removed, by action of sulfatases or exoglycosidases. Examples of some of these enzymes and the bonds they hydrolyze are shown in Figure 14.12. (Note that endo- and

exoglycosidases are also involved in the lysosomal degradation of glycoproteins and glycolipids. Deficiencies in these enzymes result in the accumulation of partially degraded carbohydrates, causing tissue damage.)

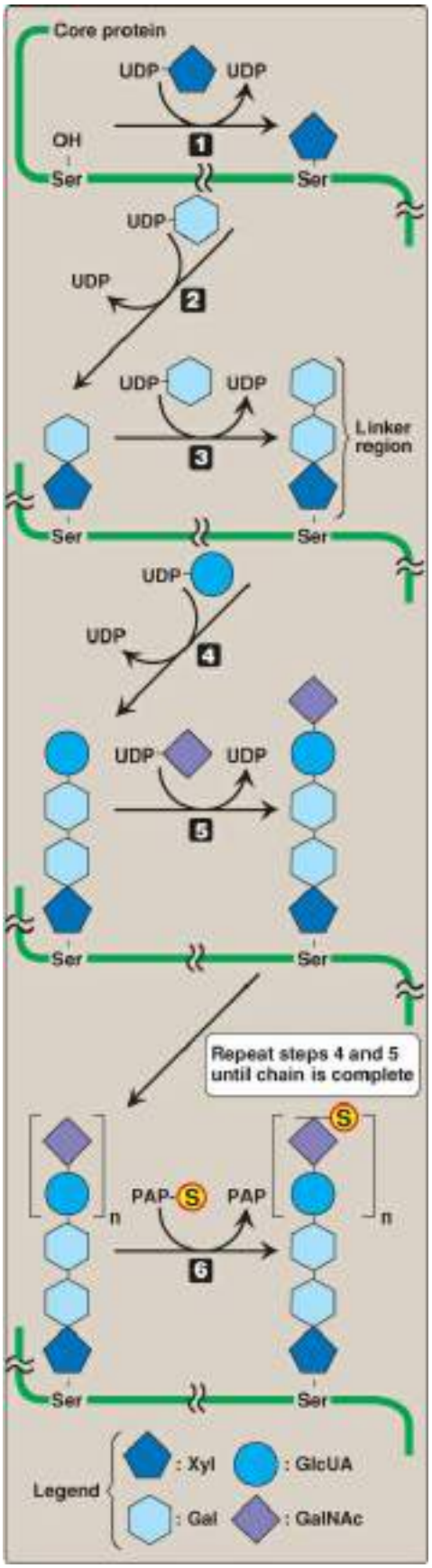


Figure 14.11
Synthesis of chondroitin sulfate. PAP-(S) = 3′-phosphoadenosyl-5′-phosphosulfate; Ser = serine.

Multiple sulfatase deficiency (Austin disease) is a rare lysosomal storage disease in which all sulfatases are nonfunctional because of a defect in the formation of formylglycine, an amino acid derivative required at the active site for enzymatic activity to occur.

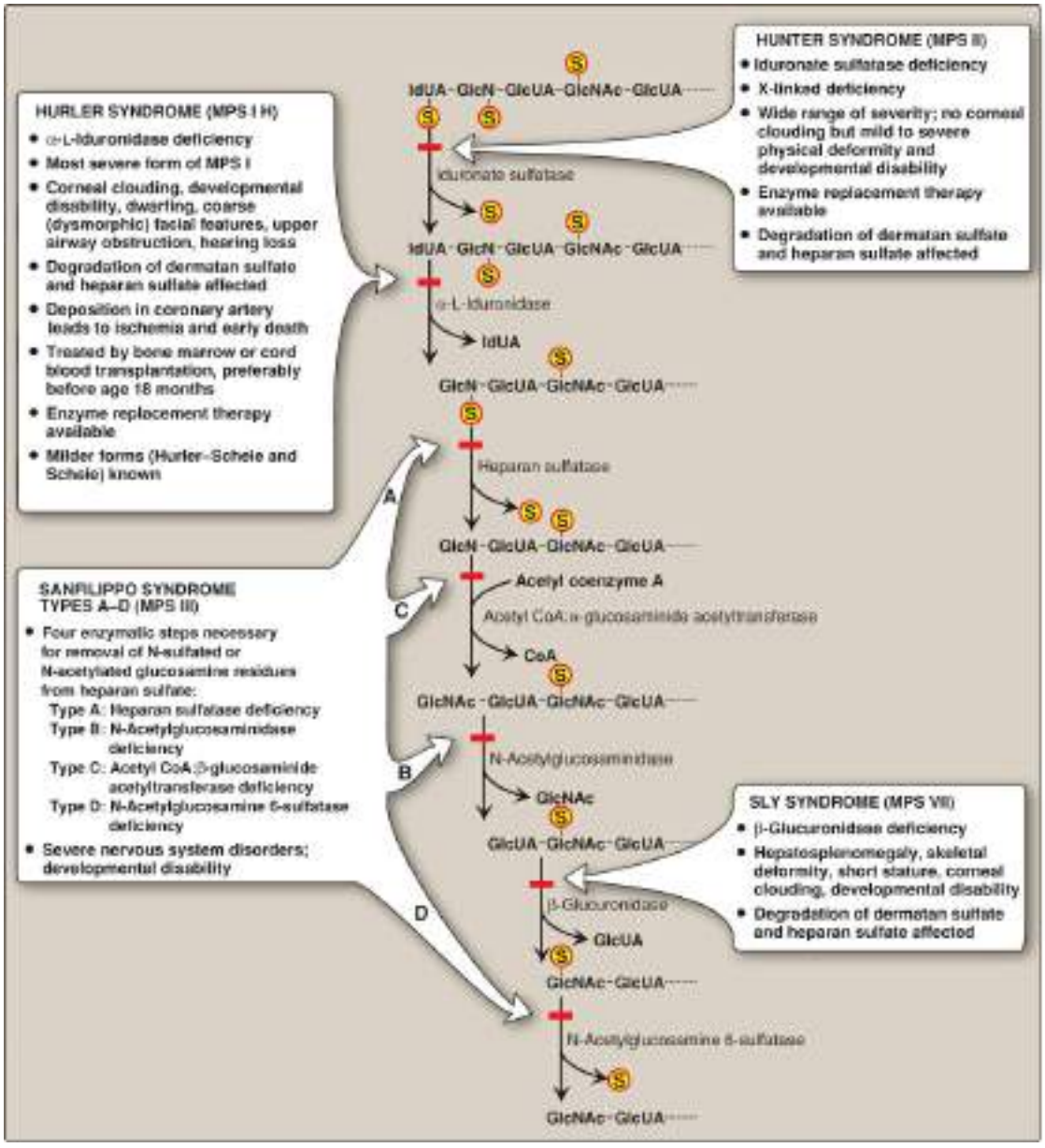


Figure 14.12
Degradation of the glycosaminoglycan heparan sulfate by lysosomal enzymes, indicating sites of enzyme deficiencies in some representative mucopolysaccharidoses (MPS). (Note: Deficiencies in galactosamine 6-sulfatase and β-galactosidase that degrade keratan sulfate result in Morquio syndrome [MPS IV], [**A** and **B**], respectively. Deficiencies in arylsulfatase B that degrades dermatan sulfate result in Maroteaux–Lamy syndrome [MPS VI].) GlcUA and IdUA = glucuronic and iduronic acids; GalNAc = N-

acetylgalactosamine; GlcNAc = N-acetylglucosamine; GlcN = glucosamine; = sulfate.

V. MUCOPOLYSACCHARIDOSES

Mucopolysaccharidoses are hereditary diseases (approximately 1:25,000 live births) caused by a deficiency of any one of the lysosomal hydrolases normally involved in the degradation of heparan sulfate, dermatan sulfate, and/or keratin sulfate (summarized in Fig. 14.12). They are progressive disorders characterized by lysosomal accumulation of GAGs in various tissues, causing a range of symptoms, such as skeletal and ECM deformities, and intellectual disability. All are autosomal-recessive disorders except Hunter syndrome, which has X-linked inheritance.

Children homozygous for any one of these diseases are apparently normal at birth and then gradually deteriorate. In severe deficiencies, death occurs in childhood. There currently is no cure. Incomplete lysosomal degradation of GAGs results in the presence of oligosaccharides in the urine. These fragments can be used to diagnose the specific mucopolysaccharidosis by identifying the structure present on the nonreducing end of the oligosaccharide, because that residue would have been the substrate for the missing enzyme. Diagnosis is confirmed by measuring the patient's cellular level of the lysosomal hydrolases. Bone marrow and cord blood transplants, in which transplanted macrophages produce the enzymes that degrade GAGs, have been used to treat Hurler and Hunter syndromes, with limited success. Enzyme replacement therapy is available for both syndromes but does not prevent neurologic damage.

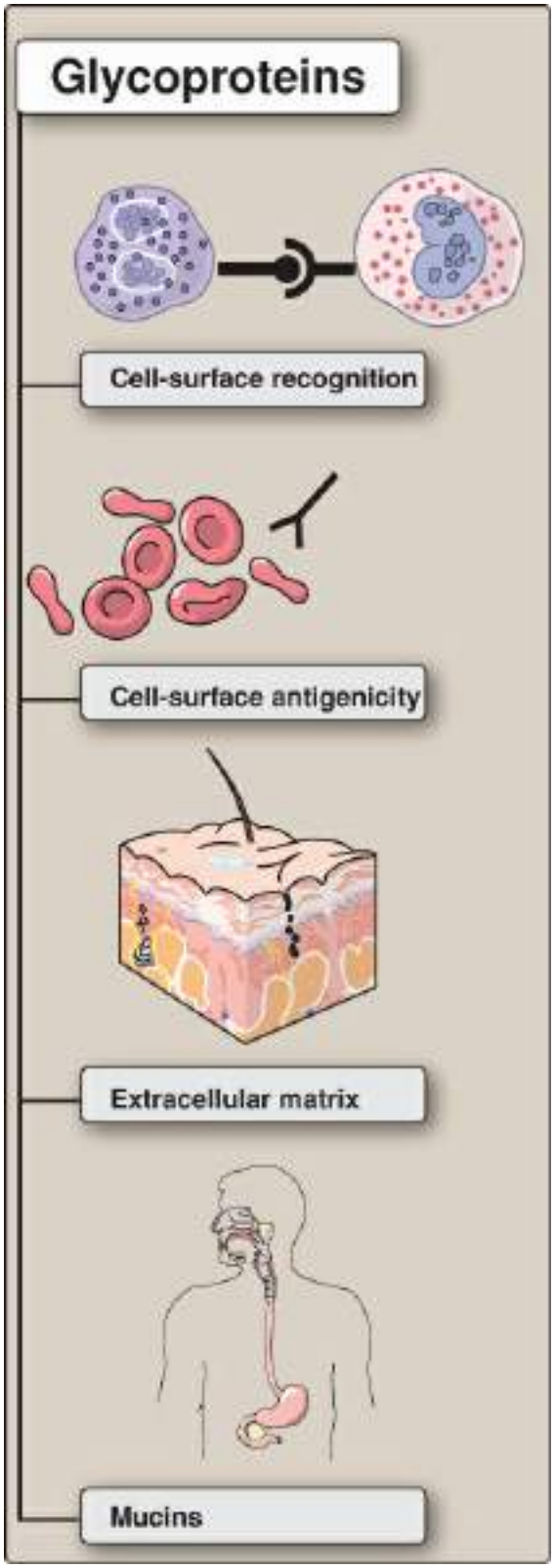


Figure 14.13
Functions of glycoproteins.

VI. GLYCOPROTEIN OVERVIEW

Glycoproteins are proteins to which oligosaccharides (glycans) are covalently attached; *glycosylation* is the most common posttranslational modification of proteins. (Note: Nonenzymatic addition of carbohydrate to proteins is known as glycation.) Glycoproteins contain highly variable amounts of carbohydrate but typically much less than that of proteoglycans. For example, the glycoprotein immunoglobulin G (IgG) contains <4% of its mass as carbohydrate, whereas the proteoglycan aggrecan contains >80%. In glycoproteins, the glycan is relatively short, usually 2 to 10 sugar residues in length, is often branched instead of linear; and may or may not be negatively charged. Membrane-bound glycoproteins participate in a broad range of cellular phenomena, including cell-surface recognition by other cells, hormones, and viruses, cell-surface antigenicity (such as the blood group antigens), and as components of the ECM and of the mucins of the gastrointestinal and urogenital tracts, where they act as protective biologic lubricants. In addition, almost all of the globular proteins present in human plasma are glycoproteins, although albumin is an exception. Figure 14.13 summarizes some glycoprotein functions.

VII. OLIGOSACCHARIDE STRUCTURE

The oligosaccharide (glycan) components of glycoproteins are generally branched heteropolymers composed primarily of D-hexoses, with the addition in some cases of neuraminic acid (a nonose) and of L-fucose, a 6-deoxyhexose.

A. Carbohydrate–protein linkage

The glycan may be attached to the protein through an N- or an O-glycosidic link (see p. 95). In the former case, the sugar chain is attached to the amide group of an asparagine side chain and, in the latter case, to the hydroxyl group of either a serine or threonine side chain. In the case of collagen, there is an O-glycosidic linkage between galactose or glucose and the hydroxyl group of hydroxylysine.

B. N- and O-linked oligosaccharides

A glycoprotein may contain only one type of glycosidic linkage (N or O linked) or may have both types within the same molecule.

1. O linked: The O-linked glycans may have one or more of a wide variety of sugars arranged in either a linear or a branched pattern. Many are found in extracellular glycoproteins or as membrane glycoprotein components. For example, O-linked oligosaccharides on the surface of red blood cells help provide the ABO blood group determinants. If the terminal sugar on the glycan is GalNAc, the blood group is A. If it is galactose, the blood group is B. If neither GalNAc nor galactose is present, the blood group is O.

2. N linked: The N-linked glycans fall into two broad classes: complex oligosaccharides and high-mannose oligosaccharides. Both contain the same pentasaccharide core shown in Figure 14.14, but the complex oligosaccharides contain a diverse group of additional sugars, for example, GlcNAc, GalNAc, L-fucose, and NANA, whereas the high-mannose oligosaccharides contain primarily mannose.

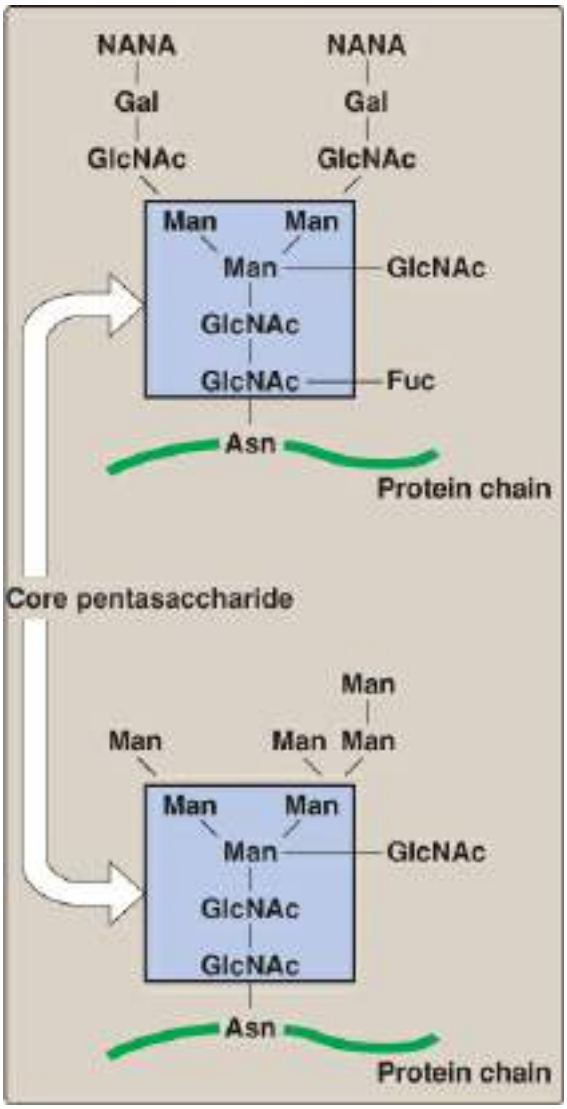


Figure 14.14
Complex (**top**) and high-mannose (**bottom**) N-linked oligosaccharides. (NOTE: Members of each class contain the same pentasaccharide core [shown inside the *box*].) NANA = N-acetylneuraminic acid; Gal = galactose; GlcNAc = N-acetylglucosamine; Man = mannose; Fuc = fucose; Asn = asparagine.

VIII. GLYCOPROTEIN SYNTHESIS

Proteins destined to function in the cytoplasm are synthesized on free cytosolic

ribosomes. However, proteins, including glycoproteins, that are destined for cellular membranes, lysosomes, or to be exported from the cell, are synthesized on ribosomes attached to the endoplasmic reticulum. These proteins contain specific signal sequences that act as molecular addresses, targeting the proteins to their proper destinations. An N-terminal hydrophobic sequence initially directs these proteins to the ER, allowing the growing polypeptide to be extruded into the lumen (see p. 505). The proteins are then transported via secretory vesicles to the Golgi, which acts as a sorting center (Fig. 14.15). In the Golgi, those glycoproteins that are to be secreted from the cell or targeted for lysosomes are packaged into vesicles that fuse with the plasma or lysosomal membrane and release their contents. Those that are destined to become components of the cell membrane are integrated into the Golgi membrane, which buds off, forming vesicles that add their membrane-bound glycoproteins to the cell membrane and are oriented with the carbohydrate portion facing toward the outside of the cell (see Fig. 14.15).

A. Carbohydrate components

The precursors of the carbohydrate components of glycoproteins are nucleotide sugars, which include UDP-glucose, UDP-galactose, UDP-GlcNAc, and UDP-GalNAc. In addition, guanosine diphosphate (GDP)-mannose, GDP-L-fucose (synthesized from GDP-mannose), and CMP-NANA may donate sugars to the growing chain. When the acidic NANA is present, the oligosaccharide has a negative charge at physiologic pH. The oligosaccharides are covalently attached to the side chains of specific amino acids in the protein, where the three-dimensional structure of the protein determines whether or not a specific amino acid is glycosylated.

B. O-Linked glycoprotein synthesis

Synthesis of the O-linked glycoproteins is very similar to that of the GAGs. First, the protein to which sugars are to be attached is synthesized on the RER and extruded into its lumen. Glycosylation begins with the transfer of GalNAc (from UDP-GalNAc) to the hydroxyl group of a specific serine or threonine residues. The glycosyltransferases responsible for the stepwise synthesis (from individual sugars) of the oligosaccharides are bound to the membranes of the Golgi. They act in a specific order, without using a template as is required for DNA, ribonucleic acid (RNA), and protein synthesis (see Unit VII), but instead by recognizing the actual structure of the growing oligosaccharide as the appropriate substrate.

C. N-Linked glycoprotein synthesis

Synthesis of N-linked glycoproteins occurs in the lumen of the RER and requires the participation of the phosphorylated form of dolichol (dolichol pyrophosphate), a lipid of the RER membrane (Fig. 14.16). The initial product is processed in the RER and Golgi.

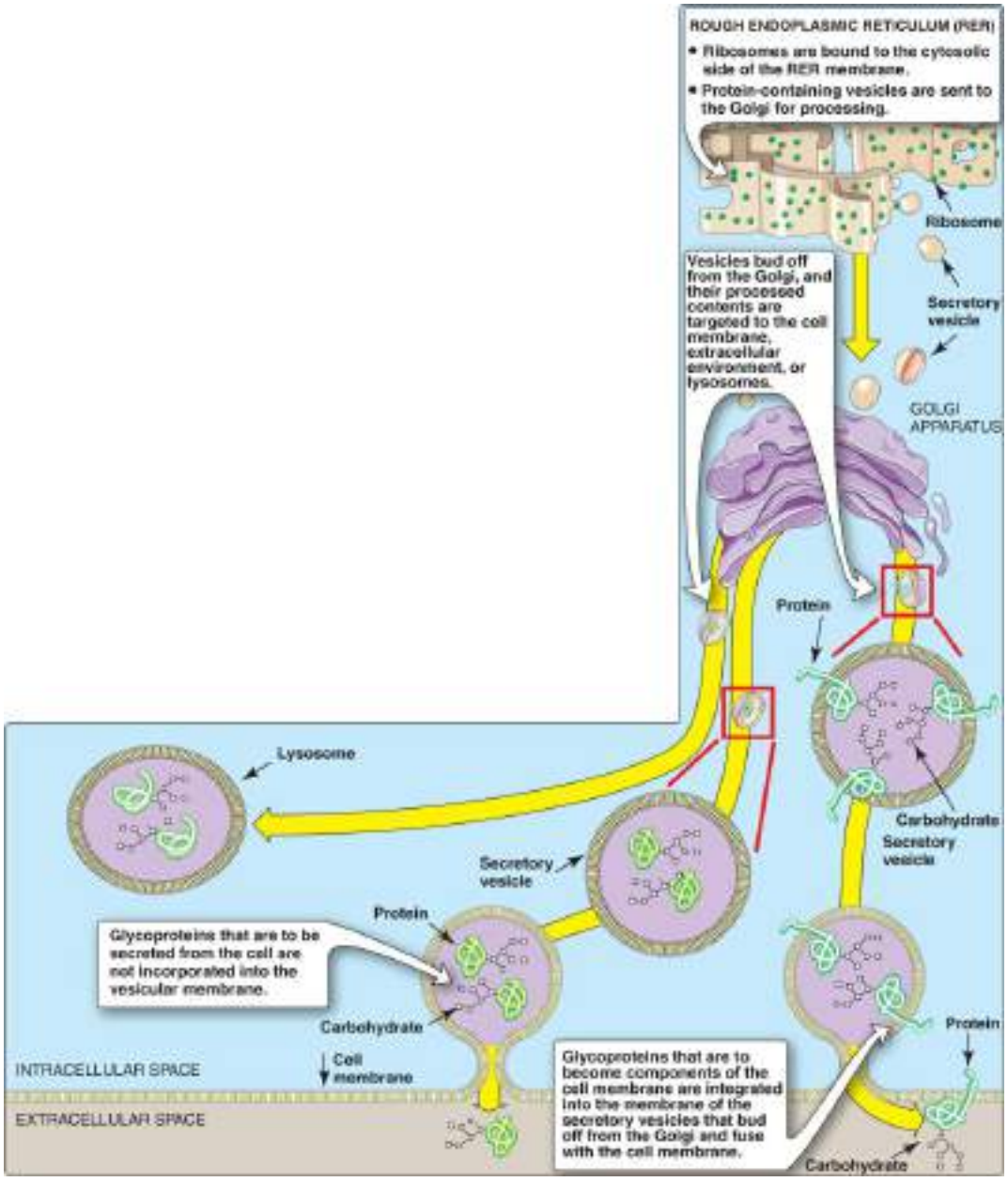


Figure 14.15
Transport of glycoproteins to and through the Golgi and their subsequent secretion or incorporation into a lysosome or the cell membrane.

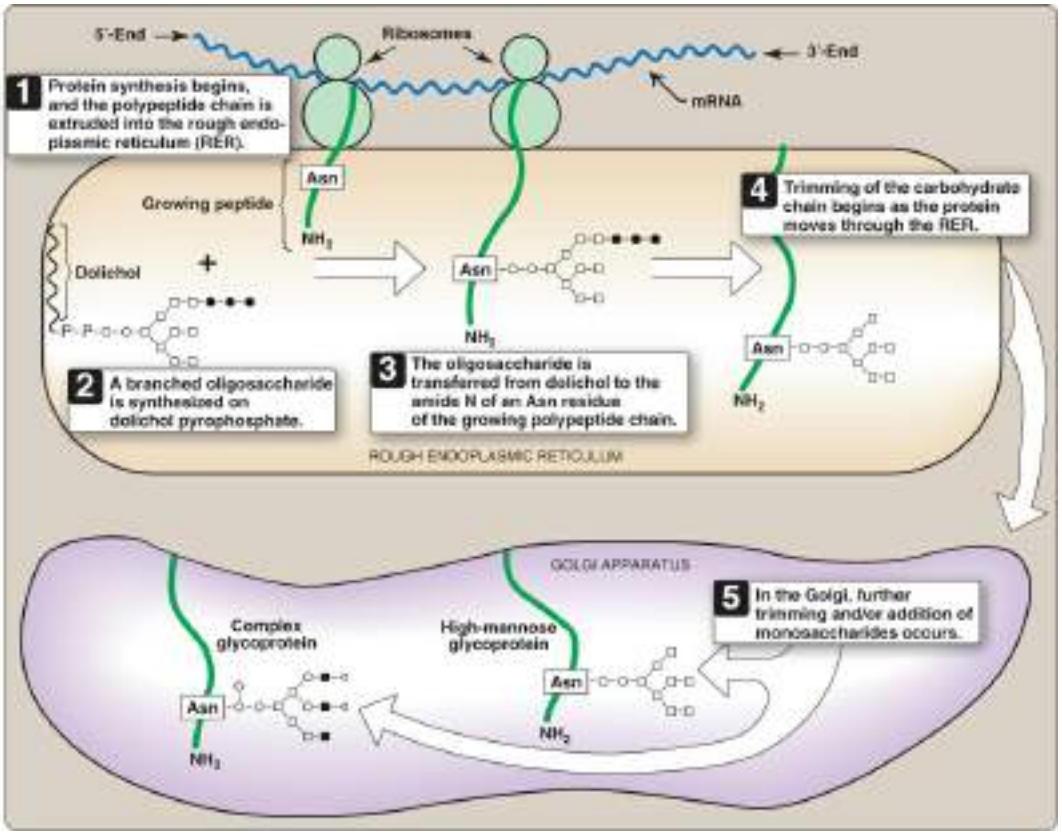


Figure 14.16
Synthesis of N-linked glycoproteins. ○ = N-acetylglucosamine; □ = mannose; • = glucose; ▪ = galactose; ◇ or ◅ = terminal group (fucose or N-acetylneuraminic acid); mRNA = messenger RNA; Asn = asparagine.

1. Dolichol-linked oligosaccharide synthesis: As with the O-linked glycoproteins, the protein is synthesized on the RER and enters its lumen. However, it does not become glycosylated with individual sugars. Instead, a lipid-linked oligosaccharide is first constructed. This consists of dolichol, an RER membrane lipid made from an intermediate of cholesterol synthesis (see p. 245) attached through a pyrophosphate linkage to an oligosaccharide containing GlcNAc, mannose, and glucose. The sugars to be added sequentially to the dolichol by membrane-bound glycosyltransferases are first GlcNAc, followed by mannose and glucose (see Fig. 14.16). The entire 14-sugar oligosaccharide is then transferred from dolichol to the amide nitrogen of an asparagine residue in the protein to be glycosylated by a protein–oligosaccharide transferase present in the RER. (Note: The antibiotic Tunicamycin inhibits N-linked glycosylation.)

Congenital disorders of glycosylation (CDG) are syndromes caused primarily by defects in the N-linked glycosylation of proteins, either oligosaccharide assembly (type I) or processing (type II).

2. N-Linked oligosaccharide processing: After addition to the protein, the N-linked oligosaccharide is processed by the removal of specific mannosyl and glucosyl residues as the glycoprotein moves through the RER. Finally, the oligosaccharide chains are completed in the Golgi by addition of a variety of sugars (e.g., GlcNAc, GalNAc, and additional mannoses and then fucose or NANA as terminal groups) to produce a complex glycoprotein. Alternatively, they are not processed further, leaving branched, mannose-containing chains in a high-mannose glycoprotein (see Fig. 14.16). The ultimate fate of N-linked glycoproteins is the same as that of the O-linked glycoproteins (e.g., they can be released by the cell or become part of a cell membrane). In addition, N-linked glycoproteins can be targeted to the lysosomes.

3. Lysosomal enzymes: N-Linked glycoproteins being processed in the Golgi can be phosphorylated on carbon 6 of one or more mannosyl residues. UDP-GlcNAc provides the phosphate in a reaction catalyzed by a phosphotransferase. Receptors, located in the Golgi membrane, bind the mannose 6-phosphate (M6P) residues of these proteins, which are then packaged into vesicles and sent to the lysosomes (Fig. 14.17).

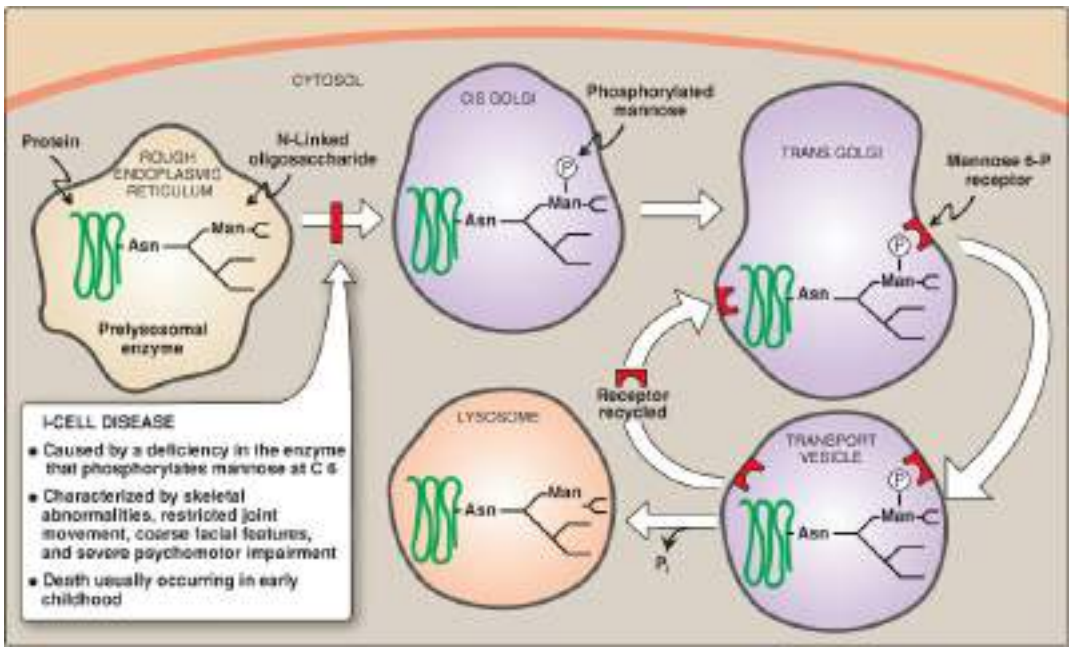


Figure 14.17
Mechanism for transport of N-linked glycoproteins to the lysosomes. Asn = asparagine; Man = mannose; Ⓟ = phosphate; P_i = inorganic phosphate.

Clinical Application 14.2: I-Cell Disease

I-Cell disease is a rare lysosomal storage disease named for large inclusion bodies seen in cells of patients with the disease. GlcNAc phosphotransferase is deficient and mannose 6-phosophate is not generated on proteins destined for lysosomes. Lack of M6P on amino acid residues causes precursor acid hydrolases to

traffic to the plasma membrane and be secreted constitutively, instead of trafficking to lysosomes. Consequently, the acid hydrolases are absent from lysosomes, and the macromolecule substrates for these digestive enzymes accumulate within the lysosomes, generating the inclusion bodies that define the disorder. In addition, high concentrations of lysosomal enzymes are found in the patient's plasma and urine, indicating that the targeting process to lysosomes is deficient.

I-Cell disease is characterized by skeletal abnormalities, restricted joint movement, coarse (dysmorphic) facial features, and severe psychomotor impairment. Because I-cell disease has features in common with the mucopolysaccharidoses and sphingolipidoses, it is termed a mucolipidosis (ML II). Currently, there is no cure, and death from cardiopulmonary complications usually occurs in early childhood. Pseudo-Hurler polydystrophy (ML III) is a less severe mucolipidosis form of I-cell disease, in which the phosphotransferase maintains some residual enzymatic activity, and it symptomatically resembles a mild form of Hurler syndrome.

IX. LYSOSOMAL GLYCOPROTEIN DEGRADATION

Degradation of glycoproteins is similar to that of the GAGs (see p. 179). The lysosomal acid hydrolases are each generally specific for the removal of one component of the glycoprotein. They are primarily exoenzymes that remove their respective groups in the reverse order of their incorporation (last on, first off). If any of the degradative enzyme is missing, degradation by the other exoenzymes cannot continue.

A group of very rare autosomal-recessive diseases called the glycoprotein storage diseases (oligosaccharidoses), caused by a deficiency of any one of the degradative enzymes, results in accumulation of partially degraded structures in the lysosomes. For example, α-mannosidosis type 3 is a severe, progressive, fatal deficiency of the enzyme α-mannosidase. Presentation is similar to Hurler syndrome, but immune deficiency is also seen. Mannose-rich oligosaccharide fragments appear in the urine. Diagnosis is by enzyme activity assay.

X. Chapter Summary

- **GAGs** are synthesized in the Golgi as **long**, **negatively charged**, **unbranched**, **heteropolysaccharide chains** generally composed of a **repeating disaccharide unit** (acidic sugar–amino sugar)$_n$.
- The **amino sugar** is either **D-glucosamine** or **D-galactosamine** and the **acidic sugar** is either **D-glucuronic acid** or its C-5 epimer **L-iduronic acid**.
- GAGs bind water, thereby producing the gel-like matrix that forms the basis of the body's **ground substance** and the lubricating properties of mucous secretions.
- There are six major types of GAGs: **chondroitin 4-** and **6-sulfates**, **keratan sulfate**, **dermatan sulfate**, **heparin**, **heparan sulfate**, and **hyaluronic acid**.
- All GAGs, except hyaluronic acid, are found covalently attached to a **core protein**, forming **proteoglycan monomers**. Many proteoglycan monomers associate with a molecule of **hyaluronic acid** to form **proteoglycan aggregates**.
- The completed proteoglycans are secreted into the **ECM** or remain associated with the outer surface of cells.
- GAGs are degraded by **lysosomal acid hydrolases**. A deficiency of any one of the hydrolases results in a **mucopolysaccharidosis** in which GAGs accumulate in tissues, causing symptoms such as **skeletal** and **ECM deformities** and **intellectual disability**. Examples of these genetic diseases include **Hunter** (X-linked) and **Hurler syndromes**.
- **Glycoproteins** are synthesized in the RER and Golgi and are proteins to which **oligosaccharides** (**glycans**) are covalently attached.
- **Membrane-bound** glycoproteins participate in **cell-surface recognition**, **cell-surface antigenicity**, and as components of the ECM and of the **mucins** of the gastrointestinal and urogenital tracts, where they act as protective biologic lubricants. Almost all of the globular proteins present in human plasma are glycoproteins.
- Precursors of carbohydrate components of glycoproteins are **nucleotide sugars**. **O-Linked glycoproteins** are produced in the Golgi by the sequential transfer of sugars from their nucleotide carriers to the hydroxyl group of a Ser or Thr residue in the protein. **N-Linked glycoproteins** are created by the transfer of a preformed oligosaccharide from its RER membrane lipid carrier, **dolichol pyrophosphate**, to the amide N of an Asn residue in the protein. They contain varying amounts of **mannose**.
- A deficiency in **N-acetylglucosamine phosphotransferase** that phosphorylates mannose residues at carbon 6 in N-linked glycoprotein enzymes destined for the lysosomes results in **I-cell disease**.
- Glycoproteins are normally degraded in lysosomes by **acid hydrolases**. A deficiency of any one of these enzymes results in a **lysosomal glycoprotein storage disease**, resulting in accumulation of partially degraded glycoproteins in the lysosome and causing a range of symptoms including skeletal deformity and intellectual disability.

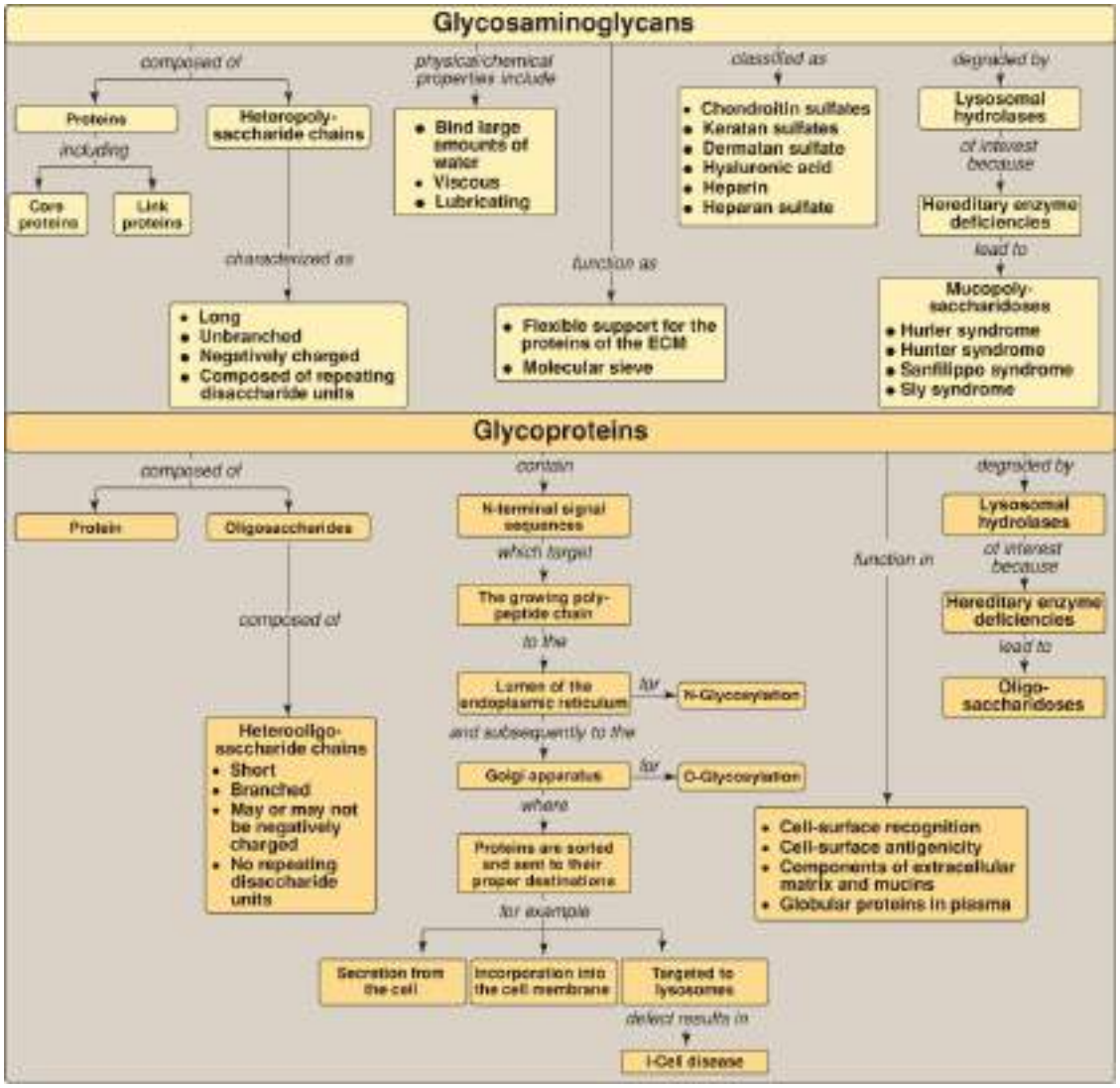


Figure 14.18
Key concept map for glycosaminoglycans and glycoproteins. ECM = extracellular matrix.

Study Questions

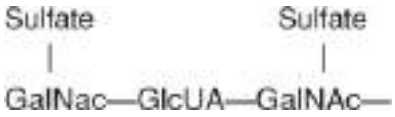
Choose the ONE best answer.

14.1 Mucopolysaccharidoses are hereditary lysosomal storage diseases caused by:

A. defects in the degradation of glycosaminoglycans.
B. abnormal targeting of acid hydrolase enzymes to lysosomes.
C. an increased rate of synthesis of the carbohydrate component of proteoglycans.
D. an insufficient rate of synthesis of proteolytic enzymes.
E. the synthesis of abnormally small amounts of core proteins.

Correct answer = A. The mucopolysaccharidoses are caused by deficiencies in any one of the lysosomal acid hydrolases responsible for the degradation of glycosaminoglycans (not proteins). The enzyme is correctly targeted to the lysosome, so blood levels of the enzyme do not increase, but it is nonfunctional. In these diseases, synthesis of the protein and carbohydrate components of proteoglycans is unaffected, in terms of both structure and amount.

14.2 The presence of the following compound in the urine of a patient suggests a deficiency in which one of the enzymes listed below?



A. Galactosidase
B. Glucuronidase
C. Iduronidase
D. Mannosidase
E. Sulfatase

Correct answer = E. Degradation of glycoproteins follows the rule: last on, first off. Because sulfation is the last step in the synthesis of this sequence, a sulfatase is required for the next step in the degradation of the compound shown.

14.3 An 8-month-old male has coarse facial features, skeletal abnormalities, and delays in both growth and development. I-cell disease is suspected. Which of the following will be observed in this patient if that diagnosis is correct?

A. Decreased production of cell surface O-linked glycoproteins.
B. Elevated levels of acid hydrolases in the blood.
C. Inability to N-glycosylate proteins.
D. Increased synthesis of proteoglycans.
E. Oligosaccharides in the urine.

Correct answer = B. I-Cell disease is a lysosomal storage disease caused by deficiency of the phosphotransferase needed for synthesis of the mannose 6-phosphate signal that targets acid hydrolases to the lysosomal matrix. This results in secretion of these enzymes from the cell and accumulation of materials within the lysosome because of impaired degradation. None of the other choices relates to I-cell disease or lysosomal function. Oligosaccharides in the urine are characteristic of the muco- and polysaccharidoses but not I-cell disease (a type II mucolipidosis).

14.4 An infant with corneal clouding has dermatan sulfate and heparan sulfate in his urine. Decreased activity of which of the enzymes listed below would confirm the suspected diagnosis of Hurler syndrome?

A. α-L-Iduronidase
B. α-Glucuronidase
C. Glycosyltransferase
D. Iduronate sulfatase

Correct answer = A. Hurler syndrome, a defect in the lysosomal degradation of glycosaminoglycans (GAGs) with corneal clouding, is due to a deficiency in α-L-iduronidase. β-Glucuronidase is deficient in Sly syndrome, and iduronate sulfatase is deficient in Hunter syndrome. Glycosyltransferases are enzymes of GAGs synthesis.

14.5 A 67-year-old male presents for evaluation of pain and stiffness in his left knee and is diagnosed with osteoarthritis. Decreases in which of the following contribute to his symptoms?

A. Lysosomal acid hydrolases
B. Cartilage proteoglycans
C. Cell-surface O-linked glycoproteins
D. Golgi phosphotransferase

Correct answer = B. Proteoglycans contribute to the resilience of cartilage. In osteoarthritis, cartilage has

degraded and the protection normally provided by proteoglycans is lost. The disease is not caused by lysosomal defects including trafficking or function of acid hydrolases.

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[a] For more information on ECM, see *LIR Cell and Molecular Biology*, 2nd Edition, Chapter 2.

[b] For more information on lysosomes, see *LIR Cell and Molecular Biology*, 2nd Ed. Chapter 5.

UNIT III:
Lipid Metabolism

Dietary Lipid Metabolism 15

I. OVERVIEW

Lipids are a heterogeneous group of water-insoluble (hydrophobic) organic molecules (Fig. 15.1). Because of their insolubility in aqueous solutions, body lipids are generally found compartmentalized, as in the case of membrane-associated lipids or droplets of triacylglycerol (TAG) in adipocytes, or transported in blood in association with protein, as in lipoprotein particles or on albumin. Lipids are a major source of energy for the body, and they also provide the hydrophobic barrier that permits partitioning of the aqueous contents of cells and subcellular structures. Lipids serve additional functions in the body (e.g., some fat-soluble vitamins have regulatory or coenzyme functions, and the prostaglandins and steroid hormones play major roles in the control of the body's homeostasis). Deficiencies or imbalances of lipid metabolism can lead to some of the major clinical problems encountered by physicians, such as atherosclerosis, diabetes, and obesity.

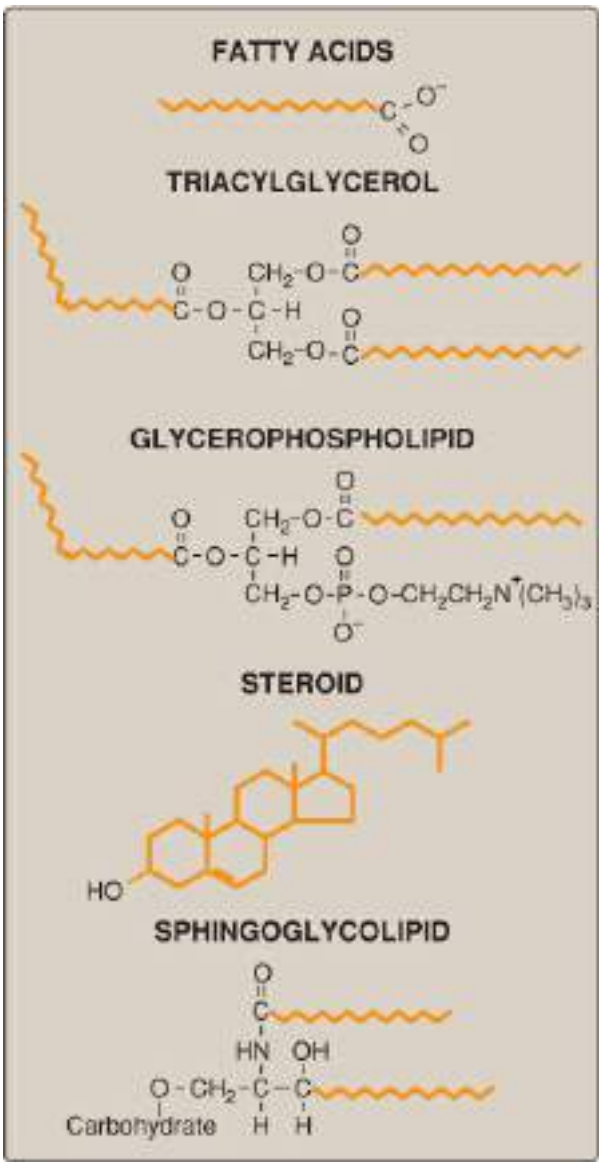


Figure 15.1
Structures of some common classes of lipids. Hydrophobic portions of the molecules are shown in orange.

II. DIGESTION, ABSORPTION, SECRETION, AND UTILIZATION

The average daily intake of lipids by U.S. adults is ~78 g, of which >90% is TAG, also known as triglyceride (TG), that consists of three fatty acids (FA) esterified to a glycerol backbone (see Fig. 15.1). The remainder of the dietary lipids consists primarily of cholesterol, cholesteryl esters, phospholipids, and nonesterified (free) FA (FFA). The digestion of dietary lipids begins in the stomach and is completed in the small intestine. The process is summarized in Figure 15.2.

A. Digestion in the stomach

Lipid digestion in the stomach is limited. It is catalyzed by lingual lipase that originates from glands at the back of the tongue and gastric lipase that is secreted by the gastric mucosa. Both enzymes are relatively acid stable, with optimal pH values of 4 to 6. These acid lipases hydrolyze FA from TAG molecules, particularly those containing short- or medium-chain-length (≤12 carbons) FA such as are found in milk fat. Consequently, these lipases play a particularly important role in lipid digestion in infants for whom milk fat is the primary source of calories. They also become important digestive enzymes in individuals with pancreatic insufficiency such as those with cystic fibrosis (CF). Lingual and gastric lipases aid these patients in degrading TAG molecules (especially those with short- to medium-chain FA) despite a near or complete absence of pancreatic lipase (see Section D.1. below).

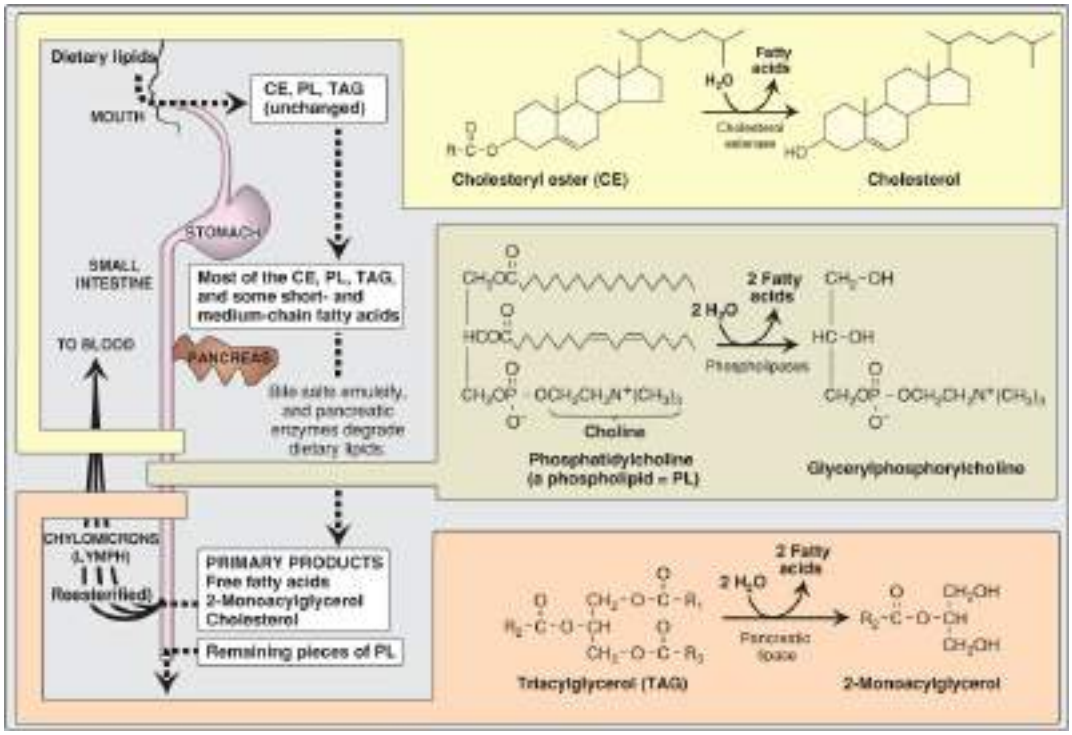


Figure 15.2
Overview of lipid digestion.

B. Cystic fibrosis

CF is the most common lethal genetic disease in Caucasians of Northern European ancestry and has a prevalence of ~1:3,300 births in the United States. CF is an autosomal-recessive disorder caused by mutations to the gene for the CF transmembrane conductance regulator (CFTR) protein that functions as a chloride channel on epithelium in the pancreas, lungs, testes, and sweat glands. Defective CFTR results in decreased secretion of chloride and increased uptake of sodium and water. In the pancreas, the depletion of water on the cell surface results in thickened mucus that clogs the pancreatic ducts, preventing pancreatic enzymes from reaching the intestine, thereby leading to pancreatic insufficiency. Treatment

includes replacement of these enzymes and supplementation with fat-soluble vitamins. (Note: CF also causes chronic lung infections with progressive pulmonary disease and male infertility.)

C. Emulsification in the small intestine

The critical process of dietary lipid emulsification occurs in the duodenum. Emulsification increases the surface area of the hydrophobic lipid droplets so that the digestive enzymes, which work at the interface of the droplet and the surrounding aqueous solution, can act effectively. Emulsification is accomplished by two complementary mechanisms, namely, use of the detergent properties of the conjugated bile salts and mechanical mixing due to peristalsis. Bile salts, made in the liver and stored in the gallbladder, are amphipathic derivatives of cholesterol. Conjugated bile salts consist of a hydroxylated sterol ring structure with a side chain to which a molecule of glycine or taurine is covalently attached by an amide linkage (Fig. 15.3). These emulsifying agents interact with the dietary lipid droplets and the aqueous duodenal contents, thereby stabilizing the droplets as they become smaller from peristalsis and preventing them from coalescing. (Note: See Chapter 18 for a more complete discussion of bile salt metabolism.)

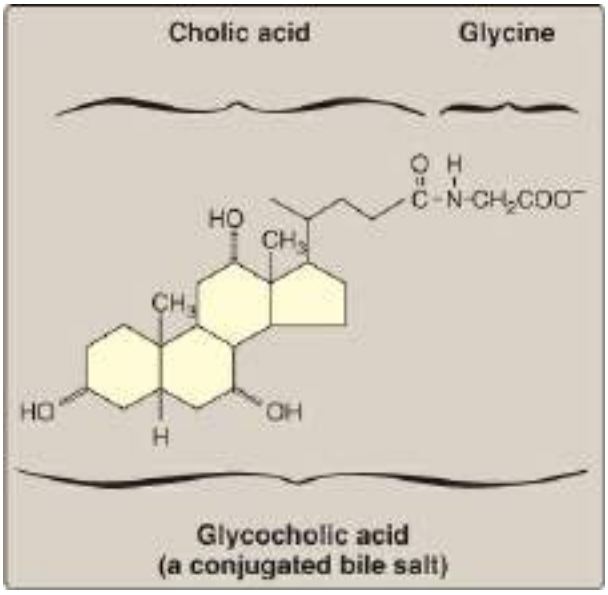


Figure 15.3
Structure of glycocholic acid.

D. Degradation by pancreatic enzymes

The dietary TAG, cholesteryl esters, and phospholipids are enzymatically degraded (digested) in the small intestine by pancreatic enzymes, whose secretion is hormonally controlled.

1. Triacylglycerol degradation: TAG molecules are too large to be taken up

efficiently by the mucosal cells (enterocytes) of the intestinal villi. Therefore, they are hydrolyzed by an esterase, pancreatic lipase, which preferentially removes the FA at carbons 1 and 3. The primary products of hydrolysis are, thus, a mixture of 2-monoacylglycerol (2-MAG) and FFA (see Fig. 15.2). (Note: Pancreatic lipase is found in high concentrations in pancreatic secretions [2% to 3% of the total protein present], and it is highly efficient catalytically, thus ensuring that only severe pancreatic deficiency, such as that seen in CF, results in significant malabsorption of fat.) A second protein, colipase, also secreted by the pancreas, binds the lipase at a ratio of 1:1 and anchors it at the lipid–aqueous interface. Colipase restores activity to lipase in the presence of inhibitory substances like bile salts that bind the micelles. (Note: Colipase is secreted as the zymogen, procolipase, which is activated in the intestine by trypsin.) Orlistat, an antiobesity drug, inhibits gastric and pancreatic lipases, thereby decreasing fat absorption, resulting in weight loss.

2. Cholesteryl ester degradation: Most dietary cholesterol is present in the free (nonesterified) form, with 10% to 15% present in the esterified form. Cholesteryl esters are hydrolyzed by pancreatic cholesteryl ester hydrolase (cholesterol esterase), which produces cholesterol plus FFA (see Fig. 15.2). Activity of this enzyme is greatly increased in the presence of bile salts.

3. Phospholipid degradation: Pancreatic juice is rich in the proenzyme of phospholipase A_2 that, like procolipase, is activated by trypsin and, like cholesteryl ester hydrolase, requires bile salts for optimum activity. Phospholipase A_2 removes one FA from carbon 2 of a phospholipid, leaving a lysophospholipid. For example, phosphatidylcholine (the predominant phospholipid of digestion) becomes lysophosphatidylcholine. The remaining FA at carbon 1 can be removed by lysophospholipase, leaving a glycerylphosphoryl base (e.g., glycerylphosphorylcholine, see Fig. 15.2) that may be excreted in the feces, further degraded, or absorbed.

4. Control: Pancreatic secretion of the hydrolytic enzymes that degrade dietary lipids in the small intestine is hormonally controlled (Fig. 15.4). Enteroendocrine cells found throughout the small intestine secrete several hormones such as cholecystokinin (CCK) and secretin. Enteroendocrine I cells in the mucosa of the lower duodenum and jejunum produce the peptide hormone CCK, in response to the presence of lipids and partially digested proteins entering these regions of the upper small intestine. CCK acts on the gallbladder (causing it to contract and release bile, a mixture of bile salts, phospholipids, and free cholesterol) and on the exocrine cells of the pancreas (causing them to release digestive enzymes). It also decreases gastric motility, resulting in a slower release of gastric contents into the small intestine. Enteroendocrine S cells produce another peptide hormone, secretin, in response to the low pH of the chyme entering the intestine from the stomach. Secretin causes the pancreas to release a solution rich in bicarbonate that helps neutralize the pH of the

intestinal contents, bringing them to the appropriate pH for digestive activity by pancreatic enzymes.

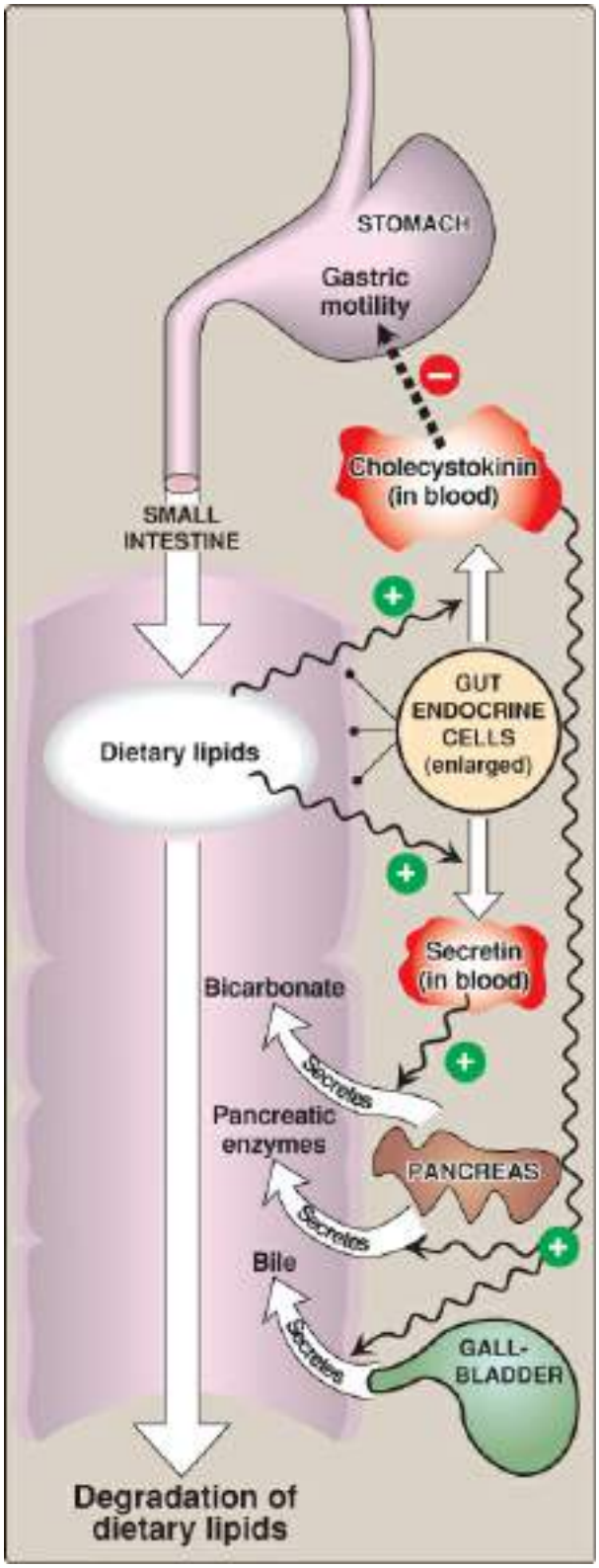


Figure 15.4
Hormonal control of lipid digestion in the small intestine. (Note: The small intestine is divided into three parts: the duodenum [upper 5%], the jejunum, and the ileum [lower 55%].)

E. Absorption by enterocytes

FFA, free cholesterol, and 2-MAG are the primary products of lipid digestion in the jejunum. These, plus bile salts and fat-soluble vitamins (A, D, E, and K), form mixed micelles (i.e., disc-shaped clusters of a mixture of amphipathic lipids that coalesce with their hydrophobic groups on the inside and their hydrophilic groups on the outside). Therefore, mixed micelles are soluble in the aqueous environment of the intestinal lumen (Fig. 15.5). These particles approach the primary site of lipid absorption, the brush border membrane of the enterocytes. This microvilli-rich apical membrane is separated from the liquid contents of the intestinal lumen by an unstirred water layer that mixes poorly with the bulk fluid. The hydrophilic surface of the micelles facilitates the transport of the hydrophobic lipids through the unstirred water layer to the brush border membrane where they are absorbed. Bile salts are absorbed in the terminal ileum, with <5% being lost in the feces. (Note: Cholesterol and plant sterols are taken up by the enterocytes through the Niemann-Pick C1-like 1 (NPC1L1) protein in the brush border cells. Ezetimibe, a cholesterol-lowering drug, inhibits NPC1L1 reducing cholesterol absorption in the small intestine.) Because short- and medium-chain FA are water soluble, they do not require the assistance of mixed micelles for absorption by the intestinal mucosa.

F. Triacylglycerol and cholesteryl ester resynthesis

The mixture of lipids absorbed by the enterocytes migrates to the smooth endoplasmic reticulum (SER) where biosynthesis of complex lipids takes place. The long-chain FA are first converted into their activated form by fatty acyl coenzyme A (CoA) synthetase (thiokinase), as shown in Figure 15.6. Using the fatty acyl CoA derivatives, the 2-MAG absorbed by the enterocytes are converted to TAG through sequential reacylations by two acyltransferases, acyl CoA: MAG acyltransferase and acyl CoA:diacylglycerol acyltransferase. Lysophospholipids are reacylated to form phospholipids by a family of acyltransferases, and cholesterol is acylated primarily by acyl CoA:cholesterol acyltransferase. (Note: Virtually all long-chain FA entering the enterocytes are used in this fashion to form TAG, phospholipids, and cholesteryl esters. Short- and medium-chain FA are not converted to their CoA derivatives and are not reesterified to 2-MAG. Instead, they are released into the portal circulation, where they are carried by serum albumin to the liver.)

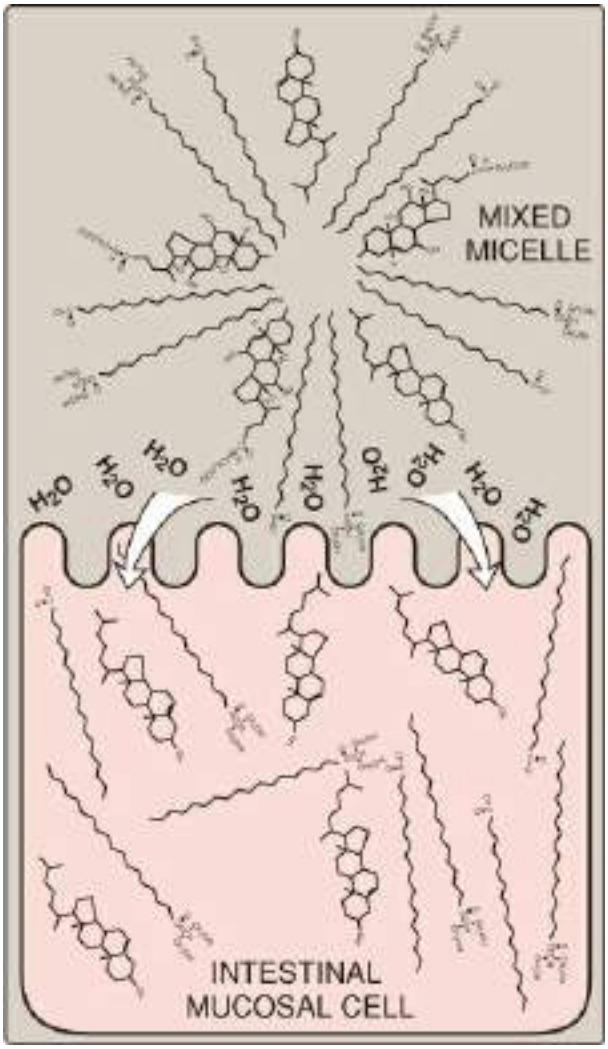


Figure 15.5
Absorption of lipids contained in a mixed micelle by an intestinal mucosal cell. The micelle itself is not absorbed. (Note: Short- and medium-chain-length fatty acids do not require incorporation into micelles.)

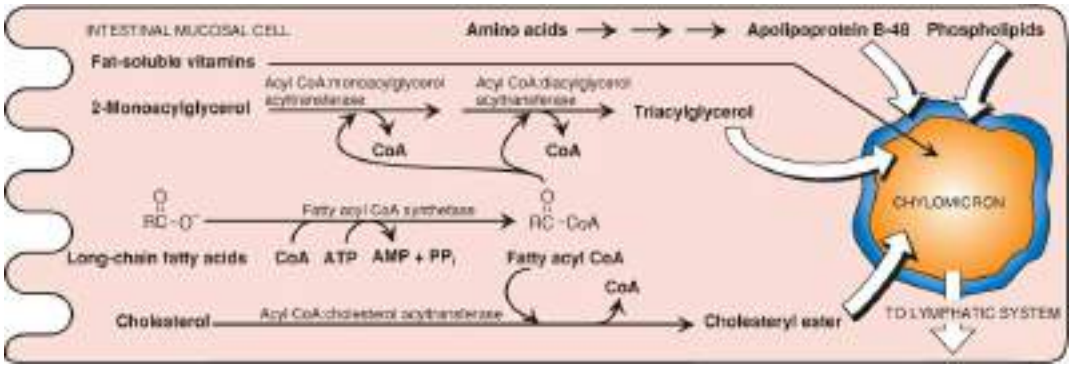


Figure 15.6
Assembly and secretion of chylomicrons by intestinal mucosal cells. (Note: Short- and medium-chain-length fatty acids do not require incorporation into chylomicrons and directly enter into the blood.) CoA = coenzyme A; AMP = adenosine monophosphate; PP_i = pyrophosphate.

G. Secretion from enterocytes

The newly resynthesized TAG and cholesteryl esters are very hydrophobic and aggregate in an aqueous environment. Therefore, they must be packaged as particles of lipid droplets surrounded by a thin layer composed of phospholipids, nonesterified cholesterol, and a molecule of the protein apolipoprotein (apo) B-48. This layer stabilizes the particle and increases its solubility, thereby preventing multiple particles from coalescing. (Note: Microsomal TG transfer protein is essential for the assembly of all TAG-rich apo B–containing particles in the ER.) The lipoprotein particles are released by exocytosis from enterocytes into the lacteals (lymphatic vessels in the villi of the small intestine). The presence of these particles in the lymph after a lipid-rich meal gives it a milky appearance. This lymph is called chyle (as opposed to chyme, the name given to the semifluid mass of partially digested food that passes from the stomach to the duodenum), and the particles are named chylomicrons. Chylomicrons follow the lymphatic system to the thoracic duct and are then conveyed to the left subclavian vein, where they enter the blood. The steps in the production of chylomicrons are summarized in Figure 15.6. (Note: Once released into blood, the nascent [immature] chylomicrons pick up apolipoproteins E and C-II from high-density lipoproteins and mature. [For a more detailed description of chylomicron structure and metabolism, see Chapter 18.])

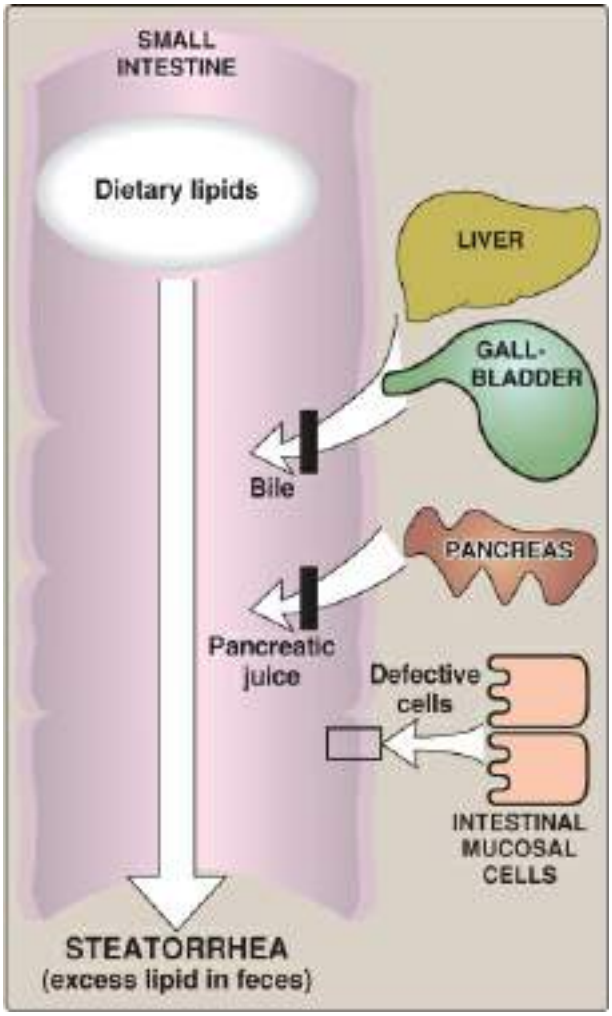


Figure 15.7
Possible causes of steatorrhea.

H. Lipid malabsorption

Lipid malabsorption, resulting in increased lipid (including the fat-soluble vitamins and essential FA, see Chapter 16) in the feces, a condition known as steatorrhea, can be caused by disturbances in lipid digestion and/or absorption (Fig. 15.7). Such disturbances can result from several conditions, including CF (causing poor digestion), short bowel syndrome (causing decreased absorption), and bariatric surgery (insufficient secretion of pancreatic enzymes).

> The ability of short- and medium-chain FA to be taken up by enterocytes without the aid of mixed micelles has made them important in medical nutrition therapy for individuals with malabsorption disorders.

I. Use by the tissues

Most of the TAG contained in chylomicrons is broken down in the capillary beds of skeletal and cardiac muscle and adipose tissue. The TAG is degraded to FFA and glycerol by lipoprotein lipase (LPL). This enzyme is synthesized and secreted primarily by adipocytes and muscle cells. Secreted LPL is anchored to the luminal surface of endothelial cells in the capillaries of muscle and adipose tissues. LPL is activated when bound to the cofactor, ApoCII which resides on the circulating lipoprotein particles. (Note: Familial chylomicronemia [type I Hyperlipoproteinemia] is a rare, autosomal-recessive disorder caused by a deficiency of LPL or its coenzyme apo C-II [see Chapter 18]. The result is fasting chylomicronemia and severe hypertriacylglycerolemia, which can cause pancreatitis.)

1. Fate of free fatty acids: The FFA derived from the hydrolysis of TAG may either directly enter adjacent muscle cells and adipocytes or be transported in the blood in association with serum albumin until they are taken up by cells. (Note: Human serum albumin is a large protein secreted by the liver. It transports a number of primarily hydrophobic compounds in the circulation, including FFA and some drugs.) Most cells can oxidize FA to produce energy. Adipocytes can also reesterify FFA to produce TAG molecules, which are stored until the FA are needed by the body.

2. Fate of glycerol: Glycerol released from TAG is taken up from the blood and phosphorylated by hepatic glycerol kinase to produce glycerol 3-phosphate, which can enter either glycolysis or gluconeogenesis by oxidation to dihydroxyacetone phosphate or be used in TAG synthesis (see Chapter 16).

3. Fate of chylomicron remnants: After most of the TAG has been removed, the chylomicron remnants (which contain cholesteryl esters, phospholipids, apolipoproteins, fat-soluble vitamins, and a small amount of TAG) bind to receptors on the liver (apo E is the ligand; see Chapter 18) and are endocytosed. The intracellular remnants are hydrolyzed to their component parts. Cholesterol and the nitrogenous bases of phospholipids (e.g., choline) can be recycled by the body. (Note: If removal of remnants by the liver is decreased because of impaired binding to their receptor, they accumulate in the plasma. This is seen in the rare type III hyperlipoproteinemia [also called familial dysbetalipoproteinemia or broad beta disease].)

III. Chapter Summary

- **Dietary lipid digestion** begins in the stomach and continues in the small intestine (Fig. 15.8).
- **Cholesteryl esters**, **phospholipids**, and **TAG** containing **long-chain-length FA** are degraded in the **small intestine** by **pancreatic enzymes**. The most important of these enzymes are **cholesterol esterase**, **phospholipase A_2**, and **pancreatic lipase**. In **CF**, thickened mucus prevents these enzymes reaching the intestine.
- TAGs in **milk fat** contain **short-** to **medium-chain-length FA** and are degraded in the **stomach** by **acid lipases** (**lingual lipase** and **gastric lipase**).
- The **hydrophobic** nature of lipids requires that dietary lipids be **emulsified** for efficient degradation. Emulsification occurs in the small intestine using **peristaltic action** (mechanical mixing) and **bile salts** (detergents).
- The primary products of dietary lipid degradation are **2-MAG**, nonesterified (free) **cholesterol**, and **free FA**. These compounds, plus the **fat-soluble vitamins**, form **mixed micelles** that facilitate dietary lipid absorption by **intestinal mucosal cells** (**enterocytes**). These cells use activated long-chain FA to regenerate TAG and cholesteryl esters and also synthesize protein **apo B-48**, all of which are then assembled with the fat-soluble vitamins into **lipoprotein particles** called **chylomicrons**. Short- and medium-chain FA enter blood directly.
- Chylomicrons are first released into the **lymph** and then enter the **blood**, where their lipid core is degraded by **LPL** (with **apo C-II** as the coenzyme) in the **capillaries** of **muscle** and **adipose** tissues. Thus, dietary lipids are made available to the peripheral tissues.
- A deficiency in the ability to degrade chylomicron components, or remove chylomicron remnants after TAG has been degraded, results in accumulation of these particles in blood.
- **Fat maldigestion** or malabsorption causes **steatorrhea** (lipid in the feces).

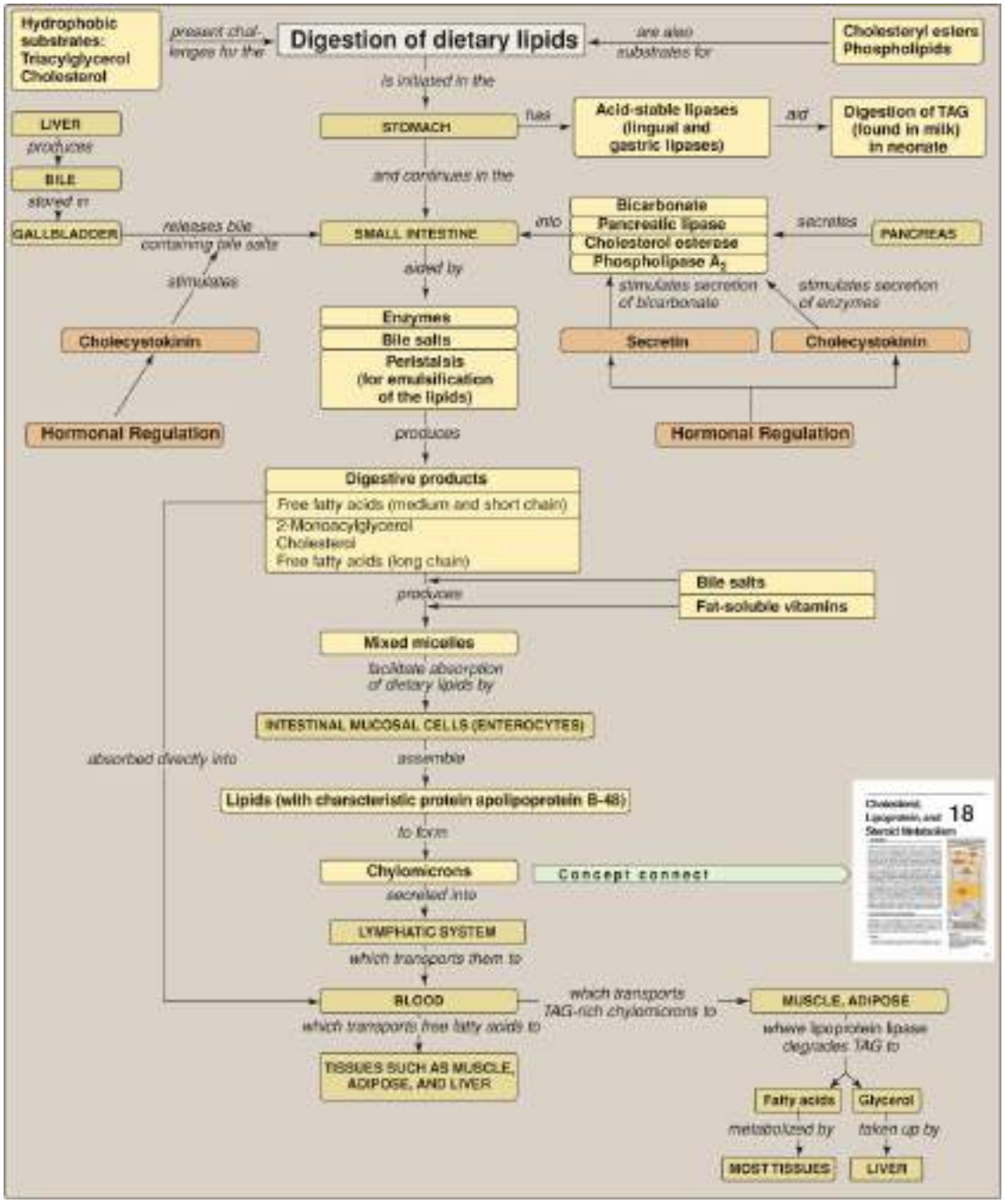


Figure 15.8
Key concept map for metabolism of dietary lipids. TAG = triacylglycerols.

Study Questions

Choose the ONE best answer.

15.1 Which one of the following statements about lipid digestion is correct?

A. Large lipid droplets are emulsified (have their surface area increased) in the mouth through the act of chewing (mastication).

B. The enzyme colipase facilitates the binding of bile salts to mixed micelles, maximizing the activity of pancreatic lipase.
C. The peptide hormone secretin causes the gallbladder to contract and release bile.
D. Patients with cystic fibrosis have difficulties with digestion because their pancreatic secretions are less able to reach the small intestine, the primary site of lipid digestion.
E. Formation of triacylglycerol-rich chylomicrons is independent of protein synthesis in the intestinal mucosa.

Correct answer = D. Patients with cystic fibrosis, a genetic disease resulting in a deficiency of a functional chloride transporter, have thickened mucus that impedes the flow of pancreatic enzymes into the duodenum. Emulsification occurs through peristalsis, which provides mechanical mixing, and bile salts that function as detergents. Colipase restores activity to pancreatic lipase in the presence of inhibitory bile salts that bind the micelles. Cholecystokinin is the hormone that causes contraction of the gallbladder and release of stored bile, and secretin causes release of bicarbonate. Chylomicron formation requires synthesis of apolipoprotein B-48.

15.2 Which one of the following statements about lipid absorption from the intestine is correct?

A. Dietary triacylglycerol must be completely hydrolyzed to free fatty acids and glycerol before absorption.
B. The triacylglycerol carried by chylomicrons is degraded by lipoprotein lipase, producing fatty acids that are taken up by muscle and adipose tissues and glycerol that is taken up by the liver.
C. Fatty acids that contain ≤12 carbon atoms are absorbed and enter the circulation primarily via the lymphatic system.
D. Deficiencies in the ability to absorb fat result in excessive amounts of chylomicrons in the blood.

Correct answer = B. The triacylglycerols (TAG) in chylomicrons are degraded to fatty acids (FA) and glycerol by lipoprotein lipase on capillary endothelial surfaces in muscle and adipose tissue, thus providing a source of FA to these tissues for degradation or storage and providing glycerol for hepatic metabolism. In the duodenum, TAG are degraded to one 2-monoacylglycerol + two free FA that get absorbed. Medium- and short-chain FA enter directly into blood (not lymph), and they neither require micelles nor get packaged into chylomicrons. Because chylomicrons contain dietary lipids that were digested and absorbed, a defect in fat absorption would result in decreased production of chylomicrons.

15.3 A 2-year-old female is brought to the physician because of recurrent respiratory tract infections, weight loss, and foul-smelling diarrhea. This patient most likely has a defective secretion in which of the following?

A. Cholecystokinin
B. Pancreatic enzymes
C. Chylomicron
D. Secretin

Correct answer: B. This patient most likely has cystic fibrosis (CF) which causes defective secretion of pancreatic enzymes such as lipase and colipase due to mutations in the cystic fibrosis transmembrane conductance receptor (CFTR). These enzymes are important for digestion and absorption of lipids. Cholecystokinin and secretin are released from enteroendocrine cells. Although they are important for lipid digestion and absorption, they are not defective in CF. Chylomicron formation and release into lymphatic system is not affected in CF.

15.4 A 45-year-old female is brought to the emergency department due to acute pain, nausea, and vomiting. Computed tomography indicates acute pancreatitis that leads to an increased activation of trypsin. Which of the following is most likely activated in this condition?

A. Gastric lipase
B. Pancreatic lipase
C. Lysophospholipase
D. Colipase

Correct answer: D. Colipase is secreted as the zymogen, procolipase, which is activated in the intestine by trypsin. Colipase is important for pancreatic lipase for hydrolyzing triacylglycerols. Gastric lipase hydrolyze short and medium chain fatty acids in milk, especially important for infants and patients with pancreatic insufficiency.

Lysophospholipase is important for the digestion of phospholipids.

15.5 A 22-month-old child is brought to the physician by her parents because of refusal to feed, chronic diarrhea, abdominal distension, and weight loss. She is diagnosed with chylomicron retention disease, which prevents the release chylomicrons into the lymphatics. This patient most likely has a deficiency in which of the following vitamins?

A. Ascorbic acid
B. Beta-carotene
C. Folate
D. Pyridoxine

Correct answer: B. Chylomicron is important for the absorption of fat-soluble vitamins: vitamin A, D, E, and K. Beta-carotene is a provitamin A that is packaged into chylomicron before its release into the lymphatics. Ascorbic acid is vitamin C, folate is vitamin B9, and pyridoxine is vitamin B6. These three vitamins are water soluble.

Fatty Acid, Triacylglycerol, and Ketone Body Metabolism 16

I. OVERVIEW

Fatty acids exist free in the body (i.e., they are nonesterified) and as fatty acyl esters in more complex molecules such as triacylglycerols (TAGs). Low levels of free fatty acids (FFA) occur in all tissues, but substantial amounts can sometimes be found in the plasma, particularly during fasting. Plasma FFA (transported on serum albumin) are in route from their point of origin (TAG of adipose tissue or circulating lipoproteins) to their site of consumption (most tissues). FFA can be oxidized by many tissues, particularly liver and muscle, to provide energy and, in the liver, to provide the substrate for ketone body synthesis. Fatty acids are also structural components of membrane lipids, such as phospholipids and glycolipids (see Chapter 17). Fatty acids attached to certain proteins enhance the ability of those proteins to associate with membranes. Fatty acids are also precursors of the hormone-like prostaglandins (see Chapter 17). Esterified fatty acids, in the form of TAG stored in white adipose tissue (WAT), serve as the major energy reserve of the body. Alterations in fatty acid metabolism are associated with obesity and diabetes. Figure 16.1 illustrates the metabolic pathways of fatty acid synthesis and degradation and their relationship to carbohydrate metabolism.

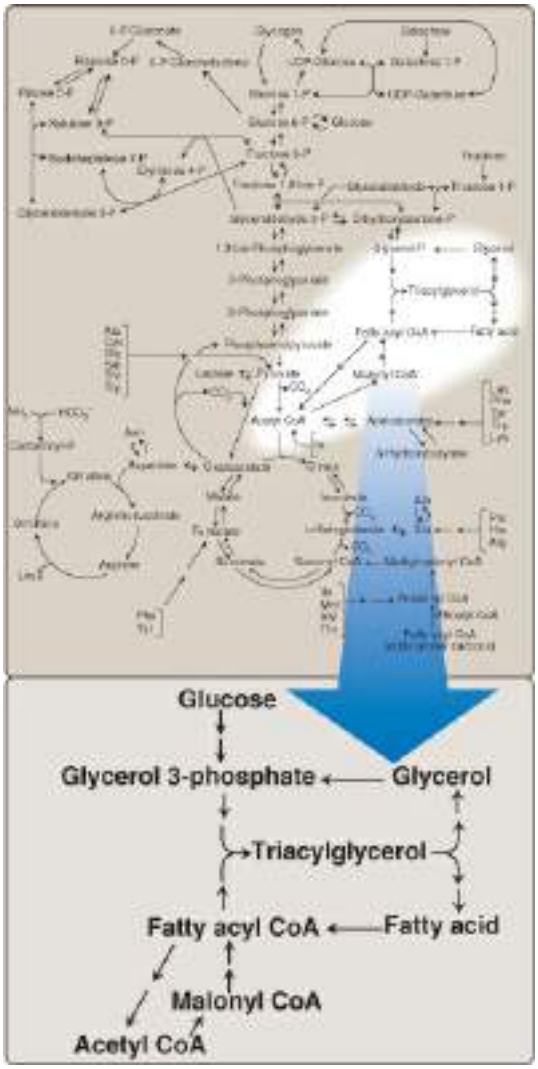


Figure 16.1
Triacylglycerol synthesis and degradation. CoA = coenzyme A.

II. FATTY ACID STRUCTURE

A fatty acid consists of a hydrophobic hydrocarbon chain with a terminal carboxyl group that has a pK_a (see p. 6) of ~4.8 (Fig. 16.2). At physiologic pH, the terminal carboxyl group (–COOH) ionizes, becoming $–COO^-$. (Note: When the pH is above the pK, the deprotonated form predominates [see p. 7].) This anionic group has an affinity for water, giving the fatty acid its amphipathic nature (having both a hydrophilic and a hydrophobic region). However, for long-chain-length fatty acids (LCFA), the hydrophobic portion is predominant. These molecules are highly water insoluble and must be transported in the circulation in association with protein. More than 90% of the fatty acids found in plasma are in the form of fatty acid esters (primarily TAG, cholesteryl esters, and phospholipids) contained in circulating lipoprotein particles (see Chapter 18). FFA are transported in the circulation in association with albumin, the most abundant protein in

serum.

Figure 16.2
Structure of a fatty acid. The carbon next to carbonyl group is designated as alpha (α). The next carbon is the beta carbon (β). When the chain is longer, the last carbon in the chain is designated as the ω carbon.

A. Fatty acid saturation

Fatty acid chains may contain no double bonds (i.e., be saturated) or contain one or more double bonds (i.e., be mono- or polyunsaturated). In humans, the majority are saturated or monounsaturated. When double bonds are present, they are nearly always in the cis rather than in the trans configuration. The introduction of a cis double bond causes the fatty acid to bend or kink at that position (Fig. 16.3). If the fatty acid has two or more double bonds, they are always spaced at three-carbon intervals. (Note: In general, addition of double bonds decreases the melting temperature [T_m] of a fatty acid, whereas increasing the chain length increases the T_m. Because membrane lipids typically contain LCFA, the presence of double bonds in some fatty acids helps maintain the fluid nature of those lipids. See p. 407 for a discussion of the dietary occurrence of cis and trans unsaturated fatty acids.)

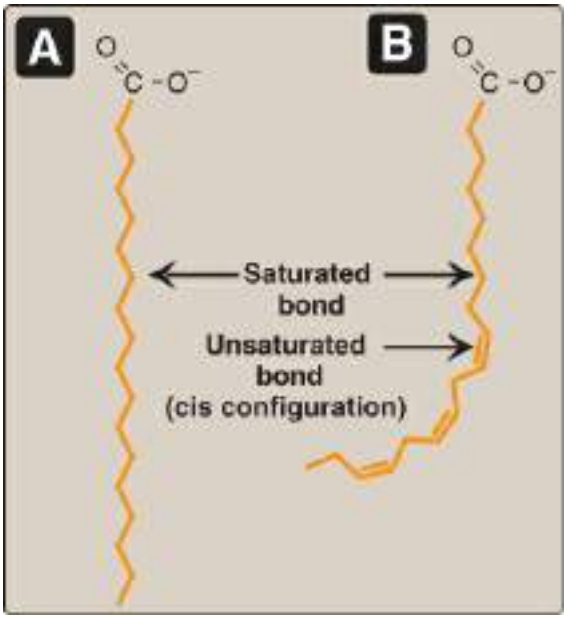


Figure 16.3
A saturated (**A**) and an unsaturated (**B**) fatty acid. Orange denotes hydrophobic portions of the molecules. (Note: Cis double bonds cause a fatty acid to kink.)

B. Fatty acid chain length and double-bond positions

The common names and structures of some fatty acids of physiologic importance are listed in Figure 16.4. In humans, fatty acids with an even number of carbon atoms (16, 18, or 20) predominate, with longer fatty acids (>22 carbons) being found in the brain. The carbon atoms are numbered, beginning with the carbonyl carbon as carbon 1. The number before the colon indicates the number of carbons in the chain, and those after the colon indicate the numbers and positions (relative to the carboxyl end) of double bonds. For example, as denoted in Figure 16.4, arachidonic acid, 20:4(5,8,11,14), is 20 carbons long and has four double bonds (between carbons 5–6, 8–9, 11–12, and 14–15). (Note: Carbon 2, the carbon to which the carboxyl group is attached, is also called the α-carbon, carbon 3 is the β-carbon, and carbon 4 is the γ-carbon. The carbon of the terminal methyl group is called the ω-carbon regardless of the chain length.) The double bonds in a fatty acid can also be referenced relative to the ω (methyl) end of the chain. Arachidonic acid is referred to as an ω-6 fatty acid because the terminal double bond is six bonds from the ω end (Fig. 16.5A). (Note: The equivalent designation of n-6 may also be used [Fig. 16.5B].) Another ω-6 fatty acid is the essential linoleic acid 18:2(9,12). In contrast, α-linolenic acid, 18:3(9,12,15), is an essential ω-3 fatty acid.

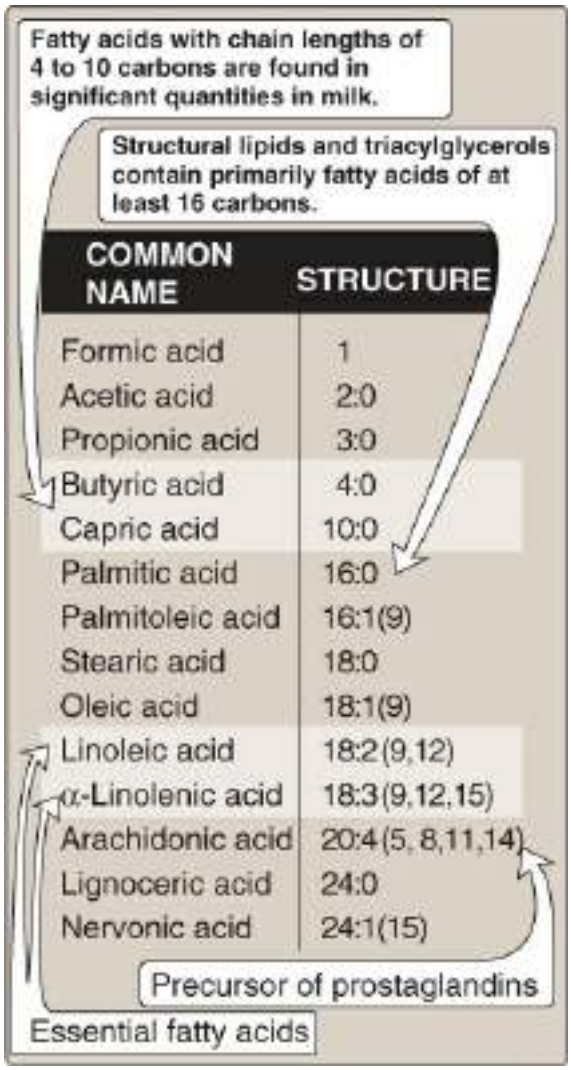


Figure 16.4
Some fatty acids of physiologic importance. (Note: A fatty acid containing 2 to 4 carbons is considered short; 6 to 12, medium; 14 to 20, long; and ≥22, very long.)

C. Essential fatty acids

Linoleic acid, the precursor of ω-6 arachidonic acid that is the substrate for prostaglandin synthesis, and α-linolenic acid, the precursor of ω-3 fatty acids that are important for growth and development, are dietary essentials in humans because we lack the enzymes that can form carbon–carbon double bonds after the 9th carbon from the methyl (ω) end of a fatty acid. Plants provide us with these essential fatty acids. (Note: Arachidonic acid becomes essential if linoleic acid is deficient in the diet. See Chapter 27 for a discussion of the nutritional significance of ω-3 and ω-6 fatty acids.)

Essential fatty acid deficiency (rare) can result in a dry, scaly dermatitis as a result of an inability to synthesize molecules that provide the water barrier in skin (see p. 228).

III. FATTY ACID *DE NOVO* SYNTHESIS

Carbohydrates and proteins obtained from the diet in excess of the body's needs for these nutrients can be converted to fatty acids. In adults, *de novo* fatty acid synthesis occurs primarily in the liver and lactating mammary glands and, to a lesser extent, in adipose tissue. This cytosolic process is endergonic and reductive. It incorporates carbons from acetyl coenzyme A (CoA) into the growing fatty acid chain, using ATP and reduced nicotinamide adenine dinucleotide phosphate (NADPH). (Note: Dietary TAG also supply fatty acids. See Chapter 24 for a discussion of the metabolism of dietary nutrients in the well-fed state.)

A. Cytosolic acetyl CoA production

The first step in fatty acid synthesis is the transfer of acetate units from mitochondrial acetyl CoA to the cytosol. Mitochondrial acetyl CoA is produced by the oxidation of pyruvate (see Chapter 9) and by the catabolism of certain amino acids (see Chapter 20). However, the CoA portion of acetyl CoA cannot cross the inner mitochondrial membrane, and only the acetyl portion enters the cytosol. It does so as part of citrate produced by the condensation of acetyl CoA with oxaloacetate (OAA) by citrate synthase (Fig. 16.6). (Note: The transport of citrate to the cytosol occurs when the mitochondrial citrate concentration is high. This is observed when isocitrate dehydrogenase of the tricarboxylic acid [TCA] cycle is inhibited by the presence of large amounts of ATP, causing citrate and isocitrate to accumulate. Therefore, cytosolic citrate may be viewed as a high-energy signal. Because a large amount of ATP is needed for fatty acid synthesis, the increase in both ATP and citrate enhances this pathway.) In the cytosol, citrate is cleaved to OAA and acetyl CoA by ATP citrate lyase.

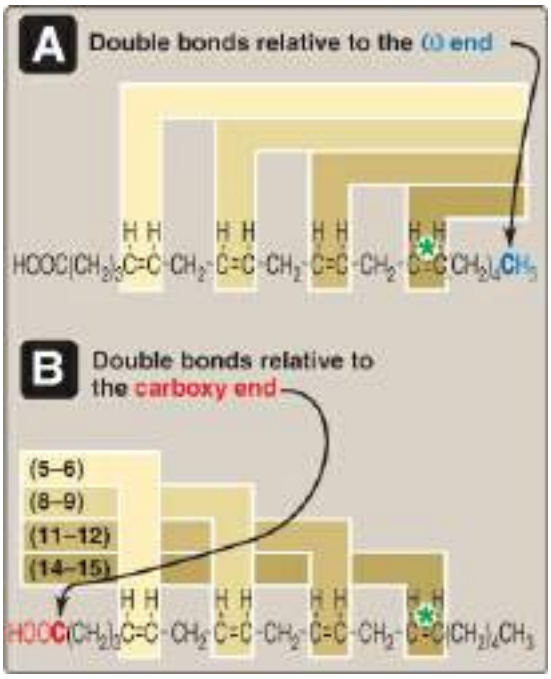


Figure 16.5
Arachidonic acid, 20:4(5,8,11,14), illustrating the position of the double bonds. **A:** Arachidonic acid is an ω-6 fatty acid because the first double bond from the ω end is 6 carbons from that end. **B:** It is also referred to as an n-6 fatty acid because the last double bond from the carboxyl end is 14 carbons from that end: 20 − 14 = 6 = n. Thus, the "ω" and "n" designations are equivalent (see*).

B. Acetyl CoA carboxylation to malonyl CoA

The energy for the carbon-to-carbon condensations in fatty acid synthesis is supplied by the carboxylation and then decarboxylation of acyl groups in the cytosol. The carboxylation of acetyl CoA to malonyl CoA is catalyzed by acetyl CoA carboxylase (ACC) (Fig. 16.7). ACC transfers carbon dioxide (CO_2) from bicarbonate (HCO_3^-) in an ATP-requiring reaction. The coenzyme is biotin (vitamin B_7), which is covalently bound to a lysyl residue of the carboxylase (see Fig. 28.16). ACC carboxylates the bound biotin, which transfers the activated carboxyl group to acetyl CoA.

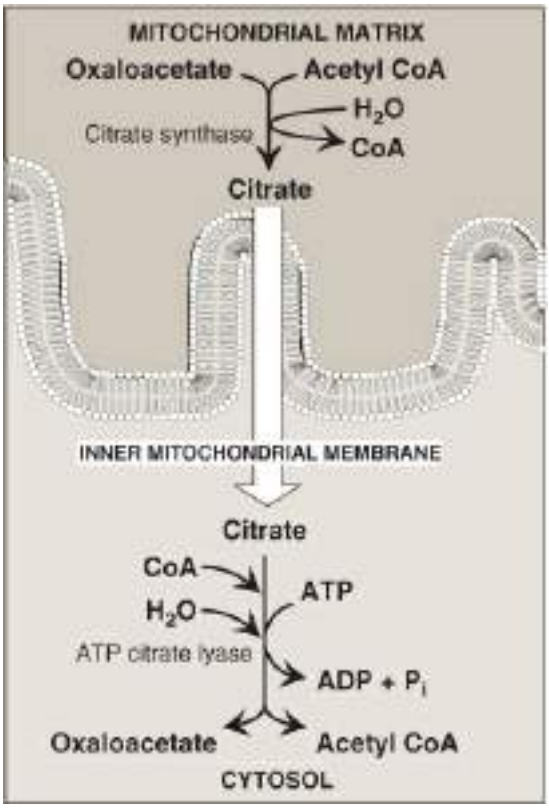


Figure 16.6
Production of cytosolic acetyl coenzyme A (CoA). (Note: Citrate is transported by the tricarboxylate transporter system.) ADP = adenosine monophosphate; P_i = inorganic phosphate.

1. Acetyl CoA carboxylase short-term regulation: This carboxylation is both the rate-limiting and the regulated step in fatty acid synthesis (see Fig. 16.7). The inactive form of ACC is a protomer (complex of ≥2 polypeptides). The enzyme is allosterically activated by citrate, which causes protomers to polymerize, and allosterically inactivated by palmitoyl CoA (the end product of the pathway), which causes depolymerization. A second mechanism of short-term regulation is by reversible phosphorylation. Adenosine monophosphate–activated protein kinase (AMPK) phosphorylates and inactivates ACC. AMPK itself is activated allosterically by AMP and covalently by phosphorylation via several kinases. At least one of these AMPK kinases is activated by cyclic AMP (cAMP)–dependent protein kinase A (PKA). Thus, in the presence of counterregulatory hormones, such as epinephrine and glucagon, ACC is phosphorylated and inactive (Fig. 16.8). In the presence of insulin, ACC is dephosphorylated and active. (Note: This is analogous to the regulation of glycogen synthase [see Chapter 11].)

2. Acetyl CoA carboxylase long-term regulation: Prolonged consumption of a diet containing excess calories (particularly high-carbohydrate, low-fat diets) causes an increase in ACC synthesis, thereby increasing fatty acid synthesis. A low-calorie or a high-fat, low-carbohydrate diet has the opposite effect. (Note: ACC synthesis is upregulated by carbohydrate [specifically glucose] via the

transcription factor carbohydrate response element–binding protein [ChREBP] and by insulin via the transcription factor sterol regulatory element–binding protein-1c [SREBP-1c]. Fatty acid synthase (FAS)[see C. below] is similarly regulated. The function and regulation of SREBP are described in Chapter 18.) Metformin, used in the treatment of type 2 diabetes, lowers plasma TAG through activation of AMPK, resulting in inhibition of ACC activity (by phosphorylation) and inhibition of ACC and FAS expression (by decreasing SREBP-1c). Metformin lowers blood glucose by increasing AMPK-mediated glucose uptake by muscle.

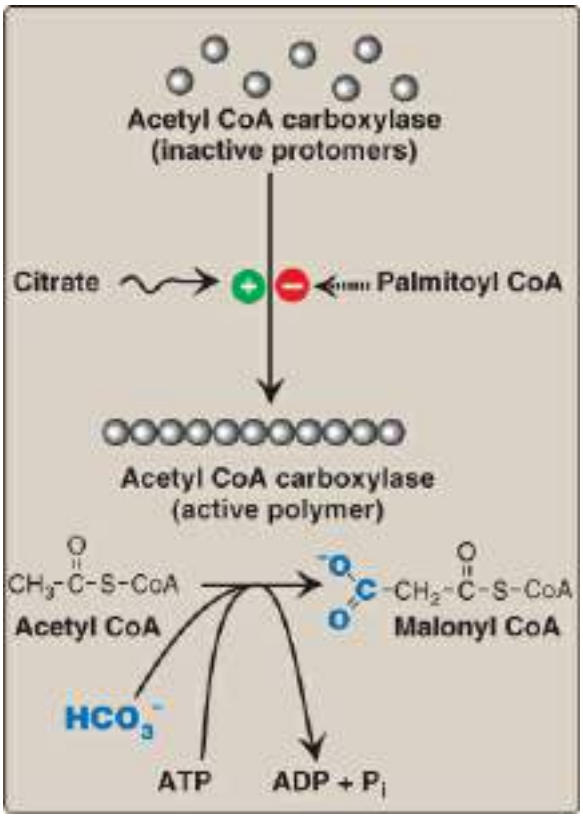


Figure 16.7
Allosteric regulation of malonyl coenzyme A (CoA) synthesis by acetyl CoA carboxylase. The carboxyl group contributed by bicarbonate (HCO_3^-) is shown in *blue*. P_i = inorganic phosphate; ADP = adenosine diphosphate.

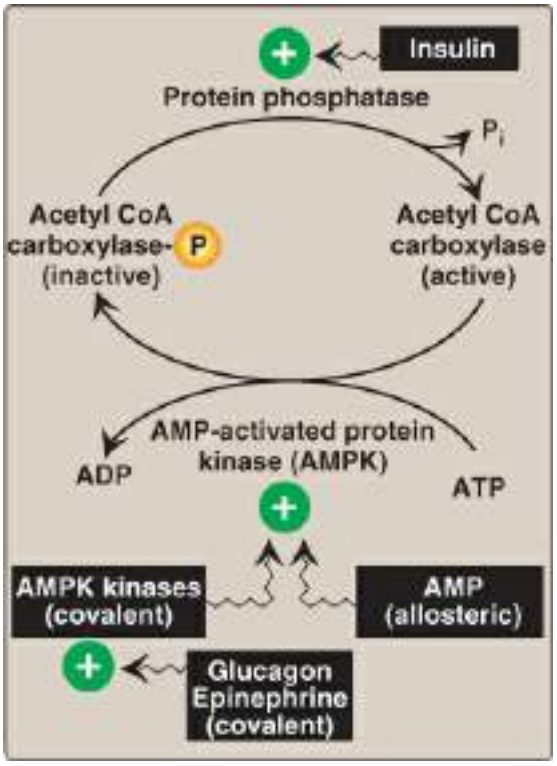


Figure 16.8
Covalent regulation of acetyl CoA carboxylase by AMPK, which itself is regulated both covalently and allosterically. CoA = coenzyme A; ADP and AMP = adenosine di- and monophosphates; P = phosphate; P_i = inorganic phosphate.

C. Eukaryotic fatty acid synthase

The remaining series of reactions of fatty acid synthesis in eukaryotes is catalyzed by the multifunctional, homodimeric enzyme FAS. The process involves the addition of two carbons from malonyl CoA to the carboxyl end of a series of acyl acceptors. Each FAS monomer is a multicatalytic polypeptide with six different enzymic domains plus a 4′-phosphopantetheine-containing acyl carrier protein (ACP) domain. 4′-Phosphopantetheine, a derivative of pantothenic acid (vitamin B_5, see Chapter 28), carries acyl units on its terminal thiol (–SH) group and presents them to the catalytic domains of FAS during fatty acid synthesis. It also is a component of CoA. The reaction numbers in brackets below refer to Figure 16.9.

(1) An acetyl group is transferred from acetyl CoA to the –SH group of the ACP.

(2) Next, this two-carbon fragment is transferred to a temporary holding site.

(3) The now-vacant ACP accepts a three-carbon malonyl group from malonyl CoA.

(4) The acetyl group on the cysteine residue condenses with the malonyl group on ACP with the release of CO_2, which was originally added by ACC. The result is a four-carbon unit attached to the ACP domain.

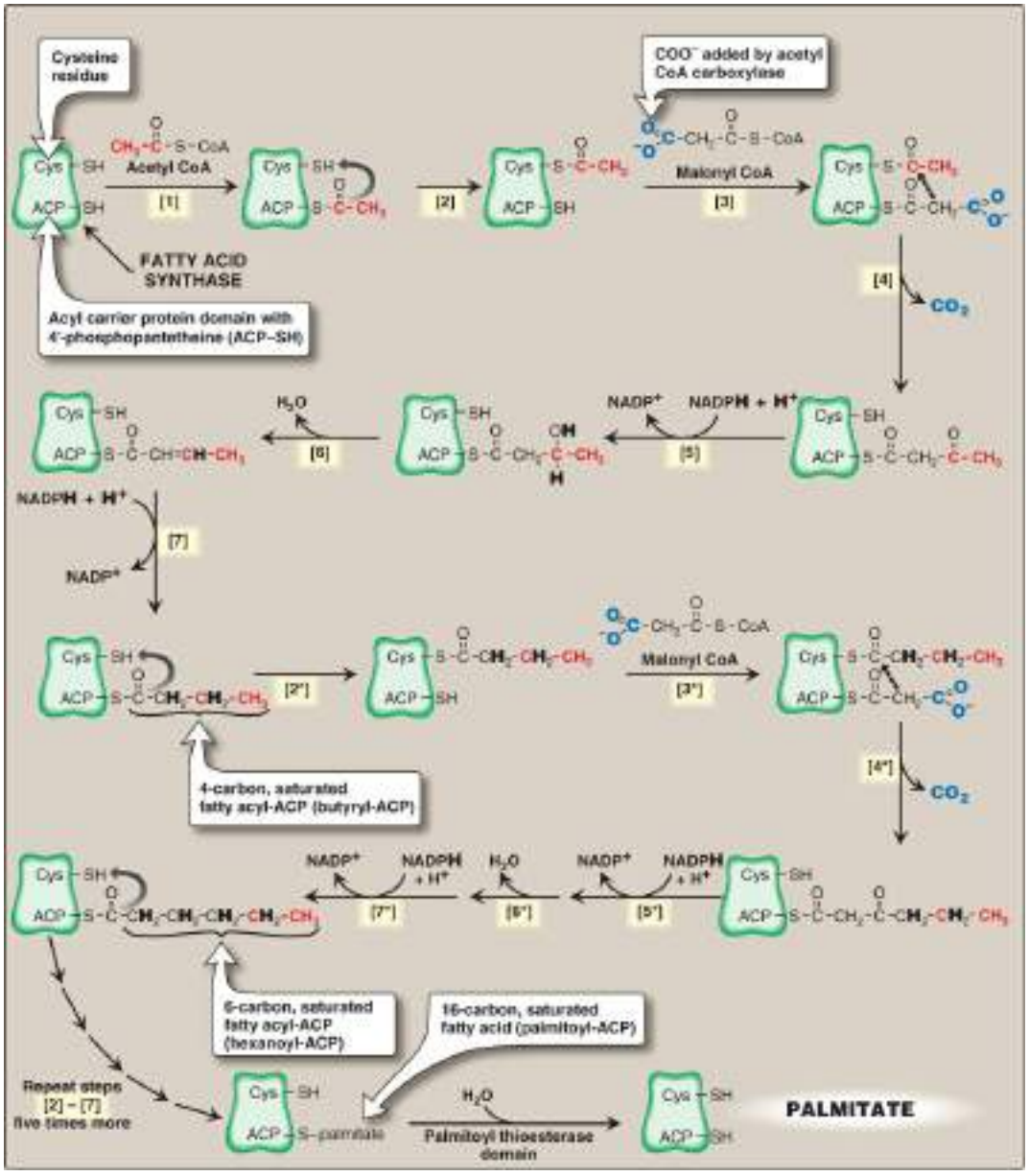


Figure 16.9
Synthesis of palmitate (16:0) by multifunctional fatty acid synthase. (Note: Numbers in brackets correspond to bracketed numbers in the text. A second repetition of the steps is indicated by numbers with an asterisk [*]. Carbons provided directly by acetyl coenzyme A [CoA] are shown in *red*.) ACP = acyl carrier protein domain; CO_2 = carbon dioxide; NADP(H) = nicotinamide adenine dinucleotide phosphate.

The next three reactions convert the 3-ketoacyl group to the corresponding saturated acyl group by a pair of NADPH-requiring reductions and a dehydration step.

(5) The keto group is reduced to an alcohol.

(6) A molecule of water is removed, creating a trans double bond between carbons 2 and 3 (the α- and β-carbons).

(7) The double bond is reduced.

This sequence of steps results in the production of a four-carbon group (butyryl) whose three terminal carbons are fully saturated and which remains attached to the ACP domain. The steps are repeated (indicated by an asterisk), beginning with the transfer of the butyryl unit from the ACP to the cysteine residue [2*], the attachment of a malonyl group to the ACP [3*], and the condensation of the two groups liberating CO_2 [4*]. The carbonyl group at the β-carbon (carbon 3, the third carbon from the sulfur) is then reduced [5*], dehydrated [6*], and reduced [7*], generating hexanoyl-ACP. This cycle of reactions is repeated until the fatty acid reaches a length of 16 carbons. The final catalytic activity of FAS, cleaves the thioester bond, releasing a fully saturated molecule of palmitate (16:0). (Note: All the carbons in palmitic acid have passed through malonyl CoA except the two donated by the original acetyl CoA [the first acyl acceptor], which are found at the methyl [ω] end of the fatty acid. This underscores the rate-limiting nature of the ACC reaction.) Shorter-length fatty acids are produced only in the lactating mammary gland.

D. Reductant sources

The synthesis of one palmitate requires 14 NADPH, a reductant (reducing agent). The pentose phosphate pathway (see Chapter 13) is a major supplier of the NADPH. Two NADPH are produced for each molecule of glucose 6-phosphate that enters this pathway. The cytosolic conversion of malate to pyruvate, in which malate is oxidized and decarboxylated by cytosolic malic enzyme ($NADP^+$-dependent malate dehydrogenase), also produces cytosolic NADPH (and CO_2), as shown in Figure 16.10. (Note: Malate can arise from the reduction of OAA by cytosolic NADH-dependent malate dehydrogenase [see Fig. 16.10]. One source of the cytosolic NADH required for this reaction is glycolysis. OAA, in turn, can arise from citrate cleavage by ATP citrate lyase.) A summary of the interrelationship between glucose metabolism and palmitate synthesis is shown in Figure 16.11.

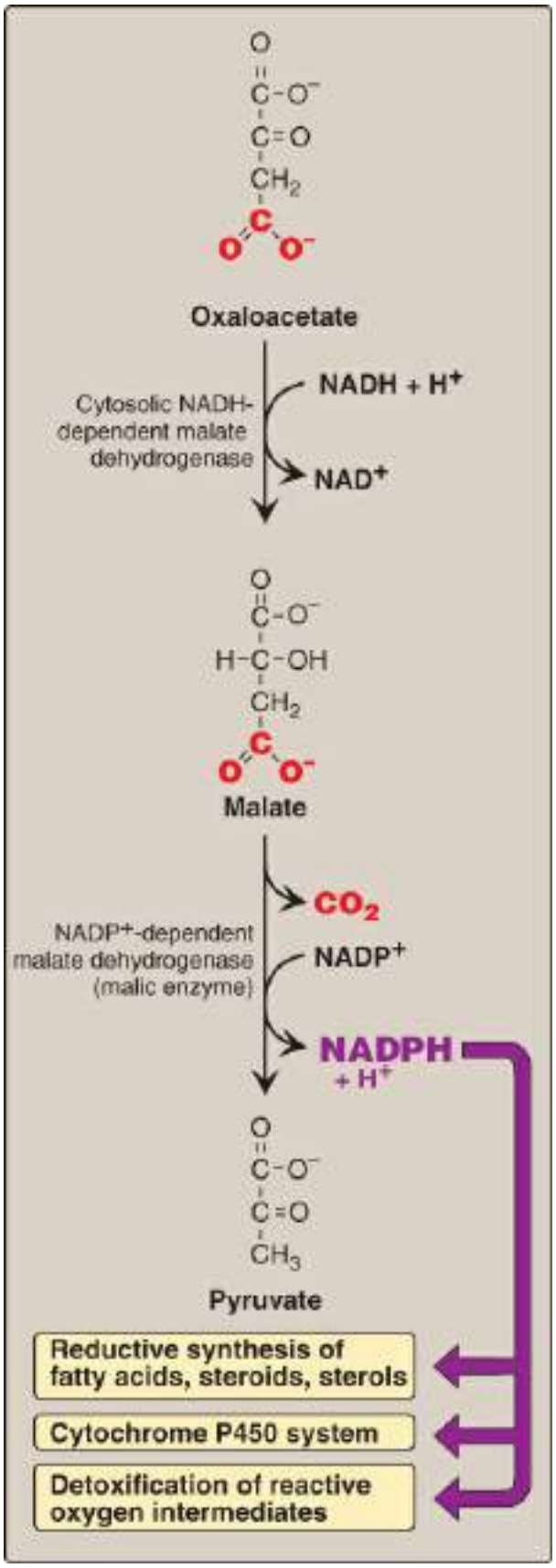


Figure 16.10
Cytosolic conversion of oxaloacetate to pyruvate with the generation of nicotinamide adenine dinucleotide phosphate (NADPH). (Note: The pentose phosphate pathway is also a source of NADPH.) NAD(H) = nicotinamide adenine dinucleotide; CO_2 = carbon dioxide.

E. Further elongation

Although palmitate, a 16-carbon, fully saturated LCFA (16:0), is the primary end product of FAS activity, it can be further elongated by the addition of two-carbon units to the carboxylate end primarily in the smooth endoplasmic reticulum (SER). Elongation requires a system of separate enzymes rather than a multifunctional enzyme. Malonyl CoA is the two-carbon donor, and NADPH supplies the electrons. The brain has additional elongation capabilities, allowing it to produce the very–long-chain fatty acids ([VLCFA] over 22 carbons) that are required for synthesis of brain lipids.

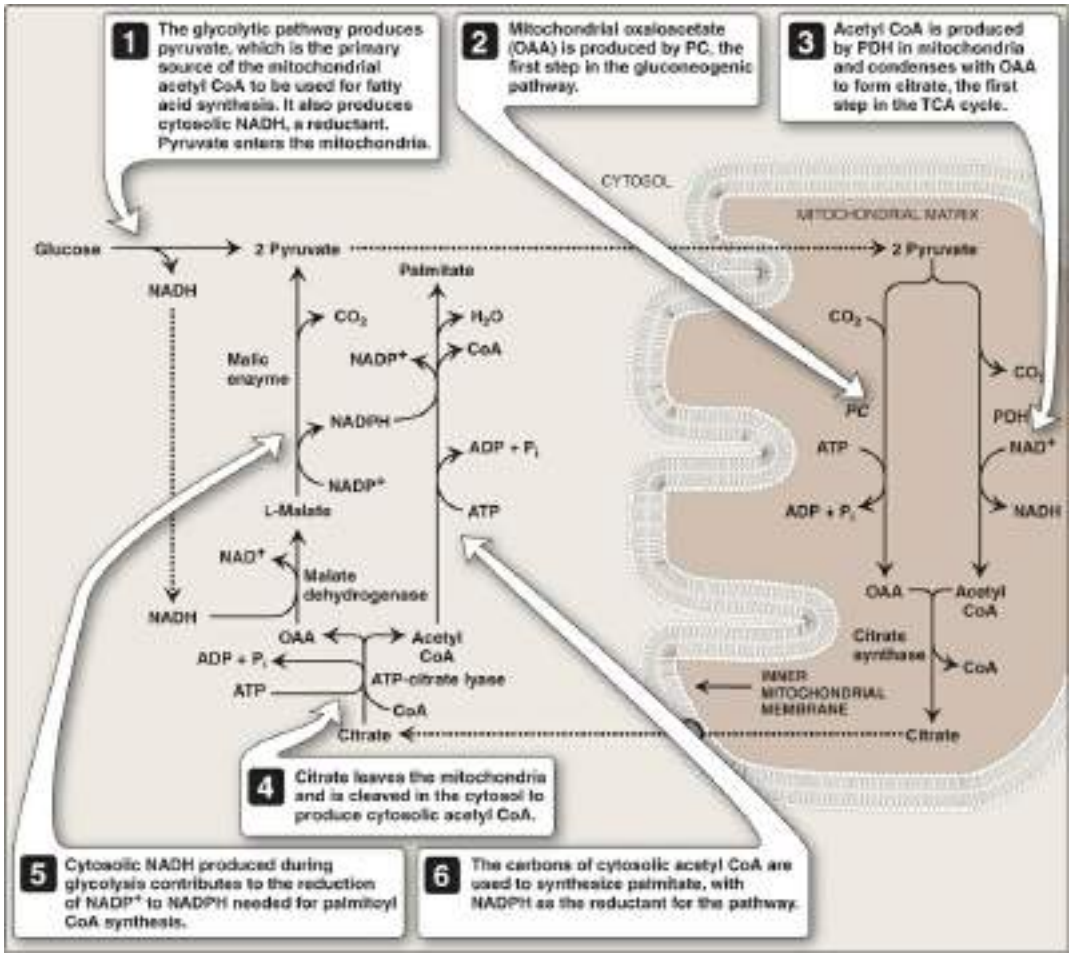


Figure 16.11
Interrelationship between glucose metabolism and palmitate synthesis. CoA = coenzyme A; NAD(H) = nicotinamide adenine nucleotide; NADP(H) = nicotinamide adenine dinucleotide phosphate; ADP = adenosine diphosphate; P_i = inorganic phosphate; CO_2 = carbon dioxide; TCA = tricarboxylic acid; PC = pyruvate carboxylase; PDH = pyruvate dehydrogenase.

F. Chain desaturation

Enzymes (fatty acyl CoA desaturases) also present in the SER are responsible for desaturating LCFA (i.e., adding cis double bonds). The desaturation reactions require oxygen (O_2), NADH, cytochrome b_5, and its flavin adenine dinucleotide (FAD)-linked reductase. The fatty acid and the NADH get oxidized as the O_2 gets reduced to H_2O. The first double bond is typically inserted between carbons 9 and 10, producing primarily oleic acid, 18:1(9), and small amounts of palmitoleic acid, 16:1(9). A variety of polyunsaturated fatty acids can be made through additional desaturation combined with elongation.

Humans have carbon 9, 6, 5, and 4 desaturases but lack the ability to introduce double bonds from carbon 10 to the ω end of the chain. This is the basis for the nutritional essentiality of the polyunsaturated ω-6 linoleic acid and ω-3 linolenic acid.

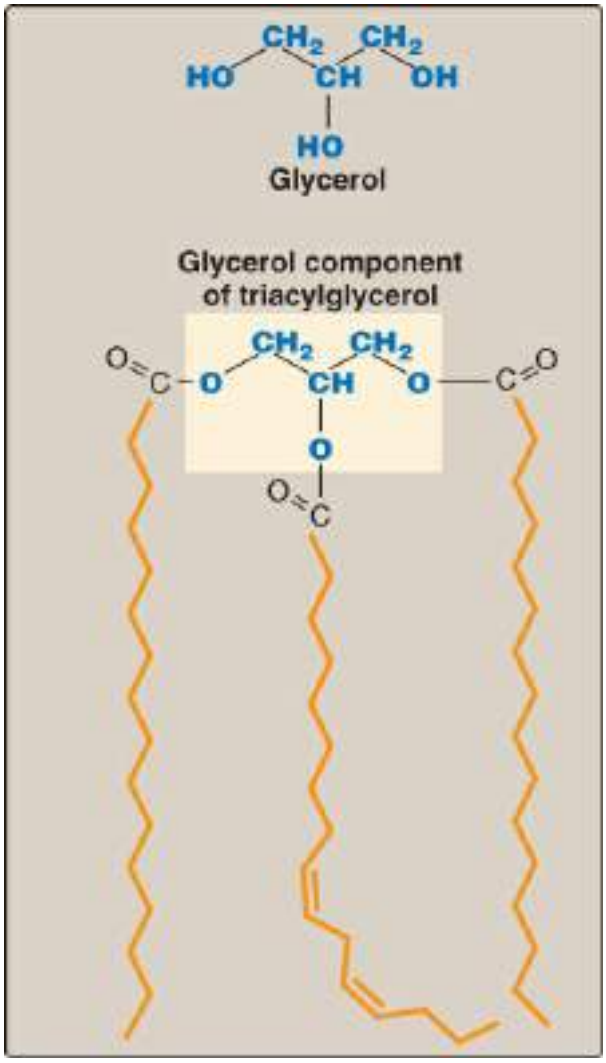


Figure 16.12

A triacylglycerol with an unsaturated fatty acid on carbon 2. *Orange* denotes the hydrophobic portions of the molecule.

G. Storage as TAG components

Mono-, di-, and triacylglycerols consist of one, two, or three molecules of fatty acid esterified to a molecule of glycerol. Fatty acids are esterified through their carboxyl groups, resulting in a loss of negative charge and formation of neutral fat. (Note: An acylglycerol that is solid at room temperature is called a fat. If liquid, it is an oil.)

1. Arrangement: The three fatty acids esterified to a glycerol molecule to form a TAG are usually not of the same type. The fatty acid on carbon 1 is typically saturated, that on carbon 2 is typically unsaturated, and that on carbon 3 can be either. Recall that the presence of the unsaturated fatty acid(s) decrease(s) the T_m of the lipid. An example of a TAG molecule is shown in Figure 16.12.

2. Triacylglycerol storage and function: Because TAG are only slightly soluble in water and cannot form stable micelles by themselves, they coalesce within white adipocytes to form large oily droplets that are nearly anhydrous. These cytosolic lipid droplets are the major energy reserve of the body. (Note: TAG stored in brown adipocytes serve as a source of heat through nonshivering thermogenesis [see Chapter 6].)

3. Glycerol 3-phosphate synthesis: Glycerol 3-phosphate is the initial acceptor of fatty acids during TAG synthesis. There are two major pathways for its production (Fig. 16.13). (Note: A third process [glyceroneogenesis] is described in Section IV A3.) In both liver (the primary site of TAG synthesis) and adipose tissue, glycerol 3-phosphate can be produced from glucose, first using the reactions of the glycolytic pathway to produce dihydroxyacetone phosphate [DHAP]. DHAP is reduced by glycerol 3-phosphate dehydrogenase to glycerol 3-phosphate. A second pathway found in the liver, but not in adipose tissue, uses glycerol kinase to convert free glycerol to glycerol 3-phosphate (see Fig. 16.13). (Note: The glucose transporter in adipocytes [GLUT-4] is insulin dependent [see Chapter 23]. Thus, when plasma glucose levels are low, adipocytes have only a limited ability to synthesize glycerol phosphate and cannot produce TAG *de novo*.)

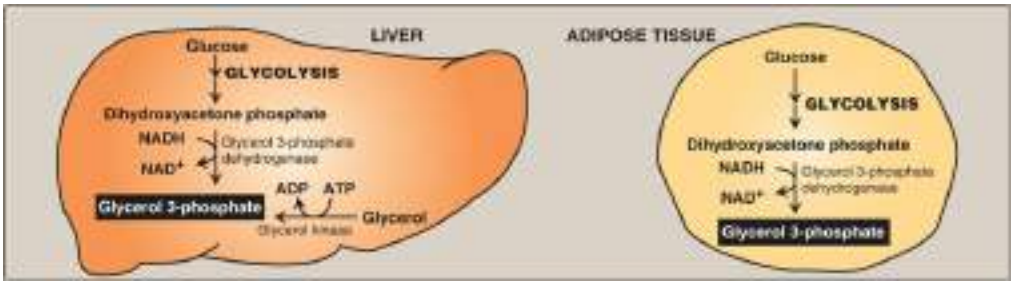


Figure 16.13
Pathways for production of glycerol 3-phosphate in liver and adipose tissue. (Note: Glycerol 3-

phosphate can also be generated by glyceroneogenesis.) NAD(H) = nicotinamide adenine dinucleotide; ADP = adenosine diphosphate.

4. Fatty acid activation: A FFA must be converted to its activated form (bound to CoA through a thioester link) before it can participate in metabolic processes such as TAG synthesis. This reaction, illustrated in Figure 15.6, is catalyzed by a family of fatty acyl CoA synthetases (thiokinases).

5. Triacylglycerol synthesis: This pathway from glycerol 3-phosphate involves four reactions, shown in Figure 16.14. These include the sequential addition of two fatty acids from fatty acyl CoA, the removal of phosphate, and the addition of the third fatty acid.

H. Triacylglycerol fate in liver and adipose tissue

In WAT, TAG is stored in a nearly anhydrous form as fat droplets in the cytosol of the cells. The fat droplets are coated with a family of proteins known as perilipins that sequester and protect TAG from lipolysis until the body requires fatty acids for fuel. (Perilipins may play a role in pathologic conditions such as type 2 diabetes, atherosclerosis, and cardiovascular disease.) Little TAG is stored in healthy liver. Instead, most is exported, packaged with other lipids and apolipoproteins to form lipoprotein particles called very–low-density lipoproteins (VLDL). Nascent VLDL are secreted directly into the blood where they mature and function to deliver the endogenously derived lipids to the peripheral tissues. (Note: Recall from Chapter 15 that chylomicrons carry dietary [exogenously derived] lipids. Plasma lipoproteins are discussed in Chapter 18.)

IV. FAT MOBILIZATION AND FATTY ACID OXIDATION

Fatty acids stored in WAT, in the form of neutral TAG, serve as the body's major fuel storage reserve. TAGs provide concentrated stores of metabolic energy because they are highly reduced and largely anhydrous. The yield from the complete oxidation of fatty acids to CO_2 and H_2O is 9 kcal/g fat (as compared to 4 kcal/g protein or carbohydrate, see Fig. 27.5).

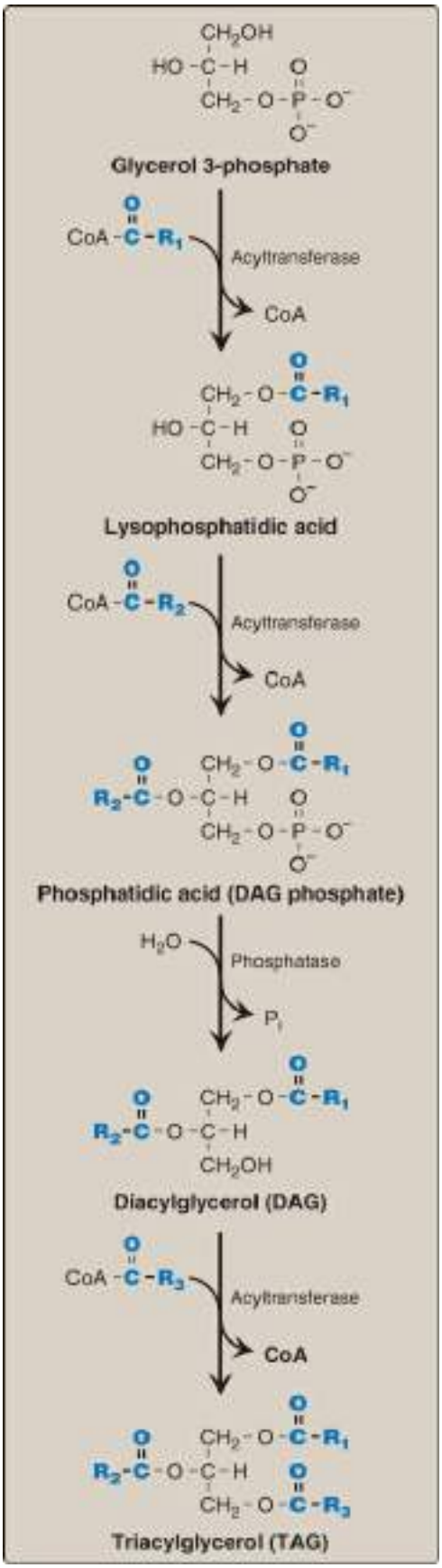


Figure 16.14
Synthesis of TAG. R1–R3 = activated fatty acids. CoA = coenzyme A; P_i = inorganic phosphate.

A. Fatty acid release from fat

The mobilization of stored fat requires the hydrolytic release of FFA and glycerol from their TAG form. This process of lipolysis is achieved by perilipins and lipases. It is initiated by adipose triglyceride lipase (ATGL), which generates a diacylglycerol that is the preferred substrate for hormone-sensitive lipase (HSL). The monoacylglycerol (MAG) product of HSL is acted upon by MAG lipase.

1. Regulation of perilipins and HSL: Both perilipins and HSL are phosphorylated by PKA, a cAMP-dependent protein kinase. cAMP is produced in the adipocyte when catecholamines (such as epinephrine) bind to cell membrane β-adrenergic receptors and activate adenylyl cyclase (Fig. 16.15). The process is similar to that of the activation of glycogen phosphorylase (see Fig. 11.9). Phosphorylation of perilipin by PKA allows the translocation and binding of phosphorylated HSL (active HSL) to the droplet. (Note: Because ACC is inhibited by hormone-directed phosphorylation, when the cAMP-mediated cascade is activated [see Fig. 16.8], fatty acid synthesis is turned off and TAG degradation is turned on.) In the presence of high plasma levels of insulin, HSL is dephosphorylated and inactivated. Insulin also suppresses expression of ATGL.

2. Fate of glycerol: The glycerol released during TAG degradation cannot be metabolized by adipocytes because they lack glycerol kinase. Rather, glycerol is transported through the blood to the liver, which has the kinase. The resulting glycerol 3-phosphate can be used to form TAG in the liver or can be converted to DHAP by reversal of the glycerol 3-phosphate dehydrogenase reaction illustrated in Figure 16.13. DHAP can participate in glycolysis or gluconeogenesis.

3. Fate of fatty acids: The FFA move through the cell membrane of the adipocyte and bind to serum albumin in the blood. They are transported to tissues such as muscle, enter cells, get activated to their CoA derivatives, and are oxidized for energy in mitochondria. Regardless of their levels, plasma FFA cannot be used for fuel by red blood cells (RBCs), which have no mitochondria. The brain does not use fatty acids for energy to any appreciable extent, but the reasons are less clear. (Note: Over 50% of the fatty acids released from adipose TAG are reesterified to glycerol 3-phosphate. WAT does not express glycerol kinase, and the glycerol 3-phosphate is produced by glyceroneogenesis, an incomplete version of gluconeogenesis: pyruvate to OAA via pyruvate carboxylase and OAA to phosphoenolpyruvate [PEP] via phosphoenolpyruvate carboxykinase. The PEP is converted [by reactions common to glycolysis and gluconeogenesis] to DHAP, which is reduced to glycerol 3-phosphate. The process decreases plasma FFA, molecules associated with insulin resistance in type 2 diabetes

and obesity [see Chapter 25].)

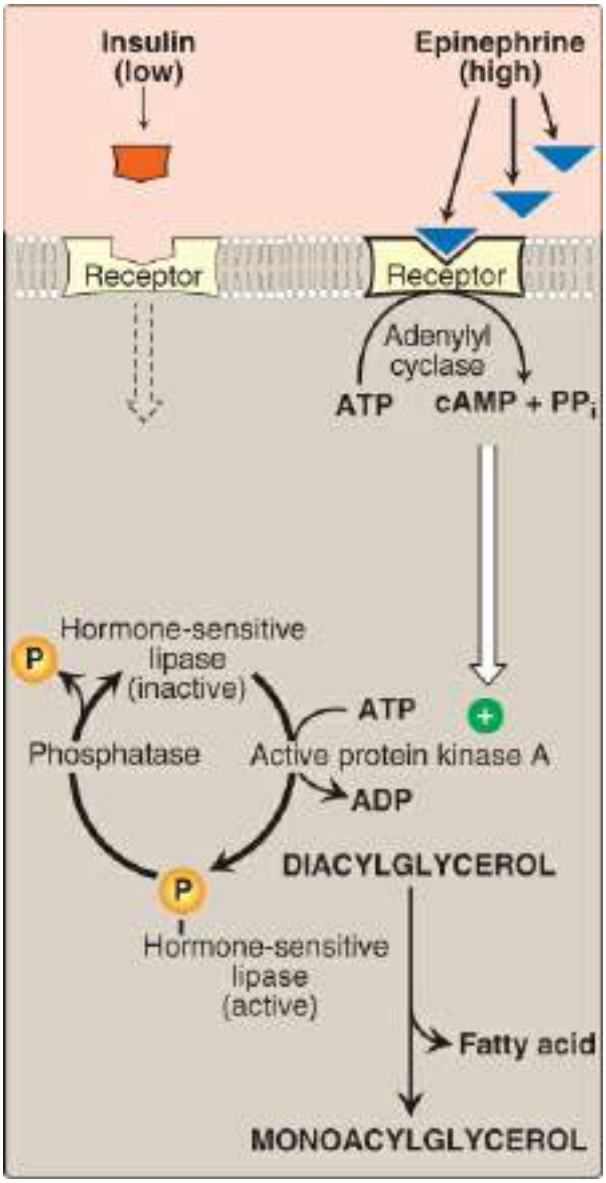


Figure 16.15
Hormonal regulation of diacylglycerol degradation in the adipocyte. (Note: Triacylglycerol is degraded to diacylglycerol by adipose triglyceride lipase.) cAMP = cyclic adenosine monophosphate; PP_i = pyrophosphate; ADP = adenosine diphosphate; (P) = phosphate.

B. Fatty acid β-oxidation

The major pathway for catabolism of fatty acids is a mitochondrial pathway called β-oxidation, in which two-carbon fragments are successively removed from the carboxyl end of the fatty acyl CoA, producing acetyl CoA, NADH, and $FADH_2$.

1. Long-chain fatty acid transport into cytosol and mitochondria: The uptake of fatty acids may occur using several different mechanisms. It may involve passive

diffusion or one of several lipid transportation proteins, such as fatty acid translocase (FAT), fatty acid–binding protein (FABP) and fatty acid transport protein (FATP). The long chain fatty acids (LCFA) are taken up by FATP. After a LCFA enters a cell, it is converted in the cytosol to its CoA derivative by long-chain fatty acyl CoA synthetase (thiokinase), an enzyme of the outer mitochondrial membrane. Because β-oxidation occurs in the mitochondrial matrix, the fatty acid must be transported across the inner mitochondrial membrane that is impermeable to CoA. Therefore, a specialized carrier transports the long-chain acyl group from the cytosol into the mitochondrial matrix. This carrier is carnitine, and this rate-limiting transport process is called the carnitine shuttle (Fig. 16.16).

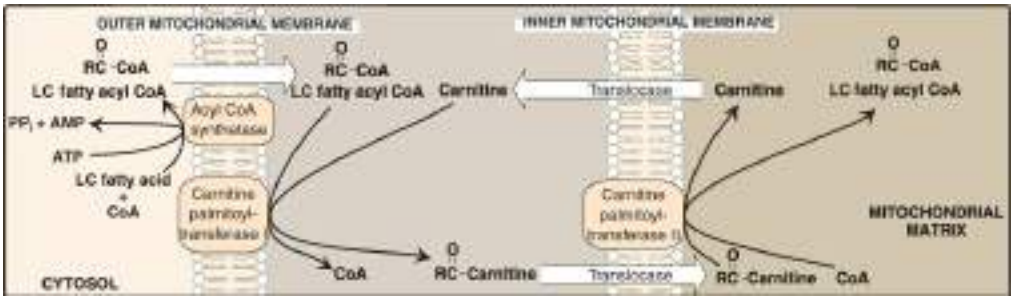


Figure 16.16
Carnitine shuttle. The net effect is that a long-chain (LC) fatty acyl coenzyme A (CoA) is transported from the outside to the inside of mitochondria. AMP = adenosine monophosphate; PP_i = pyrophosphate.

a. Translocation steps: First, the acyl group is transferred from CoA to carnitine by carnitine palmitoyltransferase I (CPT-I), an enzyme of the outer mitochondrial membrane. (Note: CPT-I is also known as CAT-I for carnitine acyltransferase I.) This reaction forms an acylcarnitine and regenerates free CoA. Second, the acylcarnitine is transported into the mitochondrial matrix in exchange for free carnitine by carnitine–acylcarnitine translocase. Carnitine palmitoyltransferase 2 (CPT-II, or CAT-II), an enzyme of the inner mitochondrial membrane, catalyzes the transfer of the acyl group from carnitine to CoA in the mitochondrial matrix, thus regenerating free carnitine.

b. Carnitine shuttle inhibitor: Malonyl CoA inhibits CPT-I, thus preventing the entry of long-chain acyl groups into the mitochondrial matrix. Therefore, when fatty acid synthesis is occurring in the cytosol (as indicated by the presence of malonyl CoA), the newly made palmitate cannot be transferred into mitochondria and degraded. (Note: Muscle tissue, although it does not synthesize fatty acids, contains the mitochondrial isozyme of ACC[ACC2], allowing regulation of β-oxidation. The liver contains both isozymes.) Fatty acid oxidation is also regulated by the acetyl CoA/CoA ratio: As the ratio increases, the CoA-requiring thiolase reaction decreases (Fig. 16.17).

c. Carnitine sources: Carnitine can be obtained from the diet, where it is found

primarily in meat products. It can also be synthesized from the amino acids lysine and methionine by an enzymatic pathway found in the liver and kidneys but not in skeletal or cardiac muscle. Therefore, these latter tissues are totally dependent on uptake of carnitine provided by endogenous synthesis or the diet and distributed by the blood. (Note: Skeletal muscle contains ~97% of all carnitine in the body.)

Carnitine enters into cells through carnitine transporters. In heart, muscle and kidney, the high-affinity transporter is organic cation transporter novel 2 (OCTN2). The liver has a different, low-affinity, high-capacity carnitine transporter. Primary carnitine deficiency develops due to a defect in OCTN2 resulting in urinary loss of carnitine and low levels of both serum and cellular carnitine.

d. Carnitine deficiencies: Such deficiencies result in decreased ability of tissues to use LCFA as a fuel. Primary carnitine deficiency is caused by defects in a membrane transporter that prevent uptake of carnitine by cardiac and skeletal muscle and the kidneys, causing carnitine to be excreted. Treatment includes carnitine supplementation. Secondary carnitine deficiency occurs primarily as a result of defects in fatty acid oxidation leading to the accumulation of acylcarnitines that are excreted in the urine, decreasing carnitine availability. Acquired secondary carnitine deficiency can be seen, for example, in patients with liver disease (decreased carnitine synthesis) or those taking the antiseizure drug valproic acid (decreased renal reabsorption). (Note: Defects in mitochondrial oxidation can also be caused by deficiencies in CPT-I and CPT-II. CPT-I deficiency affects the liver, where an inability to use LCFA for fuel greatly impairs that tissue's ability to synthesize glucose [an endergonic process] during a fast. This can lead to severe hypoglycemia, coma, and death. CPT-II deficiency can affect the liver and cardiac and skeletal muscle. The most common [and least severe] form affects skeletal muscle. It presents as muscle weakness with myoglobinemia following prolonged exercise. Treatment includes avoidance of fasting and adopting a diet high in carbohydrates and low in fat but supplemented with medium-chain TAG.)

2. Shorter-chain fatty acid entry into mitochondria: Fatty acids ≤12 carbons can cross the inner mitochondrial membrane without the aid of carnitine or the CPT system. Once inside the mitochondria, they are activated to their CoA derivatives by matrix enzymes and are oxidized. (Note: Medium-chain fatty acids are plentiful in human milk. Because their oxidation is not dependent on CPT-I, malonyl CoA is not inhibitory.)

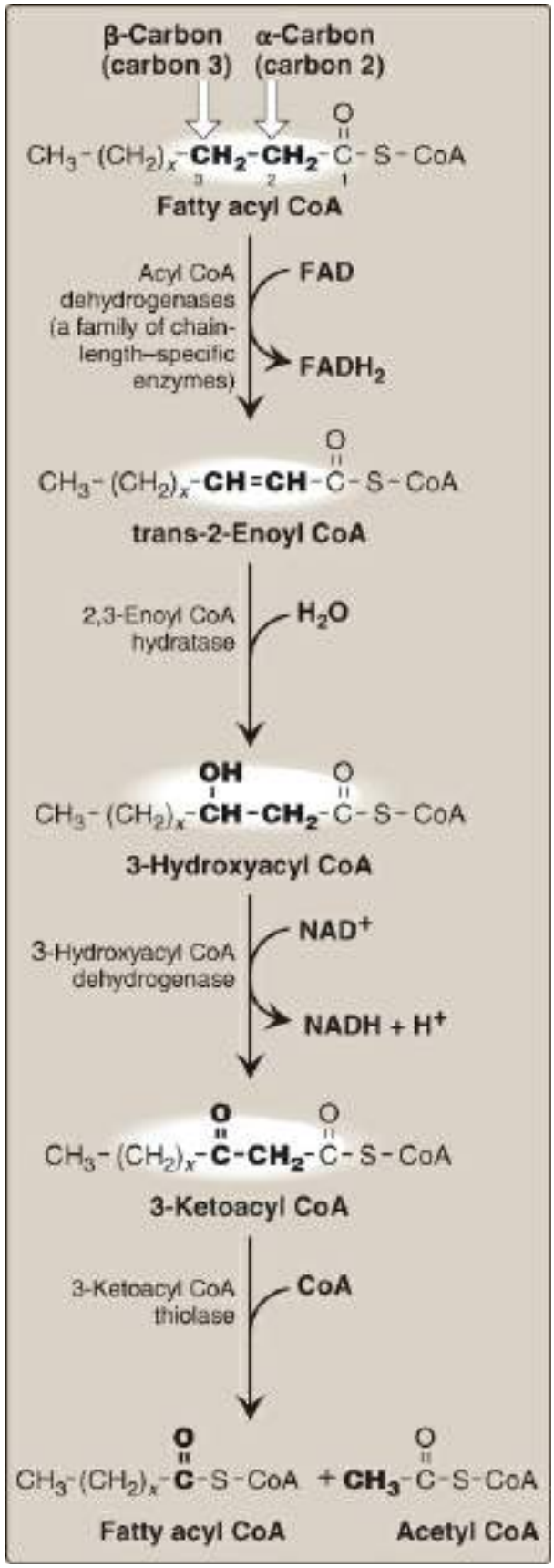


Figure 16.17
Enzymes involved in the β-oxidation of fatty acyl coenzyme A (CoA). (Note: 2,3-Enoyl CoA hydratase requires a trans double bond between carbon 2 and carbon 3.) FAD(H_2) = flavin adenine dinucleotide; NAD(H) = nicotinamide adenine dinucleotide.

3. β-Oxidation reactions: The first cycle of β-oxidation is shown in Figure 16.17. It consists of a sequence of four reactions involving the β-carbon (carbon 3) that results in shortening the fatty acid by two carbons at the carboxylate end. The steps include an oxidation that produces $FADH_2$, a hydration, a second oxidation that produces NADH, and a CoA-dependent thiolytic cleavage that releases a molecule of acetyl CoA. Each step is catalyzed by enzymes with chain-length specificity. (Note: For LCFA, the last three steps are catalyzed by a trifunctional protein.) These four steps are repeated for saturated fatty acids of even-numbered carbon chains (n/2) − 1 times (where n is the number of carbons), each cycle producing one acetyl CoA plus one NADH and one $FADH_2$. The final cycle produces two acetyl CoA. The acetyl CoA can be oxidized or used in hepatic ketogenesis (see V. below). The reduced coenzymes are oxidized by the electron transport chain, NADH by Complex I, and $FADH_2$ by coenzyme Q (see p. 82). (Note: Acetyl CoA is a positive allosteric effector of pyruvate carboxylase [see Chapter 10], thus linking fatty acid oxidation and gluconeogenesis.)

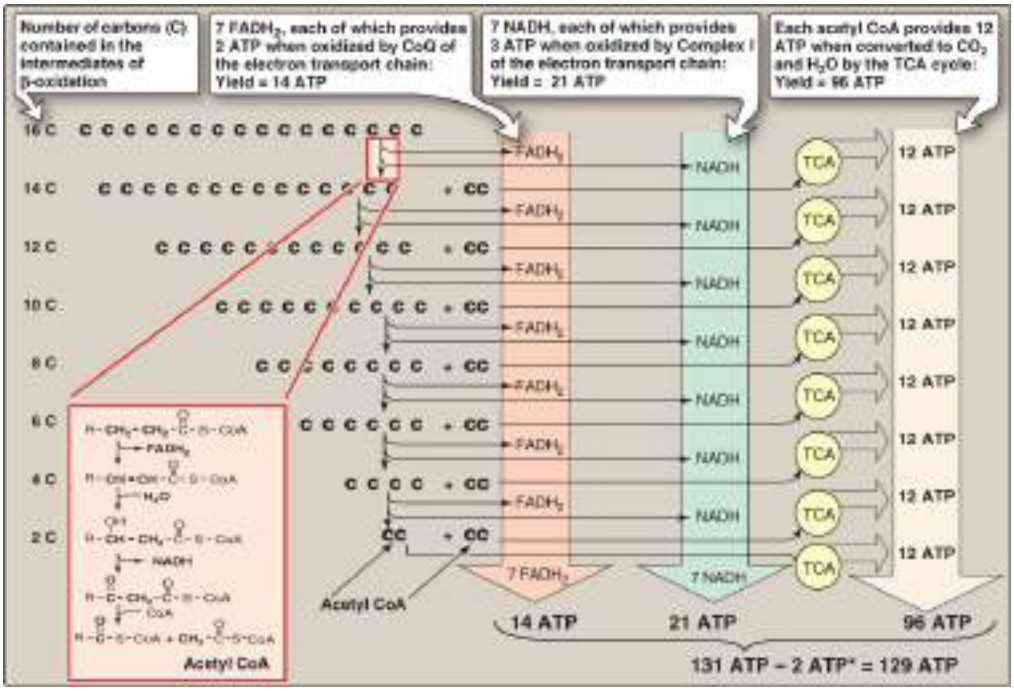


Figure 16.18
Summary of the energy yield from the oxidation of palmitoyl coenzyme A (CoA) (16 carbons). (Note: *Activation of palmitate to palmitoyl CoA requires the equivalent of 2 ATP [ATP → AMP + PP_i].) $FADH_2$ = flavin adenine dinucleotide; NADH = nicotinamide adenine dinucleotide; TCA = tricarboxylic acid; CoQ = coenzyme Q; CO_2 = carbon dioxide.

4. β-Oxidation energy yield: The energy yield from fatty acid β-oxidation is high. For example, the oxidation of a molecule of palmitoyl CoA to CO_2 and H_2O produces eight acetyl CoA, seven NADH, and seven $FADH_2$, from which 131 ATP can be generated. However, activation of the fatty acid requires two ATP. Therefore, the net yield from palmitate is 129 ATP (Fig. 16.18). A comparison of the processes of synthesis and degradation of long-chain saturated fatty acids with an even number of carbon atoms is provided in Figure 16.19.

VARIABLE	SYNTHESIS	DEGRADATION
Greatest flux through pathway	After carbohydrate-rich meal	In starvation
Hormonal state favoring pathway	High insulin/glucagon ratio	Low insulin/glucagon ratio
Major tissue site	Primarily liver	Muscle, liver
Subcellular location	Cytosol	Primarily mitochondria
Carriers of acyl/acetyl groups between mitochondria and cytosol	Citrate (mitochondria to cytosol)	Carnitine (cytosol to mitochondria)
Phosphopantetheine-containing active carriers	Acyl carrier protein domain, coenzyme A	Coenzyme A
Oxidation/reduction coenzymes	NADPH (reduction)	NAD^+, FAD (oxidation)
Two-carbon donor/product	Malonyl CoA: donor of one acetyl group	Acetyl CoA: product of β-oxidation
Activator	Citrate	–
Inhibitor	Palmitoyl CoA (inhibits acetyl CoA carboxylase)	Malonyl CoA (inhibits carnitine palmitoyltransferase-I)
Product of pathway	Palmitate	Acetyl CoA
Repetitive four-step process	Condensation, reduction dehydration, reduction	Dehydrogenation, hydration dehydrogenation, thiolysis

Figure 16.19
Comparison of the synthesis and degradation of long-chain, even-numbered, saturated fatty acids. NADPH = nicotinamide adenine dinucleotide phosphate; NAD^+ = nicotinamide adenine dinucleotide; FAD = flavin adenine dinucleotide; CoA = coenzyme A.

5. Medium-chain fatty acyl CoA dehydrogenase deficiency: In mitochondria, there are four fatty acyl CoA dehydrogenase species, each with distinct but overlapping specificity for either short–, medium–, long-, or very–long-chain fatty acids. Deficiency in each of these dehydrogenases has been observed, however medium-chain fatty acyl CoA dehydrogenase (MCAD) deficiency is the most common inborn error of β-oxidation. MCAD deficiency, an autosomal-recessive disorder, is found in 1:14,000 births worldwide, with a higher incidence in Caucasians of Northern European descent. It results in a decreased ability to oxidize fatty acids with 6 to 10 carbons, a decreased production of acetyl CoA, and an increased reliance on glucose for energy, which causes a hypoketotic hypoglycemia in patients. Laboratory urine studies show an accumulation of medium-chain acyl carnitines and medium-chain dicarboxylic acids. Treatment includes avoidance of fasting.

6. Oxidation of fatty acids with an odd number of carbons: This process proceeds by the same reaction steps as that of fatty acids with an even number of

carbons, until the final three carbons are reached. This product, propionyl CoA, is metabolized by a three-step pathway (Fig. 16.20). (Note: Propionyl CoA is also produced during the metabolism of certain amino acids [see Fig. 20.11].)

a. D-Methylmalonyl CoA synthesis: First, propionyl CoA is carboxylated, forming D-methylmalonyl CoA. The enzyme propionyl CoA carboxylase has an absolute requirement for the coenzymes biotin and ATP, as do ACC and most other carboxylases.

b. L-Methylmalonyl CoA formation: Next, the D-isomer is converted to the L-form by the enzyme methylmalonyl CoA racemase.

c. Succinyl CoA synthesis: Finally, the carbons of L-methylmalonyl CoA are rearranged, forming succinyl CoA, which can enter the TCA cycle. (Note: This is the only example of a glucogenic precursor generated from fatty acid oxidation.) The enzyme methylmalonyl CoA mutase requires a coenzyme form of vitamin B_{12} (deoxyadenosylcobalamin). The mutase reaction is one of only two reactions in the body that require vitamin B_{12} (see Chapter 28). The other vitamin B_{12}-dependent enzyme is methionine synthase, which catalyzes the synthesis of methionine from homocysteine. This reaction is essential for folate recycling and for the conversion of B_{12} into its coenzyme form. As a result, the early signs of folate and vitamin B_{12} deficiencies are similar hematologicabnormalities. At later stages in vitamin B_{12} deficiency neurologic symptoms arise, such as paresthesias, numbness, and ataxia. In patients with vitamin B_{12} deficiency, both propionic and methylmalonic acid (MMA) are excreted in the urine. Elevated serum MMA is a useful diagnostic measurement to distinguish a vitamin B_{12} deficiency from a folate deficiency. Heritable methylmalonic acidemia and aciduria may arise from a defect or deficit in either methylmalonyl CoA mutase or methionine synthase.

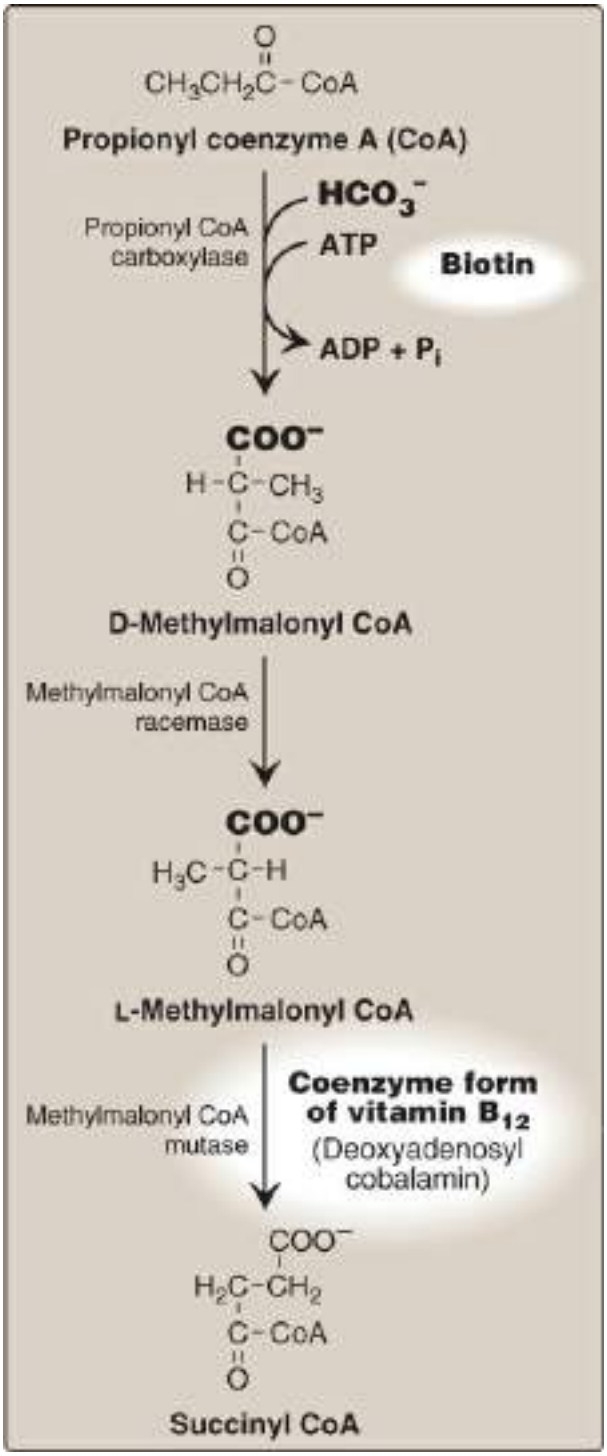


Figure 16.20
Metabolism of propionyl CoA. ADP = adenosine diphosphate; (HCO_3^-) = bicarbonate; P_i = inorganic phosphate.

7. Unsaturated fatty acid β-oxidation: The oxidation of unsaturated fatty acids generates intermediates that cannot serve as substrates for 2,3-enoyl CoA hydratase (see Fig. 16.17). Consequently, additional enzymes are required.

Oxidation of a double bond at an odd-numbered carbon, such as 18:1(9) (oleic acid), requires one additional enzyme, 3,2-enoyl CoA isomerase, which converts the 3-cis derivative obtained after three rounds of β-oxidation to the 2-trans derivative required by the hydratase. Oxidation of a double bond at an even-numbered carbon, such as 18:2(9,12) (linoleic acid), requires an NADPH-dependent 2,4-dienoyl CoA reductase in addition to the isomerase. (Note: Because unsaturated fatty acids are less reduced than saturated fatty acids, fewer reducing equivalents are produced by their oxidation.)

8. Peroxisomal β-oxidation: VLCFA ≥22 carbons in length undergo a preliminary β-oxidation in peroxisomes, because peroxisomes and not mitochondria are the primary site of the synthetase that activates fatty acids of this length. The shortened fatty acid (linked to carnitine) diffuses to a mitochondrion for further oxidation. In contrast to mitochondrial β-oxidation, the initial dehydrogenation in peroxisomes is catalyzed by a FAD-containing acyl CoA oxidase. The $FADH_2$ produced is oxidized by O_2, which is reduced to hydrogen peroxide (H_2O_2). Therefore, no ATP is generated from this step. The H_2O_2 is reduced to H_2O by catalase (see Chapter 13). (Note: Genetic defects in the ability either to target matrix proteins to peroxisomes [resulting in Zellweger syndrome, a peroxisomal biogenesis disorder] or to transport VLCFA across the peroxisomal membrane [resulting in X-linked adrenoleukodystrophy] lead to accumulation of VLCFA in the blood and tissues.)

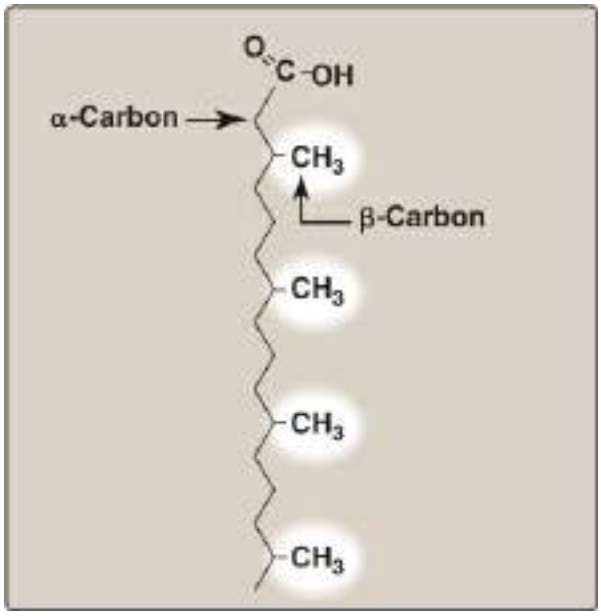


Figure 16.21
Phytanic acid, a branched-chain fatty acid 16 carbons in length.

C. Peroxisomal α-oxidation

Branched-chain phytanic acid, a product of chlorophyll metabolism, is not a

substrate for acyl CoA dehydrogenase because of the methyl group on its β-carbon (Fig. 16.21). Instead, it is hydroxylated at the α-carbon by phytanoyl CoA α-hydroxylase (PhyH); carbon 1 is released as CO_2; and the product, 15-carbon-long pristanal, is oxidized to pristanic acid, which is activated to its CoA derivative and undergoes β-oxidation. Refsum disease is a rare, autosomal-recessive disorder caused by a deficiency of peroxisomal PhyH. This results in the accumulation of phytanic acid in the plasma and tissues. The symptoms are primarily neurologic, and the treatment involves dietary restriction to halt disease progression. (Note: ω-Oxidation [at the methyl terminus] also is known and generates dicarboxylic acids. Normally a minor pathway of the SER, its upregulation is seen with conditions such as MCAD deficiency that limit fatty acid β-oxidation.)

V. KETONE BODIES: ALTERNATIVE FUEL FOR CELLS

Liver mitochondria have the capacity to convert acetyl CoA derived from fatty acid oxidation into ketone bodies. The compounds categorized as ketone bodies are acetoacetate, 3-hydroxybutyrate (also called β-hydroxybutyrate), and acetone (a nonmetabolized side product, Fig. 16.22). (Note: The two functional ketone bodies are organic acids.) Acetoacetate and 3-hydroxybutyrate are transported in the blood to the peripheral tissues. There they can be reconverted to acetyl CoA, which can be oxidized by the TCA cycle. Ketone bodies are important sources of energy for the peripheral tissues because they (1) are soluble in aqueous solution and, therefore, do not need to be incorporated into lipoproteins or carried by albumin as do the other lipids; (2) are produced in the liver during periods when the amount of acetyl CoA present exceeds the oxidative capacity of the liver; and (3) are used in proportion to their concentration in the blood by extrahepatic tissues, such as skeletal and cardiac muscle, the intestinal mucosa, and the renal cortex. Even the brain can use ketone bodies to help meet its energy needs if the blood levels rise sufficiently. Thus, ketone bodies spare glucose, which is particularly important during prolonged periods of fasting (see Chapter 24). (Note: Disorders of fatty acid oxidation present with the general picture of hypoketosis [because of decreased availability of acetyl CoA] and hypoglycemia [because of increased reliance on glucose for energy].)

A. Ketone body synthesis by the liver: ketogenesis

During a fast, the liver is flooded with fatty acids mobilized from adipose tissue. The resulting elevated hepatic acetyl CoA produced by fatty acid oxidation inhibits pyruvate dehydrogenase and activates pyruvate carboxylase (PC). The OAA produced by PC is used by the liver for gluconeogenesis rather than for the TCA cycle. Additionally, fatty acid oxidation decreases the NAD^+/NADH ratio, and the rise in NADH shifts OAA to malate (see p. 124). The decreased availability of OAA for condensation with acetyl CoA results in the increased use of acetyl CoA for ketone body synthesis. (Note: Acetyl CoA for ketogenesis is also generated by the catabolism of ketogenic amino acids.)

1. 3-Hydroxy-3-methylglutaryl CoA synthesis: The first step, formation of acetoacetyl CoA, occurs by reversal of the final thiolase reaction of fatty acid oxidation (see Fig. 16.17). Mitochondrial 3-hydroxy-3-methylglutaryl (HMG) CoA synthase combines a third molecule of acetyl CoA with acetoacetyl CoA to produce HMG CoA. HMG CoA synthase is the rate-limiting step in the synthesis of ketone bodies and is present in significant quantities only in the liver. (Note: HMG CoA is also an intermediate in cytosolic cholesterol synthesis. The two pathways are separated by location in, and conditions of, the cell.)

2. Ketone body synthesis: HMG CoA is cleaved by HMG CoA lyase to produce acetoacetate and acetyl CoA, as shown in Figure 16.22. Acetoacetate can be reduced to form 3-hydroxybutyrate with NADH as the electron donor. (Note: Because ketone bodies are not linked to CoA, they can cross the inner mitochondrial membrane.) Acetoacetate can also spontaneously decarboxylate in the blood to form acetone, a volatile, biologically nonmetabolized compound that can be detected in the breath. The equilibrium between acetoacetate and 3-hydroxybutyrate is determined by the NAD^+/NADH ratio. Because this ratio is low during fatty acid oxidation, 3-hydroxybutyrate synthesis is favored.

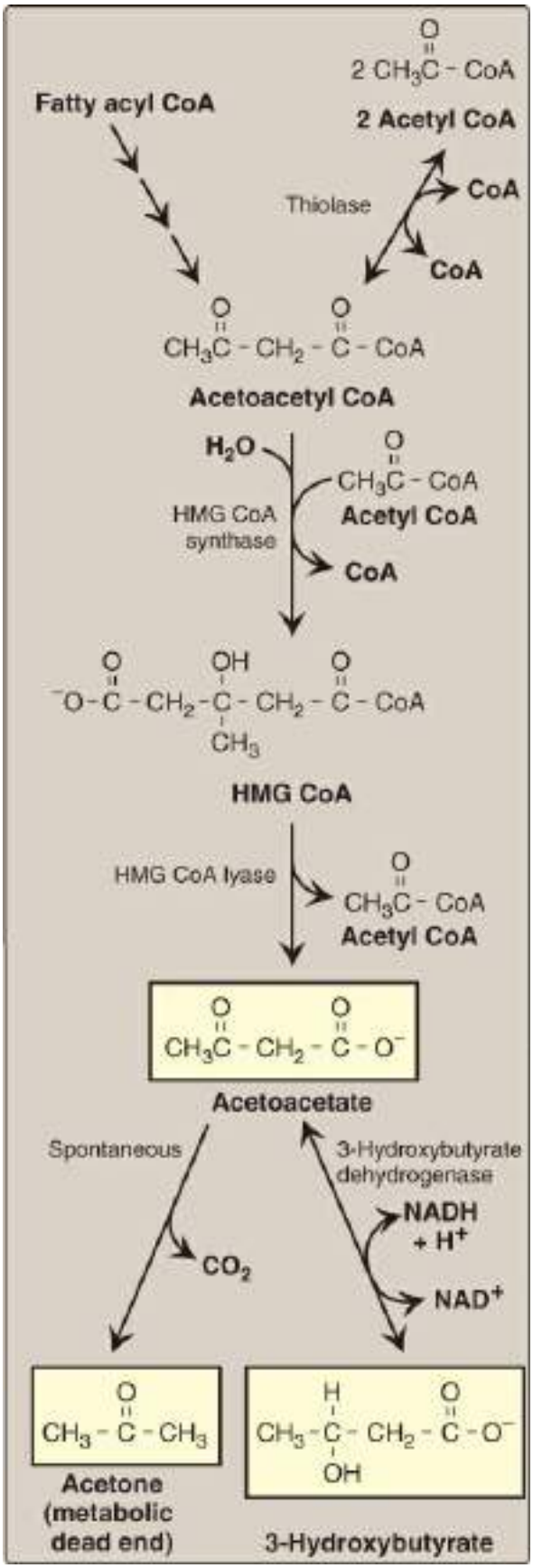


Figure 16.22
Synthesis of ketone bodies. (Note: The release of CoA in ketogenesis supports continued fatty acid oxidation.) CoA = coenzyme A; HMG = hydroxymethylglutarate; NAD(H) = nicotinamide adenine dinucleotide; CO_2 = carbon dioxide.

B. Ketone body use by the peripheral tissues: ketolysis

Although the liver constantly synthesizes low levels of ketone bodies, their production increases during fasting when ketone bodies are needed to provide energy to the peripheral tissues. 3-Hydroxybutyrate is oxidized to acetoacetate by 3-hydroxybutyrate dehydrogenase, producing NADH (Fig. 16.23). Acetoacetate is then provided with a CoA molecule taken from succinyl CoA by succinyl CoA:acetoacetate CoA transferase (thiophorase). This reaction is reversible, but the product, acetoacetyl CoA, is actively removed by its cleavage to two acetyl CoA by thiolase. This pulls the reaction forward. Extrahepatic tissues, including the brain but excluding cells lacking mitochondria (e.g., RBCs), efficiently oxidize acetoacetate and 3-hydroxybutyrate in this manner. In contrast, although the liver actively produces ketone bodies, it lacks thiophorase and, therefore, is unable to use ketone bodies as fuel.

C. Excessive ketone body production in diabetes mellitus

When the rate of formation of ketone bodies is greater than the rate of their use, their levels begin to rise in the blood (ketonemia) and, eventually, in the urine (ketonuria). This is seen most often in cases of uncontrolled type 1 diabetes mellitus (T1D), where the blood concentration of ketone bodies may reach 90 mg/dl (versus <3 mg/dl in normal individuals), and urinary excretion of ketone bodies may be as high as 5,000 mg/24 hrs. The elevation of the ketone body concentration in the blood can result in acidemia. (Note: The carboxyl group of a ketone body has a pK_a of ~4. Therefore, each ketone body loses a proton [H^+] as it circulates in the blood, which lowers the pH. Also, in uncontrolled T1D, urinary loss of glucose and ketone bodies results in dehydration. Therefore, the increased number of H^+ circulating in a decreased volume of plasma can cause a severe acidosis [ketoacidosis, Fig. 16.24] known as diabetic ketoacidosis [DKA].) A frequent symptom of DKA is a fruity odor on the breath, which results from increased production of acetone. Ketoacidosis may also be seen in cases of prolonged fasting and excessive ethanol consumption.

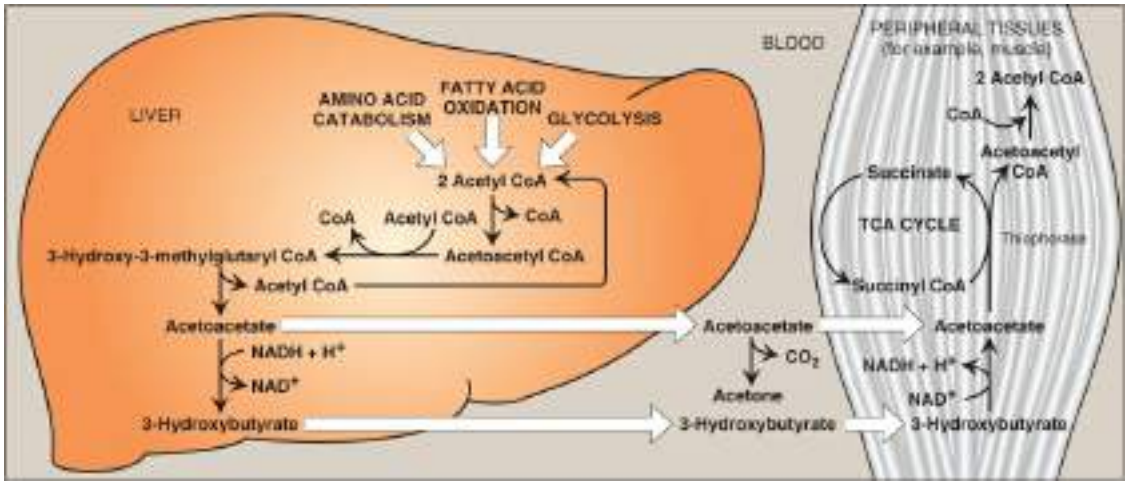


Figure 16.23
Ketone body synthesis in the liver and use in peripheral tissues. The liver and red blood cells cannot use ketone bodies. (Note: Thiophorase is also known as succinyl CoA:acetoacetate CoA transferase.) CoA = coenzyme A; NAD(H) = nicotinamide adenine dinucleotide; TCA = tricarboxylic acid; CO_2 = carbon dioxide.

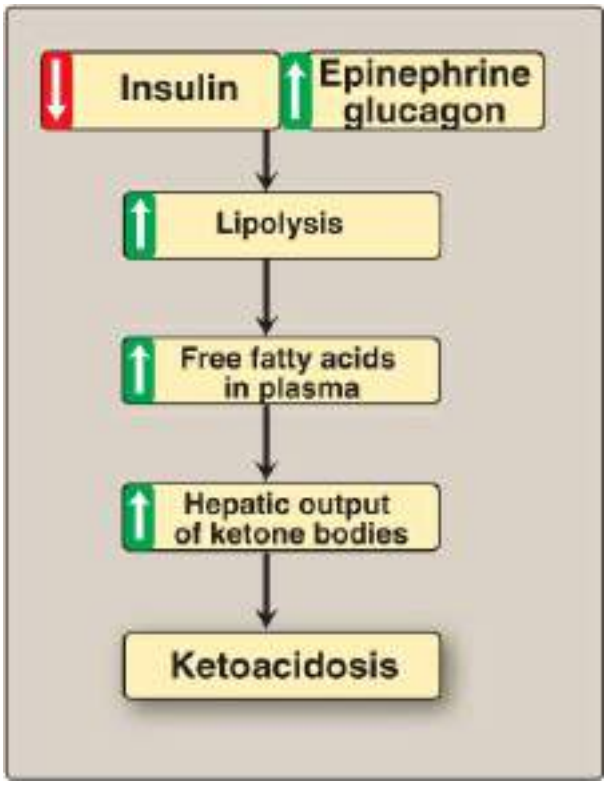


Figure 16.24
Mechanism of diabetic ketoacidosis seen in uncontrolled type 1 diabetes.

VI. Chapter Summary

- A **fatty acid**, generally a linear hydrocarbon chain with a terminal carboxyl group, can be **saturated** or **unsaturated**.
- Two unsaturated fatty acids are dietary essentials: **linoleic** and **α-linolenic acids**.
- Fatty acids are synthesized in the **liver cytosol** following a meal containing excess carbohydrate and protein.
- Carbons used to synthesize fatty acids are provided by **acetyl CoA**, energy by **ATP**, and reducing equivalents by **NADPH** (Fig. 16.25) provided by the **pentose phosphate pathway** and **malic enzyme**.
- **Citrate** carries two-carbon acetyl units from the mitochondrial matrix to the cytosol.
- The regulated step in fatty acid synthesis is the carboxylation of acetyl CoA to **malonyl CoA** by **biotin**- and **ATP**-requiring **ACC**.
- **Citrate** allosterically activates ACC, and **palmitoyl CoA** inhibits it. **ACC** can also be activated by **insulin** and inactivated by **AMPK** in response to **epinephrine**, **glucagon**, or a rise in **AMP**.
- The remaining steps in fatty acid synthesis are catalyzed **FAS**, which produces **palmitoyl CoA** by adding two-carbon units from malonyl CoA to a series of acyl acceptors.
- Fatty acids can be **elongated** and **desaturated** in the **SER**.
- When fatty acids are required for energy, HSL (**activated** by **epinephrine**, and **inhibited** by **insulin**), along with other lipases, degrades **TAG** stored in **adipocytes**.
- The fatty acids are carried by **serum albumin** to the liver and peripheral tissues, where their oxidation provides energy. The **glycerol** backbone of the degraded TAG is carried by the blood to the **liver**, where it serves as a **gluconeogenic precursor**.
- Fatty acid degradation (**β-oxidation**) occurs in **mitochondria**.
- The **carnitine shuttle** is required to transport long-chain fatty acids from the cytosol to the mitochondrial matrix. CPT-I is inhibited by **malonyl CoA**, thereby preventing simultaneous synthesis and degradation of fatty acids.
- Mitochondrial fatty acid β-oxidation produces **acetyl CoA**, **NADH**, and **$FADH_2$**.
- The first step in β-oxidation is catalyzed by one of four acyl CoA dehydrogenases, each with chain-length specificity.
- **MCADdeficiency** causes a decrease in fatty acid oxidation resulting in **hypoketonemia** and severe **hypoglycemia**.
- Oxidation of fatty acids with an **odd number** of carbons produces **propionyl CoA** which is carboxylated to **methylmalonyl CoA** (by **biotin-** and **ATP-requiring propionyl CoA carboxylase**), which is then converted to **succinyl CoA** (a gluconeogenic precursor) by **vitamin B_{12}-requiring methylmalonyl CoA mutase**.
- A genetic error in the mutase or vitamin B_{12} deficiency causes **methylmalonic acidemia** and **aciduria**. β-Oxidation of **unsaturated** fatty acids requires additional enzymes.
- β-Oxidation of **VLCFA** and α-oxidation of **branched-chain** fatty acids occur in the **peroxisome**.
- Deficiencies result in **X-linked adrenoleukodystrophy** and **Refsum disease**, respectively.
- **ω-Oxidation**, normally a minor pathway, occurs in the SER.
- Liver mitochondria can convert acetyl CoA derived from fatty acid oxidation into **acetoacetate** and **3-hydroxybutyrate** (**ketone bodies**).
- Peripheral tissues possessing mitochondria can oxidize 3-hydroxybutyrate to acetoacetate, which can be cleaved to two acetyl CoA, thereby producing energy for the cell.
- Unlike fatty acids, ketone bodies are utilized by the **brain** and, therefore, are important fuels during a fast.
- Because the liver lacks **thiophorase** required to degrade ketone bodies, it synthesizes them specifically for the peripheral tissues.
- **Ketoacidosis** occurs when the rate of ketone body formation is greater than the rate of use, as is seen in cases of uncontrolled **type 1 diabetes mellitus**.

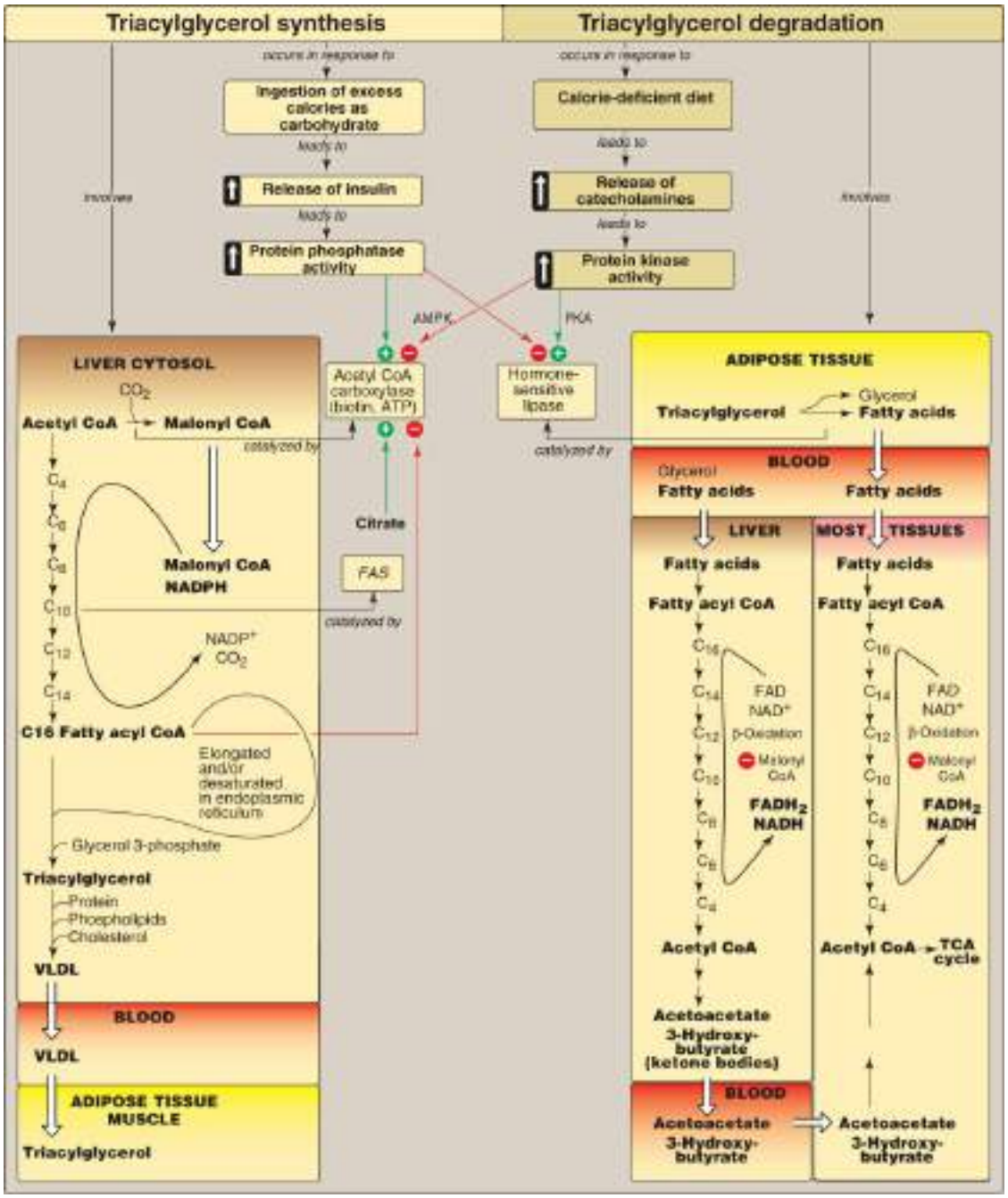


Figure 16.25
Key concept map for fatty acid and triacylglycerol metabolism. AMPK = adenosine monophosphate–activated protein kinase; PKA = protein kinase A; CoA = coenzyme A; NADP(H) = nicotinamide adenine dinucleotide phosphate; FAD(H_2) = flavin adenine dinucleotide; FAS = fatty acid synthase; CO_2 = carbon dioxide; NAD(H) = nicotinamide adenine dinucleotide; TCA = tricarboxylic acid; VLDL = very–low-density lipoprotein.

Study Questions

Choose the ONE best answer.

16.1 When oleic acid, 18:1(9), is desaturated at carbon 6 and then elongated, what is the correct representation of the product?

A. 19:2(7,9)
B. 20:2(ω-6)
C. 20:2(6,9)
D. 20:2(8,11)

Correct answer = D. Fatty acids are elongated in the smooth endoplasmic reticulum by adding two carbons at a time to the carboxylate end (carbon 1) of the molecule. This pushes the double bonds at carbon 6 and carbon 9 farther away from carbon 1. The 20:2(8,11) product is an ω-9 (n-9) fatty acid.

16.2 A 4-month-old child is being evaluated for fasting hypoglycemia. Laboratory tests at admission reveal low levels of ketone bodies (hypoketonemia), free carnitine, and long-chain acylcarnitines in the blood. Free fatty acid levels in the blood were elevated. Deficiency of which of the following would best explain these findings?

A. Adipose triglyceride lipase
B. Carnitine transporter
C. Carnitine palmitoyltransferase-I
D. Long-chain fatty acid dehydrogenase

Correct answer = B. A defect in the carnitine transporter (primary carnitine deficiency) would result in low levels of carnitine in the blood (as a result of increased urinary loss) and low levels in the tissues. In the liver, this decreases fatty acid oxidation and ketogenesis. Consequently, blood levels of free fatty acids rise. Deficiencies of adipose triglyceride lipase would decrease fatty acid availability. Deficiency of carnitine palmitoyltransferase I would result in elevated blood carnitine. Defects in any of the enzymes of β-oxidation would result in secondary carnitine deficiency, with a rise in acylcarnitines.

16.3 A teenager, concerned about his weight, attempts to maintain a fat-free diet for a period of several weeks. If his ability to synthesize various lipids are examined, which of the following is most likely to be most deficient in his ability to synthesize?

A. Cholesterol.
B. Glycolipids.
C. Phospholipids.
D. Prostaglandins.
E. Triacylglycerol.

Correct answer = D. Prostaglandins are synthesized from arachidonic acid. Arachidonic acid is synthesized from linoleic acid, an essential fatty acid obtained by humans from dietary lipids. The teenager would be able to synthesize all other compounds but, presumably, in somewhat decreased amounts.

16.4 A 6-month-old male was hospitalized following a seizure. History revealed that for several days prior, his appetite was decreased owing to a stomach virus. At admission, his blood glucose was 24 mg/dl (age-referenced normal is 60 to 100). His urine was negative for ketone bodies and positive for a variety of dicarboxylic acids. Blood carnitine levels (free and acyl bound) were normal. A tentative diagnosis of medium-chain fatty acyl coenzyme A dehydrogenase (MCAD) deficiency is made. In patients with MCAD deficiency, which of the following is most likely explanation for the fasting hypoglycemia?

A. Decreased acetyl coenzyme A production.
B. Decreased ability to convert acetyl coenzyme A to glucose.
C. Increased conversion of acetyl coenzyme A to acetoacetate.
D. Increased production of ATP and nicotinamide adenine dinucleotide.

Correct answer = A. Impaired oxidation of fatty acids <12 carbons in length results in decreased production of acetyl-coenzyme A (CoA), which is the allosteric activator of pyruvate carboxylase, a gluconeogenic enzyme; thus, glucose levels fall. Acetyl CoA can never be used for the net synthesis of glucose. Acetoacetate is a ketone

body, and with medium-chain fatty acyl CoA dehydrogenase deficiency, ketogenesis is decreased as a result of decreased production of the substrate, acetyl CoA. Impaired fatty acid oxidation means that less ATP and nicotinamide adenine dinucleotide are made, and both are needed for gluconeogenesis.

16.5 A 6-week-old female is brought to the emergency room due to hypotonia and failure to thrive. Physical examination shows dysmorphic facial features and hepatomegaly. Laboratory studies show high levels of very long-chain fatty acids and phytanic acid. Which of the following is the most likely diagnosis?

A. X-linked adrenoleukodystrophy
B. Refsum disease
C. Zellweger syndrome
D. Very–long-chain fatty acids (VLCFA) deficiency

Correct answer = C. Zellweger syndrome is caused by an inability to target matrix proteins to the peroxisome. Therefore, all peroxisomal activities are affected because functional peroxisomes are not formed. As a result, laboratory studies show elevation in both VLCA and phytanic acid in serum. Refsum disease caused by a deficiency of peroxisomal PhyH. This results in the accumulation of phytanic acid in the plasma and tissue. In X-linked adrenoleukodystrophy, the defect is an inability to transport VLCFA into the peroxisome, but other peroxisomal functions, such as α-oxidation, are normal.

Phospholipid, Glycosphingolipid, and Eicosanoid Metabolism 17

I. PHOSPHOLIPID OVERVIEW

Membrane lipids are composed of four major types: phospholipids, sphingolipids, glycolipids, and cholesterol. In this chapter, only the polar membrane lipids are discussed (Fig. 17.1A). Phospholipids are ionic compounds composed of an alcohol that is attached by a phosphodiester bond to either diacylglycerol (DAG) or sphingosine. Like fatty acids (FA), phospholipids are amphipathic in nature. That is, each has a hydrophilic head, which is the phosphate group plus whatever alcohol is attached to it (e.g., serine, ethanolamine, and choline; highlighted in blue in Fig. 17.1B), and a long, hydrophobic tail containing FA or FA-derived hydrocarbons (shown in orange in Fig. 17.1B). Phospholipids are the predominant lipids of cell membranes. In membranes, the hydrophobic portion of a phospholipid molecule is associated with the nonpolar portions of other membrane constituents, such as glycolipids, proteins, and cholesterol. The hydrophilic (polar) head of the phospholipid extends outward, interacting with the intracellular or extracellular aqueous environment (see Fig. 17.1B). Membrane phospholipids also function as a reservoir for intracellular messengers, and, for some proteins, phospholipids serve as anchors to cell membranes. Nonmembrane phospholipids serve additional functions in the body, for example, as components of lung surfactant and essential components of bile, where their detergent properties aid cholesterol solubilization.

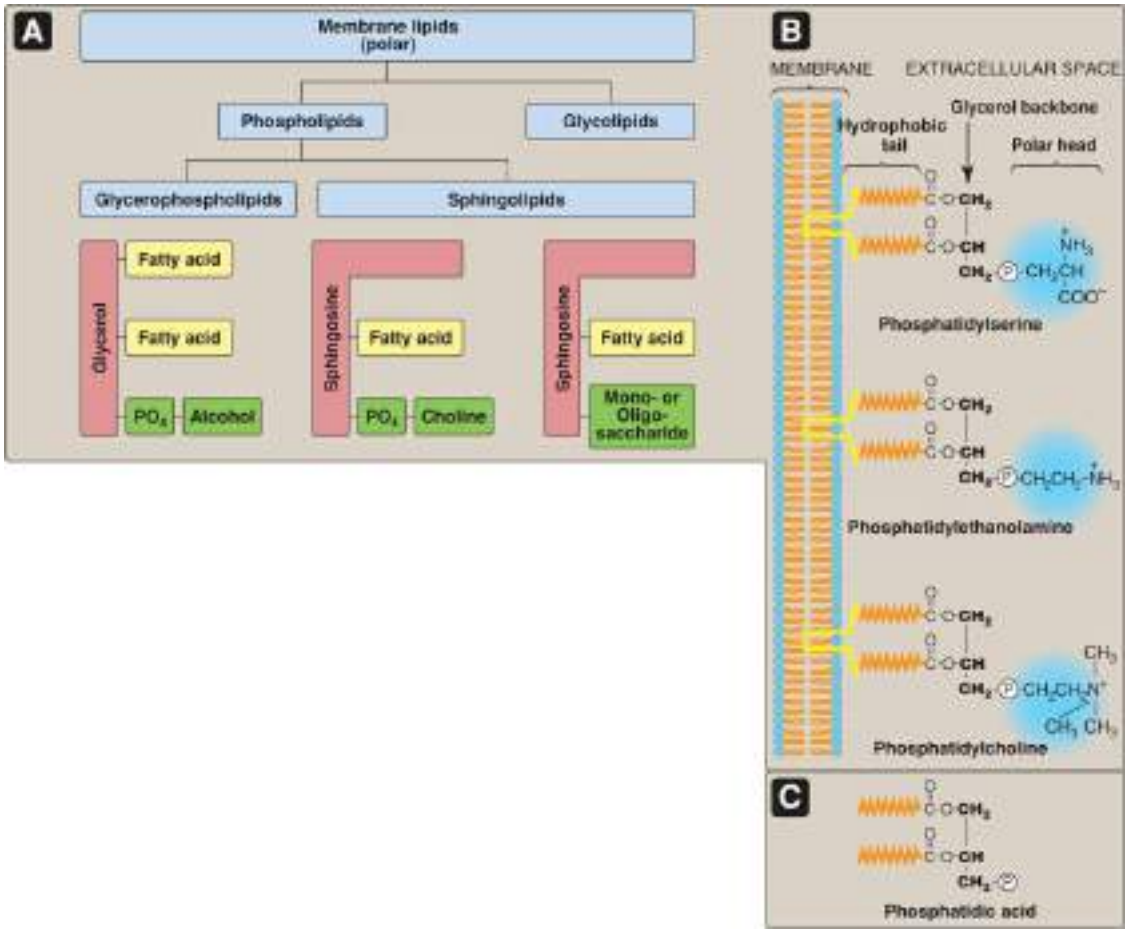


Figure 17.1
A: Polar membrane lipids. **B:** Structures of some glycerophospholipids. **C:** Phosphatidic acid. Ⓟ = phosphate (an anion).

II. PHOSPHOLIPID STRUCTURE

There are two classes of phospholipids: those that have glycerol (from glucose) as a backbone and those that have sphingosine (from serine and palmitate). Both classes are found as structural components of membranes, and both play a role in the generation of lipid-signaling molecules.

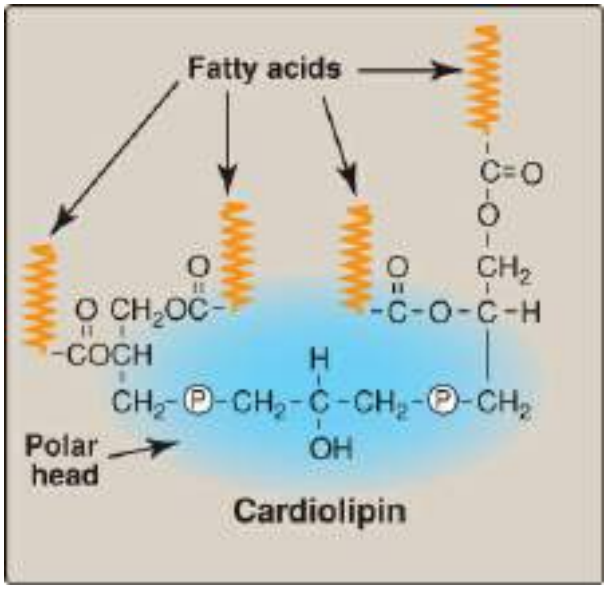


Figure 17.2
Structure of cardiolipin (diphosphatidylglycerol). Ⓟ = phosphate.

A. Glycerophospholipids

Phospholipids that contain glycerol are called glycerophospholipids (or phosphoglycerides). Glycerophospholipids constitute the major class of phospholipids and are the predominant lipids in membranes. All contain (or are derivatives of) phosphatidic acid (PA), which is DAG with a phosphate group on carbon 3 (Fig. 17.1C). Despite the apparent symmetry of the three-carbon glycerol backbone, phospholipids are directionally dependent, and C-1 is not interchangeable with C-3 of the glycerol backbone. PA is the simplest phosphoglyceride and is the precursor of the other members of this group.

1. Fromphosphatidic acid and an alcohol: The phosphate group on PA can be esterified to a compound containing an alcohol group (see Fig. 17.1). For example:

Serine	+ PA → phosphatidylserine (PS)
Ethanolamine	+ PA → phosphatidylethanolamine (PE)
Choline	+ PA → phosphatidylcholine (PC) (lecithin)
Inositol	+ PA → phosphatidylinositol (PI)
Glycerol	+ PA → phosphatidylglycerol (PG)

2. Cardiolipin: Two molecules of PA esterified through their phosphate groups to an additional molecule of glycerol form cardiolipin, or diphosphatidylglycerol (Fig. 17.2). Cardiolipin is found in membranes in prokaryotes and eukaryotes. In eukaryotes, cardiolipin is virtually exclusive to the inner mitochondrial membrane, where it maintains the structure and function of certain respiratory complexes of the electron transport chain. (Note: Cardiolipin is antigenic. A patient infected with *Treponemapallidum* [*T. pallidum*], the bacterium that

causes syphilis develops antibodies [Ab] against cardiolipin. The Wasserman test for syphilis detects Ab raised against *T. pallidum* by subjecting patient's serum to cardiolipin as an antigen. The source of antigenic response to cardiolipin is not well understood, that is, host cardiolipin due to tissue damage in the host or *T. pallidum* itself.)

3. Plasmalogens: When the FA at carbon 1 of a glycerophospholipid is replaced by an unsaturated alkyl group attached by an ether (rather than by an ester) linkage to the core glycerol molecule, an ether phosphoglyceride known as a plasmalogen is produced. For example, phosphatidylethanolamine, which is abundant in nerve tissue (Fig. 17.3A), is the plasmalogen that is similar in structure to phosphatidylethanolamine. Phosphatidylcholine abundant in heart muscle is the other quantitatively significant ether lipid in mammals. (Note: Plasmalogens have "al" rather than "yl" in their names.)

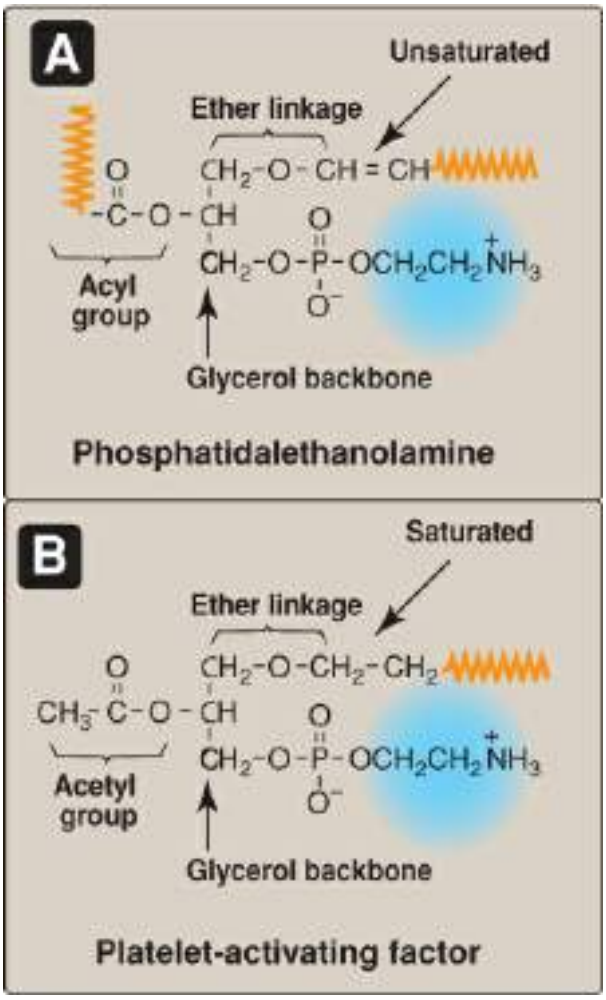


Figure 17.3
The ether glycerophospholipids. A: The plasmalogen phosphatidylethanolamine. B: Platelet-activating factor. (~~~~ is a long, hydrophobic hydrocarbon chain.)

4. Platelet-activating factor: A second example of an ether glycerophospholipid is platelet-activating factor (PAF), which has a saturated alkyl group in an ether link to carbon 1 and an acetyl residue (rather than a FA) at carbon 2 of the glycerol backbone (Fig. 17.3B). PAF is synthesized and released by a variety of cell types. It binds to surface receptors, triggering potent thrombotic and acute inflammatory events. For example, PAF activates inflammatory cells and mediates hypersensitivity, acute inflammatory, and anaphylactic reactions. It causes platelets to aggregate and activate and neutrophils and alveolar macrophages to generate superoxide radicals to kill bacteria (see Chapter 13). It also lowers blood pressure. (Note: PAF is one of the most potent bioactive molecules known, causing effects at concentrations as low as 10^{-11} mol/l.)

B. Sphingophospholipids: sphingomyelin

The backbone of sphingomyelin is the amino alcohol sphingosine, rather than glycerol (Fig. 17.4). A long-chain-length FA (LCFA) is attached to the amino group of sphingosine through an amide linkage, producing a ceramide, which can also serve as a precursor of glycolipids. The alcohol group at carbon 1 of sphingosine is esterified to phosphorylcholine, producing sphingomyelin, the only significant sphingophospholipid in humans. Sphingomyelin is an important constituent of the myelin sheath of nerve fibers and it is essential for myelin integrity and function. (Note: The myelin sheath is a layered, membranous structure that insulates and protects neuronal axons of the central nervous system [CNS]. It also allows rapid neuronal conduction along axons.)

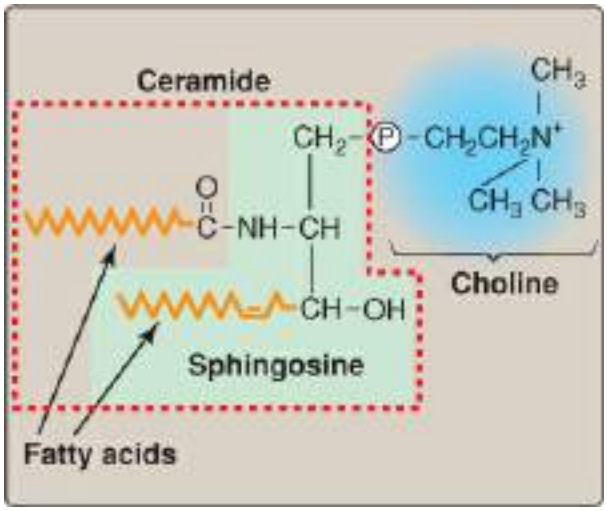


Figure 17.4
Structure of sphingomyelin, showing sphingosine (in *green box*) and ceramide components (in *dashed box*). Ⓟ = phosphate.

III. PHOSPHOLIPID SYNTHESIS

Glycerophospholipid synthesis involves either the donation of PA from

cytidinediphosphate (CDP)-DAG to an alcohol or the donation of the phosphomonoester of the alcohol from CDP-alcohol to DAG (Fig. 17.5). In both cases, the CDP-bound structure is considered an activated intermediate, and cytidine monophosphate (CMP) is released as a side product. Therefore, a key concept in glycerophospholipid synthesis is activation, of either DAG or the alcohol to be added, by linkage with CDP. (Note: This is similar in principle to the activation of sugars by their attachment to uridinediphosphate [UDP] [see Chapter 11].) The FA esterified to the glycerol alcohol groups can vary widely, contributing to the heterogeneity of this group of compounds, with saturated FA typically found at carbon 1 and unsaturated ones at carbon 2. Most phospholipids are synthesized in the smooth endoplasmic reticulum (SER). From there, they are transported to the Golgi and then to membranes of organelles or the plasma membrane or are secreted from the cell by exocytosis. (Note: Ether lipid synthesis from dihydroxyacetone phosphate begins in peroxisomes.)

A. Phosphatidic acid

PA is the precursor of other glycerophospholipids. The steps in its synthesis from glycerol 3-phosphate and two fatty acyl coenzyme A (CoA) molecules were illustrated in Figure 16.14, in which PA is shown as a precursor of triacylglycerol (TAG).

> Essentially all cells except mature erythrocytes can synthesize phospholipids, whereas TAG synthesis occurs essentially only in the liver, adipose tissue, lactating mammary glands, and intestinal mucosal cells.

B. Phosphatidylcholine and phosphatidylethanolamine

The neutral phospholipids PC and PE are the most abundant phospholipids in most eukaryotic cells. The primary route of their synthesis uses choline and ethanolamine obtained either from the diet or from the turnover of the body's phospholipids. (Note: In the liver, PC also can be synthesized from PS and PE [see 2. below].)

1. Synthesis from pre-existing choline and ethanolamine: These synthetic pathways involve the phosphorylation of choline or ethanolamine by kinases, followed by conversion to the activated form, CDP-choline or CDP-ethanolamine. Finally, choline phosphate or ethanolamine phosphate is transferred from the nucleotide (leaving CMP) to a molecule of DAG (see Fig. 17.5).

    a. Significance of choline reutilization: The reutilization of choline is important because, although humans can synthesize choline *de novo*, the amount made is insufficient for our needs. Thus, choline is an essential dietary nutrient with an adequate intake (see p. 402) of 550 mg for men and 425 mg for women. (Note: Choline is also used for the synthesis of acetylcholine, a neurotransmitter.) Although choline deficiency is rare, it may lead to muscle

damage and nonalcoholic fatty liver disease.

b. Phosphatidylcholine in lung surfactant: The pathway described above is the principal pathway for the synthesis of dipalmitoylphosphatidylcholine (DPPC or, dipalmitoyl lecithin). In DPPC, positions 1 and 2 on the glycerol are occupied by palmitate, a saturated LCFA. DPPC, made and secreted by type II pneumocytes, is a major lipid component of lung surfactant, which is the extracellular fluid layer lining the alveoli. Surfactant serves to decrease the surface tension of this fluid layer, reducing the pressure needed to reinflate alveoli, thereby preventing alveolar collapse (atelectasis). (Note: Surfactant is a complex mixture of lipids [90%] and proteins [10%], with DPPC being the major component for reducing surface tension.)

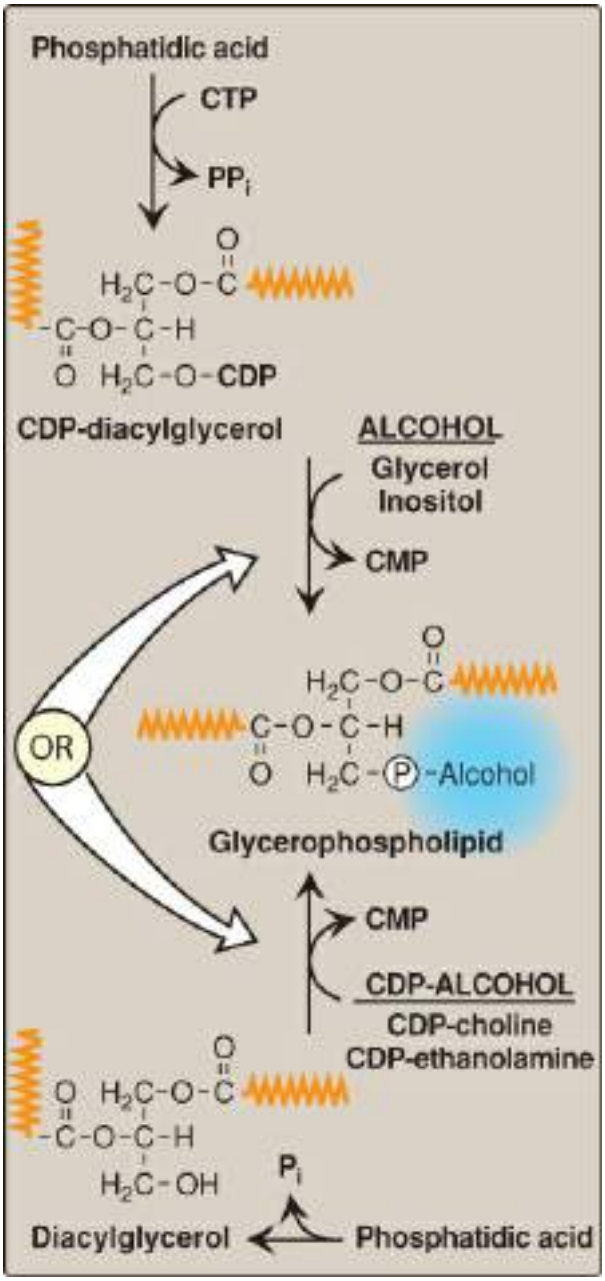


Figure 17.5
Glycerophospholipid synthesis requires activation of either diacylglycerol or an alcohol by linkage to cytidinediphosphate (CDP). CMP and CTP = cytidine mono- and triphosphates; P_i = inorganic phosphate; PP_i = pyrophosphate. (〰 is a fatty acid hydrocarbon chain.)

> Fetal lung maturity can be gauged by determining the lecithin/sphingomyelin (L/S) ratio in amniotic fluid. A value ≥2 is evidence of maturity, because it reflects the shift from sphingomyelin to DPPC synthesis that occurs in pneumocytes at ~32 weeks' gestation.

c. Lung maturity: Respiratory distress syndrome (RDS) in preterm infants is

associated with insufficient surfactant production and/or secretion and is a significant cause of all neonatal deaths in Western countries. Lung maturation can be accelerated by giving the mother glucocorticoids shortly before delivery to induce expression of specific genes. Postnatal administration of natural or synthetic surfactant (by intratracheal instillation) is also used. (Note: Acute RDS, seen in all age groups, is the result of alveolar damage [due to infection, injury, or aspiration] that causes fluid to accumulate in the alveoli, impeding the exchange of oxygen [O_2] and carbon dioxide [CO_2].)

2. Phosphatidylcholine synthesis from phosphatidylserine: The liver requires a mechanism for producing PC, even when free choline levels are low, because it exports significant amounts of PC in the bile and as a component of plasma lipoproteins. To provide the needed PC, PS is decarboxylated to PE by PS decarboxylase. PE then undergoes three methylation steps to produce PC, as illustrated in Figure 17.6. S-adenosylmethionine is the methyl group donor (see Chapter 20).

C. Phosphatidylserine

PS synthesis in mammalian tissues is provided by the base exchange reaction, in which the ethanolamine of PE is exchanged for free serine (see Fig. 17.6). This reaction, although reversible, is used primarily to produce the PS required for membrane synthesis. PS has a net negative charge. (See Chapter 35 for the role of PS in clotting.)

D. Phosphatidylinositol

Phosphatidylinositol (PI) is synthesized from free inositol and CDP-DAG, as shown in Figure 17.5. PI is an unusual phospholipid in that it most frequently contains stearic acid on carbon 1 and arachidonic acid on carbon 2 of the glycerol. Therefore, PI serves as a reservoir of arachidonic acid in membranes and, thus, provides the substrate for prostaglandin (PG) synthesis when required. Like PS, PI has a net negative charge. (Note: There is asymmetry in the phospholipid composition of the cell membrane. PS and PI, for example, are found primarily on the inner leaflet. Asymmetry is achieved by ATP-dependent enzymes known as “flippases” and “floppases.”)

1. Role in signal transduction across membranes: The phosphorylation of membrane-bound PI produces polyphosphoinositides such as phosphatidylinositol 4,5-bisphosphate ([PIP_2]; Fig. 17.7). The cleavage of PIP_2 by phospholipase C occurs in response to the binding of various neurotransmitters, hormones, and growth factors to G protein–coupled receptors (GPCRs), such as the α_1-adrenergic receptor, on the cell membrane and activation of the G_q α-subunit (Fig. 17.8). The products of this cleavage,

inositol 1,4,5-trisphosphate (IP_3) and DAG, mediate the mobilization of intracellular calcium and the activation of protein kinase C, which act synergistically to evoke specific cellular responses. Signal transduction across the membrane is, thus, accomplished.

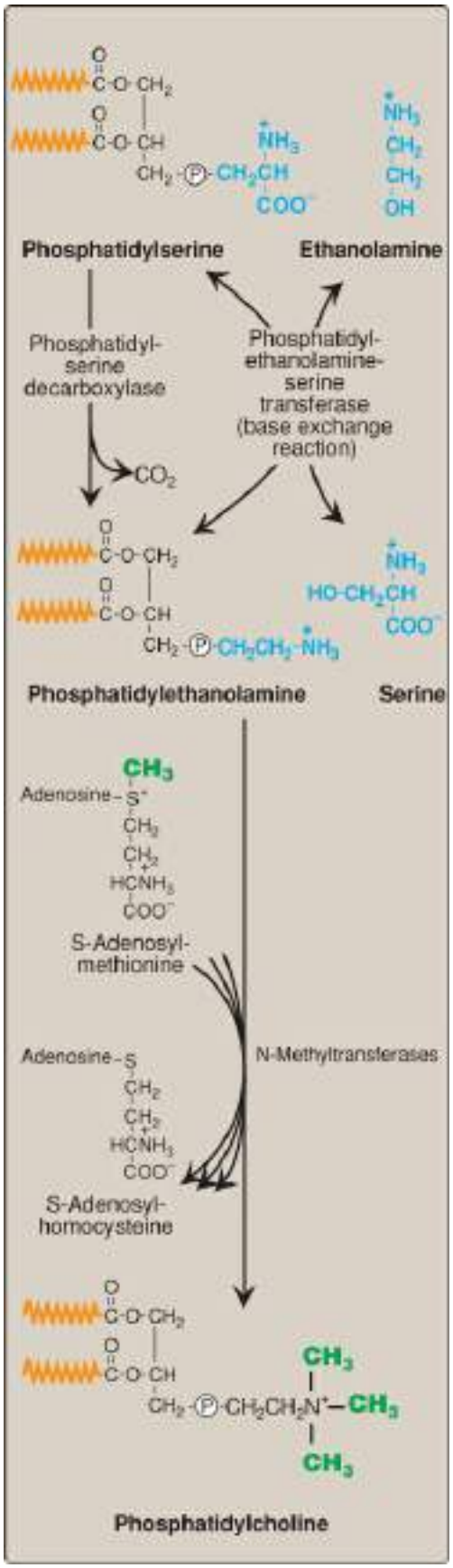


Figure 17.6
Synthesis of phosphatidylcholine from phosphatidylserine in the liver. (is a fatty acid hydrocarbon chain.) Ⓟ = phosphate; CO_2 = carbon dioxide.

2. Role in membrane protein anchoring: Specific proteins can be covalently attached through a carbohydrate bridge to membrane-bound PI (Fig. 17.9). For example, lipoprotein lipase, an enzyme that degrades TAG in lipoprotein particles (see p. 254), is attached to capillary endothelial cells by a glycosyl phosphatidylinositol (GPI) anchor. (Note: GPI-linked proteins are also found in a variety of parasitic protozoans, such as trypanosomes and leishmania.) Being attached to a membrane lipid (rather than being an integral part of the membrane) allows GPI-anchored proteins increased lateral mobility on the extracellular surface of the plasma membrane. The protein can be cleaved from its anchor by the action of phospholipase C (see Fig. 17.9). (Note: A deficiency in the synthesis of GPI in hematopoietic cells results in the hemolytic disease paroxysmal nocturnal hemoglobinuria. Some of the GPI-anchored proteins protect blood cells from the immune system which recognizes foreign substances such as viruses and bacteria in the body. The lack of GPI-anchored proteins on the red blood cells are no longer recognized as “self” and are susceptible to destruction by complement-mediated lysis.)

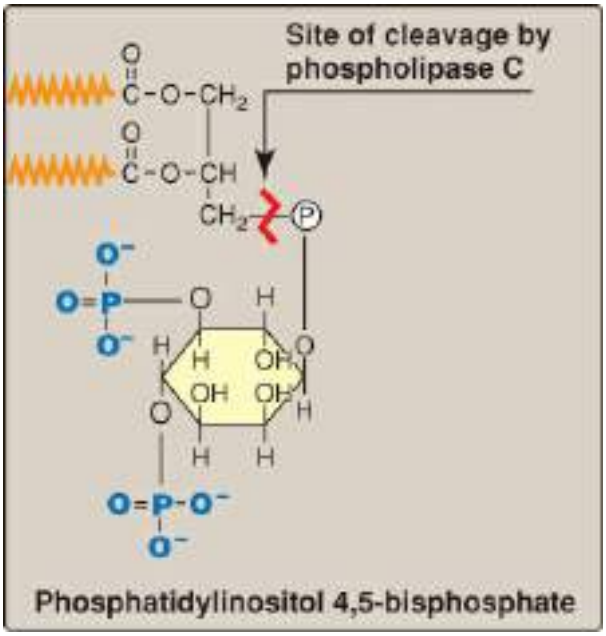


Figure 17.7
Structure of phosphatidylinositol 4,5-bisphosphate (PIP_2). Cleavage by *phospholipase C* produces inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol. (is a fatty acid hydrocarbon chain.) Ⓟ = phosphate.

E. Phosphatidylglycerol and cardiolipin

Phosphatidylglycerol is found in relatively large concentrations in mitochondrial

membranes and is a precursor of cardiolipin (diphosphatidylglycerol). It is synthesized from CDP-DAG and glycerol 3-phosphate. Cardiolipin (see Fig. 17.2) is synthesized by the transfer of DAG 3-phosphate from CDP-DAG to a pre-existing molecule of phosphatidylglycerol.

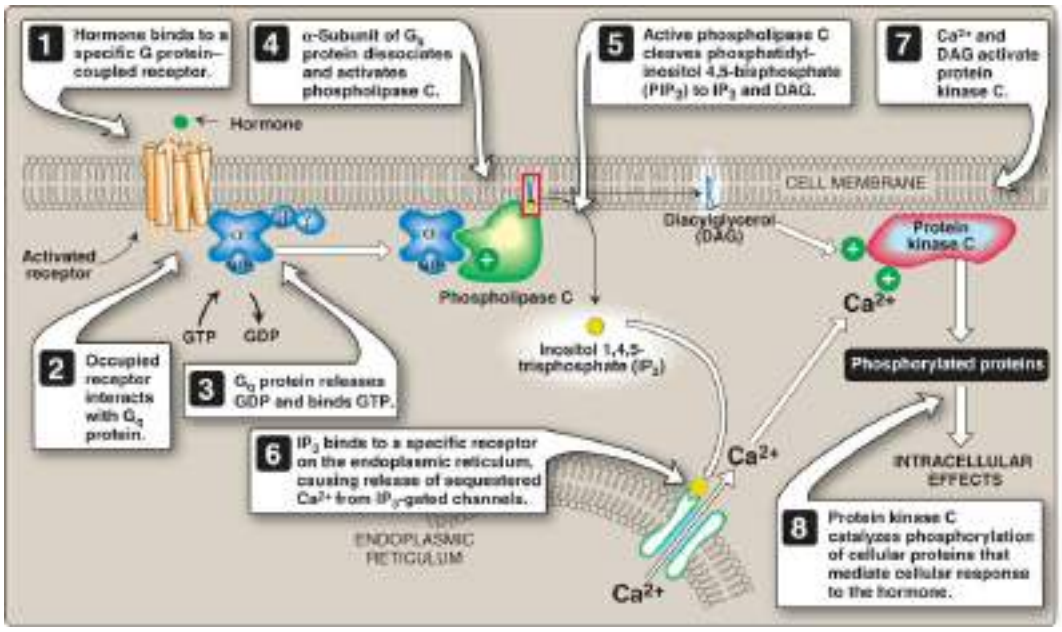


Figure 17.8
Role of inositol triphosphate and diacylglycerol in cell signaling. GDP and GTP = guanosine di- and triphosphates; Ca^{2+} = calcium.

F. Sphingomyelin

Sphingomyelin, a sphingosine-based phospholipid, is found in cell membranes and in the myelin sheath. The synthesis of sphingomyelin is shown in Figure 17.10. Briefly, palmitoyl CoA condenses with serine, as CoA and the carboxyl group (as CO_2) of serine are lost. (Note: This reaction, like the decarboxylation reactions involved in the synthesis of PE from PS and of regulators from amino acids [e.g., the catecholamines from tyrosine; see Chapter 21], requires pyridoxal phosphate [a derivative of vitamin B_6] as a coenzyme.) The product is reduced in a nicotinamide adenine dinucleotide phosphate (NADPH)-requiring reaction to sphinganine (dihydrosphingosine). The sphinganine is acylated at the amino group with one of a variety of LCFA and then desaturated to produce a ceramide, the immediate precursor of sphingomyelin (and other sphingolipids, as described in section V.).

> Ceramides play a key role in maintaining the skin's water-permeability barrier. Decreased ceramide levels are associated with a number of skin diseases.

Phosphorylcholine from PC is transferred to the ceramide, producing sphingomyelin

and DAG. (Note: Sphingomyelin of the myelin sheath contains predominantly longer-chain FA such as lignoceric acid and nervonic acid, whereas gray matter of the brain has sphingomyelin that contains primarily stearic acid.)

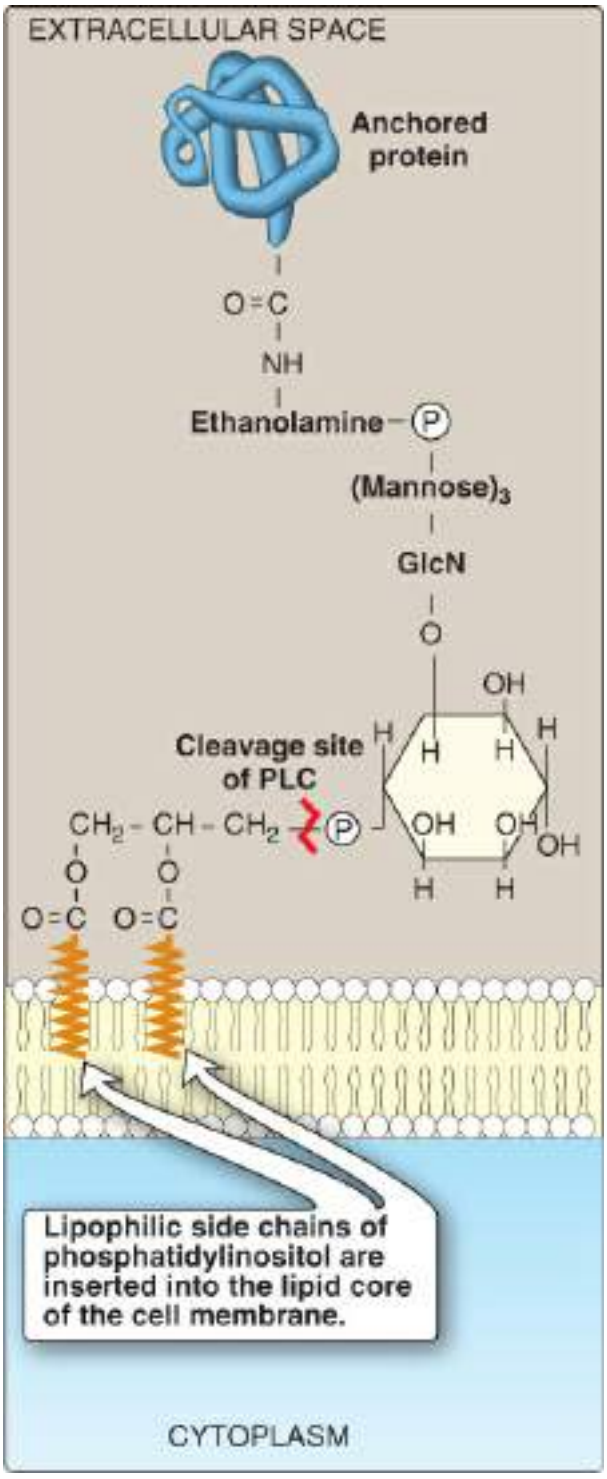


Figure 17.9
Example of a glycosyl phosphatidylinositol (GPI) membrane protein anchor. GlcN = glucosamine; P

= phosphate; PLC = phospholipase C.

IV. PHOSPHOLIPID DEGRADATION

The degradation of phosphoglycerides is performed by phospholipases found in all tissues and pancreatic juice. (Note: For a discussion of phospholipid digestion, see Chapter 15. Section D.3.) A number of toxins and venoms have phospholipase activity, and several pathogenic bacteria produce phospholipases that dissolve cell membranes and allow the spread of infection. Sphingomyelin is degraded by the lysosomal phospholipase, sphingomyelinase (see B. below).

A. Phosphoglycerides

Phospholipases hydrolyze the phosphodiester bonds of phosphoglycerides, with each enzyme cleaving the phospholipid at a specific site. The major phospholipases are shown in Figure 17.11. (Note: Removal of the FA from carbon 1 or 2 of a phosphoglyceride produces a lysophosphoglyceride, which is the substrate for lysophospholipases.) Phospholipases release molecules that can serve as second messengers (e.g., DAG and IP_3) or that are the substrates for synthesis of messengers (e.g., arachidonic acid). Phospholipases are responsible not only for degrading phospholipids but also for remodeling them. For example, phospholipases A_1 and A_2 remove specific FA from membrane-bound phospholipids, which can be replaced with different FA using fatty acyl CoA transferase. This mechanism is used as one way to create the unique lung surfactant DPCC and to ensure that carbon 2 of PI (and sometimes of PC) is bound to arachidonic acid. (Note: Barth syndrome, a rare X-linked disorder characterized by cardiomyopathy, muscle weakness, and neutropenia, is the result of defects in cardiolipin remodeling.)

B. Sphingomyelin

Sphingomyelin is degraded by sphingomyelinase, a lysosomal enzyme that removes phosphorylcholine, leaving a ceramide. The ceramide is, in turn, cleaved by ceramidase into sphingosine and a free FA (Fig. 17.12). (Note: The released ceramide and sphingosine regulate signal transduction pathways, in part by influencing the activity of protein kinase C and, thus, the phosphorylation of its protein substrates. They also promote apoptosis.) Niemann–Pick disease (types A and B) is an autosomal-recessive disorder caused by the inability to degrade sphingomyelin due to a deficiency of sphingomyelinase, a type of phospholipase C.

In the severe infantile form (type A, which shows <1% of normal enzymic activity), the liver and spleen are the primary sites of lipid deposits and therefore, hepatosplenomegaly develops. The lipid consists primarily of the sphingomyelin that cannot be degraded (Fig. 17.13). Macrophages of the reticuloendothelial system become engorged with sphingomyelin, which gives them a foamy histologic

appearance. Infants with this lysosomal storage disease experience rapid and progressive neurodegeneration as a result of deposition of sphingomyelin in the CNS. As a result, a cherry-red spot in the macula of the eye develops due to lipid deposition and edema in the retinal ganglion cells. These infants die in early childhood. A less severe variant (type B, which shows up to 10% of normal activity) with a later age of onset and a longer survival time causes little to no damage to neural tissue, but lungs, spleen, liver, and bone marrow are affected, resulting in a chronic form of the disease. Although Niemann–Pick disease occurs in all ethnic groups, type A occurs with greater frequency in the Ashkenazi Jewish population. (Note: Niemann–Pick disease Type C [NPC] results from mutations in either the NPC1 or NPC2, genes important in processing endocytosed cholesterol, and leads to both cholesterol and sphingomyelin accumulation.)

V. GLYCOLIPID OVERVIEW

Glycolipids are molecules that contain both carbohydrate and lipid components. Like the phospholipid sphingomyelin, glycolipids are derivatives of ceramides in which a LCFA is attached to the amino alcohol sphingosine. Therefore, they are more precisely called glycosphingolipids. (Note: Thus, ceramides are the precursors of both phosphorylated and glycosylated sphingolipids.) Like the phospholipids, glycosphingolipids are essential components of all membranes in the body, but they are found in greatest amounts in nerve tissue. They are located in the outer leaflet of the plasma membrane, where they interact with the extracellular environment. As such, they play a role in the regulation of cellular interactions (e.g., adhesion and recognition), growth, and development.

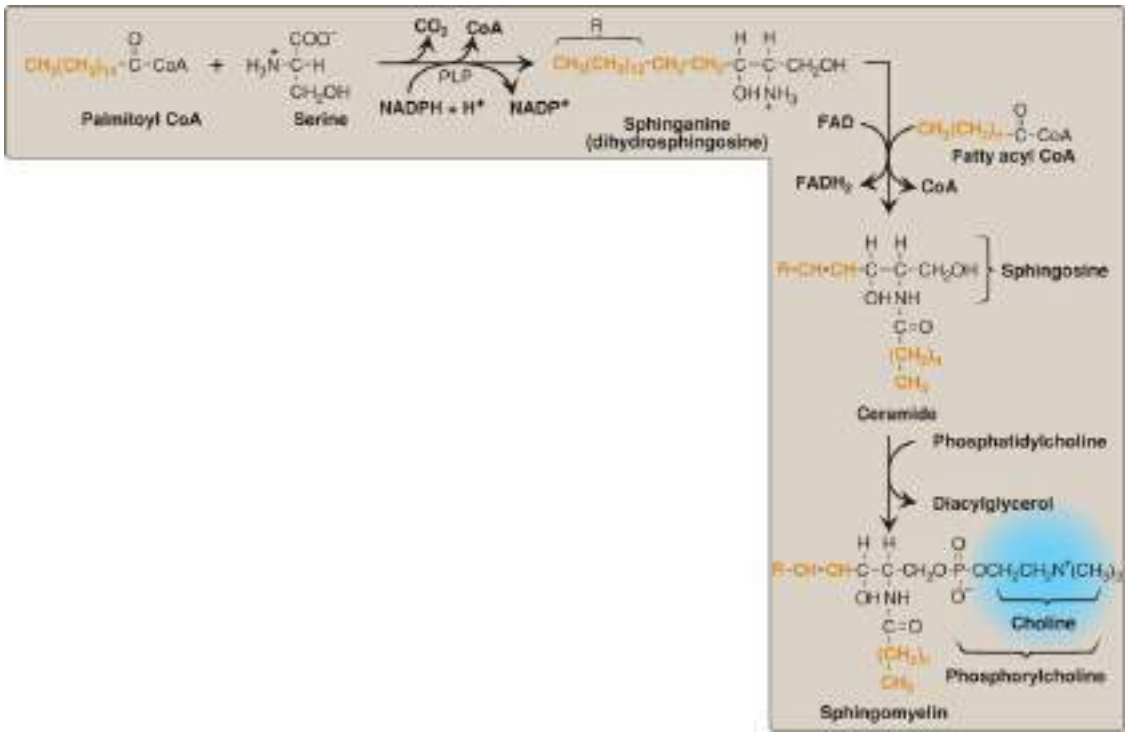


Figure 17.10
Synthesis of sphingomyelin. PLP = pyridoxal phosphate; NADP(H) = nicotinamide adenine dinucleotide phosphate; FAD(H_2) = flavin adenine dinucleotide; CoA = coenzyme A.

Membrane glycosphingolipids associate with cholesterol and GPI-anchored proteins to form lipid rafts, laterally mobile microdomains of the plasma membrane that function to organize and regulate membrane signaling and trafficking functions.

Glycosphingolipids are antigenic and are the source of ABO blood group antigens (Chapter 4, Section VII. B), various embryonic antigens specific for particular stages of fetal development, and some tumor antigens. (Note: The carbohydrate portion of a glycolipid is the antigenic determinant and the lipid portion serves as the membrane anchor.) They have been co-opted for use as cell surface receptors for cholera and tetanus toxins as well as for certain viruses and microbes. Genetic disorders associated with an inability to properly degrade the glycosphingolipids result in lysosomal accumulation of these compounds. (Note: Changes in the carbohydrate portion of glycosphingolipids [and glycoproteins] are characteristic of transformed cells [cells with dysregulated growth].)

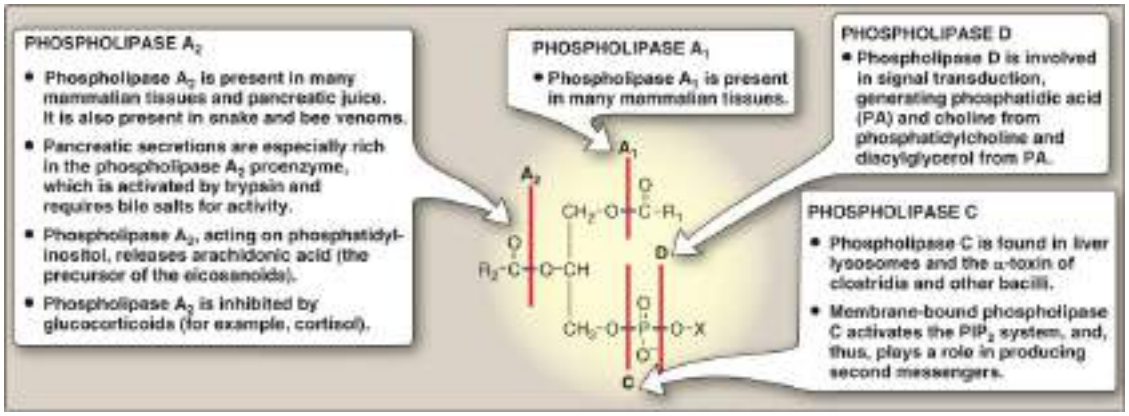


Figure 17.11
Degradation of glycerophospholipids by phospholipases. PIP_2 = phosphatidylinositol 4,5-bisphosphate; R_1 and R_2 = fatty acids; X = an alcohol.

VI. GLYCOSPHINGOLIPID STRUCTURE

The glycosphingolipids differ from sphingomyelin in that they do not contain phosphate, and the polar head function is provided by a monosaccharide or oligosaccharide attached directly to the ceramide by an O-glycosidic bond (Fig. 17.14). The number and type of carbohydrate moieties present determine the type of glycosphingolipid.

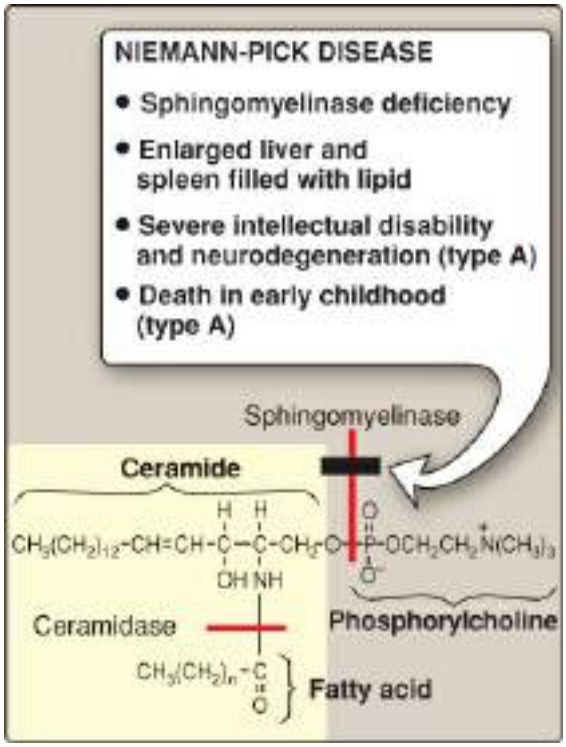


Figure 17.12
Degradation of sphingomyelin. (Note: Type B is the nonneuropathic form. It has a later age of onset and a longer survival time than type A.)

A. Neutral glycosphingolipids

The simplest neutral glycosphingolipids are the cerebrosides. These are ceramide monosaccharides that contain either a molecule of galactose (forming ceramide-galactose or galactocerebroside, the most common cerebroside found in myelin, as shown in Fig. 17.14) or glucose (forming ceramide-glucose or glucocerebroside, an intermediate in the synthesis and degradation of the more complex glycosphingolipids). (Note: Members of a group of galacto- or glucocerebrosides may also differ from each other in the type of FA attached to the sphingosine.) As their name implies, cerebrosides are found predominantly in the brain and peripheral nerves, with high concentrations in the myelin sheath. Ceramide oligosaccharides (or globosides) are produced by attaching additional monosaccharides to a glucocerebroside, for example, ceramide-glucose-galactose (also known as lactosylceramide). The additional monosaccharides can include substituted sugars such as *N*-acetylgalactosamine.

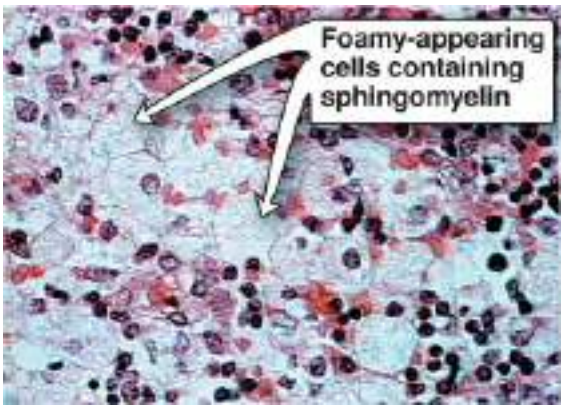


Figure 17.13
Accumulation of lipids in spleen cells from a patient with Niemann–Pick disease.

B. Acidic glycosphingolipids

Acidic glycosphingolipids are negatively charged at physiologic pH. The negative charge is provided by N-acetylneuraminic acid ([NANA], a sialic acid, as shown in Fig. 17.15) in gangliosides or by sulfate groups in sulfatides.

1. Gangliosides: These are the most complex glycosphingolipids and are found primarily in the ganglion cells of the CNS, particularly at the nerve endings. They are derivatives of ceramide oligosaccharides and contain one or more molecules of NANA (from CMP-NANA). The notation for these compounds is G (for ganglioside) plus a subscript M, D, T, or Q to indicate whether there is one (mono), two (di), three (tri), or four (quatro) molecules of NANA in the ganglioside, respectively. Additional numbers and letters in the subscript designate the monomeric sequence of the carbohydrate attached to the ceramide. (See Fig. 17.15 for the structure of G_{M2}.) Gangliosides are of medical interest because several lipid storage disorders involve the accumulation of NANA-containing glycosphingolipids in cells (see Fig. 17.19).
2. Sulfatides: These sulfoglycosphingolipids are sulfated galactocerebrosides that are negatively charged at physiologic pH. Sulfatides are found predominantly in the brain and kidneys.

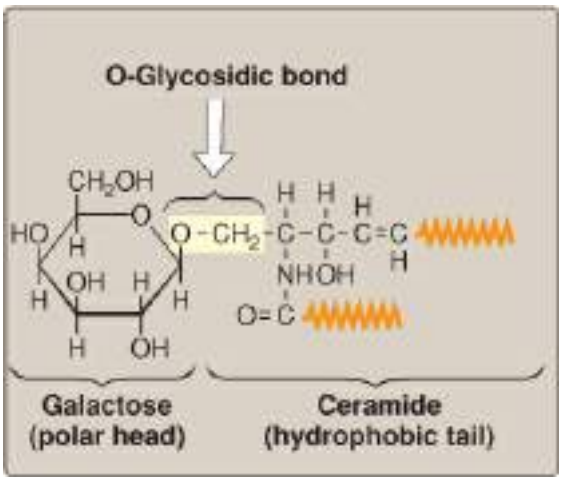


Figure 17.14
Structure of a neutral glycosphingolipid, galactocerebroside. (is a hydrophobic hydrocarbon chain.)

VII. GLYCOSPHINGOLIPID SYNTHESIS AND DEGRADATION

Synthesis of glycosphingolipids occurs primarily in the Golgi by sequential addition of glycosyl monomers transferred from UDP-sugar donors to the acceptor molecule. The mechanism is similar to that used in glycoprotein synthesis (see Chapter 14).

A. Enzymes involved in synthesis

The enzymes involved in the synthesis of glycosphingolipids are glycosyltransferases that are specific for the type and location of the glycosidic bond formed. (Note: These enzymes can recognize both glycosphingolipids and glycoproteins as substrates.)

B. Sulfate group addition

A sulfate group from the sulfate carrier 3′-phosphoadenosine-5′-phosphosulfate ([PAPS], Fig. 17.16) is added by a sulfotransferase to the 3′-hydroxyl group of the galactose in a galactocerebroside, forming the sulfatidegalactocerebroside 3-sulfate (Fig. 17.17). (Note: PAPS is also the sulfur donor in glycosaminoglycan synthesis [see p. 178] and steroid hormone catabolism [see Chapter 18].) An overview of the synthesis of sphingolipids is shown in Figure 17.18.

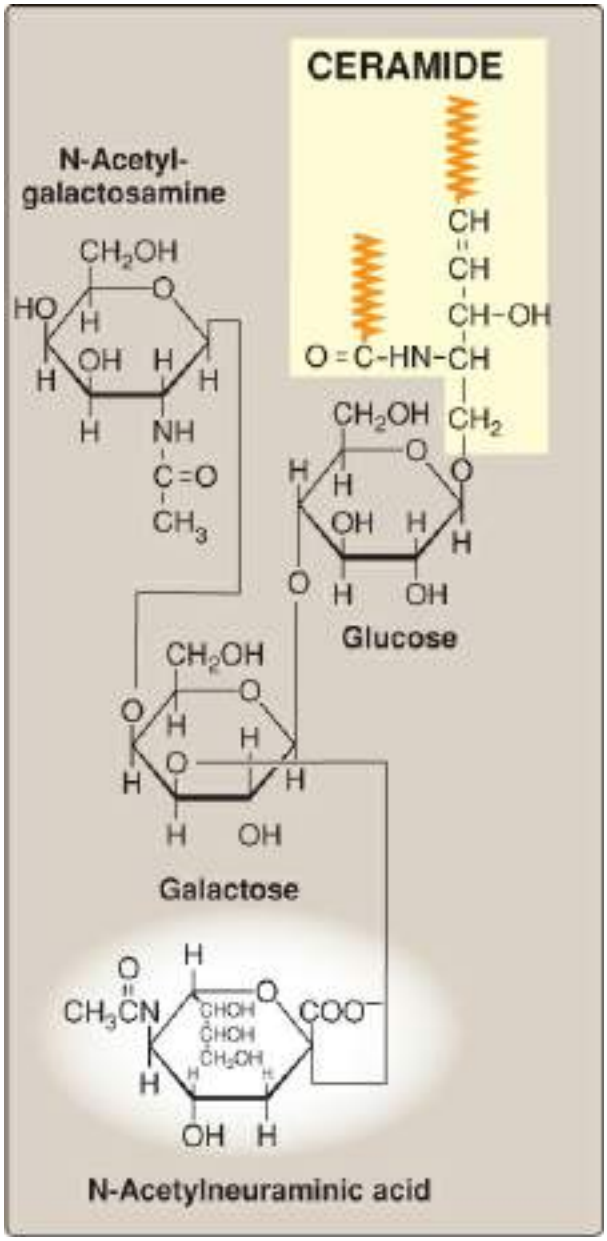


Figure 17.15
Structure of the ganglioside G_{M2}. (WWWWW is a hydrophobic hydrocarbon chain.)

C. Glycosphingolipid degradation

Glycosphingolipids are internalized by phagocytosis as described for the glycosaminoglycans (see Chapter 14). All of the enzymes required for the degradative process are present in lysosomes, which fuse with the phagosomes. The lysosomal enzymes hydrolytically and irreversibly cleave specific bonds in the glycosphingolipid. As seen with the glycosaminoglycans and glycoproteins, degradation is a sequential process following the rule “last on, first off,” in which the last group added during synthesis is the first group removed in degradation. Therefore, defects in the degradation of the polysaccharide chains in these three

glycoconjugates result in lysosomal storage diseases.

D. Sphingolipidoses

In a normal individual, synthesis and degradation of glycosphingolipids are balanced, so that the amount of these compounds present in membranes is constant. If a specific lysosomal acid hydrolase required for degradation is partially or totally missing, a sphingolipid accumulates. Lysosomal lipid storage diseases caused by these deficiencies are called sphingolipidoses. The result of a specific acid hydrolase deficiency may be seen dramatically in nerve tissue, where neurologic deterioration can lead to early death. Figure 17.19 provides an outline of the pathway of sphingolipid degradation and descriptions of some sphingolipidoses. (Note: Some sphingolipidoses can also result from defects in lysosomal activator proteins [e.g., the saposins] that facilitate access of the hydrolases to short carbohydrate chains as degradation proceeds.)

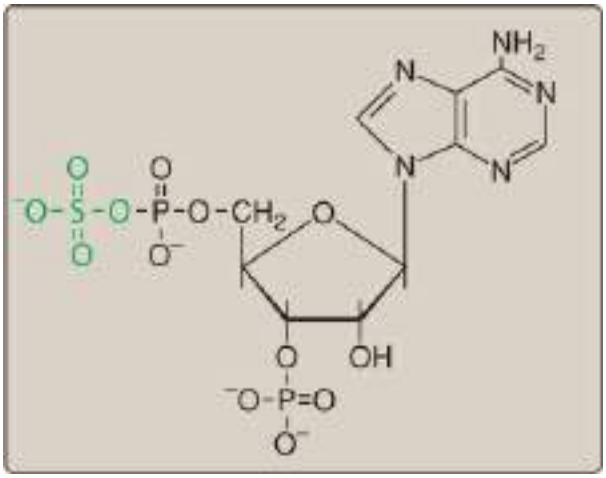


Figure 17.16
Structure of 3′-phosphoadenosine-5′-phosphosulfate.

1. Common properties: A specific lysosomal hydrolytic enzyme is deficient in the classic form of each disorder. Therefore, usually, only a single sphingolipid (the substrate for the deficient enzyme) accumulates in the involved organs in each disease. (Note: The rate of biosynthesis of the accumulating lipid is normal.) The disorders are progressive and, although many are fatal in childhood, extensive phenotypic variability is seen leading to the designation of different clinical types, such as types A and B in Niemann–Pick disease. Genetic variability is also seen because a given disorder can be caused by any one of a variety of mutations within a single gene. The sphingolipidoses are autosomal-recessive disorders, except for Fabry disease, which is X linked. The incidence of the sphingolipidoses is low in most populations, except for Gaucher and Tay–Sachs diseases, which, like Niemann–Pick disease, show a high frequency in the Ashkenazi Jewish population. (Note: Tay–Sachs also has a high frequency in Irish American, French Canadian, and Louisiana Cajun populations.)

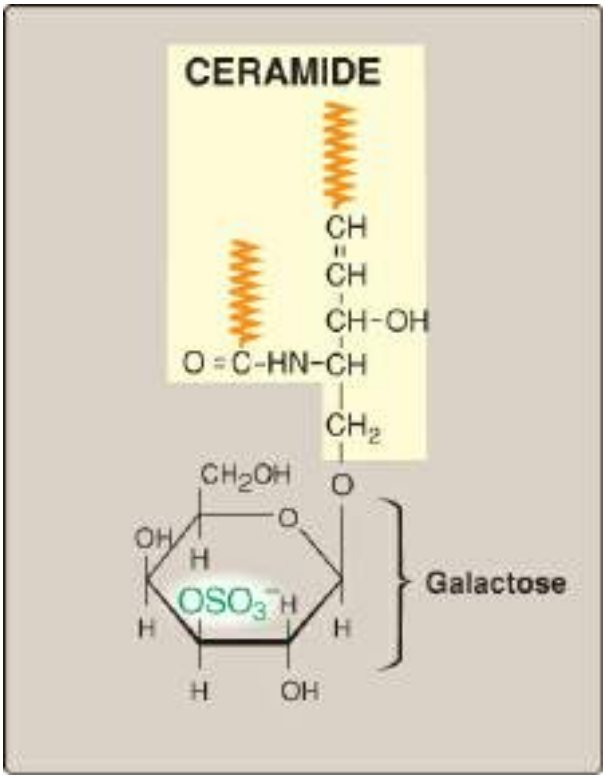


Figure 17.17
Structure of galactocerebroside 3-sulfate. (is a hydrophobic hydrocarbon chain.)

2. Diagnosis and treatment: A specific sphingolipidosis can be diagnosed by measuring enzyme activity in patient cultured fibroblasts or peripheral leukocytes or by analyzing patient DNA (see Chapter 34). Histologic examination of the affected tissue is also useful. (Note: Shell-like inclusion bodies are seen in Tay–Sachs, and a crumpled tissue paper appearance of the cytosol is seen in Gaucher disease [Fig. 17.20].) Prenatal diagnosis, using cultured amniocytes or chorionic villi, is available. Gaucher disease, in which macrophages become engorged with glucocerebroside, and Fabry disease, in which globosides accumulate in the vascular endothelial lysosomes of the brain, heart, kidneys, and skin, are treated by recombinant human enzyme replacement therapy, but the monetary cost is extremely high. Gaucher has also been treated by bone marrow transplantation (because macrophages are derived from hematopoietic stem cells). It is also treated pharmacologically using miglustat, which reduces the substrate (glucosylceramide) for the deficient enzyme. This strategy is known as substrate reduction therapy.

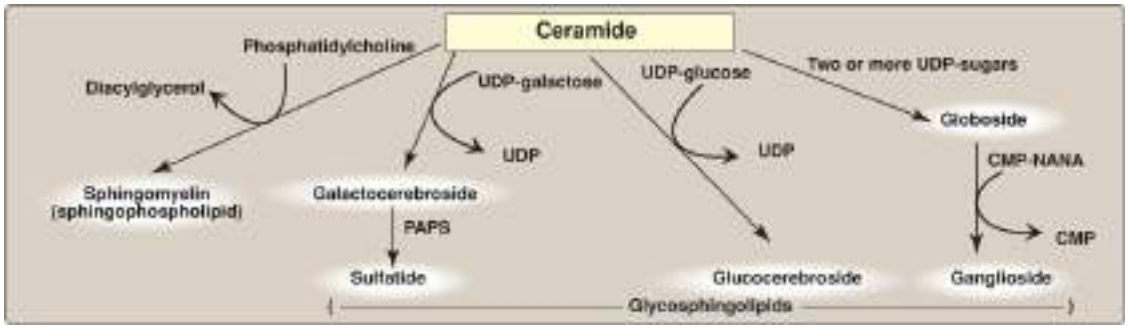


Figure 17.18
Overview of sphingolipid synthesis. UDP = uridinediphosphate; CMP = cytidine monophosphate; NANA = N-acetylneuraminic acid; PAPS = 3′-phosphoadenosine-5′-phosphosulfate.

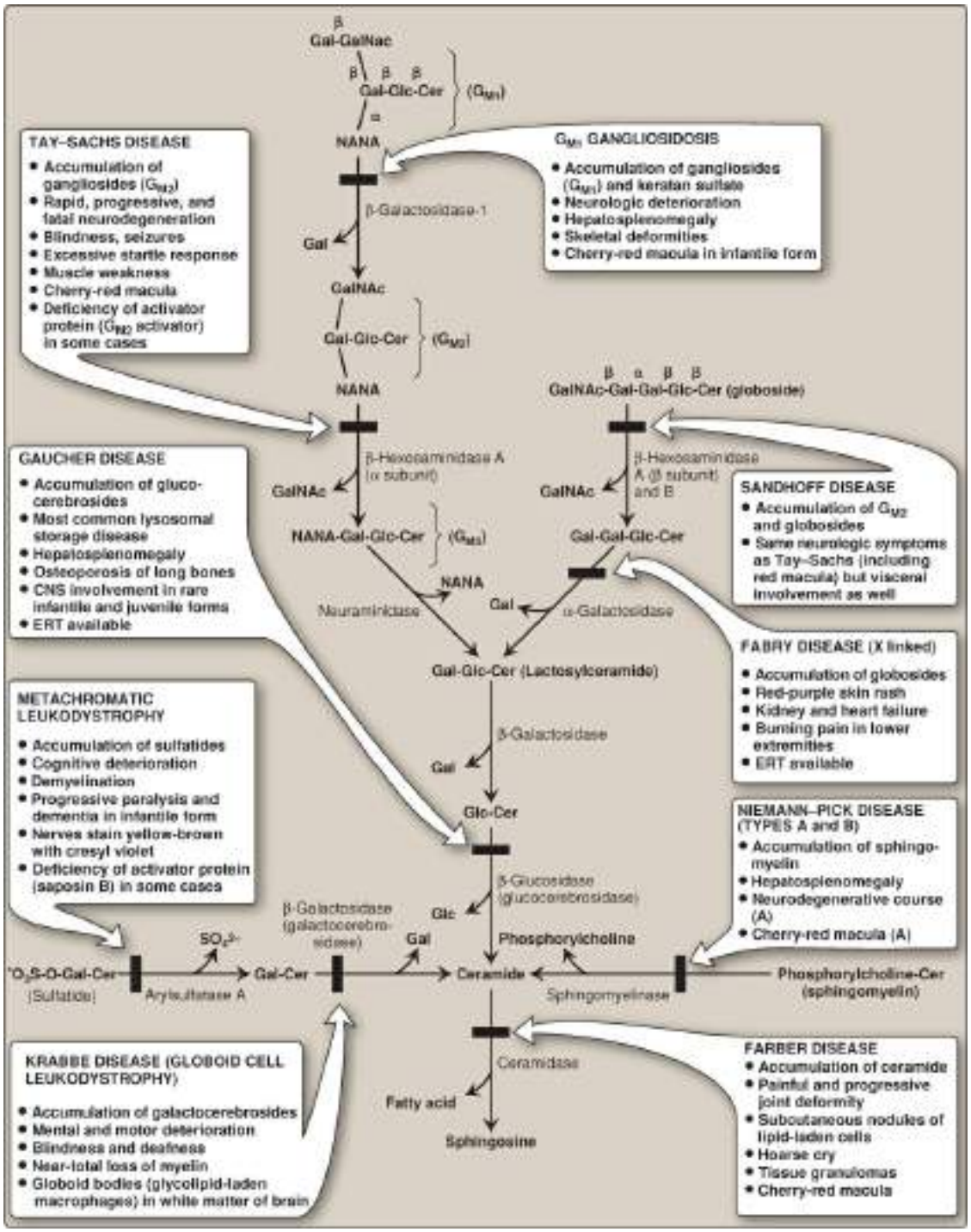


Figure 17.19
Degradation of sphingolipids showing the lysosomal enzymes affected in related genetic diseases, the sphingolipidoses. All are autosomal-recessive diseases except Fabry, which is X linked, and all can be fatal in early life. Cer = ceramide; Gal = galactose; Glc = glucose; GalNAc = N-acetylgalactosamine; NANA = N-acetylneuraminic acid; CNS = central nervous system. SO_4^{2-} = sulfate; ERT = enzyme replacement therapy.

VIII. EICOSANOIDS: PROSTAGLANDINS, THROMBOXANES, AND

LEUKOTRIENES

PGs, thromboxanes (TXs), and leukotrienes (LTs) are collectively known as eicosanoids to reflect their origin from ω-3 and ω-6 polyunsaturated FA with 20 carbons (eicosa = 20). They are extremely potent compounds that elicit a wide range of responses, both physiologic (inflammatory response) and pathologic (hypersensitivity). They ensure gastric integrity and renal function, regulate smooth muscle contraction (the intestine and uterus are key sites) and blood vessel diameter, and maintain platelet homeostasis. Although they have been compared to hormones in terms of their actions, eicosanoids differ from endocrine hormones in that they are produced in very small amounts in almost all tissues rather than in specialized glands and act locally rather than after transport in the blood to distant sites. Eicosanoids are not stored, and they have an extremely short half-life, being rapidly metabolized to inactive products. Their biologic actions are mediated by plasma membrane GPCRs (see p. 103), which are different in different organ systems and typically result in changes in cyclic adenosine monophosphate production. Examples of eicosanoid structures are shown in Figure 17.21.

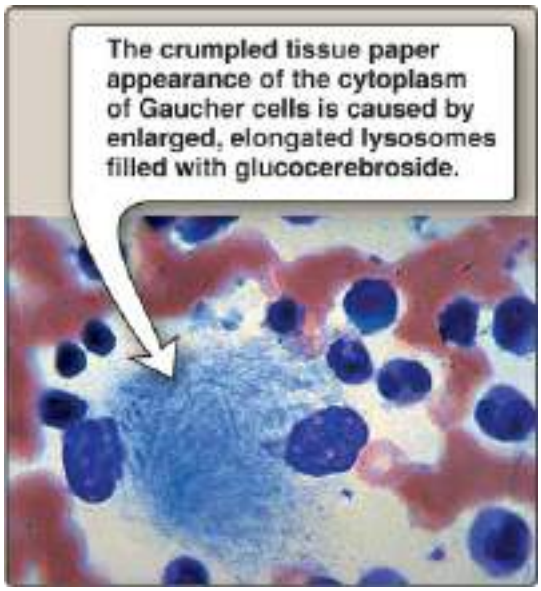


Figure 17.20
Aspirated bone marrow cells from a patient with Gaucher disease.

A. Prostaglandin and thromboxane synthesis

Arachidonic acid, an ω-6 FA containing 20 carbons and four double bonds (an eicosatetraenoic FA), is the immediate precursor of the predominant type of human PG (series 2 or those with two double bonds, as shown in Fig. 17.22). It is derived by the elongation and desaturation of the essential FA linoleic acid, also an ω-6 FA. Arachidonic acid is incorporated into membrane phospholipids (typically PI) at carbon 2, from which it is released by phospholipase A_2 (Fig. 17.23) in response to

a variety of signals, such as a rise in calcium. (Note: Series 1 PGs contain one double bond and are derived from an ω-6 eicosatrienoic FA, dihomo-γ-linolenic acid, whereas series 3 PGs contain three double bonds and are derived from eicosapentaenoic acid [EPA], an ω-3 FA. See p. 292.)

1. Prostaglandin H2 synthase: The first step in PG and TX synthesis is the oxidative cyclization of free arachidonic acid to yield PGH_2 by PGH_2 synthase (or, prostaglandin endoperoxide synthase). This enzyme is an ER membrane-bound protein that has two catalytic activities: fatty acid cyclooxygenase (COX), which requires two molecules of O_2, and peroxidase, which requires reduced glutathione (see Chapter 13). PGH_2 is converted to a variety of PGs and TXs, as shown in Figure 17.23, by cell-specific synthases. (Note: PGs contain a five-carbon ring, whereas TXs contain a heterocyclic six-membered oxane ring [see Fig. 17.21].) Two isozymes of PGH_2 synthase, usually denoted as COX-1 and COX-2, are known. COX-1 is made constitutively in most tissues and is required for maintenance of healthy gastric tissue, renal homeostasis, and platelet aggregation. COX-2 is inducible in a limited number of tissues in response to products of activated immune and inflammatory cells. (Note: The increase in PG synthesis subsequent to the induction of COX-2 mediates the pain, heat, redness, and swelling of inflammation and the fever of infection.)

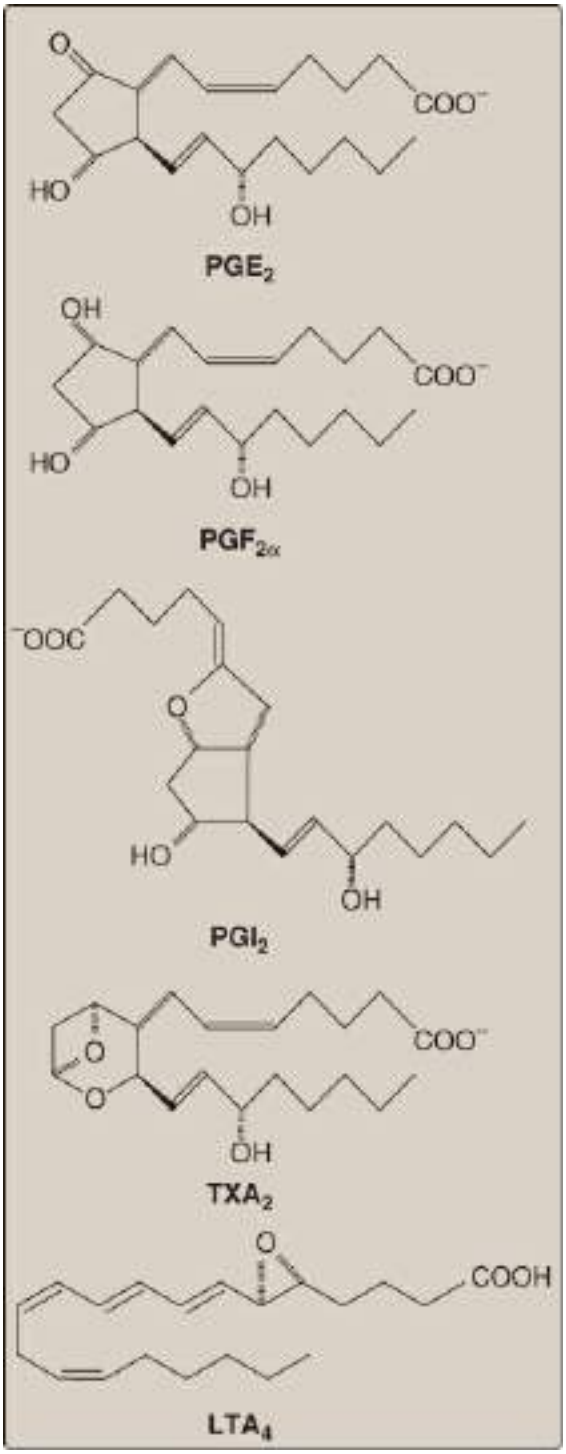


Figure 17.21
Examples of eicosanoid structures. (Note: Prostaglandins are named as follows: PG plus a third letter (e.g., E), which designates the type and arrangement of functional groups in the molecule. The subscript number indicates the number of double bonds in the molecule. PGI_2 is also known as prostacyclin. Thromboxanes are designated by TXs and leukotrienes by LTs.)

2. Synthesis inhibition: The synthesis of PG and TX can be inhibited by unrelated

compounds. For example, cortisol (a steroidal anti-inflammatory agent) inhibits phospholipase A_2 activity (see Fig. 17.23) and, therefore, arachidonic acid, the substrate for PG and TX synthesis, is not released from membrane phospholipids. Aspirin, indomethacin, and phenylbutazone (all nonsteroidal anti-inflammatory drugs [NSAIDs]) inhibit both COX-1 and COX-2 and, thus, prevent the synthesis of the parent molecule, PGH_2. (Note: Systemic inhibition of COX-1, with subsequent damage to the stomach and the kidneys and impaired clotting of blood, is the basis of aspirin's toxicity.) Aspirin (but not other NSAIDs) also induces synthesis of lipoxins (anti-inflammatory mediators made from arachidonic acid) and resolvins and protectins (inflammation-resolving mediators made from EPA). Inhibitors specific for COX-2 (the coxibs) are designed to reduce the pathologic inflammatory processes mediated by COX-2 while maintaining the physiologic functions of COX-1. Currently, celecoxib is the only FDA-approved coxib.

B. Thromboxanes and prostaglandins in platelet homeostasis

Thromboxane A_2 (TXA_2) is produced by COX-1 in activated platelets. It promotes platelet adhesion and aggregation and contraction of vascular smooth muscle, thereby promoting formation of blood clots (thrombi). (See online Chapter 35.) Prostacyclin (PGI_2), produced by COX-2 in vascular endothelial cells, inhibits platelet aggregation and stimulates vasodilation and, so, impedes thrombogenesis. The opposing effects of TXA_2 and PGI_2 limit thrombi formation to sites of vascular injury. (Note: Aspirin has an antithrombogenic effect. It inhibits TXA_2 synthesis by COX-1 in platelets and PGI_2 synthesis by COX-2 in endothelial cells through irreversible acetylation of these isozymes [Fig. 17.24]. COX-1 inhibition cannot be overcome in platelets, which lack nuclei. However, COX-2 inhibition can be overcome in endothelial cells because they have a nucleus and, therefore, can generate more of the enzyme. This difference is the basis of low-dose aspirin therapy used to lower the risk of stroke and heart attacks by decreasing formation of thrombi.)

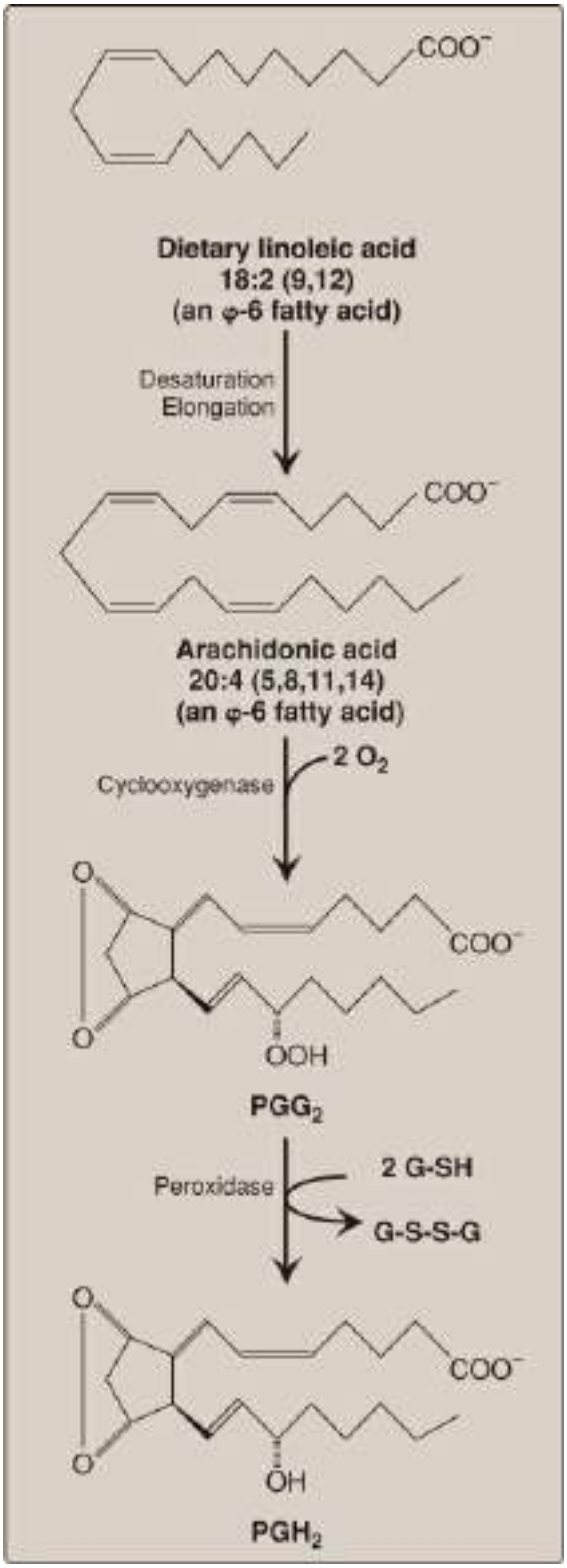


Figure 17.22
Oxidation and cyclization of arachidonic acid by the two catalytic activities (cyclooxygenase and peroxidase) of PGH_2 synthase (prostaglandin endoperoxide synthase). G-SH = reduced glutathione; G-S-S-G = oxidized glutathione; PG = prostaglandin.

C. Leukotriene synthesis

Arachidonic acid is converted to a variety of linear hydroperoxy (–OOH) acids by a separate pathway involving a family of lipoxygenases (LOXs). For example, 5-LOX converts arachidonic acid to 5-hydroperoxy-6,8,11,14 eicosatetraenoic acid ([5-HPETE]; see Fig. 17.23). 5-HPETE is converted to a series of LTs containing four double bonds, the nature of the final products varying according to the tissue. LTs are mediators of allergic response and inflammation. Inhibitors of 5-LOX and LT-receptor antagonists are used in the treatment of asthma. (Note: LT synthesis is inhibited by cortisol and not by NSAIDs. Aspirin-exacerbated respiratory disease is a response to LT overproduction with NSAID use in ~10% of individuals with asthma.)

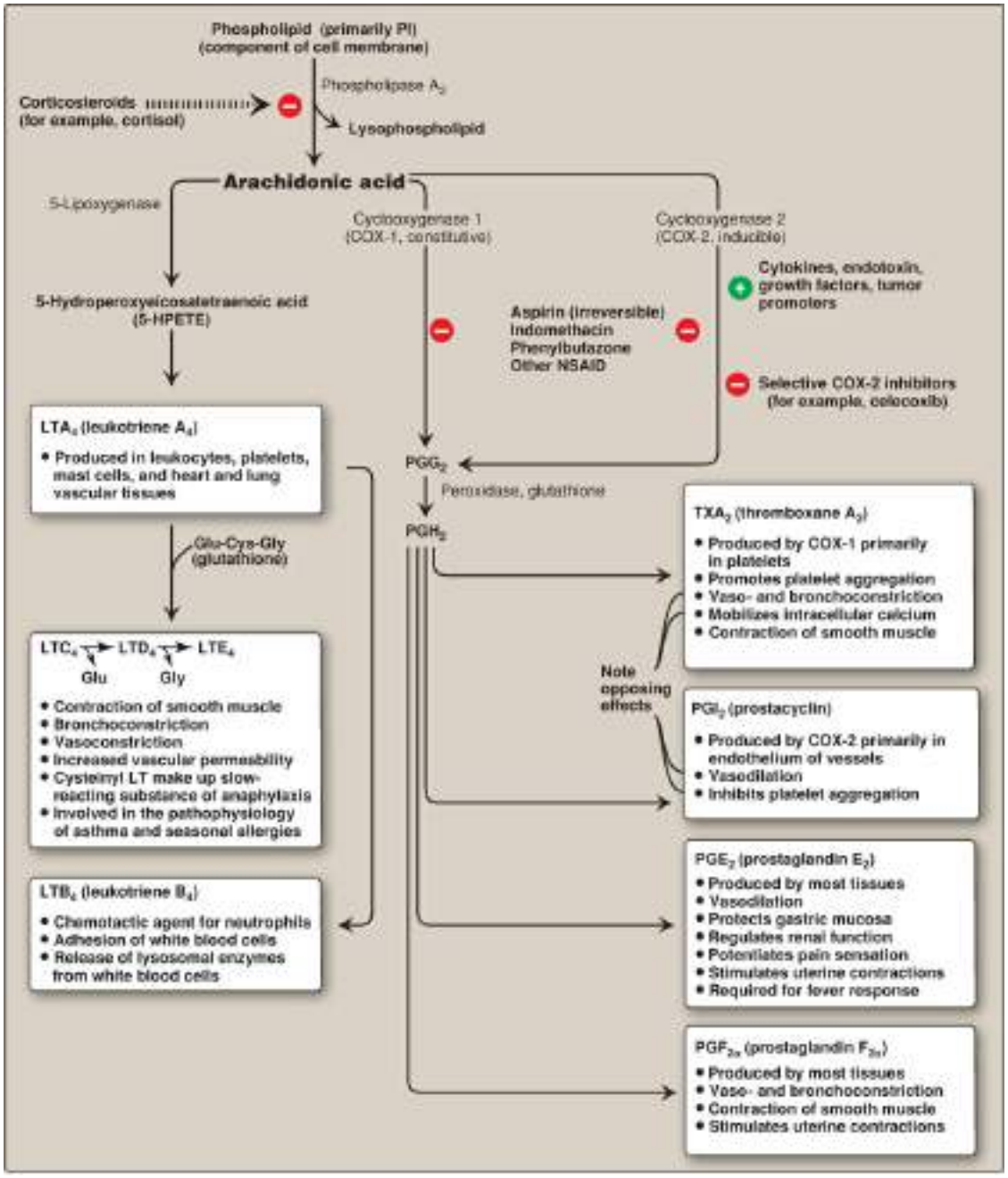


Figure 17.23
Overview of the biosynthesis and function of some important prostaglandins (PGs), leukotrienes (LT), and a thromboxane (TX) from arachidonic acid. (Note: The arachidonic acid in the membrane phospholipid was derived from the ω-6 essential fatty acid [FA], linoleic, also an ω-6 FA.) PI = phosphatidylinositol; NSAID = nonsteroidal anti-inflammatory drugs; Glu = glutamate; Cys = cysteine; Gly = glycine.

IX. Chapter Summary

- **Phospholipids** are polar, ionic compounds composed of an **alcohol** (e.g., **choline** or **ethanolamine**) attached by a phosphodiester bond either to **DAG**, producing **phosphatidylcholine** or **phosphatidylethanolamine**, or to the amino alcohol **sphingosine** (Fig. 17.25).
- Addition of a long-chain fatty acid to sphingosine produces a **ceramide**.
- Addition of **phosphorylcholine** produces the phospholipid **sphingomyelin**.
- Phospholipids are the predominant lipids of **cell membranes**.
- Nonmembrane phospholipids serve as components of **lung surfactant** and **bile**.
- **Dipalmitoylphosphatidylcholine**, also called **dipalmitoyl lecithin**, is the major lipid component of **lung surfactant**.
- Insufficient surfactant production causes **RDS**.
- **PI** serves as a reservoir for **arachidonic acid** in membranes.
- The phosphorylation of membrane-bound PI produces **PIP_2**. This compound is degraded by **phospholipase C** in response to the binding of various neurotransmitters, hormones, and growth factors to membrane **GPCRs**.
- The products of **phospholipase C**, **IP_3**, and **DAG,** mediate the mobilization of intracellular **calcium** and the activation of **protein kinase C**, which act synergistically to evoke cellular responses.
- Specific proteins can be covalently attached via a carbohydrate bridge to membrane-bound PI, forming a **GPIanchor**. A deficiency in GPI synthesis in hematopoietic cells results in the hemolytic disease **paroxysmal nocturnal hemoglobinuria**.
- The degradation of phosphoglycerides is performed by **phospholipases** found in all tissues and pancreatic juice.
- **Sphingomyelin** is degraded to a ceramide plus phosphorylcholine by the lysosomal enzyme **sphingomyelinase**, a deficiency of which causes **Niemann–Pick** (**A and B**) **disease**.
- **Glycosphingolipids** are derivatives of **ceramides** to which carbohydrates have been attached. Adding one sugar molecule to the ceramide produces a **cerebroside**, adding an oligosaccharide produces a **globoside**, and adding an acidic **NANA** molecule produces a **ganglioside**.
- Glycosphingolipids are found predominantly in cell membranes of the **brain** and **peripheral nervous tissue**, with high concentrations in the **myelin sheath**. They are **antigenic**. Glycolipids are degraded in the **lysosomes** by **acid hydrolases**. A deficiency of any one of these enzymes causes a **sphingolipidosis**, in which a characteristic sphingolipid accumulates.
- **PGs**, **TXs**, and **LTs**, the **eicosanoids**, are produced in very small amounts in almost all tissues, act locally, and have an extremely short half-life.
- **Eicosanoids** serve as mediators of the **inflammatory response**. **Arachidonic acid** is the immediate precursor of the predominant class of human PGs (those with two double bonds). It is derived by the elongation and desaturation of the essential fatty acid **linoleic acid** and is stored in the membrane as a component of a phospholipid, generally PI.
- Arachidonic acid is released from the phospholipid by **phospholipase A_2** (inhibited by **cortisol**).
- Synthesis of the **PG** and **TX** begins with the oxidative cyclization of free arachidonic acid to yield PGH_2 by **PGH_2 synthase** (or, **prostaglandin endoperoxide synthase**), an endoplasmic reticular membrane protein that has two catalytic activities: **fatty acid COX** and **peroxidase**.
- There are two isozymes of PGH_2 synthase: **COX-1** (constitutive) and **COX-2** (inducible).
- **Aspirin** irreversibly inhibits both **COX-1** and **COX-2**. Opposing effects of PGI_2 and TXA_2 limit clot formation.
- **LTs** are linear molecules produced from arachidonic acid by the **5-LOX** pathway. They mediate allergic response. Their synthesis is inhibited by cortisol and not by aspirin.

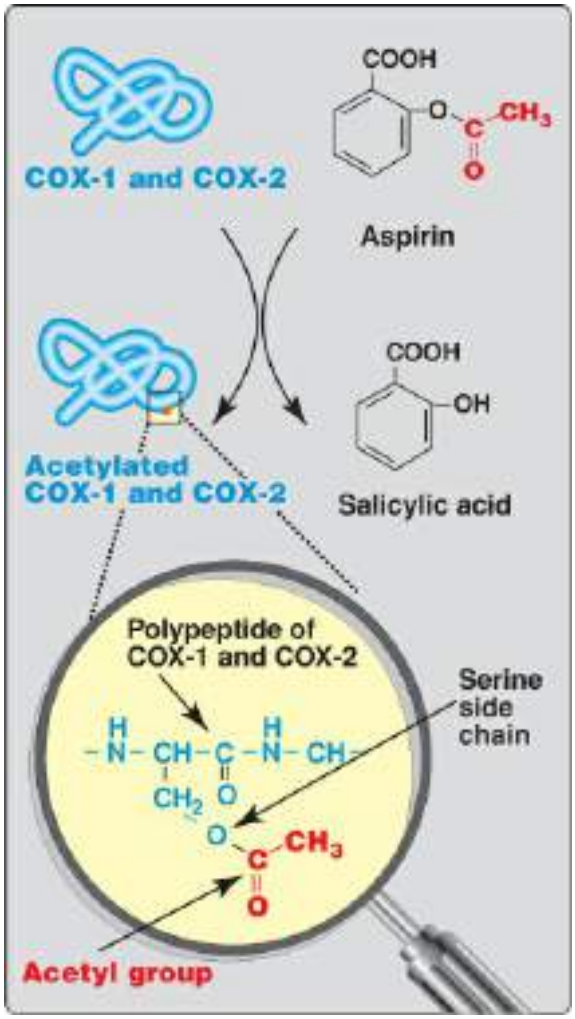


Figure 17.24
Irreversible acetylation of cyclooxygenase (COX)-1 and COX-2 by aspirin.

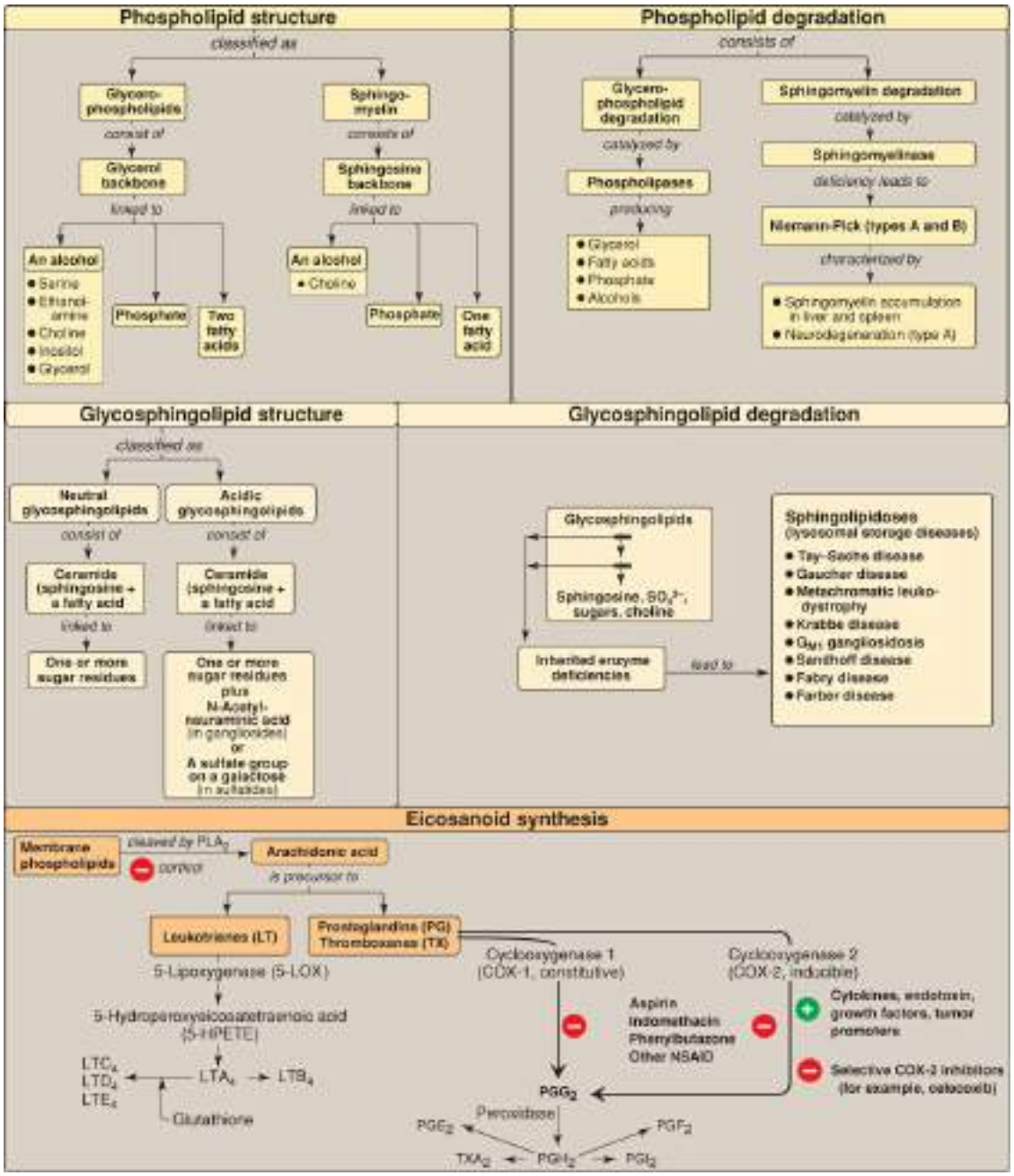


Figure 17.25
Key concept map for phospholipids, glycosphingolipids, and eicosanoids. PLA_2 = phospholipase A_2; SO_4^{2-} = sulfate ion; NSAID = nonsteroidal anti-inflammatory drugs.

Study Questions

Choose the ONE best answer.

17.1 Aspirin-exacerbated respiratory disease (AERD) is a severe reaction to nonsteroidal anti-inflammatory drugs (NSAIDs) characterized by bronchoconstriction 30 minutes to several hours after ingestion. Which of the following statements about NSAIDs best explains the symptoms seen in patients with AERD?

A. Inhibition of the activity of the cystic fibrosis transmembrane conductance regulator protein, resulting in thickened mucus that block airways.
B. Inhibition of cyclooxygenase but not lipoxygenase, resulting in the flow of arachidonic acid to leukotriene synthesis.
C. Activation of the cyclooxygenase activity of prostaglandin H_2 synthase, resulting in increased synthesis of prostaglandins that promote vasodilation.
D. Activation phospholipases, resulting in decreased amounts of dipalmitoylphosphatidylcholine and alveolar collapse (atelectasis).

Correct answer = B. NSAIDs inhibit cyclooxygenase but not lipoxygenase, so any arachidonic acid available is used for the synthesis of bronchoconstricting leukotrienes. NSAIDs have no effect on the cystic fibrosis (CF) transmembrane conductance regulator protein, defects in which are the cause of CF. Steroids, not NSAIDs, inhibit phospholipase A_2. Cyclooxygenase is inhibited by NSAIDs, not activated. NSAIDs have no effect on phospholipases.

17.2 An infant, born at 28 weeks' gestation, rapidly gave evidence of respiratory distress. Clinical laboratory and imaging results supported the diagnosis of infant respiratory distress syndrome. Which of the following is the most accurate statement about this syndrome?

A. It is unrelated to the baby's premature birth.
B. It is a consequence of too few type II pneumocytes.
C. The lecithin/sphingomyelin ratio in the amniotic fluid is likely to be high (>2).
D. The concentration of dipalmitoylphosphatidylcholine in the amniotic fluid would be expected to be lower than that of a full-term baby.
E. It is an easily treated disorder with low mortality.
F. It is treated by administering surfactant to the mother just before she gives birth.

Correct answer = D. Dipalmitoylphosphatidylcholine (DPPC or, dipalmitoyl lecithin) is the lung surfactant found in mature, healthy lungs. Respiratory distress syndrome (RDS) can occur in lungs that make too little of this compound. If the lecithin/sphingomyelin (L/S) ratio in amniotic fluid is ≥2, a newborn's lungs are considered to be sufficiently mature (premature lungs would be expected to have a ratio <2). The RDS would not be due to too few type II pneumocytes, which would simply be secreting sphingomyelin rather than DPPC at 28 weeks' gestation. The mother is given a glucocorticoid, not surfactant, prior to giving birth (antenatally). Surfactant would be administered to the baby postnatally to reduce surface tension.

17.3 A 10-year-old male was evaluated for burning sensations in his feet and clusters of small, red-purple spots on his skin. Laboratory studies revealed protein in his urine. Enzymatic analysis revealed a deficiency of α-galactosidase, and enzyme replacement therapy was recommended. Which of the following is the most likely working diagnosis?

A. Fabry disease
B. Farber disease
C. Gaucher disease
D. Krabbe disease
E. Niemann–Pick disease

Correct answer = A. Fabry disease, a deficiency of α-galactosidase, is the only X-linked sphingolipidosis. It is characterized by pain in the extremities, a red-purple skin rash (generalized angiokeratomas), and kidney and cardiac complications. Protein in his urine indicates kidney damage. Enzyme replacement therapy is available.

17.4 A 5-year-old child is brought to the pediatrician by his mother due to abdominal distention and pain in his leg. The mother states that her son started having difficulty walking and began to fall repeatedly. Physical examination shows developmental delay and hepatosplenomegaly. Fundoscopic examination shows cherry-red spots in the macula. Which of the following histologic finding of the affected tissue is most likely to confirm the diagnosis?

A. Shell-like inclusion bodies in neuronal cells

B. Crumpled tissue paper appearance of the cytosol
C. Foamy macrophages in the bone marrow
D. Globoid bodies in macrophages

Correct answer = C. Niemann–Pick disease type B is the most likely diagnosis due to the presence of hepatomegaly, neurologic defects leading to falls and cherry-red areas in the macula. The histologic finding is the foamy appearance of macrophages of the reticuloendothelial system because of sphingomyelin accumulation.

17.5 Current medical advice for individuals experiencing chest pain is to call emergency medical services and chew a regular strength, noncoated aspirin. What is the basis for recommending aspirin?

Aspirin has an antithrombogenic effect: It prevents formation of blood clots that could occlude heart vessels. Aspirin inhibits thromboxane A_2 synthesis by cyclooxygenase-1 in platelets through irreversible acetylation, thereby inhibiting platelet activation and vasoconstriction. Chewing a noncoated aspirin increases the rate of its absorption.

Cholesterol, Lipoprotein, and Steroid Metabolism 18

I. OVERVIEW

Cholesterol, the major steroid alcohol in animals, performs a number of essential functions in the body. For example, cholesterol is a structural component of all cell membranes, modulating their fluidity, and, in specialized tissues, cholesterol is a precursor of bile acids, steroid hormones, and vitamin D. Therefore, it is critically important that the cells of the body be assured an appropriate supply of cholesterol. To meet this need, a complex series of transport, biosynthetic, and regulatory mechanisms has evolved. The liver plays a central role in the regulation of the body's cholesterol homeostasis. For example, cholesterol enters the hepatic cholesterol pool from a number of sources including dietary cholesterol as well as cholesterol synthesized *de novo* by extrahepatic tissues and by the liver itself. Cholesterol is eliminated from the liver as unmodified cholesterol in the bile, or it can be converted to bile salts that are secreted into the intestinal lumen. It can also serve as a component of plasma lipoproteins that carry lipids to the peripheral tissues. In humans, the balance between cholesterol influx and efflux is not precise, resulting in a gradual deposition of cholesterol in the tissues, particularly in the endothelial linings of blood vessels. This is a potentially life-threatening occurrence when the lipid deposition leads to plaque formation, causing the narrowing of blood vessels (atherosclerosis) and increased risk of cardio-, cerebro-, and peripheral vascular disease. Figure 18.1 summarizes the major sources of liver cholesterol and the routes by which cholesterol leaves the liver.

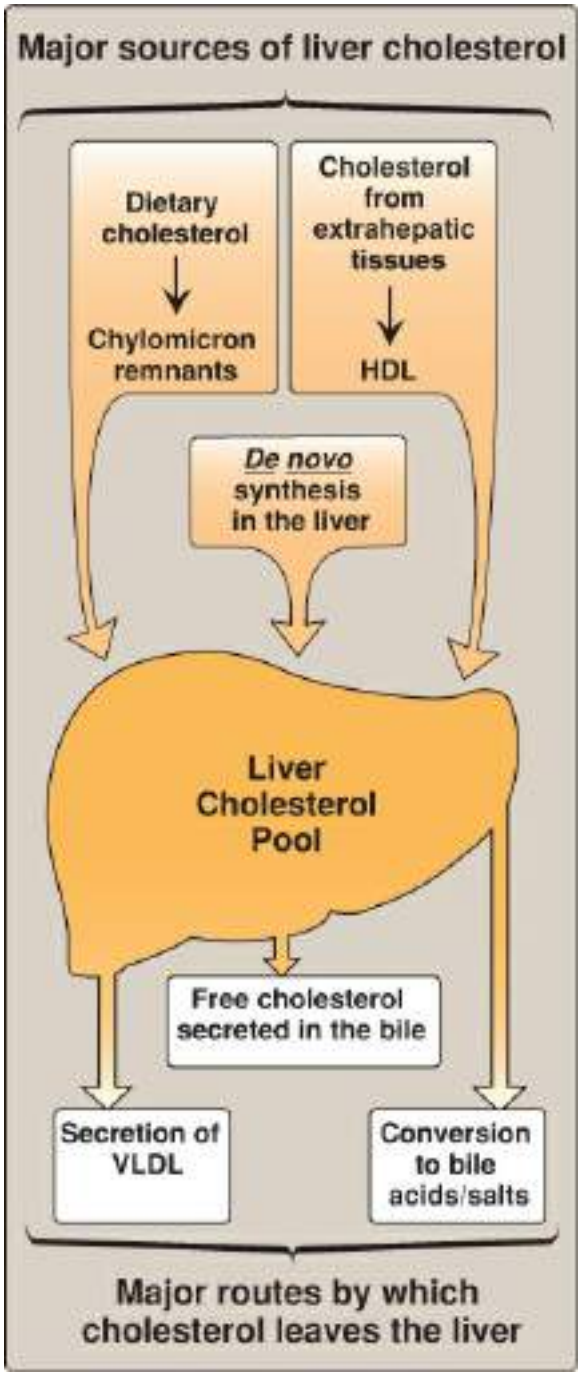


Figure 18.1
Sources of liver cholesterol (influx) and routes by which cholesterol leaves the liver (efflux). HDLs and VLDLs = high- and very–low-density lipoproteins.

II. CHOLESTEROL STRUCTURE

Cholesterol is a very hydrophobic compound. It consists of four fused hydrocarbon rings (A–D) called the steroid nucleus, and it has an eight-carbon, branched hydrocarbon chain attached to carbon 17 of the D ring. Ring A has a hydroxyl group at carbon 3, and

ring B has a double bond between carbon 5 and carbon 6 (Fig. 18.2).

A. Sterols

Steroids with 8 to 10 carbon atoms in the side chain at carbon 17 and a hydroxyl group at carbon 3 are classified as sterols. Cholesterol is the major sterol in animal tissues. It arises from *de novo* synthesis and absorption of dietary cholesterol. Intestinal uptake of cholesterol is mediated by the Niemann–Pick C1-like 1 protein, the target of the drug ezetimibe that reduces absorption of dietary cholesterol (see Chapter 15). (Note: Plant sterols [phytosterols], such as β-sitosterol, are poorly absorbed by humans [5% absorbed as compared to 40% for cholesterol]. After entering the enterocytes, they are actively transported back into the intestinal lumen. Defects in the efflux transporter [ABCG5/8] result in the rare condition of sitosterolemia in which plant sterols accumulate in the blood and tissues reducing blood flow and increasing the risk of a heart attack, stroke, or sudden death. Because some cholesterol is transported back as well, plant sterols reduce the absorption of dietary cholesterol. Daily ingestion of plant sterol esters supplied, e.g., in spreads, is one of a number of dietary strategies to reduce plasma cholesterol levels [see Chapter 27].)

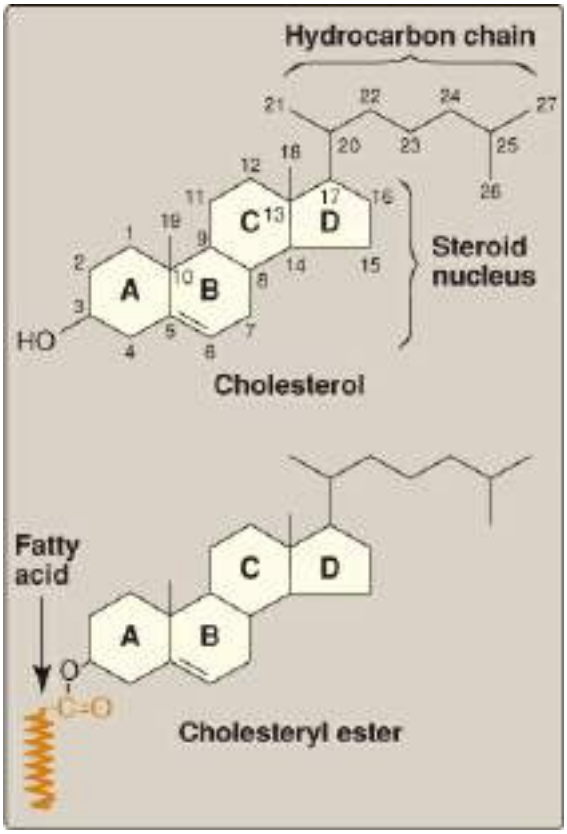


Figure 18.2
Structure of cholesterol and its ester.

B. Cholesteryl esters

Most plasma cholesterol is in an esterified form (with a fatty acid [FA] attached at carbon 3, as shown in Fig. 18.2), which makes the structure even more hydrophobic than free (nonesterified) cholesterol. Cholesteryl esters are not found in membranes and are normally present only in low levels in most cells. Because of their hydrophobicity, cholesterol and its esters must be transported in association with protein as a component of a lipoprotein particle or be solubilized by phospholipids and bile salts in the bile.

III. CHOLESTEROL SYNTHESIS

Cholesterol is synthesized by virtually all tissues in humans, although liver, intestine, adrenal cortex, and reproductive tissues, including ovaries, testes, and placenta, make the largest contributions to the cholesterol pool. As with FA, all the carbon atoms in cholesterol are provided by acetyl coenzyme A (CoA), and nicotinamide adenine dinucleotide phosphate (NADPH) provides the reducing equivalents. The pathway is endergonic, being driven by hydrolysis of the high-energy thioester bond of acetyl CoA and the terminal phosphate bond of ATP. Synthesis requires enzymes in the cytosol, the membrane of the smooth endoplasmic reticulum (SER), and the peroxisome. The pathway is responsive to changes in cholesterol concentration, and regulatory mechanisms exist to balance the rate of cholesterol synthesis against the rate of cholesterol excretion. An imbalance in this regulation can lead to an elevation in circulating levels of plasma cholesterol, with the potential for vascular disease.

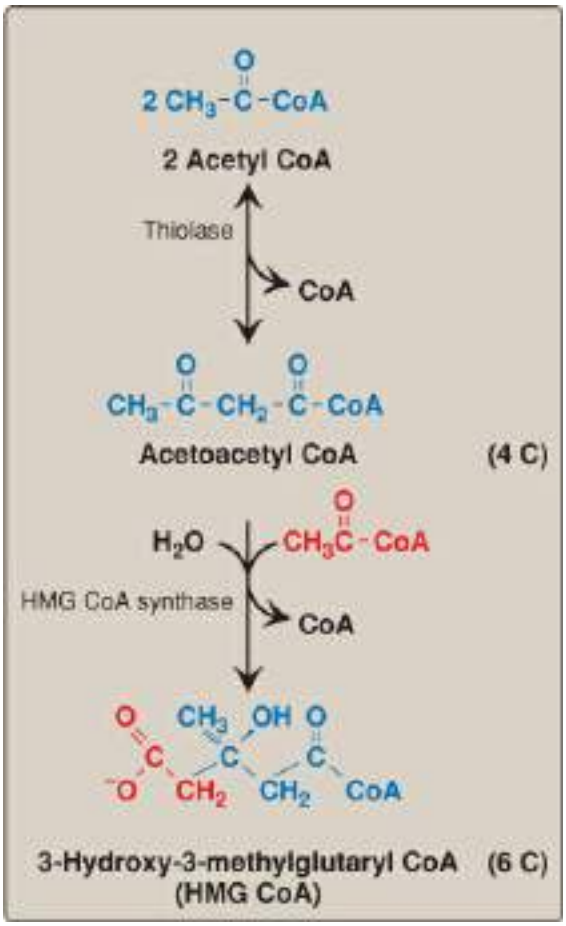


Figure 18.3
Synthesis of HMG CoA. CoA = coenzyme A.

A. 3-Hydroxy-3-methylglutaryl coenzyme A synthesis

The first two reactions in the cholesterol biosynthetic pathway are similar to those in the pathway that produces ketone bodies (see Fig. 16.22). They result in the production of 3-hydroxy-3-methylglutaryl CoA ([HMG CoA], Fig. 18.3). First, two acetyl CoA molecules condense to form acetoacetyl CoA. Next, a third molecule of acetyl CoA is added by HMG CoA synthase, producing HMG CoA, a six-carbon compound. (Note: Liver parenchymal cells contain two isoenzymes of the synthase. The cytosolic enzyme participates in cholesterol synthesis, whereas the mitochondrial enzyme functions in the pathway for ketone body synthesis.)

B. Mevalonate synthesis

HMG CoA is reduced to mevalonate by HMG CoA reductase. This is the rate-limiting and key regulated step in cholesterol synthesis. It occurs in the cytosol, uses two molecules of NADPH as the reducing agent, and releases CoA, making the reaction irreversible (Fig. 18.4). (Note: HMG CoA reductase is an integral membrane protein of the SER, with its catalytic domain projecting into the cytosol.

Regulation of reductase activity is discussed in D. below.)

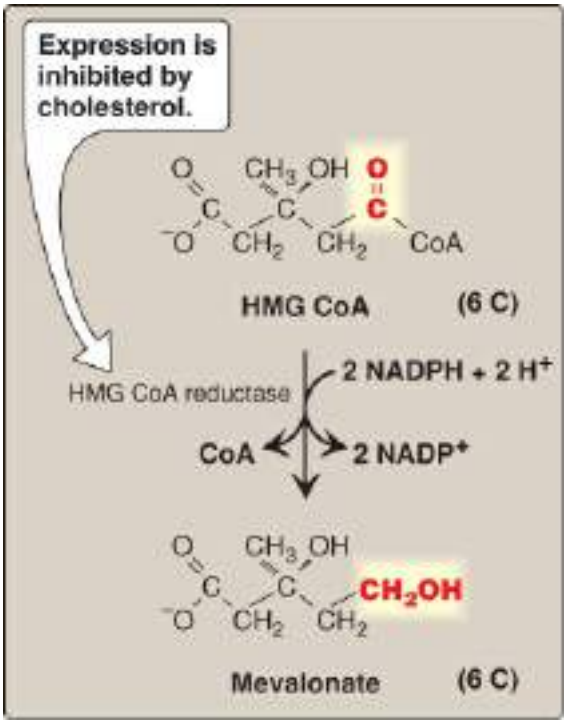


Figure 18.4
Synthesis of mevalonate. HMG CoA = hydroxymethylglutaryl coenzyme A; NADP(H) = nicotinamide adenine dinucleotide phosphate.

C. Cholesterol synthesis from mevalonate

The reactions and enzymes involved in the synthesis of cholesterol from mevalonate are illustrated in Figure 18.5. (Note: The numbers shown in brackets below correspond to numbered reactions shown in this figure.)

[1] Mevalonate is converted to 5-pyrophosphomevalonate in two steps, each of which transfers a phosphate group from ATP.

[2] A five-carbon isoprene unit, isopentenyl pyrophosphate (IPP), is formed by the decarboxylation of 5-pyrophosphomevalonate. The reaction requires ATP. (Note: IPP is the precursor of a family of molecules with diverse functions, the isoprenoids. Cholesterol is a sterol isoprenoid. Nonsterolisoprenoids include dolichol and ubiquinone [or, coenzyme Q].)

[3] IPP is isomerized to 3,3-dimethylallyl pyrophosphate (DPP).

[4] IPP and DPP condense to form 10-carbon geranyl pyrophosphate (GPP).

[5] A second molecule of IPP then condenses with GPP to form 15-carbon farnesyl pyrophosphate (FPP). (Note: Covalent attachment of farnesyl to proteins, a process known as prenylation, is one mechanism for anchoring proteins [e.g., ras] to the inner face of plasma membranes.)

[6] Two molecules of FPP combine, releasing pyrophosphate, and are reduced,

forming the 30-carbon compound squalene. (Note: Squalene is formed from six isoprenoid units. Because 3 ATP are hydrolyzed per mevalonate residue converted to IPP, a total of 18 ATP are required to make the polyisoprenoid squalene.)

[7] Squalene is converted to the sterol lanosterol by a sequence of two reactions catalyzed by SER-associated enzymes that use molecular oxygen (O_2) and NADPH. The hydroxylation of linear squalene triggers the cyclization of the structure to lanosterol.

[8] The conversion of lanosterol to cholesterol is a multistep process involving shortening of the side chain, oxidative removal of methyl groups, reduction of double bonds, and migration of a double bond. Smith–Lemli–Opitz syndrome (SLOS), an autosomal-recessive disorder of cholesterol biosynthesis, is caused by a partial deficiency in 7-dehydrocholesterol-7-reductase, the enzyme that reduces the double bond in 7-dehydrocholesterol (7-DHC), thereby converting it to cholesterol. SLOS is one of several multisystem, embryonic malformation syndromes associated with impaired cholesterol synthesis. (Note: 7-DHC is converted to vitamin D_3 in the skin [see Chapter 28].)

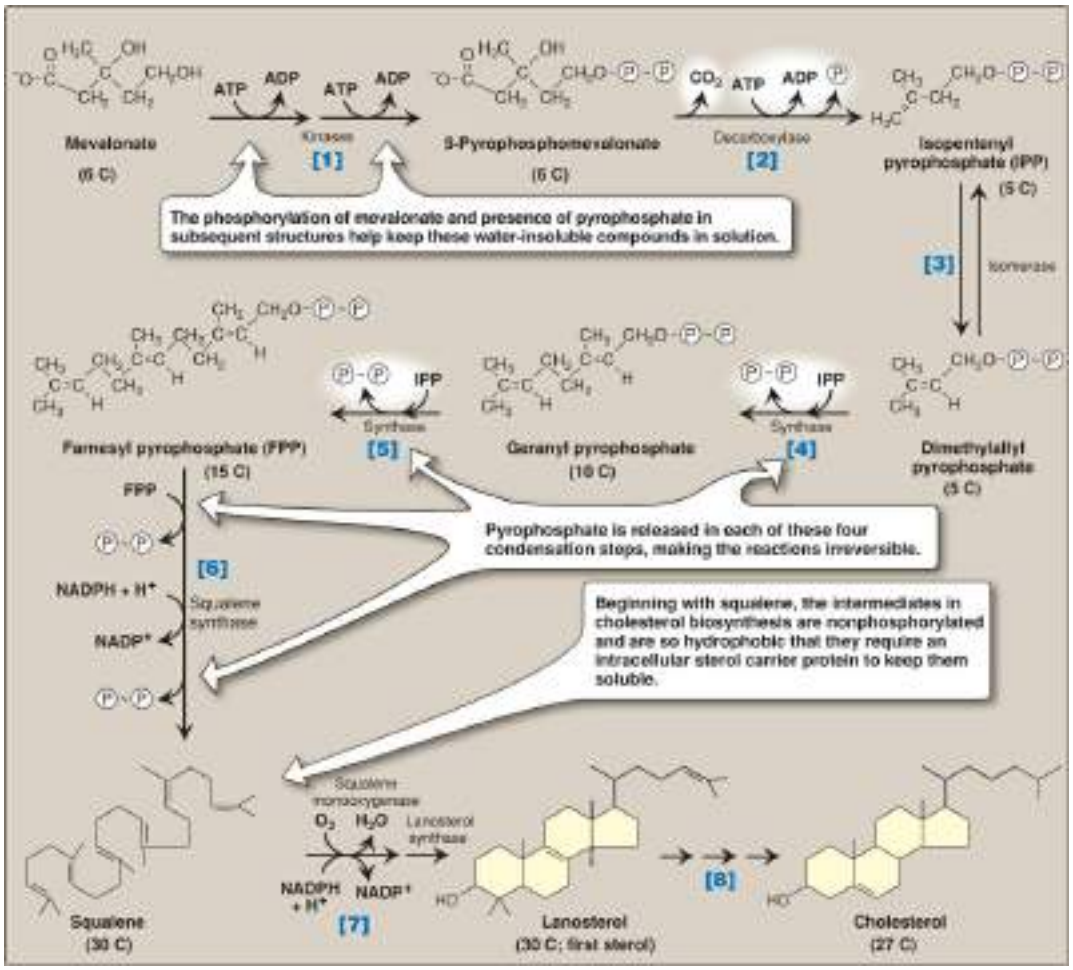


Figure 18.5
Synthesis of cholesterol from mevalonate. ADP = adenosine diphosphate; Ⓟ = phosphate; Ⓟ ~ Ⓟ = pyrophosphate; NADP(H) = nicotinamide adenine dinucleotide phosphate.

D. Branch-point reactions in the biosynthesis of cholesterol

The intermediates of cholesterol synthesis are shunted for modification of other molecules. The first branch point starts with step 2 above, the synthesis of IPP **(5C)** (Fig. 18.6). Subsequent addition of 5-carbon isoprene units results in the synthesis of geranyl-PP **(10C)**, farnesyl-PP **(15C),** and geranylgeranyl-PP **(20C)**, respectively**.** These molecules can modify proteins so that they can be anchored into the membrane lipids. Farnesylation of heme creates heme A, a specialized heme in cytochrome *a* of the electron transport chain. Farnesylation and geranylgeranylation of proteins such as ras oncogene can lead to activation of cellular signaling pathways for proliferation. Geranylgeranylation also produces dolichol, which is important for sugar transfer during glycoprotein synthesis, and ubiquinone, a lipid-soluble electron carrier in oxidative phosphorylation.

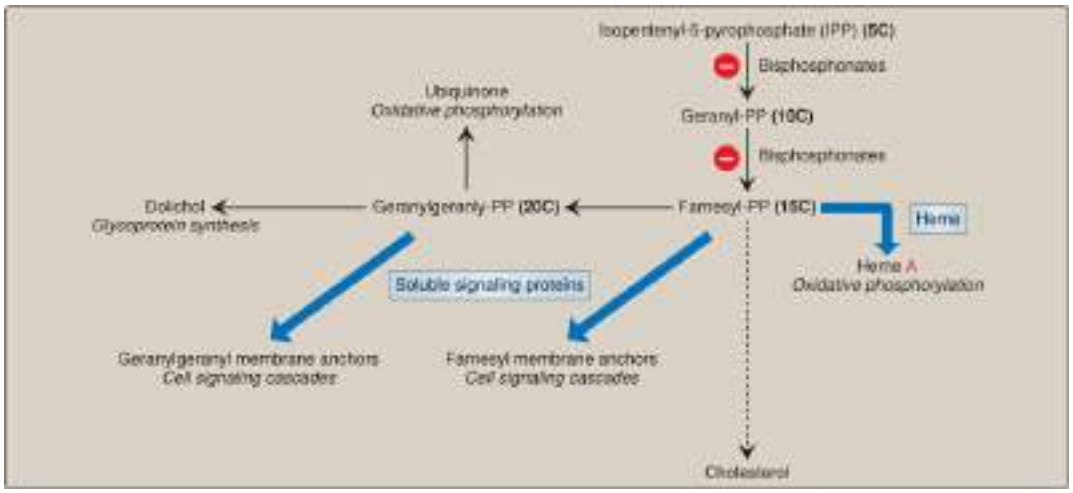


Figure 18.6
Branch-point reactions in the biosynthesis of cholesterol. The *thin arrows* indicate an enzymatic or chemical conversion to a product. *Thick arrows* indicate protein modifications. The italics below the protein modifications indicate the processes that the products are involved in. PP = pyrophosphate.

Biphosphonates are used to inhibit bone resorption in osteoporosis and Paget disease. The new generation of bisphosphonates has been shown to kill cancer cells by inhibiting the synthesis of farnesyl-PP and geranylgeranyl-PP.

E. Cholesterol synthesis regulation

HMG CoA reductase is the major control point for cholesterol biosynthesis and is subject to different kinds of metabolic control.

1. Sterol-dependent regulation of gene expression: Expression of the gene for HMG CoA reductase is controlled by the transacting factor, sterol regulatory

element–binding protein-2 (SREBP-2), which binds DNA at the cis-acting sterol regulatory element (SRE) upstream of the *reductase* gene. Inactive SREBP-2 is an integral protein of the SER membrane and associates with a second SER membrane protein, SREBP cleavage–activating protein (SCAP). When sterol levels in the SER are low, the SREBP-2–SCAP complex moves from the ER to the Golgi. In the Golgi membrane, SREBP-2 is sequentially acted upon by two proteases, which generate a soluble fragment that enters the nucleus, binds the SRE, and functions as a transcription factor. This results in increased synthesis of HMG CoA reductase and, therefore, increased cholesterol synthesis (Fig. 18.7). However, if sterols are abundant, they bind SCAP at its sterol-sensing domain and induce the binding of SCAP to yet other ER membrane proteins, the insulin-induced gene proteins (INSIGs). This results in the retention of the SCAP–SREBP complex in the SER, thereby preventing the activation of SREBP-2 and leading to downregulation of cholesterol synthesis. (Note: SREBP-1c upregulates expression of enzymes involved in FA synthesis in response to insulin.)

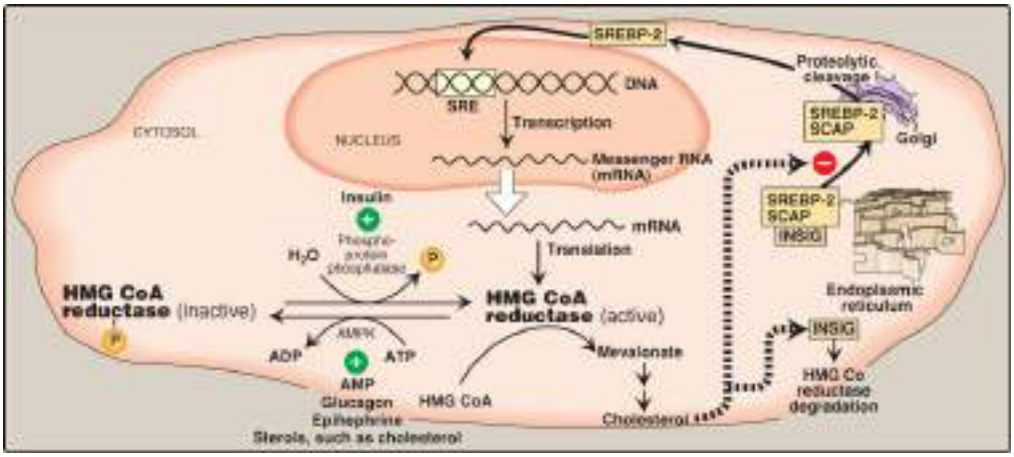


Figure 18.7
Regulation of hydroxymethylglutaryl coenzyme A (HMG CoA) reductase. SRE = sterol regulatory element; SREBP = SRE-binding protein; SCAP = SREBP cleavage–activating protein; AMPK = adenosine monophosphate–activated protein kinase; ADP = adenosine diphosphate; P = phosphate; INSIG = insulin-induced gene protein.

2. Sterol-accelerated enzyme degradation: The reductase itself is a sterol-sensing integral protein of the SER membrane. When sterol levels in the SER are high, the enzyme binds to INSIG proteins (see Fig. 18.7). Binding leads to cytosolic transfer, ubiquitination, and proteasomal degradation of the reductase (see Chapter 19).

3. Sterol-independent phosphorylation/dephosphorylation: HMG CoA reductase activity is controlled covalently through the actions of adenosine monophosphate (AMP)–activated protein kinase (AMPK) and a phosphoprotein phosphatase (see Fig. 18.7). The phosphorylated form of the enzyme is inactive, whereas the dephosphorylated form is active. (Note: Because AMPK is

activated by AMP, cholesterol synthesis, like FA synthesis, is decreased when ATP availability is decreased.)

4. Hormonal regulation: The activity of HMG CoA reductase is controlled hormonally. An increase in insulin favors dephosphorylation (activation) of the reductase, whereas an increase in glucagon and epinephrine and elevated levels of cholesterol has the opposite effect (see Fig. 18.7).

5. Drug inhibition: The statin drugs (atorvastatin, fluvastatin, lovastatin, pravastatin, rosuvastatin, and simvastatin) are structural analogs of HMG CoA and are (or are metabolized to) reversible, competitive inhibitors of HMG CoA reductase (Fig. 18.8). They are used to decrease plasma cholesterol levels in patients with hypercholesterolemia. The best recognized adverse effects of statins include muscle pain, fatigue, weakness, and rhabdomyolysis. These effects may be due to the inhibition of heme A and ubiquinone synthesis, which are both essential for oxidative phosphorylation for energy (Fig. 18.6). Genetic polymorphisms also influence the physiologic response to statins. For example, organic anion transporting polypeptide (OATP1B1, also known as SLCO1B1) has a polymorphism at nucleotide 521 T>C that is used as a biomarker for simvastatin myopathy.

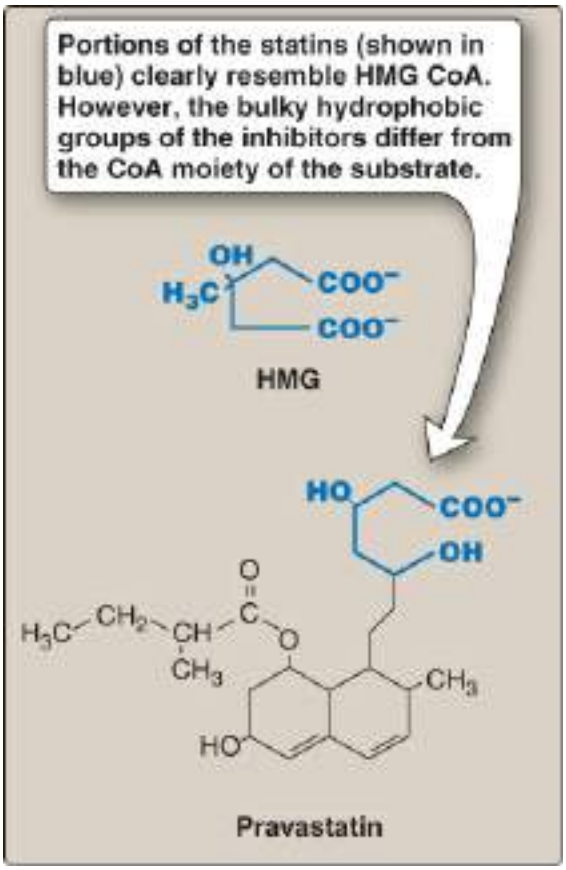


Figure 18.8
Structural similarity of hydroxymethylglutaric acid (HMG) and pravastatin, a clinically useful

cholesterol-lowering drug of the statin family. CoA = coenzyme A.

IV. CHOLESTEROL DEGRADATION

Humans cannot metabolize the cholesterol ring structure to carbon dioxide and water. Rather, the intact steroid nucleus is eliminated from the body by conversion to bile acids and bile salts, a small percentage of which is excreted in the feces, and by secretion of cholesterol into the bile, which transports it to the intestine for elimination. (Note: Some of the cholesterol in the intestine is modified by bacteria before excretion. The primary compounds made are the isomers coprostanol and cholestanol, which are reduced derivatives of cholesterol. Together with cholesterol, these compounds make up the bulk of neutral fecal sterols.)

V. BILE ACIDS AND BILE SALTS

Bile consists of a watery mixture of organic and inorganic compounds. Phosphatidylcholine (PC), or lecithin (see Chapter 17), and conjugated bile salts are quantitatively the most important organic components of bile. Bile can either pass directly from the liver, where it is synthesized, into the duodenum through the common bile duct, or be stored in the gallbladder when not immediately needed for digestion.

A. Structure

The bile acids contain 24 carbons, with two or three hydroxyl groups and a side chain that terminates in a carboxyl group (Fig. 18.9A). The carboxyl group has a pK_a of ~6. In the duodenum (pH~6), this group will be protonated in half of the molecules (the bile acids) and deprotonated in the rest (the bile salts). The terms bile acid and bile salt are frequently used interchangeably, however. Both forms have hydroxyl groups that are α in orientation (they lie below the plane of the rings) and methyl groups that are β (they lie above the plane of the rings). Therefore, the molecules have both a polar and a nonpolar surface and can act as emulsifying agents in the intestine, helping prepare dietary fat (triacylglycerol [TAG]) and other complex lipids for degradation by pancreatic digestive enzymes (Fig. 18.9B).

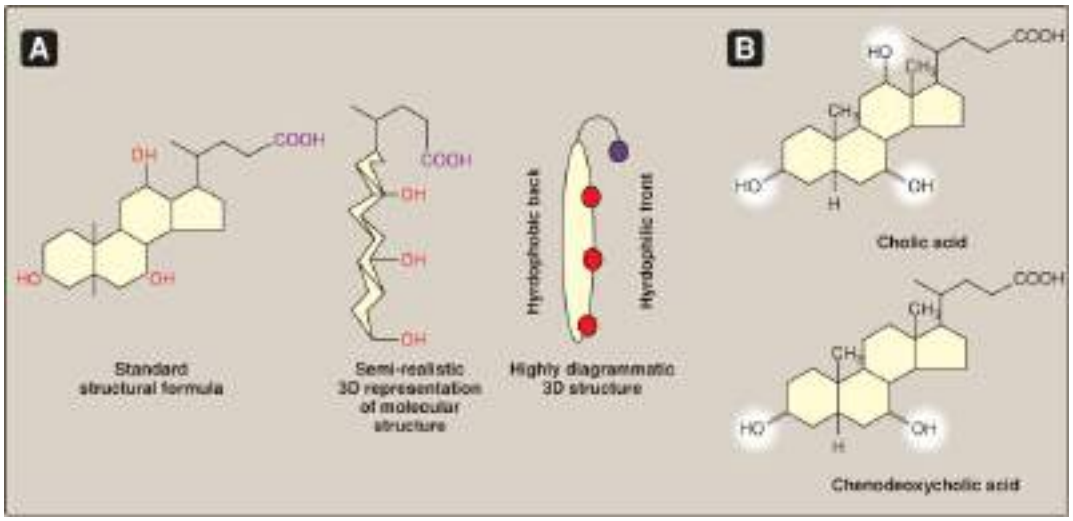


Figure 18.9
A: Structure of bile acids. The hydrophobic (nonpolar) and hydrophilic (polar) surfaces help emulsifying and the digestion of fats in the intestine surfaces. The *oval shape* indicates the cholesterol backbone. The *red balls* indicate the hydroxyl groups. The *purple ball* indicates the carboxyl group. **B:** The most abundant bile acids in human bile: cholic acid and chenodeoxycholic acid.

B. Synthesis

Bile acids are synthesized in the liver by a multistep, multiorganelle pathway in which hydroxyl groups are inserted at specific positions on the steroid structure; the double bond of the cholesterol B ring is reduced; and the hydrocarbon chain is shortened by three carbons, introducing a carboxyl group at the end of the chain. The most common resulting compounds, cholic acid (a triol) and chenodeoxycholic acid (a diol), as shown in Figure 18.9B, are called primary bile acids. The rate-limiting step in bile acid synthesis is the introduction of a hydroxyl group at carbon 7 of the steroid nucleus by 7-α-hydroxylase, an SER-associated cytochrome P450 (CYP) monooxygenase found only in liver. Expression of the enzyme is downregulated by bile acids and cholesterol (Fig. 18.10). Expression of cholesterol-7-alpha hydroxylase is upregulated by cholesterol and downregulated by bile acids. Elevated levels of cholesterol in the liver stimulate the nuclear receptor liver X factor (LXR), which increases the transcription of cholesterol-7-alpha hydroxylase. Elevated levels of bile acids activate another nuclear receptor bile acid receptor (BAR, also known as farnesoid X receptor [FXR]) which downregulates the transcription of cholesterol-7-alpha hydroxylase.

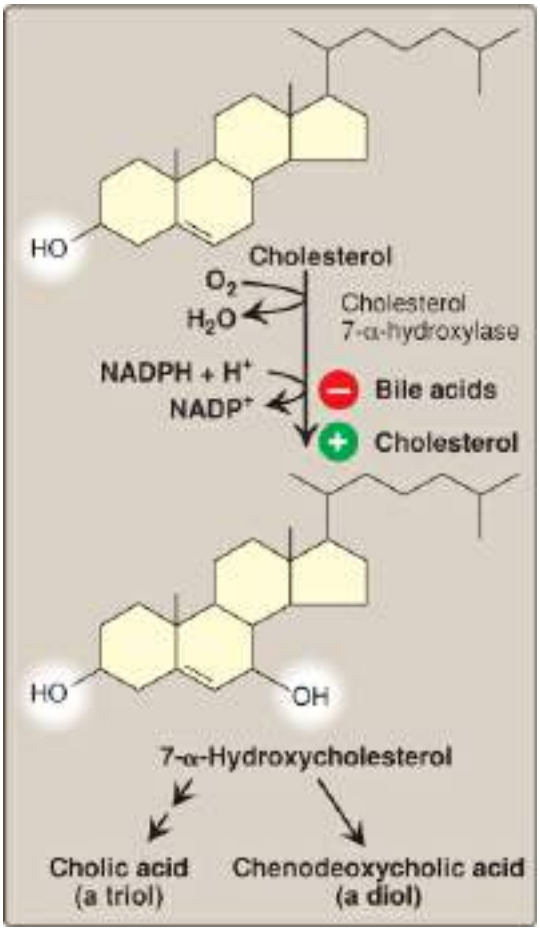


Figure 18.10
Synthesis and regulation of the bile acids, cholic acid and chenodeoxycholic acid from cholesterol.

C. Conjugation

Before the bile acids leave the liver, they are conjugated to a molecule of either glycine or taurine (an end product of cysteine metabolism) by an amide bond between the carboxyl group of the bile acid and the amino group of the added compound. These new structures include glycocholic and glycochenodeoxycholic acids and taurocholic and taurochenodeoxycholic acids (Fig. 18.11). The ratio of glycine to taurine forms in the bile is ~3/1. Addition of glycine or taurine results in the presence of a carboxyl group with a lower pK_a (from glycine) or a sulfonate group (from taurine), both of which are fully ionized (negatively charged) at the alkaline pH of bile and the duodenum. The conjugated, ionized bile salts are more effective detergents than the unconjugated ones because of their enhanced amphipathic nature. Therefore, only the conjugated forms are found in the bile. Individuals with genetic deficiencies in the conversion of cholesterol to bile acids are treated with exogenously supplied chenodeoxycholic acid.

Bile salts provide the only significant mechanism for cholesterol excretion, both as a metabolic

product of cholesterol and as a solubilizer of cholesterol in bile.

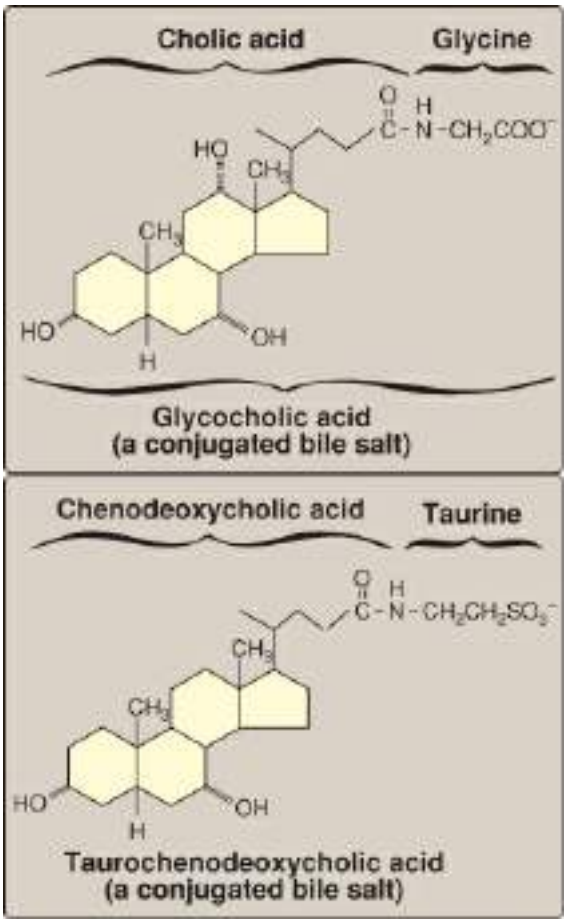


Figure 18.11
Conjugated bile salts. Note “cholic” in the names.

D. Enterohepatic circulation

Bile salts secreted into the intestine are efficiently reabsorbed (>95%) and reused. The liver actively secretes bile salts via the bile salt export pump. In the intestine, they are reabsorbed in the terminal ileum via the apical sodium (Na^+)-bile salt cotransporter and returned to the blood via a separate transport system. (Note: Lithocholic acid is only poorly absorbed.) They are efficiently taken up from the blood by the hepatocytes via an isoform of the cotransporter. (Note: Albumin binds bile salts and transports them through the blood as was seen with FA.) The continuous cycle of bile salt secretion into the bile, passage through the duodenum (where some are deconjugated then dehydroxylated to secondary bile salts), uptake in the ileum, and subsequent return to the liver (as a mixture of primary and secondary forms) is termed the enterohepatic circulation (Fig. 18.12). Between 15 and 30 g of bile salts are secreted from the liver into the duodenum each day, yet only ~0.5 g (<3%) is lost daily in the feces. Approximately 0.5 g/day is synthesized

from cholesterol in the liver to replace the amount lost. Bile acid sequestrants, such as cholestyramine, bind bile salts in the gut and prevent their reabsorption, thereby promoting their excretion. They are used in the treatment of hypercholesterolemia, because the removal of bile salts relieves the inhibition on bile acid synthesis in the liver, thereby diverting additional cholesterol into that pathway. (Note: Dietary fiber also binds bile salts and increases their excretion [see Chapter 27].)

E. Bacterial action on bile salts

After enterohepatic circulation, a small amount of secreted bile salts reaches the colon where the salts are exposed to bacterial modification by the gut microbiome. Bacteria of the intestinal microbiota can deconjugate (remove glycine and taurine) bile salts. They can also dehydroxylate carbon 7, producing secondary bile acids such as deoxycholic acid from cholic acid and lithocholic acid from chenodeoxycholic acid. A small proportion of these secondary bile acids are absorbed by the colonic epithelium and may be conjugated and hydroxylated by the liver enzymes to produce secondary bile salts. The rest are eliminated in the feces.

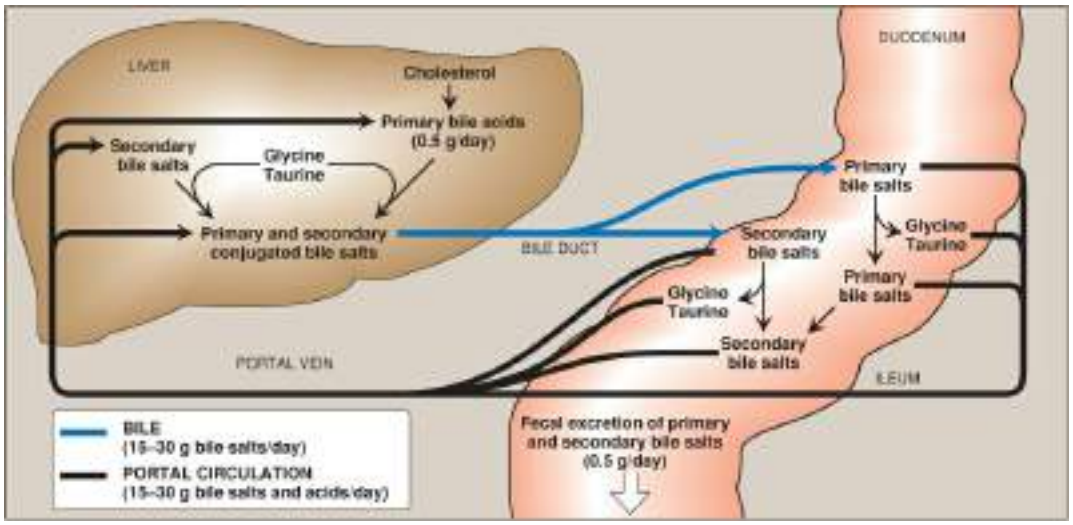


Figure 18.12
Enterohepatic circulation of bile salts. (Note: Ionized bile acids are called bile salts.)

F. Bile salt deficiency: Cholelithiasis

The movement of cholesterol from the liver into the bile must be accompanied by the simultaneous secretion of phospholipid and bile salts. If this dual process is disrupted and more cholesterol is present than can be solubilized by the bile salts and PC present, the cholesterol may precipitate in the gallbladder, leading to cholesterol gallstone disease or cholelithiasis (Fig. 18.13). This disorder is typically caused by a decrease of bile acids in the bile. Cholelithiasis also may result from increased secretion of cholesterol into bile, as seen with the use of fibrates (e.g., gemfibrozil) to reduce cholesterol (and TAG) in the blood. Laparoscopic cholecystectomy (surgical removal of the gallbladder through a small incision) is

currently the treatment of choice. However, for patients who are unable to undergo surgery, oral administration of chenodeoxycholic acid to supplement the body's supply of bile acids results in a gradual (months to years) dissolution of the gallstones. (Note: Cholesterol stones account for >85% of cases of cholelithiasis, with bilirubin and mixed stones accounting for the rest).

Figure 18.13
Gallbladder with gallstones.

VI. PLASMA LIPOPROTEINS

The plasma lipoproteins are spherical macromolecular complexes of lipids and proteins (apolipoproteins). The lipoprotein particles include chylomicrons, chylomicron remnants, very–low-density lipoproteins (VLDLs), VLDL remnants, also known as intermediate-density lipoproteins (IDLs), low-density lipoproteins (LDLs), high-density lipoproteins (HDLs) and lipoprotein (a) (Lp[a]). They differ in lipid and protein composition, size, density (Fig. 18.14), and site of origin. (Note: Because lipoprotein particles constantly interchange lipids and apolipoproteins, the actual apolipoprotein and lipid content of each class of particles is somewhat variable.) Lipoproteins function both to keep their component lipids soluble as they transport them in the plasma and to provide an efficient mechanism for transporting their lipid contents to (and from) the tissues. In humans, there is a gradual deposition of lipid (especially cholesterol) in tissues.

A. Composition

Lipoproteins are composed of a neutral lipid core (containing TAG and cholesteryl esters) surrounded by a shell of amphipathic apolipoproteins, phospholipid, and nonesterified (free) cholesterol (Fig. 18.15). These amphipathic compounds are oriented such that their polar portions are exposed on the surface of the lipoprotein, thereby rendering the particle soluble in aqueous solution. The TAG and cholesterol carried by the lipoproteins are obtained either from the diet (exogenous source) or from *de novo* synthesis (endogenous source). (Note: The cholesterol [C] content of plasma lipoproteins is now routinely measured in fasting blood. Friedewald equation

[LDL-C = Total C – HDL-C − TAG/5] is used to calculate LDL-C once the total C, HDL, and TAG are measured in serum. This formula assumes the TAG/cholesterol ratio in VLDL is 5:1 The goal value for total cholesterol is <200 mg/dl.)

1. Size and density: Chylomicrons are the lipoprotein particles lowest in density and largest in size and that contain the highest percentage of lipid (as TAG) and the lowest percentage of protein. VLDLs and LDLs are successively denser, having higher ratios of protein to lipid. HDL particles are the smallest and densest. Plasma lipoproteins can be separated on the basis of their electrophoretic mobility, as shown in Figure 18.16, or on the basis of their density by ultracentrifugation.

2. Apolipoproteins: The apolipoproteins associated with lipoprotein particles have a number of diverse functions, such as providing recognition sites for cell-surface receptors and serving as activators or coenzymes for enzymes involved in lipoprotein metabolism. Some of the apolipoproteins are required as essential structural components of the particles and cannot be removed (in fact, the particles cannot be produced without them), whereas others are transferred freely between lipoproteins. Apolipoproteins are divided by structure and function into several major classes, denoted by letters, with each class having subclasses (e.g., apolipoprotein [apo] C-I, apo C-II, and apo C-III). (Note: The functions of all the apolipoproteins are not yet known.)

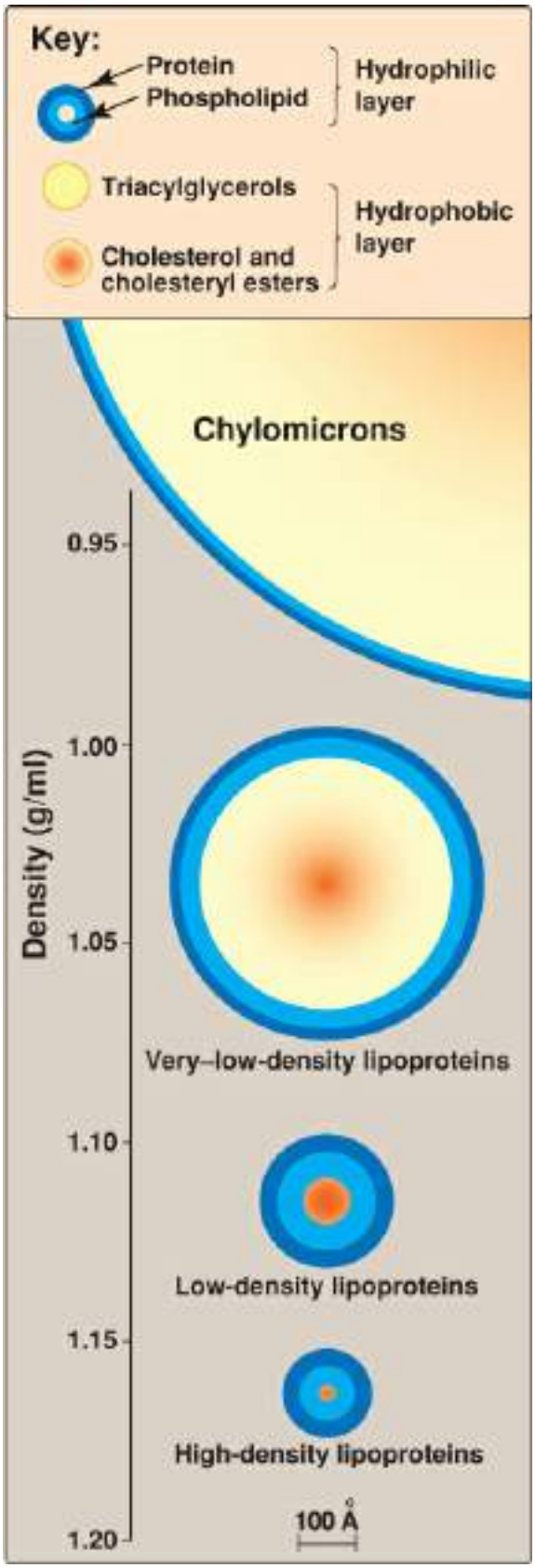


Figure 18.14

Plasma lipoprotein particles exhibit a range of sizes and densities, and typical values are shown. Ring widths approximate the amount of each component. (Note: Although cholesterol and its esters are shown as one component in the center of each particle, physically, cholesterol is on the surface, whereas cholesteryl esters are in the interior.)

B. Chylomicron metabolism

Chylomicrons are assembled in intestinal mucosal cells and carry dietary (exogenous) TAG, cholesterol, fat-soluble vitamins, and cholesteryl esters to the peripheral tissues (Fig. 18.17). (Note: TAGs account for close to 90% of the lipids in a chylomicron.)

1. Apolipoprotein synthesis: Apo B-48 is unique to chylomicrons. Its synthesis begins on the rough ER (RER), and it is glycosylated as it moves through the RER and Golgi. (Note: Apo B-48 is so named because it constitutes the N-terminal 48% of the protein encoded by the gene for apo B. Apo B-100, which is synthesized by the liver and found in VLDL and LDL, represents the entire protein encoded by this gene. Posttranscriptional editing [see Chapter 33] of a cytosine to a uracil in intestinal apo B-100 messenger RNA [mRNA] creates a nonsense [stop] codon [see Chapter 33], allowing translation of only 48% of the mRNA.)

2. Chylomicron assembly: Many enzymes involved in TAG, cholesterol, and phospholipid synthesis are located in the SER. Assembly of the apolipoprotein and lipid into chylomicrons requires microsomal triglyceride transfer protein (MTP), which loads apo B-48 with lipid. This occurs before transition from the ER to the Golgi, where the particles are packaged in secretory vesicles. These fuse with the plasma membrane releasing the lipoproteins, which then enter the lymphatic system and, ultimately, the blood. (Note: Chylomicrons leave the lymphatic system via the thoracic duct that empties into the left subclavian vein.)

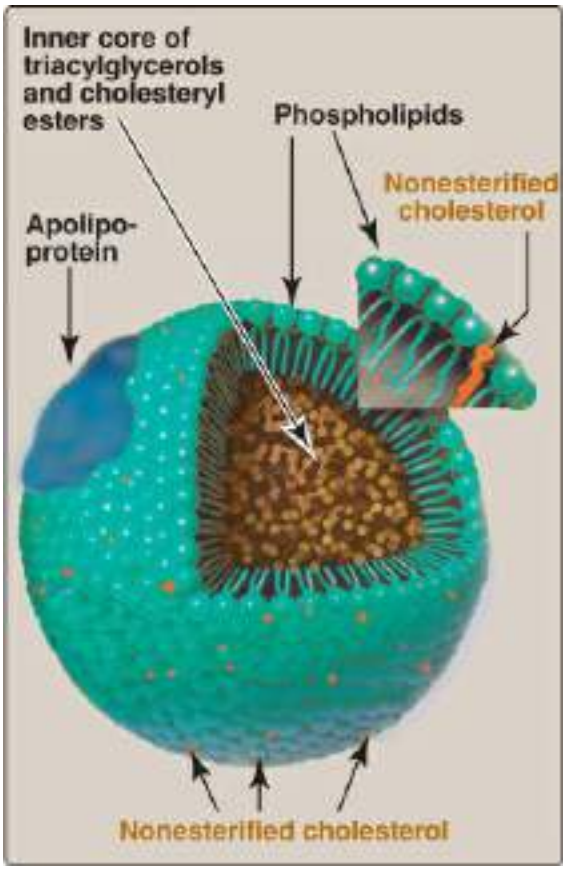


Figure 18.15
Structure of a typical lipoprotein particle.

3. Nascent chylomicron modification: The particle released by the intestinal mucosal cell is called a nascent chylomicron because it is functionally incomplete. When it reaches the plasma, the particle is rapidly modified, receiving apo E (which is recognized by hepatic receptors) and apo C. The latter includes apo C-II, which is necessary for the activation of lipoprotein lipase (LPL), the enzyme that degrades the TAG contained in the chylomicron. The source of these apolipoproteins is circulating HDL (see Fig. 18.17). (Note: Apo C-III on TAG-rich lipoproteins inhibits LPL.)

4. Triacylglycerol degradation by lipoprotein lipase: LPL is an extracellular enzyme that is anchored to the capillary walls of most tissues but predominantly those of adipose tissue and cardiac and skeletal muscle. The adult liver does not express this enzyme. (Note: A hepatic lipase is found on the surface of endothelial cells of the liver. It plays a role in TAG degradation in chylomicrons and VLDL and is important in HDL metabolism.) LPL, activated by apo C-II on circulating chylomicrons, hydrolyzes the TAG in these particles to FA and glycerol. The FA are stored (in adipose) or used for energy (in muscle). The glycerol is taken up by the liver, converted to dihydroxyacetone phosphate (an intermediate of glycolysis), and used in lipid synthesis or gluconeogenesis.

(Note: Patients with a deficiency of LPL or apo C-II [type I hyperlipoproteinemia or familial chylomicronemia] show a dramatic accumulation [≥1,000 mg/dl] of chylomicron-TAG in the plasma [hypertriacylglycerolemia] even in the fasted state. They are at increased risk for acute pancreatitis. Treatment is the reduction of dietary fat.)

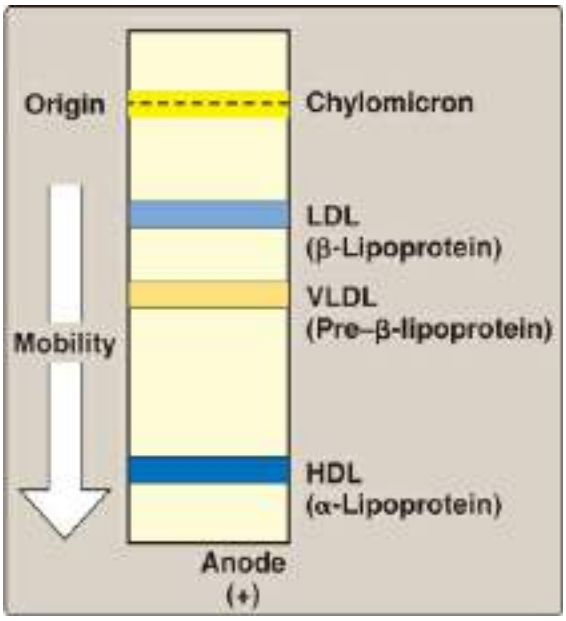


Figure 18.16
Electrophoretic mobility of plasma lipoprotein particles. (Note: The order of low-density lipoprotein [LDL] and very–low-density lipoprotein [VLDL] is reversed if ultracentrifugation is used as the separation technique.) HDL = high-density lipoprotein.

5. Lipoprotein lipase expression: LPL is synthesized by adipose tissue and by cardiac and skeletal muscle. Expression of the tissue-specific isozymes is regulated by nutritional state and hormonal level. For example, in the fed state (elevated insulin levels), LPL synthesis is increased in adipose but decreased in muscle tissue. Fasting (decreased insulin) favors LPL synthesis in muscle. (Note: The highest concentration of LPL is in cardiac muscle, reflecting the use of FA to provide much of the energy needed for cardiac function.)

6. Chylomicron remnant formation: As the chylomicron circulates, and >90% of the TAG in its core is degraded by LPL, the particle decreases in size and increases in density. In addition, the C apolipoproteins (but not apo B or E) are returned to HDL. The remaining particle, called a remnant, is rapidly removed from the circulation by the liver, whose cell membranes contain lipoprotein receptors that recognize apo E (see Fig. 18.17). Chylomicron remnants bind to these receptors and are taken into the hepatocytes by endocytosis. The endocytosed vesicle then fuses with a lysosome, and the apolipoproteins, cholesteryl esters, and other components of the remnant are hydrolytically degraded, releasing amino acids, free cholesterol, and FA. The receptor is recycled. (Note: The mechanism

of receptor-mediated endocytosis is illustrated for LDL in Fig. 18.21.)

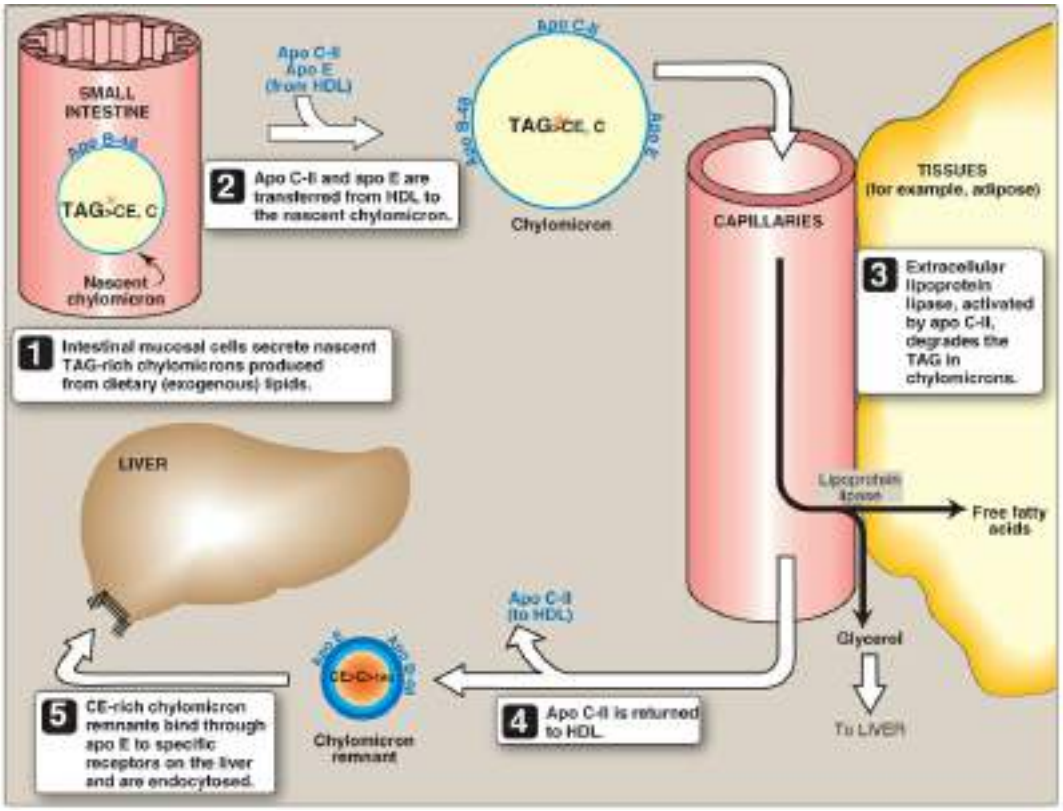


Figure 18.17
Metabolism of chylomicrons. Apo B-48, apo C-II, and apo E are apolipoproteins found as components of plasma lipoprotein particles. The particles are not drawn to scale (see Fig. 18.14 for details of their size and density). TAG = triacylglycerol; C = cholesterol; CE = cholesteryl ester; HDL = high-density lipoprotein.

C. Very–low-density lipoprotein metabolism

VLDLs are produced in the liver (Fig. 18.18). They are composed predominantly of endogenous TAG (~60%), and their function is to carry this lipid from the liver (site of synthesis) to the peripheral tissues. There, the TAG is degraded by LPL, as discussed for chylomicrons. (Note: Nonalcoholic fatty liver [hepatic steatosis] occurs in conditions in which there is an imbalance between hepatic TAG synthesis and the secretion of VLDL. Such conditions include obesity and type 2 diabetes mellitus [see Chapter 25].)

1. Release from the liver: VLDLs are secreted directly into the blood by the liver as nascent particles containing apo B-100. They must obtain apo C-II and apo E from circulating HDL (see Fig. 18.18). As with chylomicrons, apo C-II is required for activation of LPL. (Note: Abetalipoproteinemia is a rare hypolipoproteinemia caused by a defect in MTP, leading to an inability to load apo B with lipid. Consequently, few VLDLs or chylomicrons are formed, and TAG accumulates in the liver and intestine. Absorption of fat-soluble vitamins is decreased. LDLs are

low.)

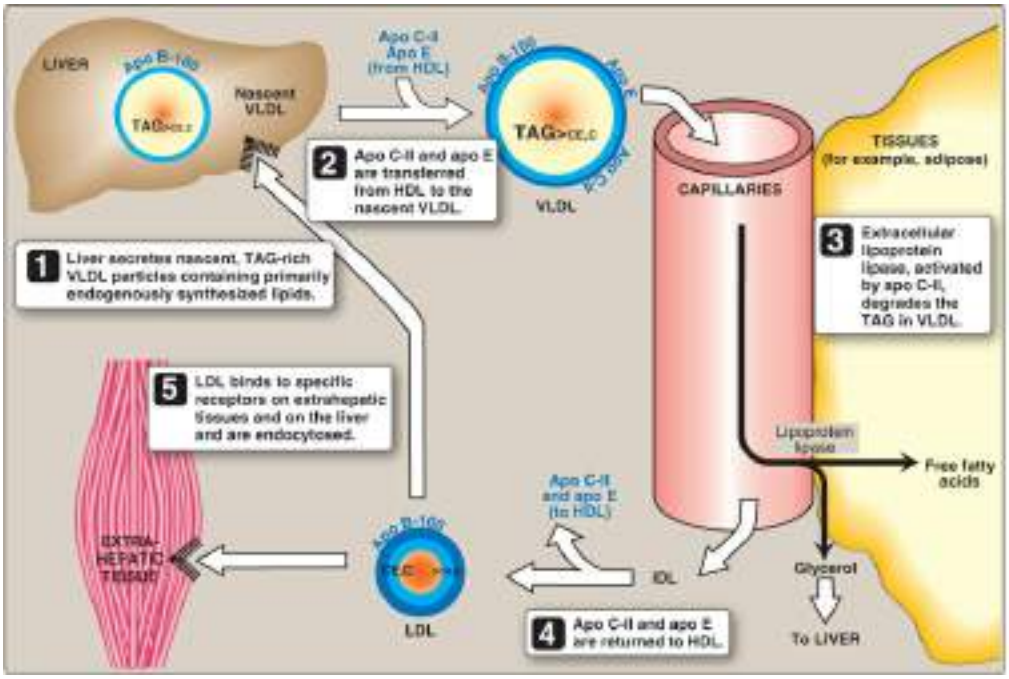


Figure 18.18
Metabolism of very–low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) particles. Apo B-100, C-II, and E are apolipoproteins found as components of plasma lipoprotein particles. The particles are not drawn to scale (see Fig. 18.14 for details of their size and density). (Note: IDL can also be taken up by liver.) TAG = triacylglycerol; HDLs and IDLs = high- and intermediate-density lipoproteins; C = cholesterol; CE = cholesteryl ester.

2. Modification in the circulation: As VLDL passes through the circulation, TAG is degraded by LPL, causing the VLDL to decrease in size and become denser. Surface components, including the C and E apolipoproteins, are returned to HDL, but the particles retain apo B-100. Additionally, some TAGs are transferred from VLDL to HDL in an exchange reaction that concomitantly transfers cholesteryl esters from HDL to VLDL. This exchange is accomplished by cholesteryl ester transfer protein (CETP), as shown in Figure 18.19.

3. Conversion to low-density lipoproteins: With these modifications, the VLDL is converted in the plasma to LDL. IDLs of varying sizes are formed during this transition. IDL can also be taken up by liver cells through receptor-mediated endocytosis that uses apo E as the ligand. Apo E is normally present in three isoforms, E-2 (the least common), E-3 (the most common), and E-4. Apo E-2 binds poorly to receptors, and patients who are homozygotic for apo E-2 are deficient in the clearance of IDL and chylomicron remnants. These individuals have familial type III hyperlipoproteinemia (familial dysbetalipoproteinemia or broad beta disease), with hypercholesterolemia and premature atherosclerosis. (Note: The apo E-4 isoform confers increased susceptibility to an earlier age of onset of the late-onset form of Alzheimer disease. The effect is dose dependent,

with homozygotes being at greatest risk. Estimates of the risk vary.)

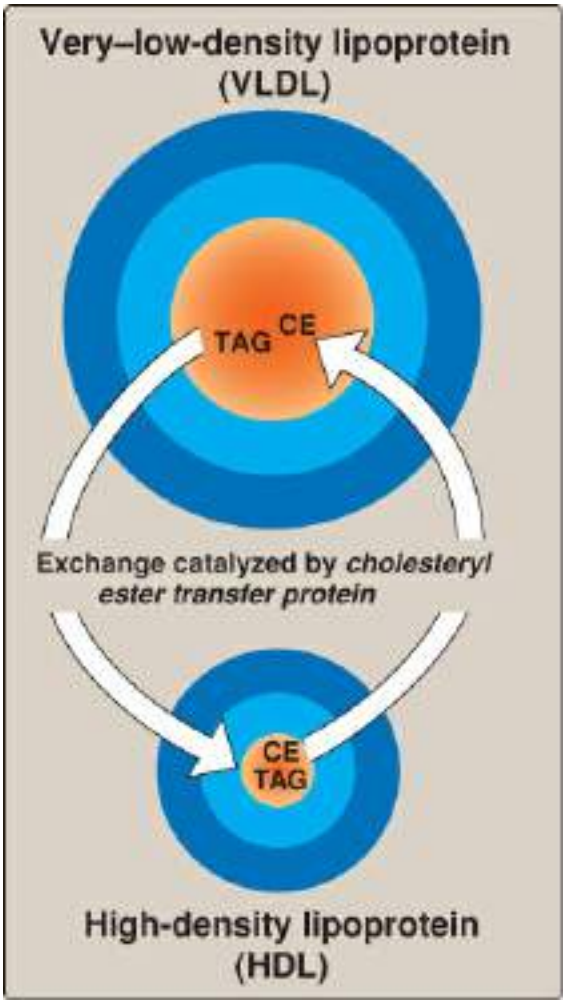


Figure 18.19
Transfer of cholesteryl ester (CE) from HDL to VLDL in exchange for triacylglycerol (TAG).

D. Low-density lipoprotein metabolism

LDL particles contain much less TAG than their VLDL predecessors and have a high concentration of cholesterol and cholesteryl esters (Fig. 18.20). About 70% of plasma cholesterol is in LDL.

1. Receptor-mediated endocytosis: The primary function of LDL particles is to provide cholesterol to the peripheral tissues (or return it to the liver). They do so by binding to plasma membrane LDL receptors that recognize apo B-100 (but not apo B-48). Because these LDL receptors can also bind apo E, they are known as apo B-100/apo E receptors. A summary of the uptake and degradation of LDL particles is presented in Figure 18.21. (Note: The numbers in brackets below refer to corresponding numbers on that figure.) A similar mechanism of receptor-mediated endocytosis is used for the uptake and

degradation of chylomicron remnants and IDLs by the liver.

[1] LDL receptors are negatively charged glycoproteins that are clustered in pits on cell membranes. The cytosolic side of the pit is coated with the protein clathrin, which stabilizes the pit.

[2] After binding, the LDL–receptor complex is endocytosed. (Note: Defects in the synthesis of functional LDL receptors causes a significant elevation in plasma LDL-C. Patients with such deficiencies have type IIa hyperlipidemia [familial hypercholesterolemia (FH)] and premature atherosclerosis. Autosomal dominant hypercholesterolemia can also be caused by defects in apo B-100 that reduce its binding to the receptor and by increased activity of a protease, proprotein convertase subtilisin/kexin type 9 [PCSK9], which promotes internalization and lysosomal degradation of the receptor. PCSK9 inhibitors are now available for the treatment of hypercholesterolemia.)

[3] The vesicle containing LDL loses its clathrin coat and fuses with other similar vesicles, forming larger vesicles called endosomes.

[4] The pH of the endosome falls (due to the proton-pumping activity of endosomal ATPase), which allows separation of the LDL from its receptor. The receptors then migrate to one side of the endosome, whereas the LDL stay free within the lumen of the vesicle.

[5] The receptors can be recycled, whereas the lipoprotein remnants in the vesicle are transferred to lysosomes and degraded by lysosomal acid hydrolases, releasing free cholesterol, amino acids, FA, and phospholipids. These compounds can be reutilized by the cell. (Note: A few of the lysosomal storage diseases result from rare autosomal-recessive deficiencies in the ability to hydrolyze lysosomal cholesteryl esters [Wolman disease] or to transport free cholesterol out of the lysosome [Niemann–Pick disease, type C].)

2. Endocytosed cholesterol and cholesterol homeostasis: The chylomicron remnant-, IDL-, and LDL-derived cholesterol affects cellular cholesterol content in several ways (Fig. 18.21). First, expression of the gene for HMG CoA reductase is inhibited by high cholesterol, and *de novo* cholesterol synthesis decreases as a result. Additionally, degradation of the reductase is accelerated. Second, synthesis of new LDL receptor protein is reduced by decreasing the expression of the LDL receptor gene, thus limiting further entry of LDL-C into cells. (Note: As was seen with the *reductase* gene, transcriptional regulation of the LDL receptor gene involves an SRE and SREBP-2. This allows coordinate regulation of the expression of these proteins.) Third, if the cholesterol is not required immediately for some structural or synthetic purpose, it is esterified by acyl CoA:cholesterol acyltransferase (ACAT). ACAT transfers a FA from a fatty acyl CoA to cholesterol, producing a cholesteryl ester that can be stored in the cell (Fig. 18.22). The activity of ACAT is enhanced in the presence of increased intracellular cholesterol.

3. Uptake by macrophage scavenger receptors: In addition to the highly specific and regulated receptor-mediated pathway for LDL uptake described above, macrophages possess high levels of scavenger receptor (SR) activity. These receptors, known as scavenger receptor class A (SR-A), can bind a broad range of ligands and mediate the endocytosis of chemically modified LDL in which the lipid or apo B component has been oxidized. Unlike the LDL receptor, the SR is not downregulated in response to increased intracellular cholesterol. Cholesteryl esters accumulate in macrophages and cause their transformation into “foam” cells, which participate in the formation of atherosclerotic plaque (Fig. 18.23). LDL-C is the primary cause of atherosclerosis.

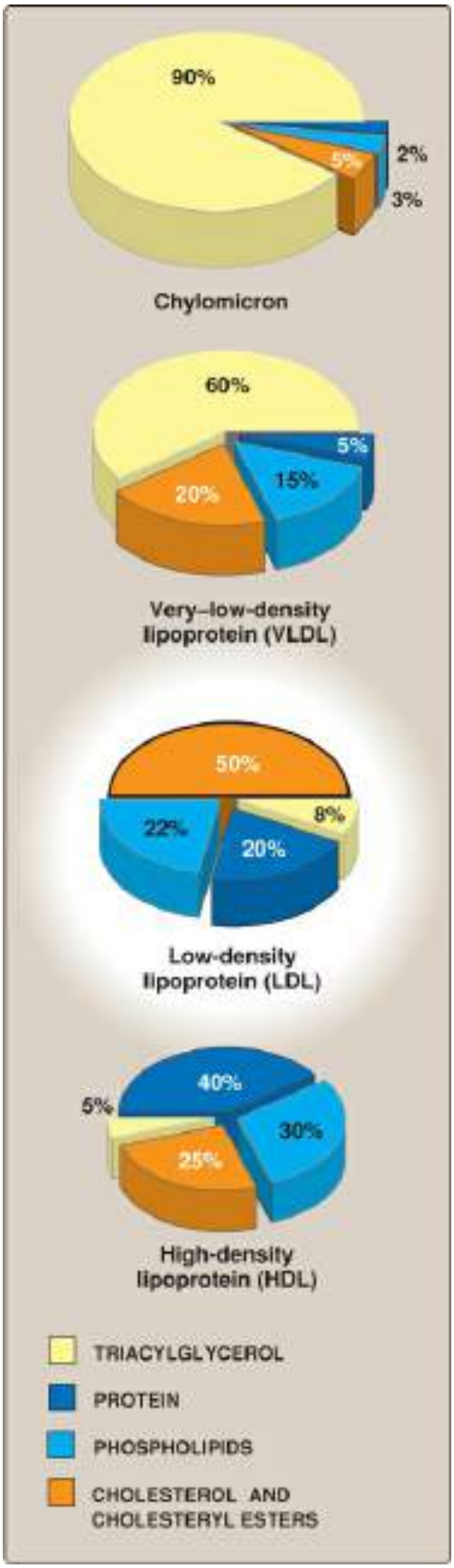


Figure 18.20
Composition of the plasma lipoprotein particles. Note the high concentration of cholesterol and cholesteryl esters in LDL.

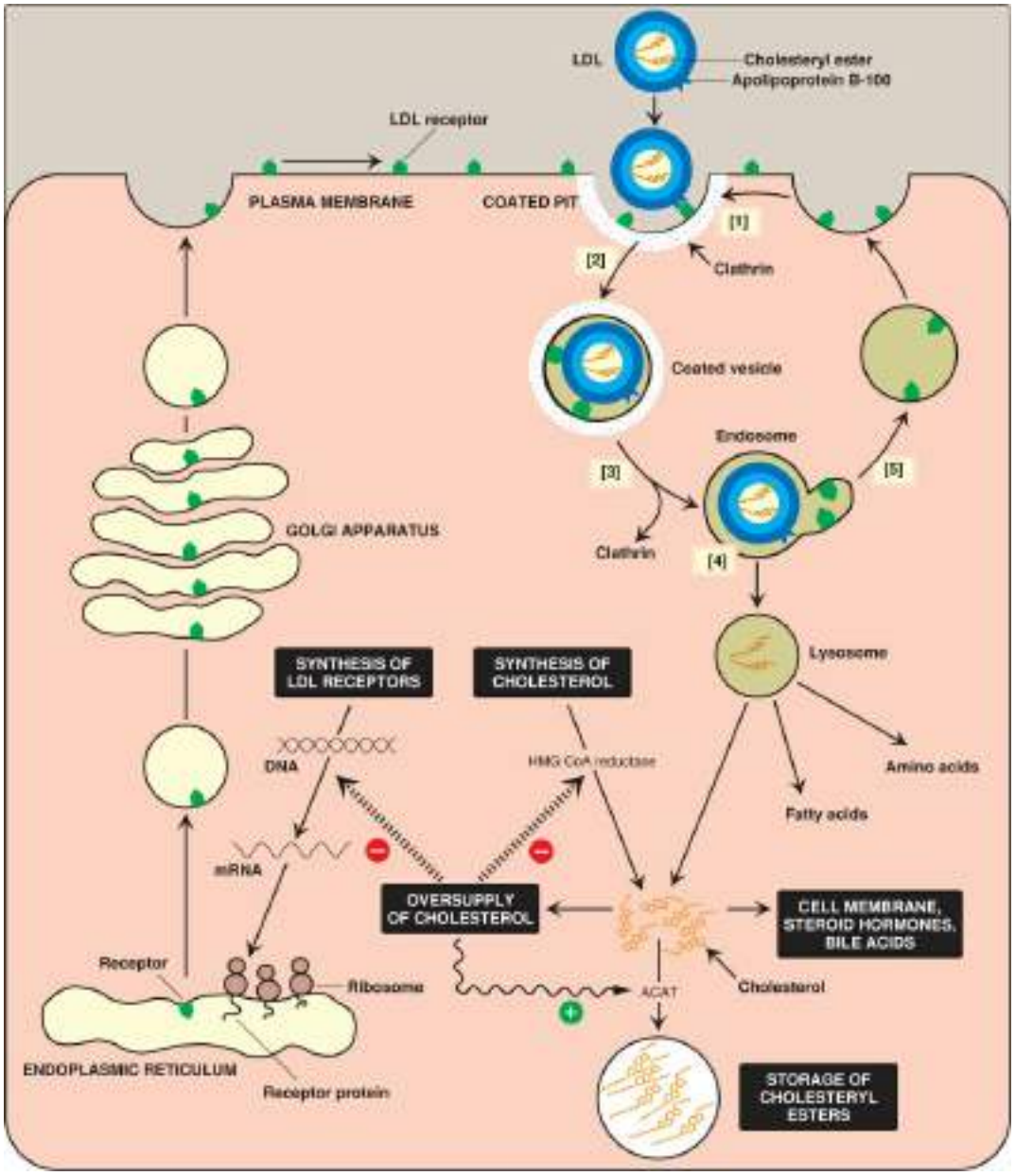


Figure 18.21
Cellular uptake and degradation of low-density lipoprotein (LDL) particles. (Note: Oversupply of cholesterol accelerates the degradation of HMG CoA reductase. It also decreases transcription of its gene as seen with the LDL receptor.) ACAT = acyl CoA:cholesterol acyltransferase; HMG CoA = hydroxymethylglutaryl coenzyme A; mRNA = messenger RNA.

E. High-density lipoprotein metabolism

HDLs comprise a heterogeneous family of lipoproteins with a complex metabolism that is not yet completely understood. HDL particles are formed in the blood by the

addition of lipid to apo A-1, an apolipoprotein made and secreted by the liver and intestine. Apo A-1 accounts for ~70% of the apolipoproteins in HDL. HDLs perform a number of important functions, including the following.

1. Apolipoprotein supply: HDL particles serve as a circulating reservoir of apo C-II (the apolipoprotein that is transferred to VLDL and chylomicrons and is an activator of LPL) and apo E (the apolipoprotein required for the receptor-mediated endocytosis of IDLs and chylomicron remnants).
2. Nonesterified cholesterol uptake: Nascent HDLs are disc-shaped particles containing primarily phospholipid (largely PC) and apo A, C, and E. They take up cholesterol from nonhepatic (peripheral) tissues and return it to the liver as cholesteryl esters (Fig. 18.24). (Note: HDL particles are excellent acceptors of nonesterified cholesterol as a result of their high concentration of phospholipids, which are important solubilizers of cholesterol.)

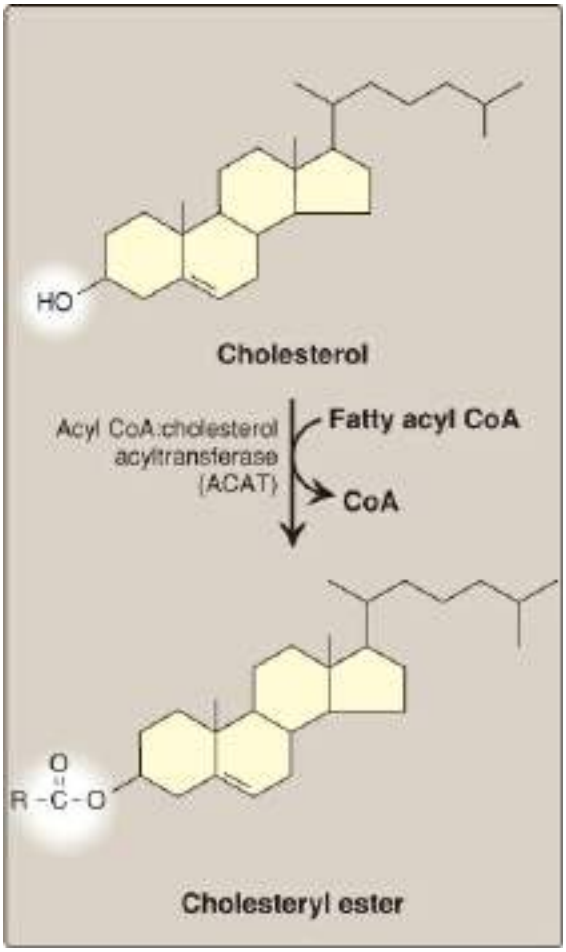


Figure 18.22
Synthesis of intracellular cholesteryl ester by ACAT. (Note: Lecithin: cholesterol acyl transferase [LCAT] is the extracellular enzyme that esterifies cholesterol using phosphatidylcholine [lecithin] as the source of the fatty acid.) CoA = coenzyme A.

3. Cholesterol esterification: The cholesterol taken up by HDL is immediately esterified by the plasma enzyme lecithin:cholesterolacyltransferase (LCAT, also known as PCAT, in which P stands for PC, the source of the FA). This enzyme is synthesized and secreted by the liver. LCAT binds to nascent HDL and is activated by apo A-I. LCAT transfers the FA from carbon 2 of PC to cholesterol. This produces a hydrophobic cholesteryl ester, which is sequestered in the core of the HDL, and lysophosphatidylcholine, which binds to albumin. (Note: Esterification maintains the cholesterol concentration gradient, allowing continued efflux of cholesterol to HDL.) As the discoidal nascent HDL accumulates cholesteryl esters, it first becomes a spherical, relatively cholesteryl ester–poor HDL3 and, eventually, a cholesteryl ester–rich HDL2 particle that carries these esters to the liver. Hepatic lipase, which degrades TAG and phospholipids, participates in the conversion of HDL2 to HDL3 (see Fig. 18.24). CETP transfers some of the cholesteryl esters from HDL to VLDL in exchange for TAG, relieving product inhibition of LCAT. Because VLDLs are catabolized to LDLs, the cholesteryl esters transferred by CETP are ultimately taken up by the liver.

4. Reverse cholesterol transport: The selective transfer of cholesterol from peripheral cells to HDL and from HDL to the liver for bile acid synthesis or disposal via the bile is a key component of cholesterol homeostasis. This process of reverse cholesterol transport (RCT) is, in part, the basis for the inverse relationship seen between plasma HDL concentration and atherosclerosis and for the designation of HDL as the “good” cholesterol carrier. (Note: Exercise and estrogen raise HDL levels.) RCT involves efflux of cholesterol from peripheral cells to HDL, esterification of the cholesterol by LCAT, binding of the cholesteryl ester–rich HDL (HDL2) to liver (and, perhaps, steroidogenic cells), selective transfer of the cholesteryl esters into these cells, and release of lipid-depleted HDL (HDL3). The efflux of cholesterol from peripheral cells is mediated primarily by the transport protein ABCA1. (Note: Tangier disease is a very rare deficiency of ABCA1 and is characterized by the virtual absence of HDL particles due to degradation of lipid-poor apo A-1.) Cholesteryl ester uptake by the liver is mediated by the cell-surface receptor scavenger receptor class B type 1 (SR-B1) that binds HDL (see Section VI D3 for SR-A receptors). The HDL particle itself is not taken up. Instead, there is selective uptake of the cholesteryl ester from the HDL particle. (Note: Low HDL-C is a risk factor for atherosclerosis.)

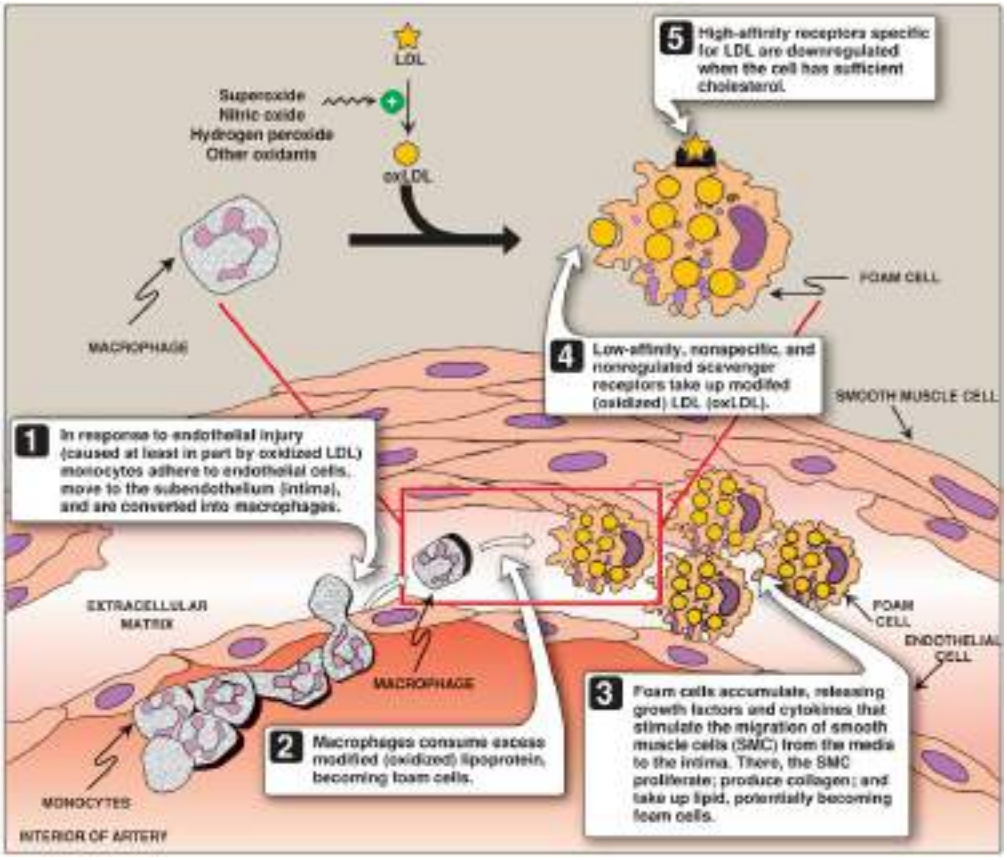


Figure 18.23
Role of oxidized low-density lipoprotein (LDL) particles in plaque formation in an arterial wall.

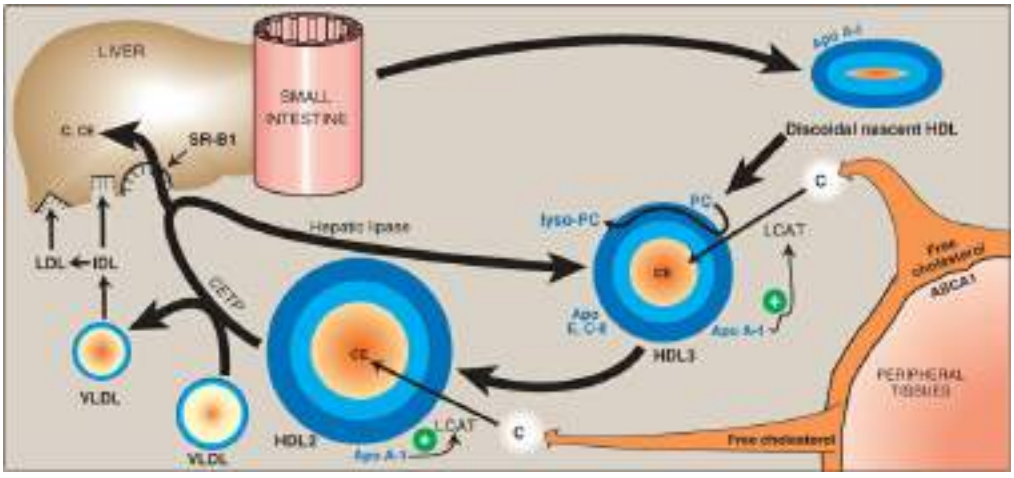


Figure 18.24
Metabolism of high-density lipoprotein (HDL) particles. Apo = apolipoprotein; ABCA1 = ATP-binding cassette transport protein; C = cholesterol; CE = cholesteryl ester; LCAT = lecithin:cholesterolacyltransferase; VLDLs, IDLs, and LDLs = very-low–, intermediate-, and low-density lipoproteins; CETP = cholesteryl ester transfer protein; SR-B1 = scavenger receptor B1.

ABCA1 is an ATP-binding cassette (ABC) protein. ABC proteins use energy from ATP hydrolysis to transport materials, including lipids, in and out of cells and across intracellular compartments. In addition to Tangier disease, defects in specific ABC proteins result in sitosterolemia, cystic fibrosis, X-linked adrenoleukodystrophy, respiratory distress syndrome due to decreased surfactant secretion, and liver disease due to decreased bile salt secretion.

F. Lipoprotein (a) and heart disease

Lp(a), is nearly identical in structure to an LDL particle. Its distinguishing feature is the presence of an additional apolipoprotein molecule, apo(a), which is covalently linked at a single site to apo B-100. Apo(a) is structurally homologous to plasminogen, the precursor of a blood protease whose target is fibrin. Fibrin is the main protein component of blood clots (see Chapter 35). Lp(a) is an independent risk factor for coronary heart disease. The apo(a) component of Lp(a) particles is indicated to promote atherogenesis. Circulating levels of Lp(a) are determined primarily by genetics. However, diet may play some role, as trans FA have been reported to increase Lp(a). On the other hand, niacin reduces Lp(a), as well as LDL-C and TAG, but raises HDL-C.

VII. STEROID HORMONES

Cholesterol is the precursor of all classes of steroid hormones: glucocorticoids (e.g., cortisol), mineralocorticoids (e.g., aldosterone), and the sex hormones (i.e., androgens, estrogens, and progestins), as shown in Figure 18.25. (Note: Glucocorticoids and mineralocorticoids are collectively called corticosteroids.) Synthesis and secretion occur in the adrenal cortex (cortisol, aldosterone, and androgens), ovaries and placenta (estrogens and progestins), and testes (testosterone). Steroid hormones are transported by the blood from their sites of synthesis to their target organs. Because of their hydrophobicity, they must be complexed with a plasma protein. Albumin can act as a nonspecific carrier and does carry aldosterone. However, specific steroid-carrier plasma proteins bind the steroid hormones more tightly than does albumin (e.g., corticosteroid-binding globulin, or transcortin, is responsible for transporting cortisol). A number of genetic diseases are caused by deficiencies in specific steps in the biosynthesis of steroid hormones. Some representative diseases are described in Figure 18.26.

A. Synthesis

Synthesis involves shortening the hydrocarbon chain of cholesterol and hydroxylating the steroid nucleus. The initial and rate-limiting reaction converts cholesterol to the 21-carbon pregnenolone. It is catalyzed by the cholesterol side-chain cleavage enzyme, a cytochrome P450 (CYP) mixed function oxidase of the inner mitochondrial membrane that is also known as $P450_{scc}$ and desmolase. NADPH and O_2 are required for the reaction. The cholesterol substrate can be newly synthesized, taken up from lipoproteins, or released by an esterase from

cholesteryl esters stored in the cytosol of steroidogenic tissues. The cholesterol moves to the outer mitochondrial membrane. An important control point is the subsequent movement from the outer to the inner mitochondrial membrane. This process is mediated by steroidogenic acute regulatory (StAR) protein. Pregnenolone is the parent compound for all steroid hormones (see Fig. 18.26). It is oxidized and then isomerized to progesterone, which is further modified to the other steroid hormones by CYP protein–catalyzed hydroxylation reactions in the SER and mitochondria. A defect in the activity or amount of an enzyme in this pathway can lead to a deficiency in the synthesis of hormones beyond the affected step and to an excess in the hormones or metabolites before that step. Because all members of the pathway have potent biologic activity, serious metabolic imbalances occur with enzyme deficiencies (see Fig. 18.26). Collectively, these disorders are known as the congenital adrenal hyperplasia (CAH), because they result in enlarged adrenals. (Note: Addison disease, due to autoimmune destruction of the adrenal cortex, is characterized by adrenocortical insufficiency.)

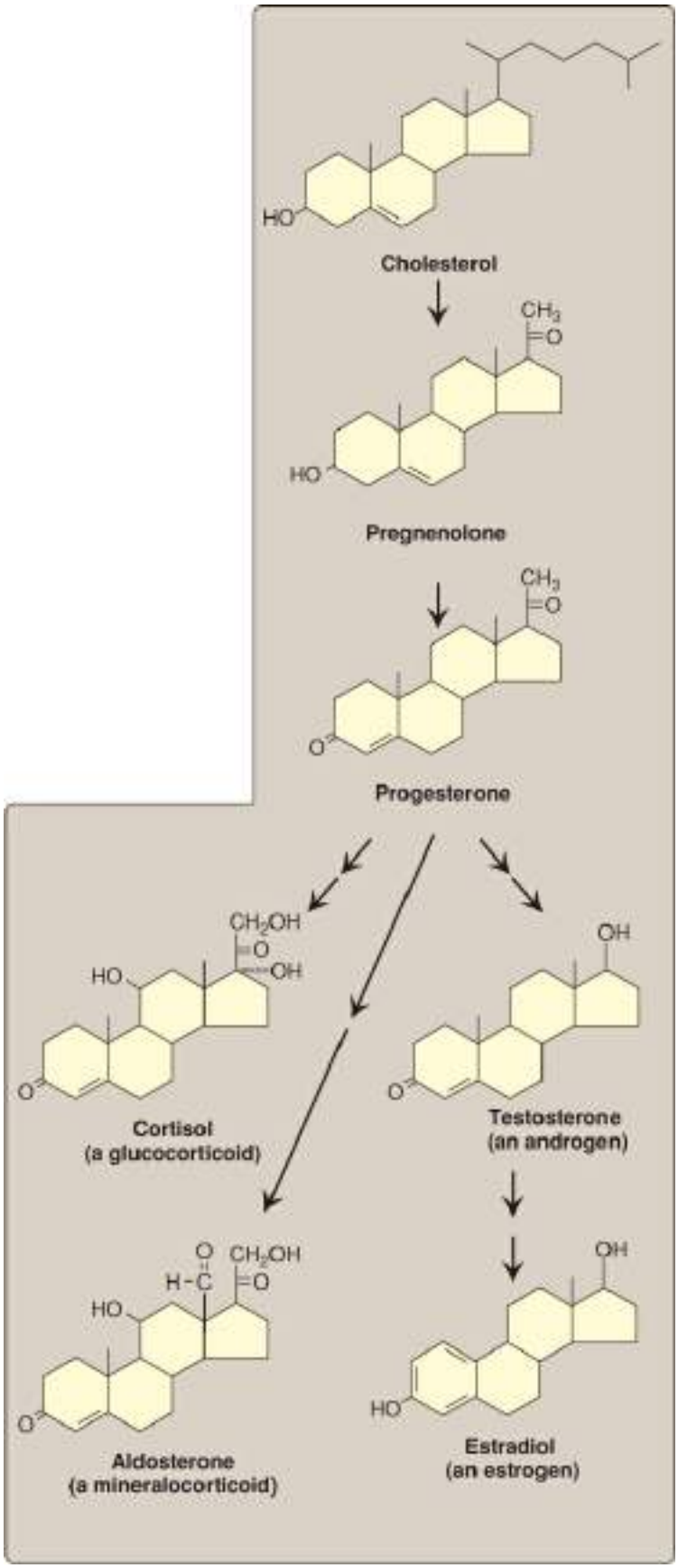


Figure 18.25
Key steroid hormones.

B. Adrenal cortical steroid hormones

Steroid hormones are synthesized and secreted in response to hormonal signals. The corticosteroids and androgens are made in different regions of the adrenal cortex and are secreted into blood in response to different signals. (Note: The adrenal medulla makes catecholamines [see Chapter 21].)

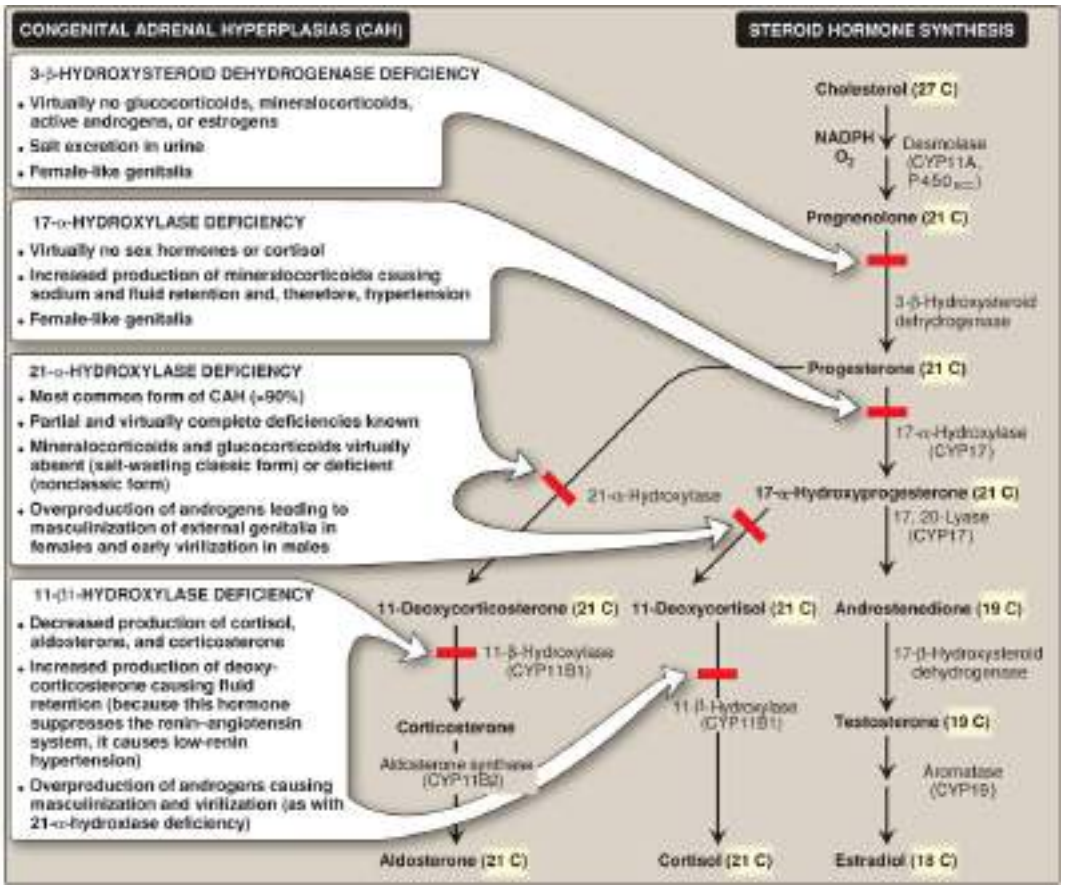


Figure 18.26
Steroid hormone synthesis and associated diseases. (Note: 3-β-Hydroxysteroid dehydrogenase, CYP17, and CYP11B2 are multifunctional enzymes. Synthesis of testosterone and the estrogens occurs primarily outside of the adrenal gland.) NADPH = nicotinamide adenine dinucleotide phosphate; CYP = cytochrome P450.

1. Cortisol: Its production in the middle layer (zona fasciculata) of the adrenal cortex is controlled by the hypothalamus, to which the pituitary gland is attached (Fig. 18.27). In response to severe stress (e.g., infection), corticotropin-releasing hormone (CRH), produced by the hypothalamus, travels through capillaries to the anterior lobe of the pituitary, where it induces the production and secretion of adrenocorticotropic hormone (ACTH), a peptide. ACTH stimulates the adrenal cortex to synthesize and secrete the glucocorticoid cortisol, the stress hormone.

(Note: ACTH binds to a membrane G protein–coupled receptor, resulting in cyclic AMP [cAMP] production and activation of protein kinase A [PKA]. PKA phosphorylates and activates both the esterase that converts cholesteryl ester to free cholesterol and StAR protein.) Cortisol allows the body to respond to stress through its effects on intermediary metabolism (e.g., increased gluconeogenesis) and the inflammatory and immune responses (which are decreased). As cortisol levels rise, the release of CRH and ACTH is inhibited. (Note: The reduction of cortisol in CAH results in a rise in ACTH that causes adrenal hyperplasia.)

2. Aldosterone: Its production in the outer layer (zona glomerulosa) of the adrenal cortex is induced by a decrease in the plasma Na^+/potassium (K^+) ratio and by the hormone angiotensin II (Ang-II). Ang-II (an octapeptide) is produced from angiotensin I ([Ang-I] a decapeptide) by angiotensin-converting enzyme (ACE), an enzyme found predominantly in the lungs but also distributed widely in the body. (Note: Ang-I is produced in the blood by cleavage of an inactive precursor, angiotensinogen, secreted by the liver. Cleavage is catalyzed by renin, made and secreted by the kidneys.) Ang-II binds to cell surface receptors. However, in contrast to ACTH, its effects are mediated through the phosphatidylinositol 4,5-bisphosphate pathway and not by cAMP. Aldosterone's primary effect is on the kidney tubules, where it stimulates Na^+ and water uptake and K^+ excretion (Fig. 18.28). (Note: An effect of aldosterone is an increase in blood pressure. Competitive inhibitors of ACE are used to treat renin-dependent hypertension.)

3. Androgens: Both the inner (zona reticularis) and middle layers of the adrenal cortex produce androgens, primarily dehydroepiandrosterone and androstenedione. Although adrenal androgens themselves are weak, they are converted by aromatase (CYP19) to testosterone, a stronger androgen, in the testes and to estrogens in the ovaries (primarily) of premenopausal women. (Note: Postmenopausal women produce estrogen at extragonadal sites such as the breast. Aromatase inhibitors are used in the treatment of estrogen-responsive breast cancer in these women.)

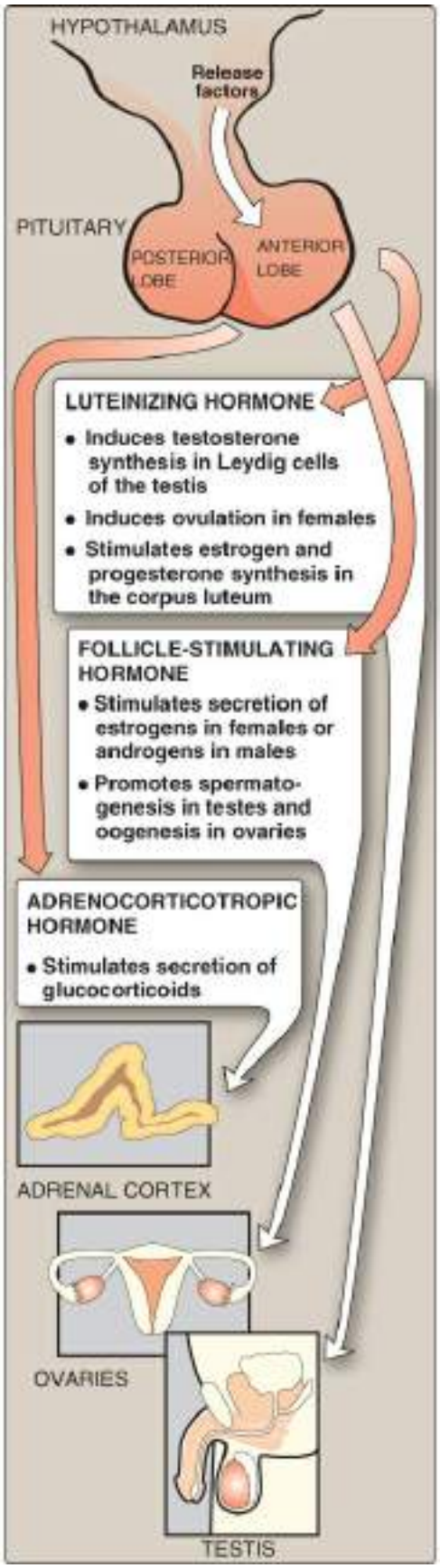


Figure 18.27
Pituitary hormone stimulation of steroid hormone synthesis and secretion.

C. Gonadal steroid hormones

The testes and ovaries (gonads) synthesize hormones necessary for sexual differentiation and reproduction. A single hypothalamic-releasing factor, gonadotropin-releasing hormone, stimulates the anterior pituitary to release the glycoproteins luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Like ACTH, LH and FSH bind to surface receptors and cause an increase in cAMP. LH stimulates the testes to produce testosterone and the ovaries to produce estrogens and progesterone (see Fig. 18.28). FSH regulates the growth of ovarian follicles and stimulates testicular spermatogenesis.

D. Mechanism

Each steroid hormone diffuses across the plasma membrane of its target cell and binds to a specific cytosolic or nuclear receptor. These receptor–ligand complexes accumulate in the nucleus, dimerize, and bind to specific regulatory DNA sequences (hormone response elements [HREs]) in association with coactivator proteins, thereby causing increased transcription of targeted genes (Fig. 18.29). An HRE is found in the promoter or an enhancer element (see Chapter 31) for genes that respond to a specific steroid hormone, thus ensuring coordinated regulation of these genes. Hormone–receptor complexes can also inhibit transcription in association with corepressors. (Note: The binding of a hormone to its receptor causes a conformational change in the receptor that uncovers its DNA-binding domain, allowing the complex to interact through a zinc finger motif with the appropriate DNA sequence. Receptors for the steroid hormones, plus those for thyroid hormone, retinoic acid, and 1,25-dihydroxycholecalciferol [vitamin D], are members of a superfamily of structurally related gene regulators that function in a similar way.)

E. Further metabolism

Steroid hormones are generally converted into inactive metabolic excretion products in the liver. Reactions include reduction of unsaturated bonds and the introduction of additional hydroxyl groups. The resulting structures are made more soluble by conjugation with glucuronic acid or sulfate (from 3′-phosphoadenosyl-5′-phosphosulfate). These conjugated metabolites are fairly water soluble and do not need protein carriers. They are eliminated in feces and urine.

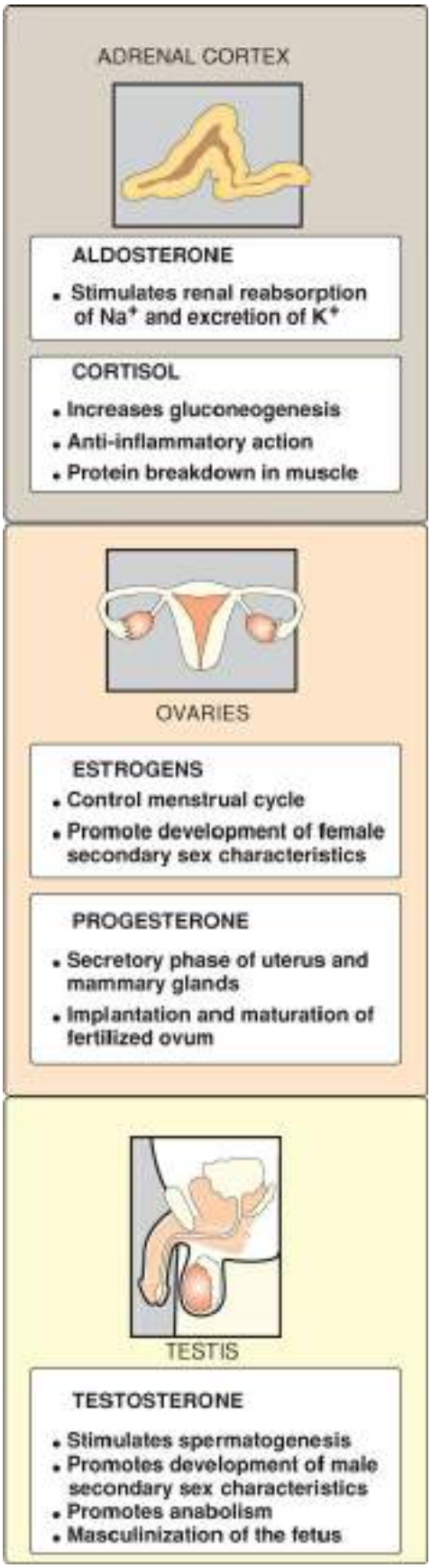


Figure 18.28
Actions of steroid hormones. Na^+ = sodium; K^+ = potassium.

VIII. Chapter Summary

- **Cholesterol** is a hydrophobic compound, with a single hydroxyl group to which a FA can be attached, producing an even more hydrophobic **cholesteryl ester**.
- Cholesterol is synthesized by virtually all human tissues, although primarily by the **liver**, **intestine**, **adrenal cortex**, and **reproductive tissues.**
- Synthesis requires enzymes of the **cytosol**, **SER**, and **peroxisomes**.
- The rate-limiting and regulated step in cholesterol synthesis is catalyzed **HMG CoA reductase**, which produces **mevalonate** from HMG CoA.
- **HMG CoA reductase** is highly regulated by a number of mechanisms: (1) via the transcription factor, **SREBP-2**; (2) accelerated **degradation** of the **protein** when cholesterol levels are high; (3) **phosphorylation** causing **inactivation** of the enzyme by **AMPK**; and (4) hormonal regulation by **insulin** and **glucagon**.
- **Statins** are **competitive inhibitors** of HMG CoA reductase. These drugs are used to decrease plasma cholesterol in patients with **hypercholesterolemia**.
- The ring structure of cholesterol cannot be degraded in humans. It is eliminated from the body either by conversion to bile salts or by secretion into the **bile**.
- The rate-limiting step in bile acid synthesis is catalyzed by **cholesterol-7-α-hydroxylase**, which is inhibited by **bile acids**.
- Before the bile acids leave the liver, they are conjugated. Conjugated bile acids are known as bile salts which are **more** ionized and **more** water **soluble** than bile acids at the alkaline pH of the bile.
- Intestinal **bacteria** modify bile salts producing the **secondary bile salts.**
- Bile salts are efficiently reabsorbed (>95%) and return to the liver by **enterohepatic circulation.**
- Enterohepatic circulation of bile salts is reduced by **bile acid sequestrants.**
- If more cholesterol enters the bile than can be solubilized by the available bile salts and PC, **cholesterol gallstone disease** (**cholelithiasis**) can occur.
- The plasma lipoproteins (see Fig. 18.30) include **chylomicrons**, **VLDLs**, **IDLs**, **LDLs**, and **HDLs**. They function to keep lipids soluble as they transport them between tissues.
- Lipoproteins are composed of **TAG** and **cholesteryl esters** in the core surrounded by a shell of **amphipathic apolipoproteins**, **phospholipid**, and **nonesterified cholesterol**.
- **Chylomicrons** are assembled in **intestinal mucosal cells** from **dietary lipids**. Each nascent chylomicron particle has one molecule of **apo B-48**.
- Due to their large size, **chylomicrons** are released from the cells into the lymphatic system and travel to the blood. Apo C-II activates endothelial **LPL**, which degrades the TAG in chylomicrons to FA and glycerol. The **FA** that are released are stored in **adipose tissue** or used for energy in **muscle**. The **glycerol** is metabolized by the **liver**.
- After most of the TAG is removed, the **chylomicron remnant**, carrying most of the **dietary cholesterol**, binds to a liver receptor that recognizes apo E.
- Patients with a **deficiency** of LPL or apo C-II show a dramatic accumulation of chylomicrons in the plasma (**type I hyperlipoproteinemia** or **familial chylomicronemia**) even if fasted.
- Nascent VLDLs are produced in the liver and are composed predominantly of TAG. They contain a single molecule of **apo B-100**. VLDLs carry hepatic TAG to the peripheral tissues where LPL degrades the lipid.
- The VLDL particle receives **cholesteryl esters** from HDL in exchange for TAG. This process is accomplished by **CETP**.
- VLDL in the plasma is first converted to IDL and then to LDL.
- Apo B-100 on LDL is recognized by the **LDL receptor** which results in the **receptor-mediated endocytosis of LDL. The contents of LDL are** degraded in the **lysosomes and the LDL receptor is recycled**. The protease **PCSK9** prevents receptor recycling.
- Defective uptake of these chylomicron remnants and IDL causes **type III hyperlipoproteinemia** or **dysbetalipoproteinemia**.
- Defects in the synthesis of functional LDL receptors causes **type II ahyperlipoproteinemia** (**FH**).

- HDLs are created by **lipidation** of **apo A-1** synthesized in the liver and intestine. They have a number of functions, including (1) serving as a circulating **reservoir** of apo C-II and apo E for chylomicrons and VLDL; (2) removing **cholesterol** from peripheral tissues via ABCA1 and esterifying it using **LCAT**, a liver-synthesized plasma enzyme that is activated by **apo A-1**; and (3) delivering these cholesteryl esters to the liver (**RCT**) for uptake via **SR-B1**.
- Cholesterol is the precursor of all classes of **steroid hormones**, which include **glucocorticoids**, **mineralocorticoids**, and the **sex hormones**. Synthesis occurs in the **adrenal cortex (the glucocorticoids, the mineralocorticoids, and the androgens)**, **gonads,** and **placenta**.
- The initial and rate-limiting step is the conversion of cholesterol to **pregnenolone** by the side-chain cleavage enzyme **$P450_{scc}$**. Deficiencies in synthesis lead to **CAH**.
- Each steroid hormone binds to a specific intracellular receptor in its target cell. These **receptor–hormone complexes** bind to specific regulatory DNA sequences (**HREs**) in association with coactivator proteins/corepressors, thereby regulating **transcription** of targeted genes.

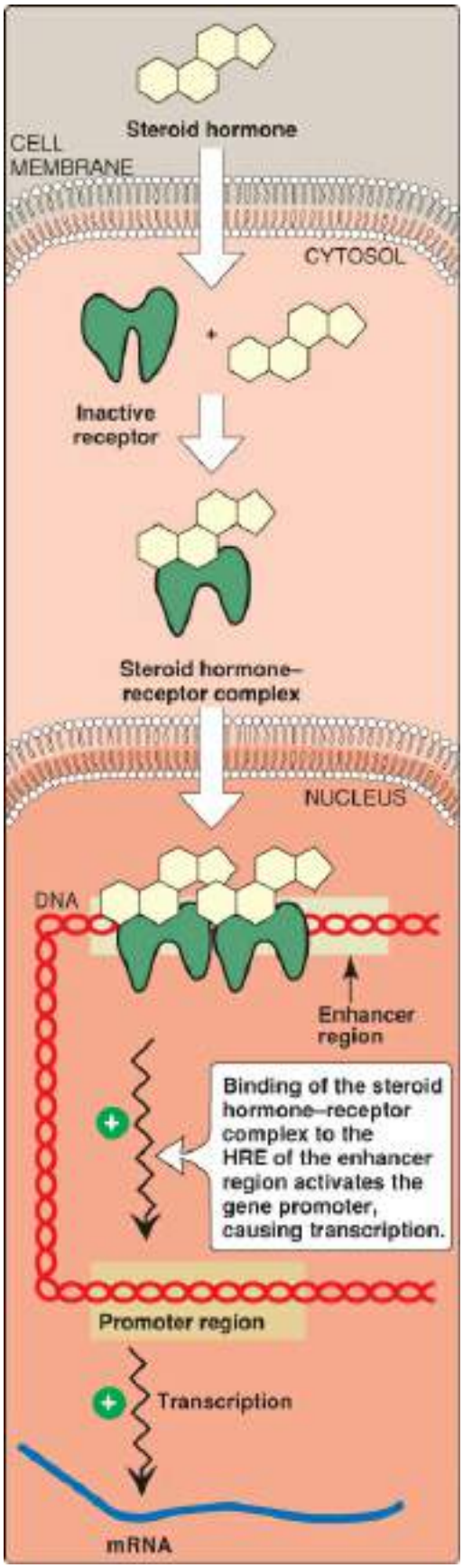


Figure 18.29
Activation of transcription by interaction of steroid hormone–receptor complex with hormone response element (HRE). The receptor contains domains that bind the hormone, DNA, and coactivating proteins. mRNA = messenger RNA.

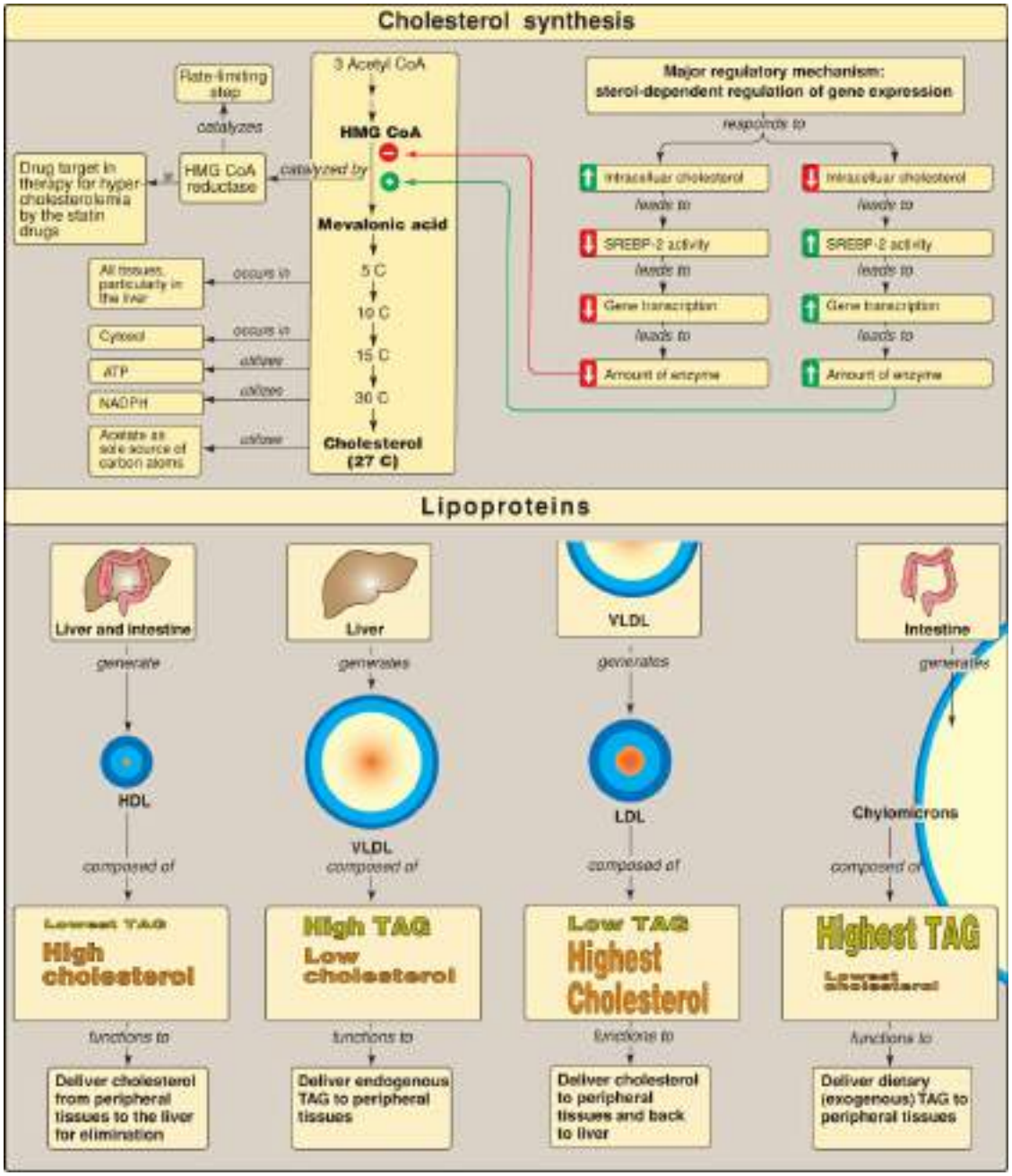


Figure 18.30
Concept map for cholesterol and the lipoproteins. HMG CoA = hydroxymethylglutaryl coenzyme A; SREBP = sterol regulatory element–binding protein; HDLs, LDLs, and VLDLs = high-, low-, and very-low-density lipoproteins; TAG = triacylglycerol; NADPH = nicotinamide adenine dinucleotide phosphate; C = carbon.

Study Questions

Choose the ONE best answer.

18.1 Mice were genetically engineered to contain hydroxymethylglutaryl coenzyme A reductase in which serine 871, a phosphorylation site, was replaced by alanine. Which of the following statements concerning the modified form of the enzyme is most likely to be correct?

A. The enzyme is nonresponsive to ATP depletion.
B. The enzyme is nonresponsive to statin drugs.
C. The enzyme is nonresponsive to the sterol response element–sterol response element–binding protein system.
D. The enzyme is unable to be degraded by the ubiquitin–proteasome system.

Correct answer = A. The reductase is regulated by covalent phosphorylation and dephosphorylation. Depletion of ATP results in a rise in adenosine monophosphate (AMP), which activates AMP kinase (AMPK), thereby phosphorylating and inactivating the reductase. In the absence of the serine, a common phosphorylation site, the enzyme cannot be phosphorylated by AMPK. The enzyme is also regulated physiologically through changes in transcription and degradation and pharmacologically by statin drugs (competitive inhibitors), but none of these depends on serine phosphorylation.

18.2 Calculate the amount of cholesterol in the low-density lipoproteins in an individual whose fasting blood gave the following lipid-panel test results: total cholesterol = 300 mg/dl, high-density lipoprotein cholesterol = 25 mg/dl, triglycerides = 150 mg/dl.

A. 55 mg/dl
B. 95 mg/dl
C. 125 mg/dl
D. 245 mg/dl

Correct answer = D. The total cholesterol in the blood of a fasted individual is equal to the sum of the cholesterol in low-density lipoproteins plus the cholesterol in high-density lipoproteins plus the cholesterol in very–low-density lipoproteins (VLDLs). This last term is calculated by dividing the triacylglycerol value by 5 because cholesterol accounts for about one-fifth of the volume of VLDL in fasted blood.

For Questions 18.3 and 18.4, use the following scenario.

A young female with a history of severe abdominal pain was taken to her local hospital at 5 AM in severe distress. Blood was drawn, and the plasma appeared milky, with the triacylglycerol level >2,000 mg/dl (normal = 4 to 150 mg/dl). The patient was placed on a diet extremely limited in fat but supplemented with medium-chain triglycerides.

18.3 Which of the following lipoprotein particles are most likely responsible for the appearance of the patient's plasma?

A. Chylomicrons
B. High-density lipoproteins
C. Intermediate-density lipoproteins
D. Low-density lipoproteins
E. Very–low-density lipoproteins

Correct answer = A. The milky appearance of her plasma was a result of triacylglycerol-rich chylomicrons. Because 5 AM is presumably several hours after her evening meal, the patient must have difficulty degrading these lipoprotein particles. Intermediate-, low-, and high-density lipoproteins contain primarily cholesteryl esters, and, if one or more of these particles was elevated, it would cause hypercholesterolemia. Very–low-density lipoproteins do not cause the described milky appearance of plasma.

18.4 Which one of the following proteins is most likely to be deficient in this patient?

A. Apolipoprotein A-I
B. Apolipoprotein B-48
C. Apolipoprotein C-II
D. Cholesteryl ester transfer protein
E. Microsomal triglyceride transfer protein

Correct answer = C. The triacylglycerol (TAG) in chylomicrons is degraded by endothelial lipoprotein lipase (LPL), which requires apolipoprotein (apo) C-II as a coenzyme. Deficiency of LPL or apo C-II results in decreased ability to degrade chylomicrons to their remnants, which get cleared (via apo E) by liver receptors. Apo A-I is the coenzyme for lecithin:cholesterolacyltransferase; apo B-48 is the characteristic structural protein of chylomicrons; cholesteryl ester transfer protein catalyzes the cholesteryl ester–TAG exchange between high-density and very–low-density lipoproteins (VLDLs); and microsomal triglyceride transfer protein is involved in the formation, not degradation, of chylomicrons (and VLDLs).

18.5 Complete the table below for an individual with classic 21-α-hydroxylase deficiency relative to a normal individual.

Variable	Increased	Decreased
Aldosterone		
Androstenedione		
Cortisol		
Blood glucose		
Adrenocorticotropic hormone		
Blood pressure		

How might the results be changed if this individual were deficient in 17-α-hydroxylase, rather than 21-α- hydroxylase?

Classic 21-α-hydroxylase deficiency causes mineralocorticoids (aldosterone) and glucocorticoids (cortisol) to be virtually absent. Because aldosterone increases blood pressure, and cortisol increases blood glucose, their deficiencies result in a decrease in blood pressure and blood glucose, respectively. Cortisol normally feeds back to inhibit adrenocorticotropic hormone (ACTH) release by the pituitary, and, so, its absence results in an elevation in ACTH. The loss of 21-α-hydroxylase pushes progesterone and pregnenolone to androgen synthesis and, therefore, causes androstenedione levels to rise. With 17-α-hydroxylase deficiency, sex hormone synthesis would be decreased. Mineralocorticoid production would be increased, leading to hypertension.

UNIT IV:
Nitrogen Metabolism

Amino Acids: Nitrogen Disposal 19

I. OVERVIEW

Unlike fats and carbohydrates, amino acids are not stored by the body. That is, no protein exists whose sole function is to maintain a supply of amino acids for future use. Therefore, amino acids must be obtained from the diet, synthesized *de novo*, or produced from the degradation of body protein. Any amino acids in excess of the biosynthetic needs of the cell are rapidly degraded. The first phase of catabolism involves the removal of the α-amino groups (usually by transamination and subsequent oxidative deamination), forming ammonia and the corresponding α-keto acids, the carbon skeletons of amino acids. A portion of the free ammonia is excreted in the urine, but most is used in the synthesis of urea (Fig. 19.1), which is quantitatively the most important route for disposing of nitrogen from the body. In the second phase of amino acid catabolism, described in Chapter 20, the carbon skeletons of the α-keto acids are converted to common intermediates of energy-producing metabolic pathways. These compounds can be metabolized to carbon dioxide (CO_2) and water (H_2O), glucose, fatty acids, or ketone bodies by the central pathways of metabolism described in Chapters 8 to 13, and 16.

II. OVERALL NITROGEN METABOLISM

Amino acid catabolism is part of the larger process of the metabolism of nitrogen-containing molecules. Nitrogen enters the body in a variety of compounds present in food, the most important being amino acids contained in dietary protein. Nitrogen leaves the body as urea, ammonia, and other products derived from amino acid metabolism (such as creatinine, see p. 320). The role of body proteins in these transformations involves two important concepts: the amino acid pool and protein turnover.

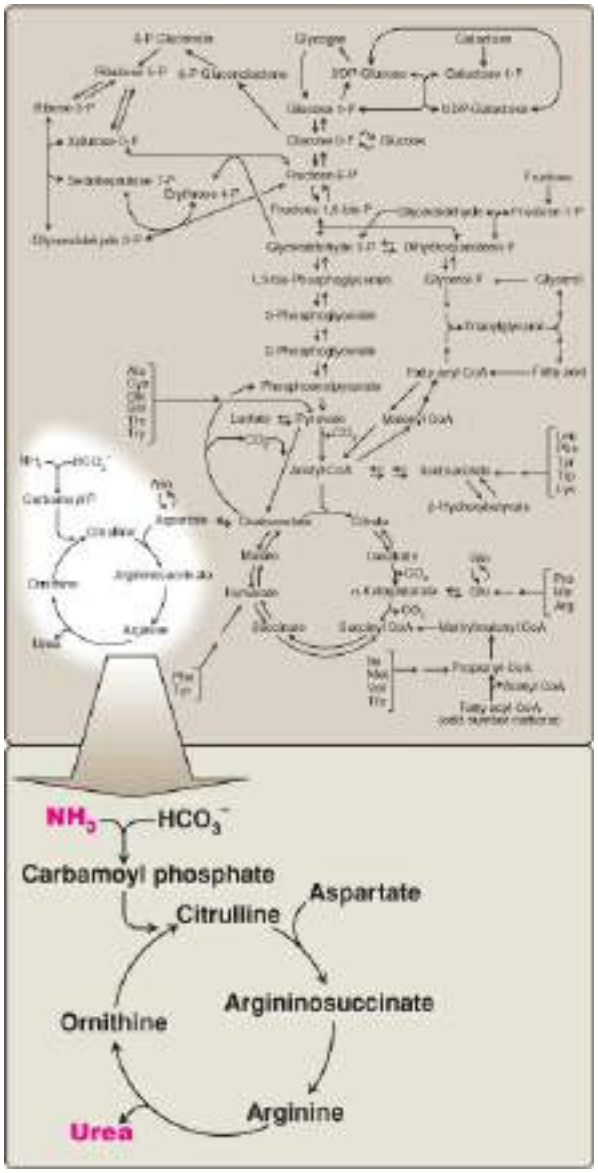


Figure 19.1
Urea cycle shown as part of the essential pathways of energy metabolism. (Note: Fig. 8.2, p. 101, for a more detailed map of metabolism.) NH_3 = ammonia; CO_2 = carbon dioxide.

A. Amino acid pool

Free amino acids are present throughout the body, such as in cells, blood, and the extracellular fluids. For the purpose of this discussion, envision all of these amino acids as if they belonged to a single entity, called the amino acid pool. This pool is supplied by three sources: (1) amino acids provided by the degradation of endogenous (body) proteins, most of which are reutilized; (2) amino acids derived from exogenous (dietary) protein; and (3) nonessential amino acids synthesized from simple intermediates of metabolism (Fig. 19.2). Conversely, the amino acid pool is depleted by three routes: (1) synthesis of body protein, (2) consumption of amino acids as precursors of essential nitrogen-containing small molecules, and (3)

conversion of amino acids to glucose, glycogen, fatty acids, and ketone bodies or oxidation to CO_2 + H_2O (Fig. 19.2). Although the amino acid pool is small (comprising ~90 to 100 g of amino acids) in comparison with the amount of protein in the body (~12 kg in a 70-kg man), it is conceptually at the center of whole-body nitrogen metabolism.

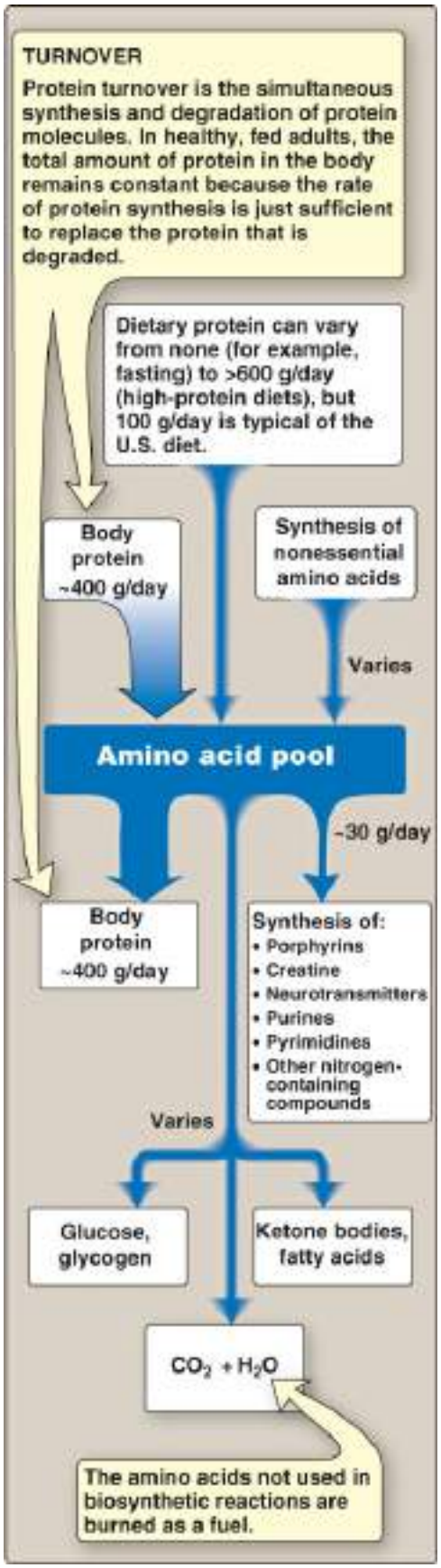


Figure 19.2
Sources and fates of amino acids. (Note: Nitrogen from amino acid degradation is released as ammonia, which is converted to urea and excreted.) CO_2 = carbon dioxide.

In healthy, well-fed individuals, the input to the amino acid pool is balanced by the output. That is, the amount of amino acids contained in the pool is constant. The amino acid pool is said to be in a steady state, and the individual is said to be in nitrogen balance (see p. 412).

B. Protein turnover

Most proteins in the body are constantly being synthesized and then degraded (turned over), permitting the removal of abnormal or unneeded proteins. For many proteins, regulation of synthesis determines the concentration of protein in the cell, with protein degradation assuming a minor role. For other proteins, the rate of synthesis is constitutive (i.e., essentially constant), and cellular levels of the protein are controlled by selective degradation.

1. Rate: In healthy adults, the total amount of protein in the body remains constant because the rate of protein synthesis is just sufficient to replace the protein that is degraded. This process, called protein turnover, leads to the hydrolysis and resynthesis of 300 to 400 g of body protein each day. The rate of protein turnover varies widely for individual proteins. Short-lived proteins (e.g., many regulatory proteins and misfolded proteins) are rapidly degraded, having half-lives measured in minutes or hours. Long-lived proteins, with half-lives of days to weeks, constitute the majority of proteins in the cell. Structural proteins, such as collagen, are metabolically stable and have half-lives measured in months or years.

2. Protein degradation: There are two major enzyme systems responsible for degrading proteins: the ATP-dependent ubiquitin (Ub)–proteasome system of the cytosol and the ATP-independent degradative enzyme system of the lysosomes. Proteasomes selectively degrade damaged or short-lived proteins. Lysosomes use acid hydrolases (see p. 178) to nonselectively degrade intracellular proteins (autophagy) and extracellular proteins (heterophagy), such as plasma proteins that are taken into the cell by endocytosis.

a. Ubiquitin–proteasome system: Proteins selected for degradation by the cytosolic Ub–proteasome system are first modified by the covalent attachment of Ub, a small, globular, nonenzymic protein that is highly conserved across eukaryotic species. Ubiquitination of the target substrate occurs through isopeptide linkage of the α-carboxyl group of the C-terminal glycine of Ub to the ε-amino group of a lysine in the protein substrate by a three-step, enzyme-catalyzed, ATP-dependent process. (Note: Enzyme 1 [E1, an activating enzyme] activates Ub, which is then transferred to E2 [a conjugating enzyme]. E3 [a ligase] identifies the protein to be degraded and

interacts with E2-Ub. There are many more E3 proteins than there are E1 or E2.) The consecutive addition of four or more Ub molecules to the target protein generates a polyubiquitin chain. Proteins tagged with Ub chains are recognized by a large, barrel-shaped, macromolecular, proteolytic complex called a proteasome (Fig. 19.3). The proteasome unfolds, deubiquitinates, and cuts the target protein into fragments that are then further degraded by cytosolic proteases to amino acids, which enter the amino acid pool. The Ub is recycled. It is noteworthy that the selective degradation of proteins by the Ub–proteosome complex (unlike simple hydrolysis by proteolytic enzymes) requires ATP hydrolysis.

b. Degradation signals: Because proteins have different half-lives, it is clear that protein degradation cannot be random but, rather, is influenced by some structural aspect of the protein that serves as a degradation signal, which is recognized and bound by an E3. The half-life of a protein is also influenced by the amino (N)-terminal residue, the so-called N-end rule, and ranges from minutes to hours. Destabilizing N-terminal amino acids include arginine and posttranslationally modified amino acids such as acetylated alanine. In contrast, serine is a stabilizing amino acid. Additionally, proteins rich in sequences containing proline, glutamate, serine, and threonine (called PEST sequences after the one-letter designations for these amino acids) are rapidly ubiquitinated and degraded and, therefore, have short half-lives.

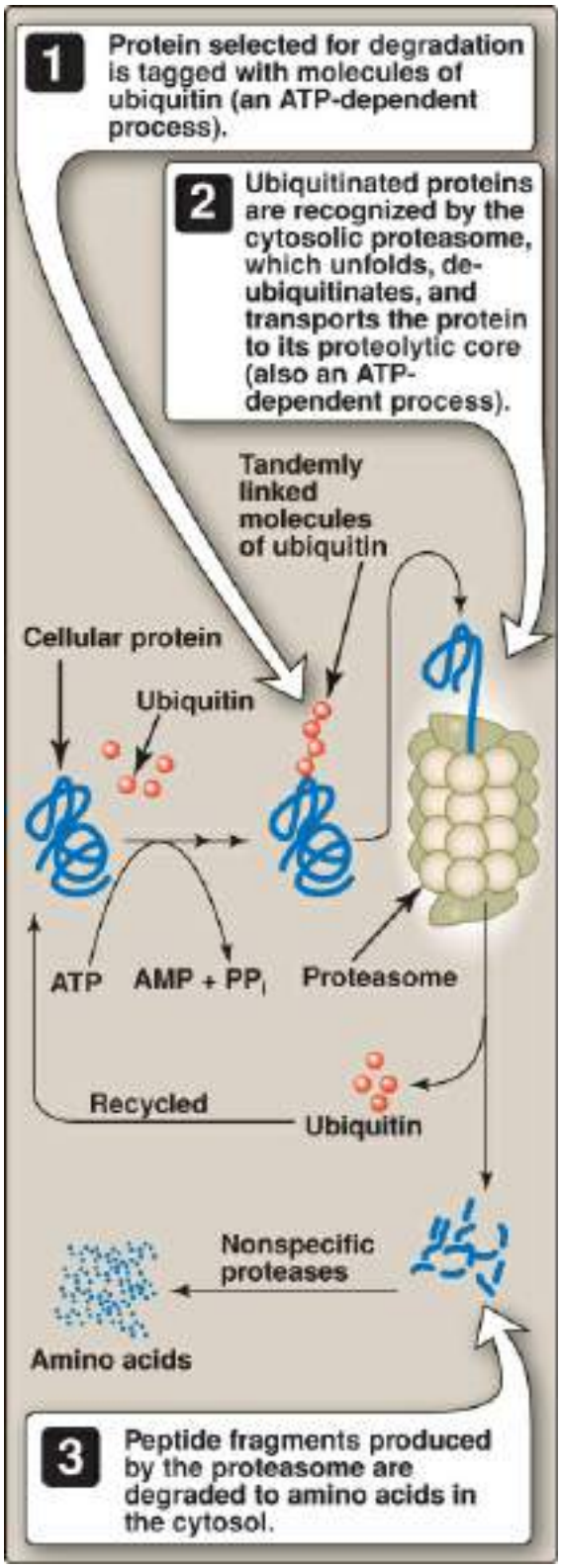


Figure 19.3
The ubiquitin–proteasome degradation pathway of proteins. AMP = adenosine monophosphate; PP_i = pyrophosphate.

III. DIETARY PROTEIN DIGESTION

Most of the nitrogen in the diet is consumed in the form of protein, typically amounting to 70 to 100 g/day in the American diet (Fig. 19.2). Proteins are generally too large to be absorbed by the intestine. (Note: An example of an exception to this rule is that newborns can take up maternal antibodies in breast milk.) Therefore, proteins must be hydrolyzed to yield di- and tripeptides as well as individual amino acids, which can be absorbed. Proteolytic enzymes responsible for degrading proteins are produced by three different organs: the stomach, the pancreas, and the small intestine (Fig. 19.4).

A. Digestion by gastric secretion

The digestion of proteins begins in the stomach, which secretes gastric juice, a unique solution containing hydrochloric acid (HCl) and the proenzyme pepsinogen.

1. Hydrochloric acid: Stomach HCl is too dilute (pH 2 to 3) to hydrolyze proteins. The acid, secreted by the parietal cells of the stomach, functions instead to kill some bacteria and to denature proteins, thereby making them more susceptible to subsequent hydrolysis by proteases.

2. Pepsin: This acid-stable endopeptidase is secreted by the chief cells of the stomach as an inactive zymogen (or proenzyme), pepsinogen. (Note: In general, zymogens contain extra amino acids in their sequences that prevent them from being catalytically active. Removal of these amino acids permits the proper folding required for an active enzyme.) In the presence of HCl, pepsinogen undergoes a conformational change that allows it to cleave itself (autocatalysis) to the active form, pepsin, which releases polypeptides and a few free amino acids from dietary proteins.

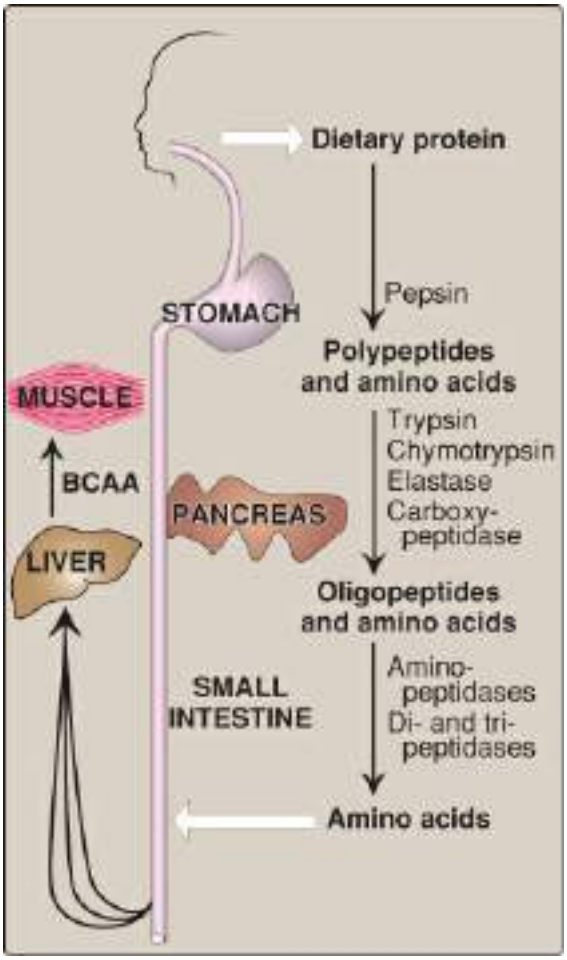


Figure 19.4
Digestion of dietary proteins by the proteolytic enzymes of the gastrointestinal tract. BCAA = branched chain amino acids.

B. Digestion by pancreatic enzymes

On entering the small intestine, the polypeptides produced in the stomach by the action of pepsin are further cleaved to oligopeptides and amino acids by a group of pancreatic proteases that include both endopeptidases (that cleave within) and exopeptidases (that cut at an end). (Note: Bicarbonate [HCO_3^-], secreted by the pancreas in response to the intestinal hormone secretin, raises the intestinal pH.)

1. Specificity: Each of these enzymes has a different specificity for the amino acid R-groups adjacent to the susceptible peptide bond (Fig. 19.5). For example, trypsin cleaves only when the carbonyl group of the peptide bond is contributed by arginine or lysine. These enzymes, like pepsin described above, are synthesized and secreted as inactive zymogens.

2. Zymogen release: The release and activation of the pancreatic zymogens are mediated by the secretion of cholecystokinin, a polypeptide hormone of the small intestine (see p. 194).

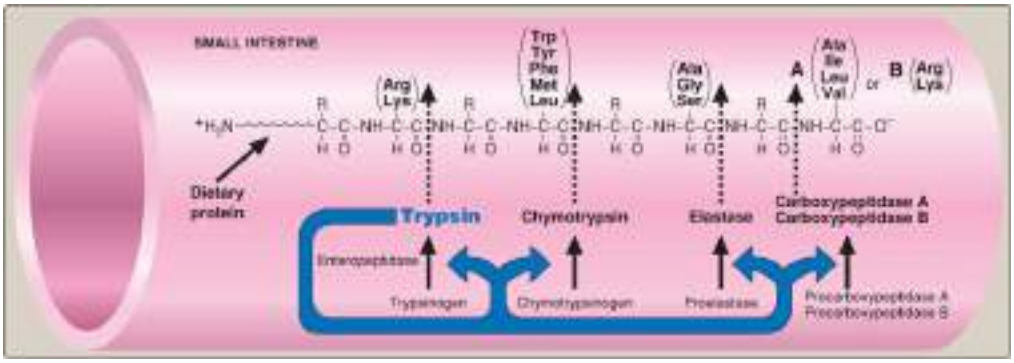


Figure 19.5
Cleavage of dietary protein in the small intestine by pancreatic proteases. The peptide bonds susceptible to hydrolysis are shown for each of the five major pancreatic proteases. (Note: The first three are serine endopeptidases, whereas the last two are exopeptidases. Each is produced from an inactive zymogen.)

3. Zymogen activation: Enteropeptidase (also called enterokinase), a serine protease synthesized by and present on the luminal (apical) surface of intestinal mucosal cells (enterocytes) of the brush border, converts the pancreatic zymogen trypsinogen to trypsin by removal of a hexapeptide from the N-terminus of trypsinogen. Trypsin subsequently converts other trypsinogen molecules to trypsin by cleaving a limited number of specific peptide bonds in the zymogen. Thus, enteropeptidase unleashes a cascade of proteolytic activity because trypsin is the common activator of all the pancreatic zymogens (Fig. 19.5).

4. Digestion abnormalities: In individuals with a deficiency in pancreatic secretion (e.g., because of chronic pancreatitis, cystic fibrosis, or surgical removal of the pancreas), the digestion and absorption of fat and protein are incomplete. This results in the abnormal appearance of lipids in the feces (a condition called steatorrhea; see p. 196) as well as undigested protein.

Celiac disease (celiac sprue) is a disease of malabsorption resulting from immune-mediated damage to the small intestine in response to ingestion of gluten (or gliadin produced from gluten), a protein found in wheat, barley, and rye.

C. Digestion of oligopeptides by small intestine enzymes

The luminal surface of the enterocytes contains aminopeptidase, an exopeptidase that repeatedly cleaves the N-terminal residue from oligopeptides to produce even smaller peptides and free amino acids.

D. Amino acid and small peptide intestinal absorption

Most free amino acids are taken into enterocytes via sodium-dependent secondary active transport by solute carrier (SLC) proteins of the apical membrane. At least

seven different transport systems with overlapping amino acid specificities are known. Di- and tripeptides, however, are taken up by a proton-linked peptide transporter (PepT1). The peptides are then hydrolyzed to free amino acids. Regardless of their source, free amino acids are released from enterocytes into the portal system by sodium-independent transporters of the basolateral membrane. Therefore, only free amino acids are found in the portal vein after a meal containing protein. These amino acids are either metabolized by the liver or released into the general circulation. (Note: Branched-chain amino acids [BCAAs] are not metabolized by the liver but, instead, are sent from the liver to muscle via the blood [Fig. 19.4].)

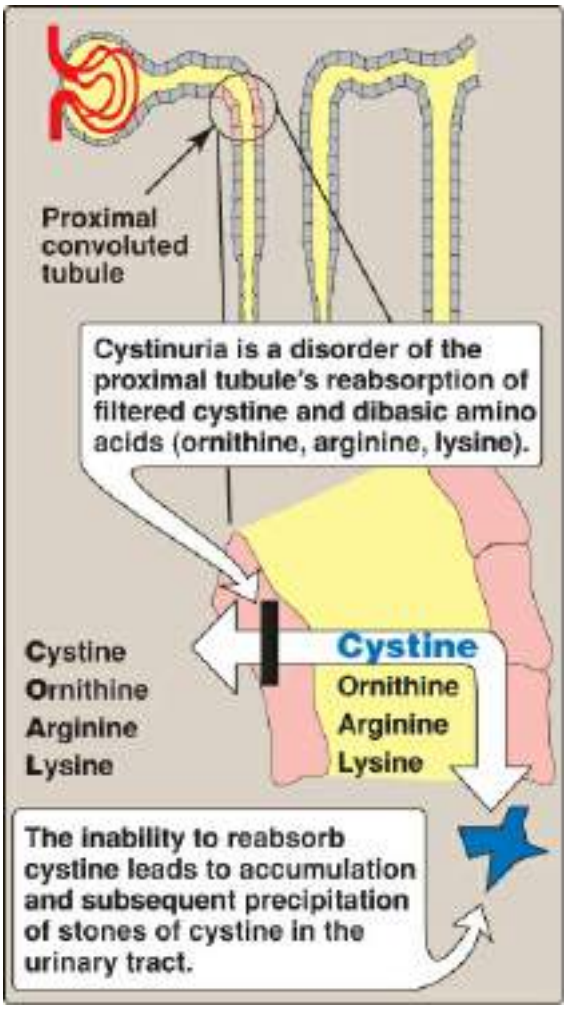


Figure 19.6
Genetic defect seen in cystinuria. (Note: Cystinuria is distinct from cystinosis, a rare defect in the transport of cystine out of lysosomes that results in the formation of cystine crystals within the lysosome and widespread tissue damage.)

E. Absorption abnormalities

The small intestine and the proximal tubules of the kidneys have common transport

systems for amino acid uptake. Consequently, a defect in any one of these systems results in an inability to absorb particular amino acids into the intestine and into the kidney tubules. For example, one system is responsible for the uptake of cystine and the dibasic amino acids ornithine, arginine, and lysine (represented as COAL). In the inherited disorder cystinuria, this carrier system is defective, and all four amino acids appear in the urine (Fig. 19.6). Cystinuria occurs at a frequency of 1 in 7,000 individuals, making it one of the most common inherited diseases and the most common genetic error of amino acid transport. The disease expresses itself clinically by the precipitation of cystine to form kidney stones (calculi), which can block the urinary tract. Oral hydration is an important part of treatment for this disorder. (Note: Defects in the uptake of tryptophan by a neutral amino acid transporter can result in Hartnup disorder and pellagra-like [see p. 430] dermatologic and neurologic symptoms.)

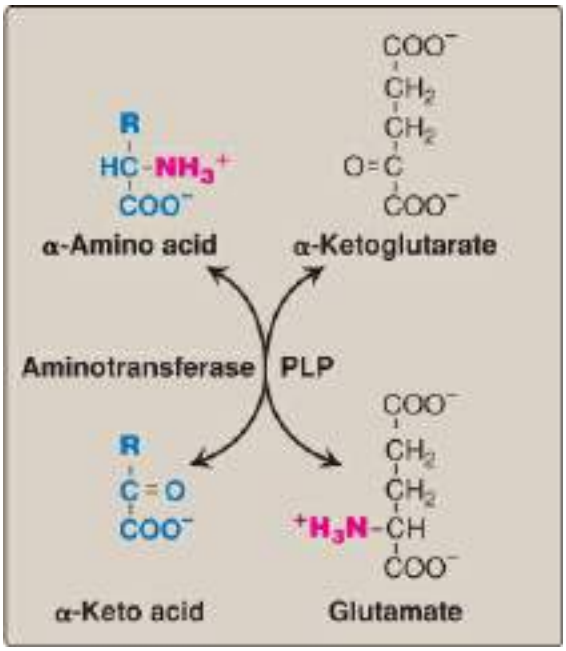


Figure 19.7
Aminotransferase reaction using α-ketoglutarate as the amino group acceptor. PLP = pyridoxal phosphate.

IV. NITROGEN REMOVAL FROM AMINO ACIDS

The presence of the α-amino group keeps amino acids safely locked away from oxidative breakdown. Removing the α-amino group is essential for producing energy from any amino acid and is an obligatory step in the catabolism of all amino acids. Once removed, this nitrogen can be incorporated into other compounds or excreted as urea, with the carbon skeletons being metabolized. This section describes transamination and oxidative deamination, reactions that ultimately provide ammonia and aspartate, the two sources of urea nitrogen (see p. 279).

A. Transamination: funneling amino groups to form glutamate

The first step in the catabolism of most amino acids is the transfer of their α-amino group to α-ketoglutarate (Fig. 19.7), producing an α-keto acid (derived from the original amino acid) and glutamate (derived from α-ketoglutarate). The citric acid cycle ketoacid intermediate α-ketoglutarate plays a pivotal role in amino acid metabolism by accepting the amino groups from most amino acids, thereby becoming its structurally related amino acid, glutamate. Glutamate produced by transamination can be oxidatively deaminated (see B. below) or used as an amino group donor in the synthesis of nonessential amino acids. This transfer of amino groups from one carbon skeleton to another is catalyzed by a family of readily reversible enzymes called aminotransferases (also called transaminases). These enzymes are found in the cytosol and mitochondria of cells throughout the body. All amino acids, with the exception of lysine and threonine, participate in transamination at some point in their catabolism. (Note: These two amino acids lose their α-amino groups by deamination [see pp. 294 and 295].)

1. Substrate specificity: Each aminotransferase is specific for one or, at most, a few amino group donors. Aminotransferases are named after the specific amino group donor, because the acceptor of the amino group is almost always α-ketoglutarate. Two important aminotransferase reactions are catalyzed by alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as shown in Figure 19.8. All aminotransferases require the coenzyme pyridoxal phosphate (a derivative of vitamin B_6; see p. 428), which is covalently linked to the ε-amino group of a specific lysine residue at the active site of the enzyme.

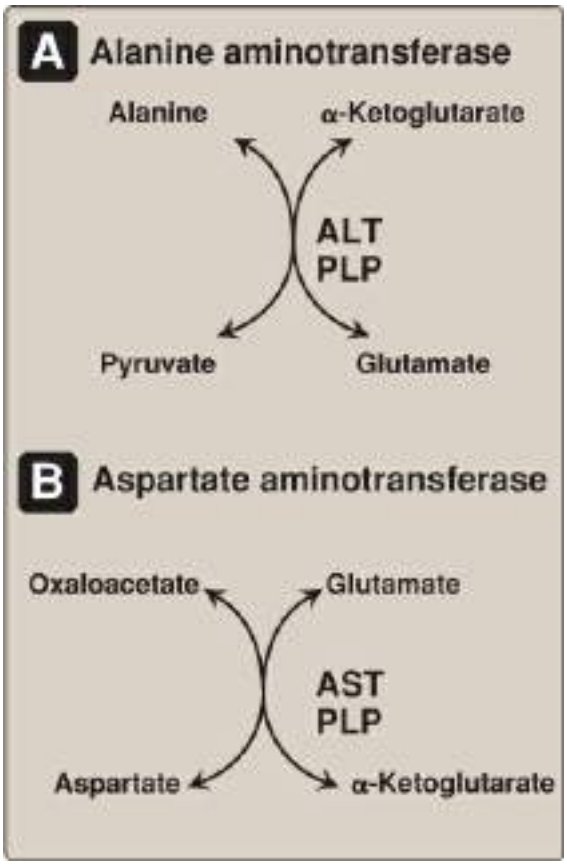


Figure 19.8
Reactions catalyzed during amino acid catabolism. A: Alanine aminotransferase (ALT). B: Aspartate aminotransferase (AST). PLP = pyridoxal phosphate.

a. Alanine aminotransferase: ALT is present in many tissues. The enzyme catalyzes the transfer of the amino group of alanine to α-ketoglutarate, resulting in the formation of pyruvate and glutamate. The reaction is readily reversible. However, during amino acid catabolism, this enzyme (like most aminotransferases) functions in the direction of glutamate synthesis. (Note: In effect, glutamate acts as a collector of nitrogen from most amino acids.)

b. Aspartate aminotransferase: AST is an exception to the rule that aminotransferases funnel amino groups to form glutamate. During amino acid catabolism, AST primarily transfers amino groups from glutamate to oxaloacetate, forming α-ketoglutarate and aspartate, respectively. Aspartate is used as a source of nitrogen in the urea cycle (see p. 281). Like other transaminations, the AST reaction is reversible.

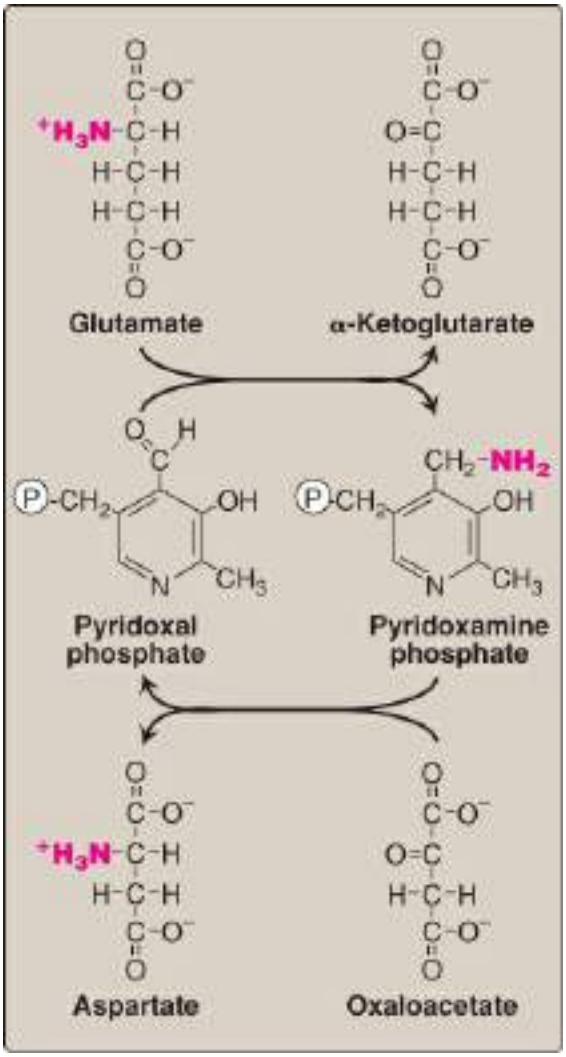


Figure 19.9
Cyclic interconversion of pyridoxal phosphate and pyridoxamine phosphate during the aspartate aminotransferase reaction. Ⓟ = phosphate group.

2. Mechanism: Figure 19.9 shows the mechanistic reactions for the transamination catalyzed by AST. Aminotransferases act by transferring the amino group of an amino acid substrate (glutamate) to the pyridoxal part of the coenzyme to generate pyridoxamine phosphate. The amino acid substrate glutamate is thus converted to an α-keto acid product (α-ketoglutarate). The pyridoxamine form of the coenzyme then reacts with an α-keto acid substrate (oxaloacetate) to form an amino acid product (aspartate), at the same time regenerating the original aldehyde form of the coenzyme.

3. Equilibrium: For most transamination reactions, the equilibrium constant is near 1. This allows the reaction to function in both amino acid degradation through removal of α-amino groups (e.g., after consumption of a protein-rich meal) and biosynthesis of nonessential amino acids through addition of amino groups to the carbon skeletons of α-keto acids (e.g., when the supply of amino acids from

the diet is not adequate to meet the synthetic needs of cells).

4. Diagnostic value: Aminotransferases are normally intracellular enzymes, with the low levels found in the plasma representing the release of cellular contents during normal cell turnover. Elevated plasma levels of aminotransferases indicate damage to cells rich in these enzymes. For example, physical trauma or a disease process can cause cell lysis, resulting in release of intracellular enzymes into the blood. Two aminotransferases, AST and ALT, are of particular diagnostic value when they are found in the plasma.

   a. Hepatic disease: Plasma AST and ALT are elevated in nearly all hepatic diseases but are particularly high in conditions that cause extensive cell necrosis, such as severe viral hepatitis, toxic injury, and prolonged circulatory collapse. ALT is more specific than AST for liver disease, but the latter is more sensitive because the liver contains larger amounts of AST. Serial measurements of AST and ALT (liver function tests) are often useful in determining the course of liver damage. Figure 19.10 shows the early release of ALT into the blood, following ingestion of a liver toxin. (Note: The elevation in bilirubin results from hepatocellular damage that decreases the hepatic conjugation and excretion of bilirubin [see p. 316].)

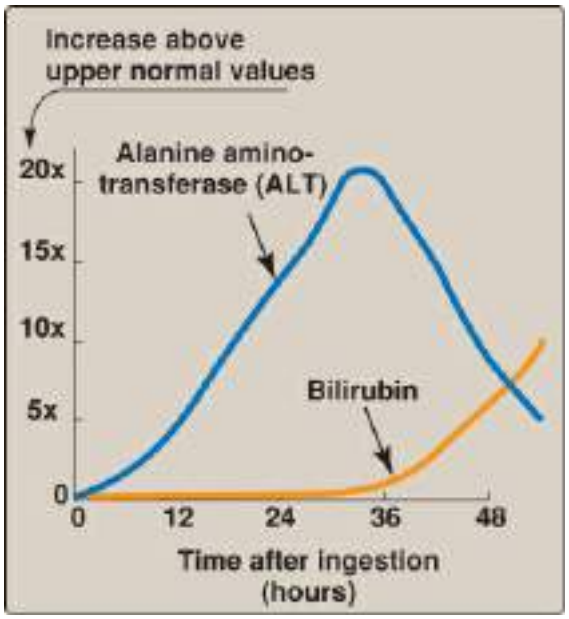


Figure 19.10
Pattern of ALT and bilirubin in the plasma, following poisoning by ingestion of the toxic mushroom *Amanita phalloides*.

   b. Nonhepatic disease: Aminotransferases may be elevated in nonhepatic diseases such as those that cause damage to cardiac or skeletal muscle. However, these disorders can usually be distinguished clinically from liver disease using additional lab tests. When muscle damage is suspected, creatine kinase, lactate dehydrogenase, and myoglobin plasma levels, in

addition to AST and ALT levels, may also be increased. Blood urea nitrogen, bilirubin, γ-glutamyl transferase (GGT) and alkaline phosphatase (ALP) levels would be in the normal range. If bone disease is suspected, ALP levels will be disproportionately higher than the AST, ALT, and GGT levels.

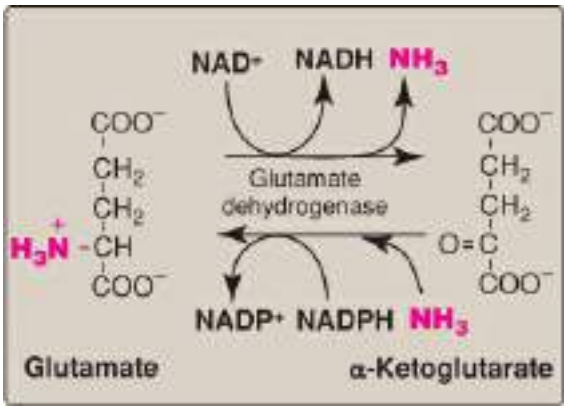


Figure 19.11
Oxidative deamination by glutamate dehydrogenase. (Note: The enzyme is unusual in that it uses both nicotinamide adenine dinucleotide [NAD^+] and nicotinamide adenine dinucleotide phosphate [NADPH].) NH_3 = ammonia.

B. Oxidative deamination: amino group removal

In contrast to transamination reactions that transfer amino groups, oxidative deamination reactions result in the liberation of the amino group as free ammonia (Fig. 19.11). These reactions occur primarily in the liver and kidney. They provide α-keto acids that can enter the central pathways of energy metabolism and ammonia, which is a source of nitrogen in hepatic urea synthesis. (Note: Ammonia exists primarily as ammonium [NH_4^+] in aqueous solution, but it is the unionized form [NH_3] that crosses membranes.)

1. Glutamate dehydrogenase: As described above, the amino groups of most amino acids are ultimately funneled to glutamate by means of transamination with α-ketoglutarate. Glutamate is unique in that it is the only amino acid that undergoes rapid oxidative deamination, a reaction catalyzed by glutamate dehydrogenase ([GDH], Fig. 19.11). Therefore, the sequential action of transamination (resulting in the transfer of amino groups from most amino acids to α-ketoglutarate to produce glutamate) and the oxidative deamination of that glutamate (regenerating α-ketoglutarate) provide a pathway whereby the amino groups of most amino acids can be released as ammonia.

a. Coenzymes: GDH, a mitochondrial enzyme, is unusual in that it can use either nicotinamide adenine dinucleotide (NAD^+) or its phosphorylated reduced form (NADPH) as a coenzyme (Fig. 19.11). NAD^+ is used primarily in oxidative deamination (the simultaneous loss of ammonia coupled with the oxidation of the carbon skeleton, as shown in Fig. 19.12A), whereas NADPH

is used in reductive amination (the simultaneous gain of ammonia coupled with the reduction of the carbon skeleton, as shown in Fig. 19.12B).

b. Reaction direction: The direction of the reaction depends on the relative concentrations of glutamate, α-ketoglutarate, and ammonia and the ratio of oxidized to reduced coenzymes. For example, after ingestion of a meal containing protein, glutamate levels in the liver are elevated, and the reaction proceeds in the direction of amino acid degradation and the formation of ammonia (Fig. 19.12A). High ammonia levels are required to drive the reaction to glutamate synthesis.

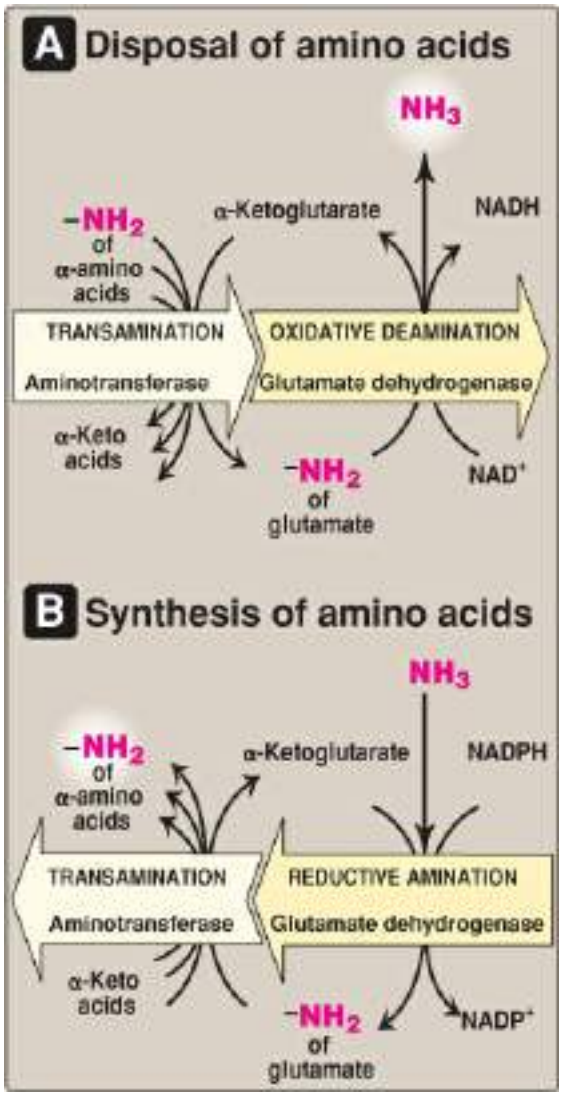


Figure 19.12
A, B: Combined actions of aminotransferase and glutamate dehydrogenase reactions. (Note: Reductive amination occurs only when ammonia [NH_3] level is high.) NAD(H) = nicotinamide adenine dinucleotide; NADP(H) = nicotinamide adenine dinucleotide phosphate.

c. Allosteric regulators: Guanosine triphosphate is an allosteric inhibitor of GDH, whereas adenosine diphosphate is an activator. Therefore, when energy levels are low in the cell, amino acid degradation by GDH is high, facilitating energy production from the carbon skeletons derived from amino acids.

2. D-Amino acid oxidase: D-Amino acids (see p. 5) are supplied by the diet but are not used in the synthesis of mammalian proteins. They are, however, efficiently metabolized to α-keto acids, ammonia, and hydrogen peroxide in the peroxisomes of liver and kidney cells by flavin adenine dinucleotide–dependent D-amino acid oxidase (DAO). The α-keto acids can enter the general pathways of amino acid metabolism and be reaminated to L-isomers or catabolized for energy. (Note: DAO degrades D-serine, the isomeric form of serine that modulates N-methyl-D-aspartate [NMDA]-type glutamate receptors. Increased DAO activity has been linked to increased susceptibility to schizophrenia. DAO also converts glycine to glyoxylate [see p. 292].) L-Amino acid oxidases are found in snake venom.

C. Ammonia transport to the liver

Two mechanisms are available in humans for the transport of ammonia from peripheral tissues to the liver for conversion to urea. Both are important in, but not exclusive to, skeletal muscle. The first uses glutamine synthetase to combine ammonia with glutamate to form glutamine, a nontoxic transport form of ammonia (Fig. 19.13). The glutamine is transported in the blood to the liver where it is cleaved by glutaminase to glutamate and ammonia (see p. 283). The glutamate is oxidatively deaminated to ammonia and α-ketoglutarate by GDH. The ammonia is converted to urea. The second transport mechanism involves the formation of alanine by the transamination of pyruvate produced from both aerobic glycolysis and metabolism of the succinyl coenzyme A (CoA) generated by the catabolism of the BCAA isoleucine and valine. Alanine is transported in the blood to the liver, where it is transaminated by ALT to pyruvate. The pyruvate is used to synthesize glucose, which can enter the blood and be used by muscle, a pathway called the glucose–alanine cycle. The glutamate product of ALT can be deaminated by GDH, generating ammonia. Thus, both alanine and glutamine carry ammonia to the liver.

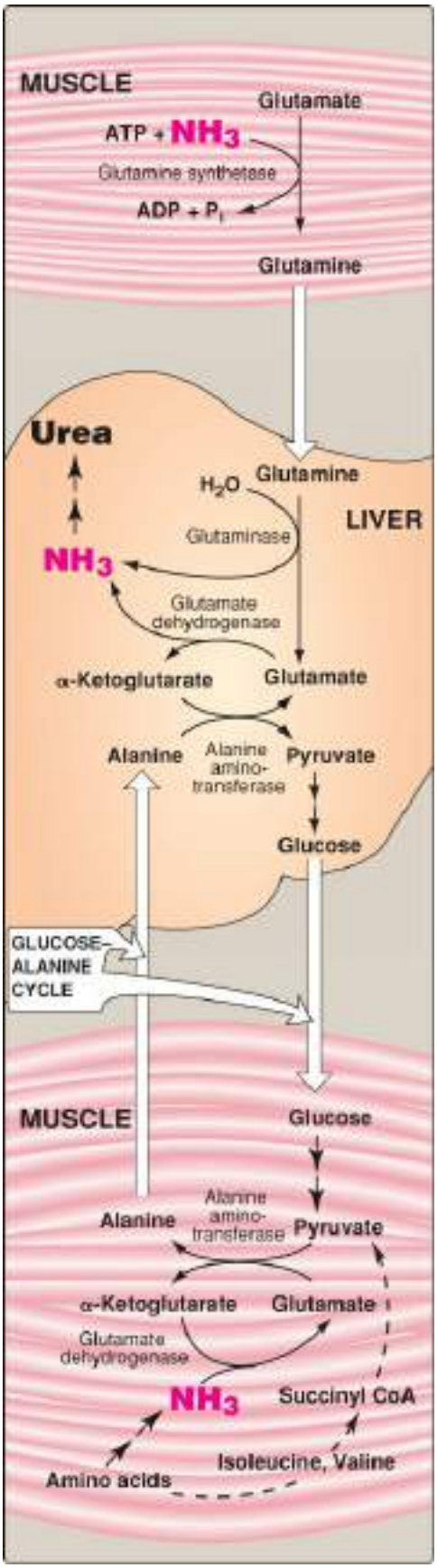


Figure 19.13
Transport of ammonia (NH_3) from muscle to the liver. ADP = adenosine diphosphate; P_i = inorganic phosphate; CoA = coenzyme A.

V. UREA CYCLE

Urea (H_2NCNH_2, with O) is the major disposal form of amino groups derived from amino acids and accounts for ~90% of the nitrogen-containing components of urine. One nitrogen of the urea molecule is supplied by free ammonia and the other nitrogen by aspartate. (Note: Glutamate is the immediate precursor of both ammonia [through oxidative deamination by GDH] and aspartate nitrogen [through transamination of oxaloacetate by AST].) The carbon and oxygen of urea are derived from CO_2 (as HCO_3^-). Urea is produced by the liver and then is transported in the blood (blood urea nitrogen) to the kidneys for excretion in the urine.

A. Reactions

The first two reactions leading to the synthesis of urea occur in the mitochondrial matrix, whereas the remaining cycle enzymes are located in the cytosol (Fig. 19.14). (Note: Gluconeogenesis [see p. 128] and heme synthesis [see p. 309] also involve both the mitochondrial matrix and the cytosol.)

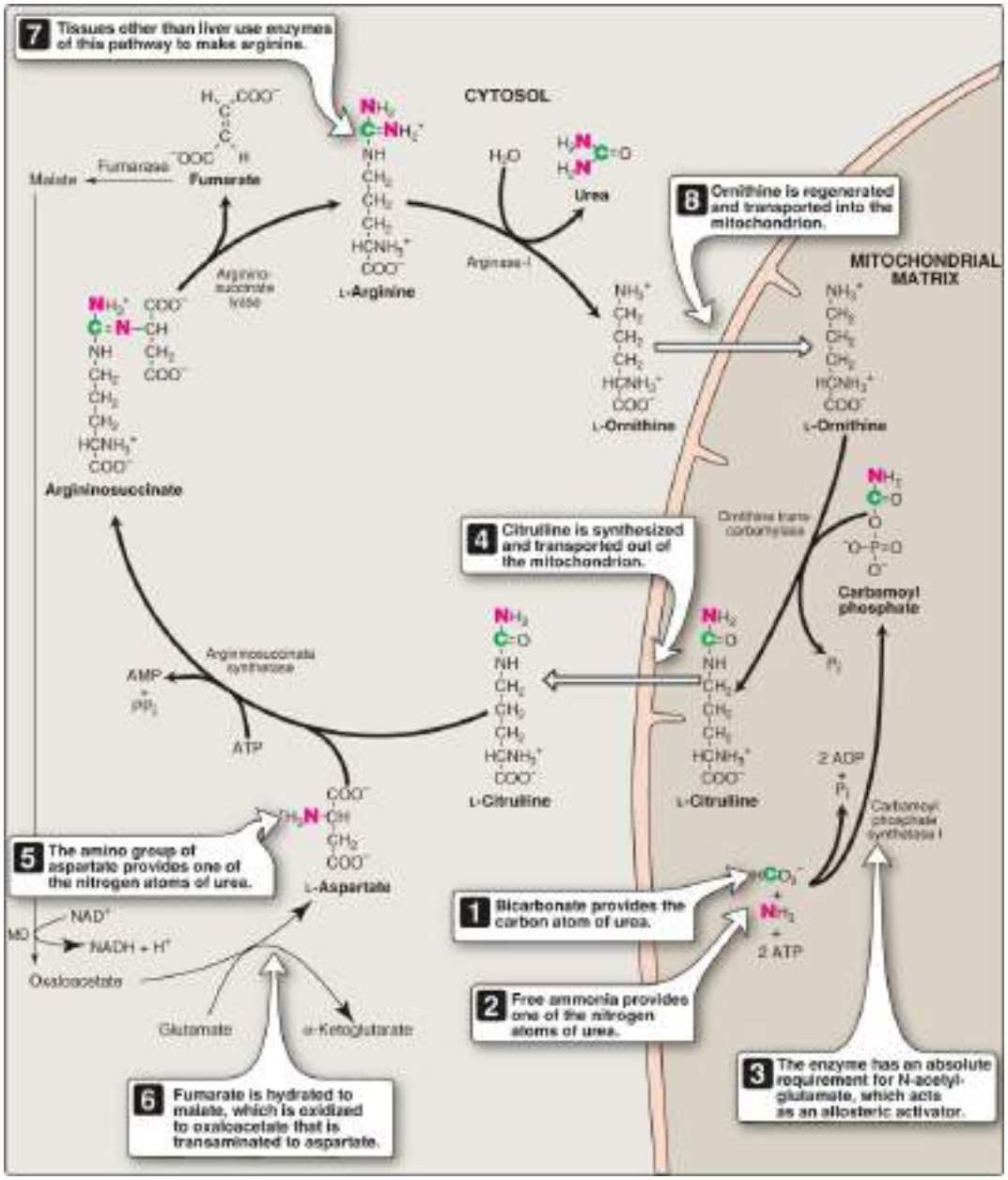


Figure 19.14
Reactions of the urea cycle. (Note: An antiporter transports citrulline and ornithine across the inner mitochondrial membrane.) ADP = adenosine diphosphate; AMP = adenosine monophosphate; PP_i = pyrophosphate; P_i = inorganic phosphate; NAD(H) = nicotinamide adenine dinucleotide; MD = malate dehydrogenase.

1. Carbamoyl phosphate formation: Formation of carbamoyl phosphate by carbamoyl phosphate synthetase I (CPS I) is driven by cleavage of two molecules of ATP. Ammonia incorporated into carbamoyl phosphate is provided primarily by the oxidative deamination of glutamate by mitochondrial GDH (Fig. 19.11). Ultimately, the nitrogen atom derived from this ammonia becomes one of the nitrogens of urea. CPS I requires N-acetylglutamate (NAG) as a positive allosteric activator (Fig. 19.14). (Note: Carbamoyl phosphate synthetase II

participates in the biosynthesis of pyrimidines [see p. 335]. It does not require NAG, uses glutamine as the nitrogen source, and occurs in the cytosol.)

2. Citrulline formation: The carbamoyl portion of carbamoyl phosphate is transferred to ornithine by ornithine transcarbamylase (OTC) as the phosphate is released as inorganic phosphate. The reaction product, citrulline, is transported to the cytosol. (Note: Ornithine and citrulline move across the inner mitochondrial membrane via an antiporter. These basic amino acids are not incorporated into cellular proteins because there are no codons for them [see p. 496].) Ornithine is regenerated with each turn of the urea cycle, much in the same way that oxaloacetate is regenerated by the reactions of the tricarboxylic acid (TCA) cycle (see p. 120).

3. Argininosuccinate formation: Argininosuccinate synthetase combines citrulline with aspartate to form argininosuccinate. The α-amino group of aspartate provides the second nitrogen that is ultimately incorporated into urea. The formation of argininosuccinate is driven by the cleavage of ATP to adenosine monophosphate and pyrophosphate. This is the third and final molecule of ATP consumed in the formation of urea.

4. Argininosuccinate cleavage: Argininosuccinate is cleaved by argininosuccinate lyase to yield arginine and fumarate. The arginine serves as the immediate precursor of urea. The fumarate is hydrated to malate, providing a link with several metabolic pathways. Malate can be oxidized by malate dehydrogenase to oxaloacetate, which can be transaminated to aspartate (Fig. 19.8) and enter the urea cycle (Fig. 19.14). Alternatively, malate can be transported into mitochondria via the malate–aspartate shuttle (see p. 87), reenter the TCA cycle, and get oxidized to oxaloacetate, which can be used for gluconeogenesis (see p. 131). (Note: Malate oxidation generates NADH for oxidative phosphorylation [see p. 84], thereby reducing the energy cost of the urea cycle.)

5. Arginine cleavage to ornithine and urea: Arginase-I hydrolyzes arginine to ornithine and urea and is virtually exclusive to the liver. Therefore, only the liver can cleave arginine, thereby synthesizing urea, whereas other tissues, such as the kidney, can synthesize arginine from citrulline. (Note: Arginase-II in kidneys controls arginine availability for nitric oxide synthesis [see p. 165].)

6. Fate of urea: Urea diffuses from the liver and is transported in the blood to the kidneys, where it is filtered and excreted in the urine (Fig. 19.19). A portion of the urea diffuses from the blood into the intestine and is cleaved to CO_2 and ammonia by bacterial urease. The ammonia is partly lost in the feces and is partly reabsorbed into the blood. In patients with kidney failure, plasma urea levels are elevated, promoting a greater transfer of urea from blood into the gut. The intestinal action of urease on this urea becomes a clinically important source of ammonia, contributing to the hyperammonemia often seen in these patients. Oral administration of antibiotics reduces the number of intestinal

bacteria responsible for this ammonia production.

B. Overall stoichiometry

$$\text{Aspartate} + NH_3 + HCO_3^- + 3ATP + H_2O \rightarrow$$
$$\text{urea} + \text{fumarate} + 2ADP + AMP + 2P_i + PP_i$$

Because four high-energy phosphate bonds are consumed in the synthesis of each molecule of urea, the synthesis of urea is irreversible, with a large, negative ΔG (see p. 78). One nitrogen of the urea molecule is supplied by free ammonia and the other nitrogen by aspartate. Glutamate is the immediate precursor of both ammonia (through oxidative deamination by GDH) and aspartate nitrogen (through transamination of oxaloacetate by AST). In effect, both nitrogen atoms of urea arise from glutamate, which, in turn, gathers nitrogen from other amino acids (Fig. 19.15).

C. Regulation

NAG is an essential activator for CPS I, the rate-limiting step in the urea cycle. It increases the affinity of CPS I for ATP. NAG is synthesized from acetyl CoA and glutamate by N-acetylglutamate synthase (NAGS), as shown in Figure 19.16, in a reaction for which arginine is an activator. The cycle is also regulated by substrate availability (short-term regulation) and enzyme induction (long term).

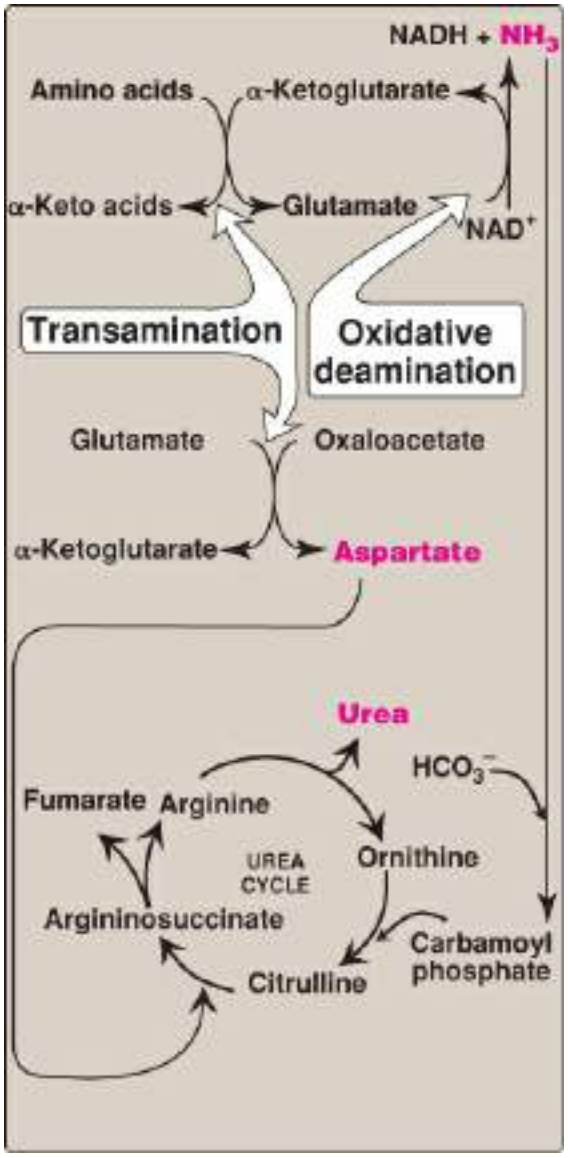


Figure 19.15
Flow of nitrogen from amino acids to urea. Amino groups for urea synthesis are collected in the form of ammonia (NH_3) and aspartate. NAD(H) = nicotinamide adenine dinucleotide; HCO_3^- = bicarbonate.

VI. AMMONIA METABOLISM

Ammonia is produced by all tissues during the metabolism of a variety of compounds, and it is disposed of primarily by formation of urea in the liver. However, the blood ammonia level must be kept very low, because even slightly elevated concentrations (hyperammonemia) are toxic to the central nervous system (CNS). Therefore, a mechanism is required for the transport of nitrogen from the peripheral tissues to the liver for ultimate disposal as urea while keeping circulating levels of free ammonia low.

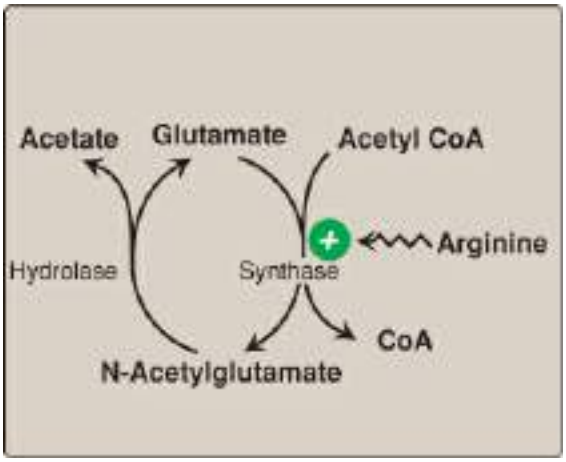


Figure 19.16
Formation and degradation of N-acetylglutamate, an allosteric activator of carbamoyl phosphate synthetase I. CoA = coenzyme A.

A. Sources

Amino acids are quantitatively the most important source of ammonia because most Western diets are high in protein and provide excess amino acids, which travel to the liver and undergo transdeamination (i.e., the linking of the aminotransferase and GDH reactions), producing ammonia. (Note: The liver catabolizes straight-chain amino acids, primarily.) However, substantial amounts of ammonia can be obtained from other sources.

1. Glutamine: An important source of plasma glutamine is from the catabolism of BCAA in skeletal muscle. This glutamine is taken up by cells of the intestine, the liver, and the kidneys. The liver and kidneys generate ammonia from glutamine by the actions of glutaminase (Fig. 19.17) and GDH. In the kidneys, most of this ammonia is excreted into the urine as NH_4^+, which provides an important mechanism for maintaining the body's acid–base balance through the excretion of protons. In the liver, the ammonia is detoxified to urea and excreted. (Note: α-Ketoglutarate, the second product of GDH, is a glucogenic precursor in the liver and kidneys.) Ammonia is also generated by intestinal glutaminase. Enterocytes obtain glutamine either from the blood or from digestion of dietary protein. (Note: Intestinal glutamine metabolism also produces alanine, which is used by the liver for gluconeogenesis, and citrulline, which is used by the kidneys to synthesize arginine.)

2. Intestinal bacteria: Ammonia is formed from urea by the action of bacterial urease in the lumen of the intestine. This ammonia is absorbed from the intestine by way of the portal vein, and virtually all is removed by the liver via conversion to urea.

3. Amines: Amines obtained from the diet and monoamines that serve as hormones or neurotransmitters give rise to ammonia by the action of

monoamine oxidase (see p. 318).

4. Purines and pyrimidines: In the catabolism of purines and pyrimidines, amino groups attached to the ring atoms are released as ammonia (see Fig. 22.15, p. 333).

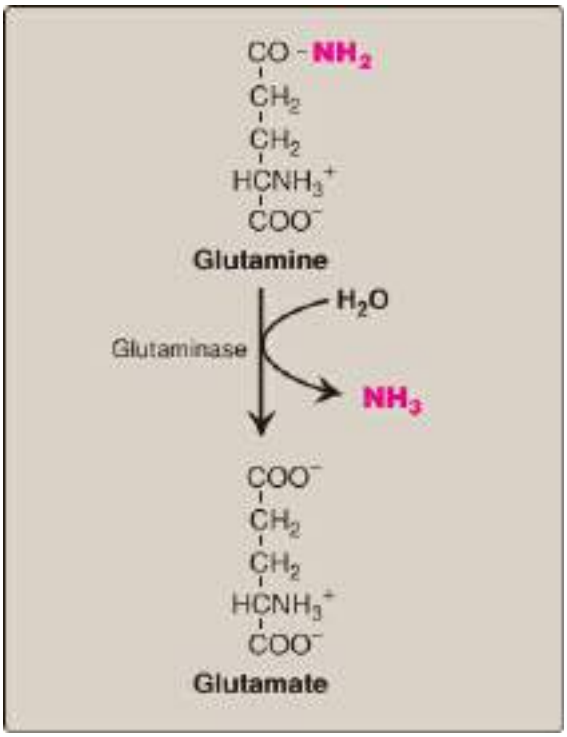


Figure 19.17
Hydrolysis of glutamine to form ammonia (NH_3).

B. Transport in the circulation

Although ammonia is constantly produced in the tissues, it is present at very low levels in blood. This is due both to the rapid removal of blood ammonia by the liver and to the fact that several tissues, particularly muscle, release amino acid nitrogen in the form of glutamine and alanine, rather than as free ammonia (Fig. 19.13).

1. Urea: Formation of urea in the liver is quantitatively the most important disposal route for ammonia. Urea travels in the blood from the liver to the kidneys, where it passes into the glomerular filtrate.

2. Glutamine: This amide of glutamate provides a nontoxic storage and transport form of ammonia (Fig. 19.18). The ATP-requiring formation of glutamine from glutamate and ammonia by glutamine synthetase occurs primarily in skeletal muscle and the liver but is also important in the CNS, where it is the major mechanism for the removal of ammonia in the brain. Glutamine is found in plasma at concentrations higher than other amino acids, a finding consistent with its transport function. (Note: The liver keeps blood ammonia levels low

through glutaminase, GDH, and the urea cycle in periportal [close to inflow of blood] hepatocytes and through glutamine synthetase as an ammonia scavenger in the perivenous hepatocytes.) Ammonia metabolism is summarized in Figure 19.19.

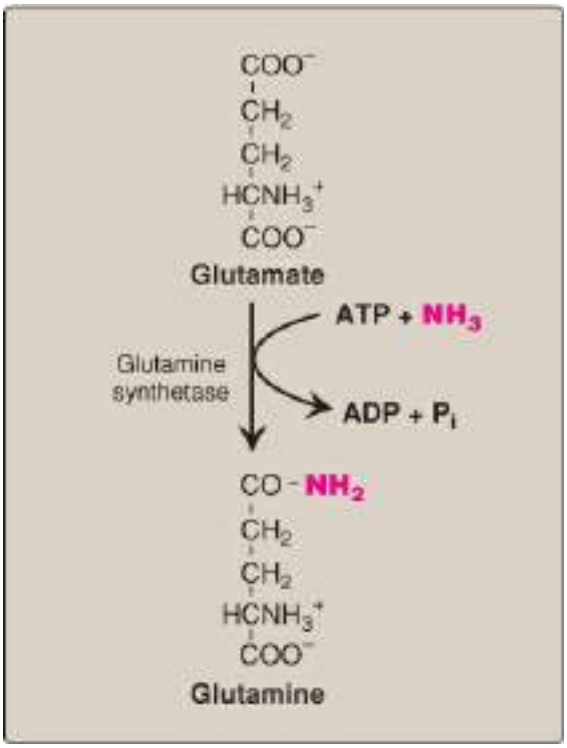


Figure 19.18
Synthesis of glutamine. ADP = adenosine diphosphate; P_i = inorganic phosphate; NH_3 = ammonia.

C. Hyperammonemia

The capacity of the hepatic urea cycle exceeds the normal rates of ammonia generation, and the levels of blood ammonia are normally low (5 to 35 µmol/l). However, when liver function is compromised, due to either genetic defects of the urea cycle or liver disease, blood levels can be >1,000 µmol/l. Such hyperammonemia is a medical emergency, because ammonia has a direct neurotoxic effect on the CNS. For example, elevated concentrations of ammonia in the blood cause the symptoms of ammonia intoxication, which include tremors, slurring of speech, somnolence (drowsiness), vomiting, cerebral edema, and blurring of vision. At high concentrations, ammonia can cause coma and death. There are two major types of hyperammonemia.

1. Acquired: Liver disease is a common cause of acquired hyperammonemia in adults and may be due, for example, to viral hepatitis or to hepatotoxins such as alcohol. Cirrhosis of the liver may result in formation of collateral circulation around the liver. As a result, portal blood is shunted directly into the systemic circulation and does not have access to the liver. Therefore, the conversion of

ammonia to urea is severely impaired, leading to elevated levels of ammonia.

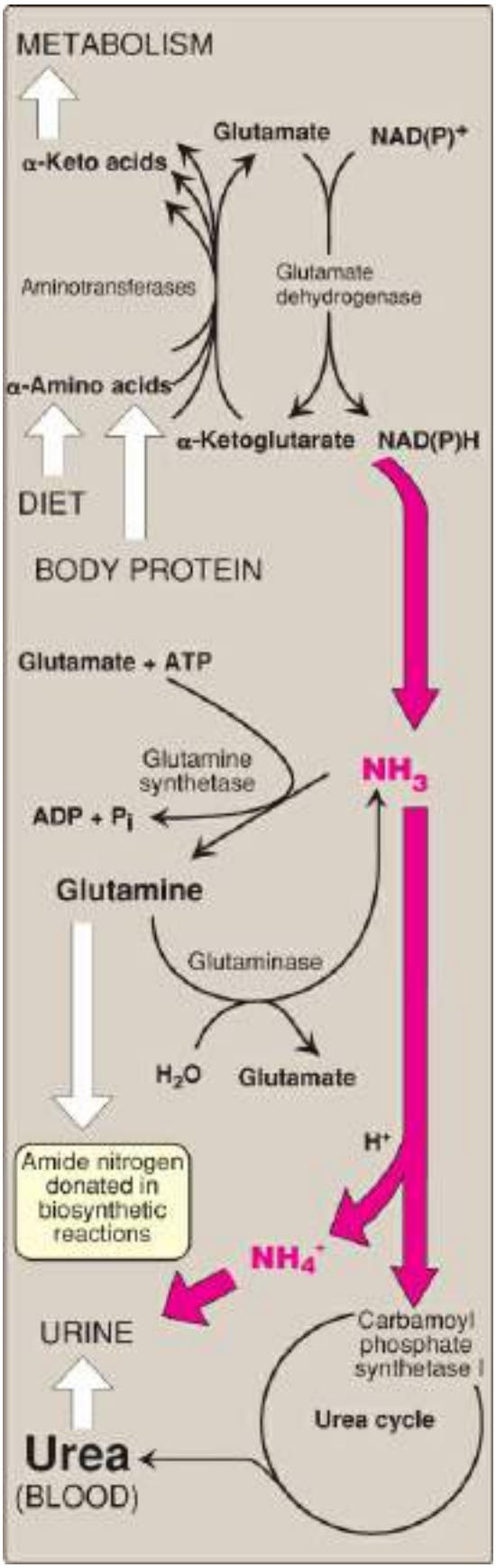


Figure 19.19
Ammonia (NH_3) metabolism. Urea content in the urine is reported as urinary urea nitrogen, or UUN. Urea in blood is reported as BUN (blood urea nitrogen). (Note: The enzymes glutamate dehydrogenase, glutamine synthetase, and carbamoyl phosphate synthetase I fix NH_3 into organic molecules.)

2. Congenital: Genetic deficiencies of each of the five enzymes of the urea cycle (and of NAGS) have been described, with an overall incidence of ~1:25,000 live births. OTC deficiency is X linked, predominantly affecting males, although female carriers may become symptomatic. All of the other urea cycle disorders follow an autosomal-recessive inheritance pattern. In each case, the failure to synthesize urea leads to hyperammonemia during the first weeks following birth. Combinations of other symptoms common to hyperammonemia (tremors, slurred speech, drowsiness, vomiting cerebral edema, blurred vision, intellectual and developmental disability, and in severe hyperammonemia, even coma and death) can also be seen in different urea cycle deficiencies. Diagnosis is based upon symptoms, laboratory testing, and genetic testing. Historically, congenital urea cycle defects have a high morbidity (neurologic manifestations) and mortality. Additional information for specific urea cycle deficiencies are summarized in the following sections.

a. Ornithine transcarbamylase deficiency: OTC deficiency is the most common urea cycle disorder. Specific laboratory test results include a decrease in reaction and the downstream products citrulline and arginine. Interestingly, there is also an increase in detectable serum and urinary orotic acid levels. Carbamoyl phosphate, one of the OTC substrates, instead becomes a substrate for pyrimidine biosynthesis, entering into the pathway downstream of the regulatory reaction (see Fig. 22.21, p. 336). As a result, orotic acid is an overproduced pyrimidine biosynthesis pathway intermediate. (Note: Elevated orotic acid is also seen in hereditary orotic aciduria, due to a pyrimidine biosynthesis enzyme deficiency in UMP synthase [UMPS]. Along with genetic testing, OTC deficiency can be differentially diagnosed from UMPS deficiency based on other symptoms. Hyperammonemia is a symptom of OTC deficiency, but not of UMPS deficiency; instead, megaloblastic anemia may be a symptom of UMPS deficiency.)

b. Argininosuccinate synthetase deficiency: This deficiency is also referred to as citrullinemia type 1, as there is an accumulation of the substrate for the reaction, citrulline, in blood and urine. There may be a neonatal acute (classic) form, a milder late-onset form, a form that begins during or after pregnancy, and an asymptomatic form. In the neonatal acute form, citrulline can be detected as part of newborn screening. This detection is critical to prevent hyperammonemia and brain damage.

c. Argininosuccinate lyase deficiency: In argininosuccinate lyase deficiency, there is an accumulation of the substrate for the reaction, argininosuccinate,

in the urine, resulting in argininosuccinic aciduria. This is diagnostic and part of the newborn screening. In more severe- and late-onset forms of the deficiency, the aciduria may be associated with neurologic abnormalities, developmental delays, and cognitive impairment.

d. Arginase-I deficiency: In arginase-I deficiency, there is an accumulation of the substrate for the reaction, arginine, in the blood and urine, and is often referred to as argininemia or hyperargininemia. The hyperammonemia seen with arginase deficiency is often less severe because arginine contains two waste nitrogens, and can be excreted in the urine. As such, patients with this deficiency may appear to be healthy at birth, and have normal development during the first 1 to 3 years. After this, the first symptoms of arginase deficiency may appear with apparent developmental delays, loss of developmental milestones, and intellectual disability. Hyperammonemia may be episodic, associated with high-protein meals or periods of stress, such as illness or fasting.

e. N-acetylglutamate synthase deficiency: Like the aginase-I deficiency, a deficiency in NAGS can result in developmental delays and intellectual disability. Less severe forms may episodic later in life, associated with periods of high-protein meals, stress, or fasting. Carglumic acid is an FDA-approved therapy for NAGS deficiency. It is a synthetic form of NAG, the positive allosteric activator of carbamoyl phosphate synthetase I.

f. Treatment for hyperammonemia: Treatment for urea cycle enzyme deficiencies involves both limiting protein intake in the diet in the presence of sufficient calories to prevent protein catabolism, and the removal of excess ammonia in the blood. This can vary depending on the enzyme deficiency and the defect severity. Patients adhere to a low-protein diet, with minimal protein levels needed to maintain good health. This can vary depending on the age and weight of the patient. Drinks with special formulas and/or medical foods can be purchased in which protein levels are tailored to the patient's needs. Nitrogen-scavenging medications, including the aromatic acids benzoate and phenylbutyrate, can reduce ammonia levels in the blood. Benzoate combines with glycine to form hippurate. Phenylbutyrate is converted to phenylacetate, and combines with glutamine to form phenylacetylglutamine (Fig. 19.20). Both end products, hippurate and phenylacetylglutamine, are readily excreted in the urine. The combined excretion of glycine and glutamine, and their subsequent biosynthesis, effectively lowers ammonia levels and the potential for hyperammonemia. During severe hyperammonemia, patients may also require dialysis, intravenous fluids, or other treatments to quickly reduce blood ammonia levels and prevent permanent brain damage.

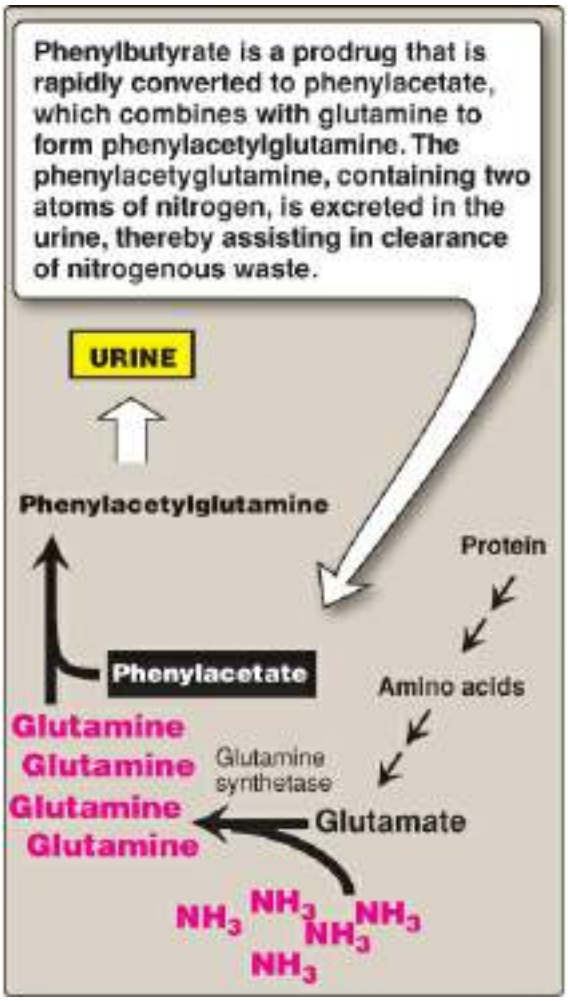


Figure 19.20
Treatment of patients with urea cycle defects by administration of phenylbutyrate to aid in excretion of ammonia (NH_3).

VII. Chapter Summary

- **Nitrogen enters** the body in a variety of compounds present in food, the most important being **amino acids** contained in **dietary protein**.
- **Nitrogen leaves** the body as **urea**, **ammonia**, and other products derived from amino acid metabolism (Fig. 19.21).
- Free amino acids in the body are produced by hydrolysis of dietary protein by **proteases** activated from their **zymogen** form in the stomach and intestine, degradation of tissue proteins, and *de novo* synthesis. This **amino acid pool** is consumed in the synthesis of body protein, metabolized for energy, or its members used as precursors for other nitrogen-containing compounds.
- Free amino acids from digestion are taken up by intestinal **enterocytes** via **sodium-dependent secondary active transport**. Small peptides are taken up via **proton-linked transport**.
- Body protein is simultaneously degraded and resynthesized, a process known as **protein turnover**. The concentration of a cellular protein may be determined by regulation of its synthesis or degradation. The ATP-dependent, cytosolic, selective **Ub–proteasome** and ATP-independent, relatively nonselective **lysosomal acid hydrolases** are the two major enzyme systems that are responsible for **degrading proteins**.
- Nitrogen cannot be stored, and amino acids in excess of the biosynthetic needs of the cell are quickly degraded. The first phase of **catabolism** involves the transfer of the α-amino groups through **transamination** by **pyridoxal phosphate**–dependent **aminotransferases** (**transaminases**), followed by **oxidative deamination of glutamate** by **GDH**, forming **ammonia** and the corresponding **α-keto acids**.
- A portion of the **free ammonia** is excreted in the **urine**. Some ammonia is used in converting glutamate to **glutamine** for safe transport, but most is used in the hepatic synthesis of **urea**, which is quantitatively the most important route for disposing of nitrogen from the body. **Alanine** also carries nitrogen to the liver for disposal as urea.
- The two major causes of **hyperammonemia** (with its neurologic effects) are acquired liver disease and congenital deficiencies of urea cycle enzymes such as X-linked **OTC**.

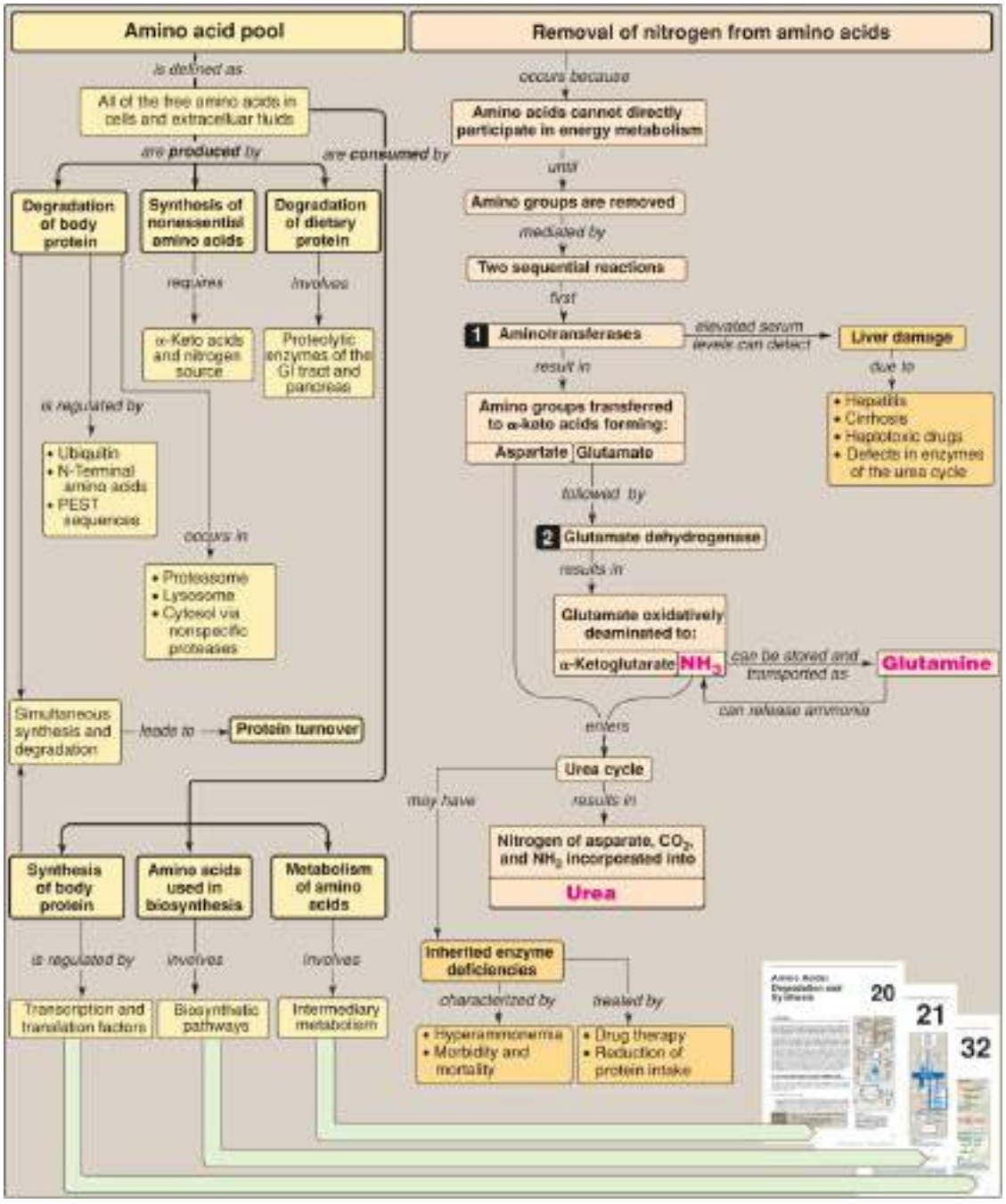
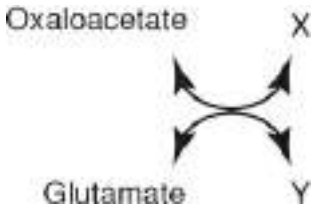


Figure 19.21
Key concept map for nitrogen metabolism. GI = gastrointestinal; PEST = proline, glutamate, serine, threonine; NH_3 = ammonia; CO_2 = carbon dioxide.

Study Questions

Choose the ONE best answer.

19.1 In this transamination reaction (right), which of the following are the products X and Y?



A. Alanine, α-ketoglutarate
B. Aspartate, α-ketoglutarate
C. Glutamate, alanine
D. Pyruvate, aspartate
E. Alanine, pyruvate

Correct answer = B. Transamination reactions always have an amino acid and an α-keto acid as substrates. The products of the reaction are also an amino acid (corresponding to the α-keto substrate) and an α-keto acid (corresponding to the amino acid substrate). Three amino acid/α-keto acid pairs commonly encountered in metabolism are alanine/pyruvate, aspartate/oxaloacetate, and glutamate/α-ketoglutarate. In this question, glutamate is deaminated to form α-ketoglutarate, and oxaloacetate is aminated to form aspartate.

19.2 Which one of the following statements about amino acids and their metabolism is correct?

A. Free amino acids are taken into the enterocytes by a single proton-linked transport system.
B. In healthy, well-fed individuals, the input to the amino acid pool exceeds the output.
C. The liver uses ammonia to buffer protons.
D. Muscle-derived glutamine is deaminated in liver and kidney tissue to ammonia + a gluconeogenic precursor.
E. The first step in the catabolism of most amino acids is their oxidative deamination.
F. The toxic ammonia generated from the amide nitrogen of amino acids is transported through blood as arginine.

Correct answer = D. Glutamine, produced by the catabolism of branched-chain amino acids in muscle, is deaminated by glutaminase to ammonia + glutamate. The glutamate is deaminated by glutamate dehydrogenase to ammonia + α-ketoglutarate, which can be used for gluconeogenesis. Free amino acids are taken into enterocytes by several different sodium-linked transport systems. Healthy, well-fed individuals are in nitrogen balance, in which nitrogen input equals output. The liver converts ammonia to urea, and the kidneys use ammonia to buffer protons. Amino acid catabolism begins with transamination that generates glutamate. The glutamate undergoes oxidative deamination. Toxic ammonia is transported as glutamine and alanine. Arginine is synthesized and hydrolyzed in the hepatic urea cycle.

For Questions 19.3 to 19.5, use the following scenario.

A female neonate appeared healthy until age ~24 hours, when she became lethargic. A sepsis workup proved negative. At 56 hours, she started showing focal seizure activity. The plasma ammonia level was found to be 887 μmol/l (normal 5 to 35 μmol/l). Quantitative plasma amino acid levels revealed a marked elevation of citrulline but not argininosuccinate.

19.3 Which one of the following enzymic activities is most likely to be deficient in this patient?

A. Arginase
B. Argininosuccinate lyase
C. Argininosuccinate synthetase
D. Carbamoyl phosphate synthetase I
E. Ornithine transcarbamylase

Correct answer = C. Genetic deficiencies of each of the five enzymes of the urea cycle, as well as deficiencies in N-acetylglutamate synthase, have been described. The accumulation of citrulline (but not argininosuccinate) in the plasma of this patient means that the enzyme required for the conversion of citrulline to argininosuccinate (argininosuccinate synthetase) is defective, whereas the enzyme that cleaves argininosuccinate

(argininosuccinate lyase) is functional.

19.4 Which one of the following would also be elevated in the blood of this patient?

A. Asparagine
B. Glutamine
C. Lysine
D. Urea
E. Arginine

Correct answer = B. Deficiencies of the enzymes of the urea cycle result in the failure to synthesize urea and lead to hyperammonemia in the first few weeks after birth. Glutamine will also be elevated because it acts as a nontoxic storage and transport form of ammonia. Therefore, elevated glutamine accompanies hyperammonemia. Asparagine, lysine, and arginine do not serve this sequestering role. Urea would be decreased because of impaired activity of the urea cycle. (Note: Alanine would also be elevated in this patient.)

19.5 Why might supplementation with arginine be of benefit to this patient?

The arginine will be cleaved by arginase to urea and ornithine. Ornithine will be combined with carbamoyl phosphate by ornithine transcarbamylase to form citrulline. Citrulline, containing one waste nitrogen, will be excreted.

Amino Acids: Degradation and Synthesis 20

I. OVERVIEW

Amino acid degradation involves removal of the α-amino group, followed by the catabolism of the resulting α-keto acids (carbon skeletons). The degradation pathways of the various amino acids converge to form seven intermediate products: oxaloacetate, pyruvate, α-ketoglutarate, fumarate, succinyl coenzyme A (CoA), acetyl CoA, and acetoacetate. The products directly enter the pathways of intermediary metabolism, resulting either in the synthesis of glucose, ketone bodies, or lipids or in the production of energy through their oxidation to carbon dioxide (CO_2) by the tricarboxylic acid (TCA) cycle. Figure 20.1 provides an overview of these pathways, with a more detailed summary presented in Figure 20.15 (see p. 299). Nonessential amino acids (Fig. 20.2) can be synthesized in sufficient amounts from the intermediates of metabolism or, as in the case of cysteine and tyrosine, from essential amino acids. In contrast, because the essential amino acids cannot be synthesized (or synthesized in sufficient amounts) by humans, they must be obtained from the diet in order for normal protein synthesis to occur. Genetic defects in the pathways of amino acid metabolism can cause serious disease.

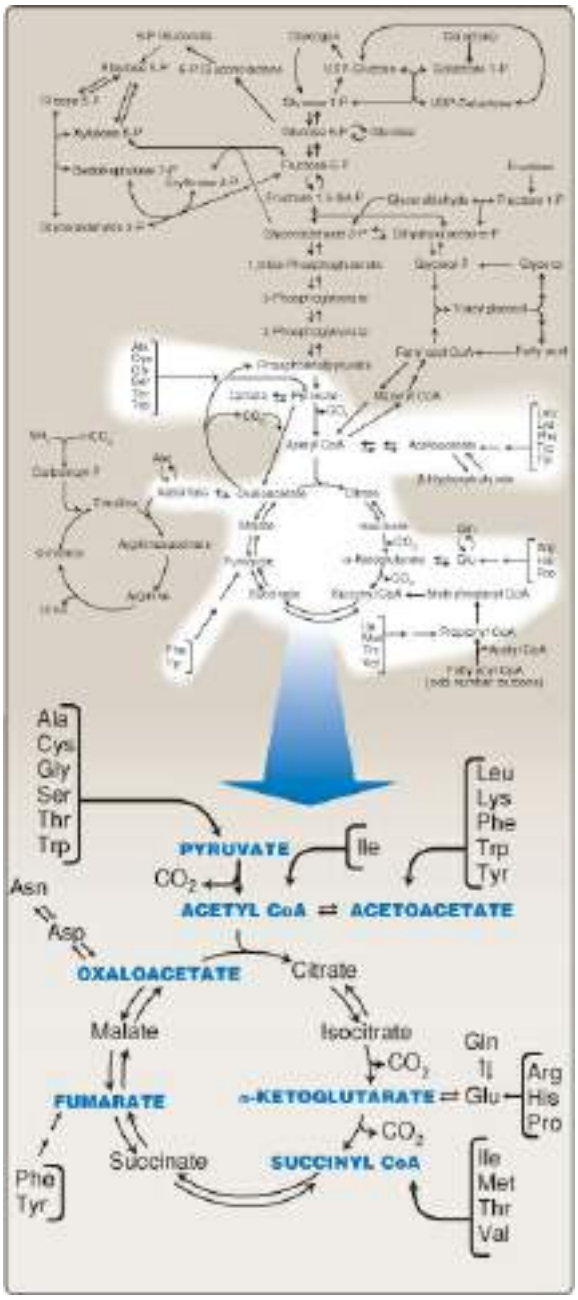


Figure 20.1
Amino acid metabolism shown as a part of the essential pathways of energy metabolism. (see Fig. 8.2 for a more detailed map of metabolism.) CoA = coenzyme A; CO_2 = carbon dioxide.

II. GLUCOGENIC AND KETOGENIC AMINO ACIDS

Amino acids can be classified as glucogenic, ketogenic, or both, based on which of the seven intermediates are produced during their catabolism (see Fig. 20.2).

A. Glucogenic amino acids

Amino acids whose catabolism yields pyruvate or one of the intermediates of the TCA cycle are termed glucogenic. Because these intermediates are substrates for gluconeogenesis (see p. 129), they can give rise to the net synthesis of glucose in the liver and kidney.

Color-coding used in this chapter:	• **BLUE CAPS TEXT = names of seven products of amino acid metabolism** • **Red text = names of glucogenic amino acids** • **Brown text = names of glucogenic and ketogenic amino acids** • **Green text = names of ketogenic amino acids** • **Cyan text = one-carbon compounds**

B. Ketogenic amino acids

Amino acids whose catabolism yields either acetyl CoA (directly, without pyruvate serving as an intermediate) or acetoacetate (or its precursor acetoacetyl CoA) are termed ketogenic (see Fig. 20.2). Acetoacetate is one of the ketone bodies, which also include 3-hydroxybutyrate and acetone (see p. 216). Leucine and lysine are the only exclusively ketogenic amino acids found in proteins. Their carbon skeletons are not substrates for gluconeogenesis and, therefore, cannot give rise to the net synthesis of glucose.

III. AMINO ACID CARBON SKELETON CATABOLISM

The pathways by which amino acids are catabolized are conveniently organized according to which one (or more) of the seven intermediates listed above is produced from a particular amino acid.

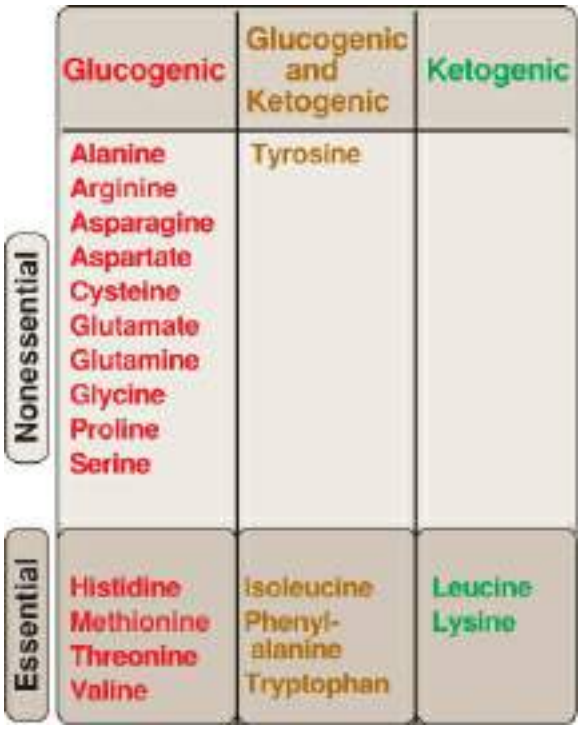


Figure 20.2
Classification of amino acids. (Note: Some amino acids can become conditionally essential; e.g., supplementation with glutamine and arginine has been shown to improve outcomes in patients with trauma, postoperative infections, and immunosuppression.)

A. Amino acids that form oxaloacetate

Asparagine is hydrolyzed by asparaginase, liberating ammonia and aspartate (Fig. 20.3). Aspartate is converted to its corresponding ketoacid by transamination to form oxaloacetate (see Fig. 20.3). (Note: Some rapidly dividing leukemic cells are unable to synthesize sufficient asparagine to support their growth. This makes asparagine an essential amino acid for these cells, which, therefore, require asparagine from the blood. Asparaginase, which hydrolyzes asparagine to aspartate, can be administered systemically to treat leukemia. Asparaginase lowers the level of asparagine in the plasma, thereby depriving cancer cells of a required nutrient.)

B. Amino acids that form α-ketoglutarate via glutamate

1. Glutamine: This amino acid is hydrolyzed to glutamate and ammonia by the enzyme glutaminase (see p. 283). Glutamate is converted to α-ketoglutarate by transamination or through oxidative deamination by glutamate dehydrogenase (see p. 278).

2. Proline: This amino acid is oxidized to glutamate. Glutamate is transaminated or oxidatively deaminated to form α-ketoglutarate.

3. Arginine: This amino acid is hydrolyzed by arginase to produce ornithine (and

urea). (Note: The reaction occurs primarily in the liver as part of the urea cycle [see p. 281].) Ornithine is subsequently converted to α-ketoglutarate, with glutamate semialdehyde as an intermediate.

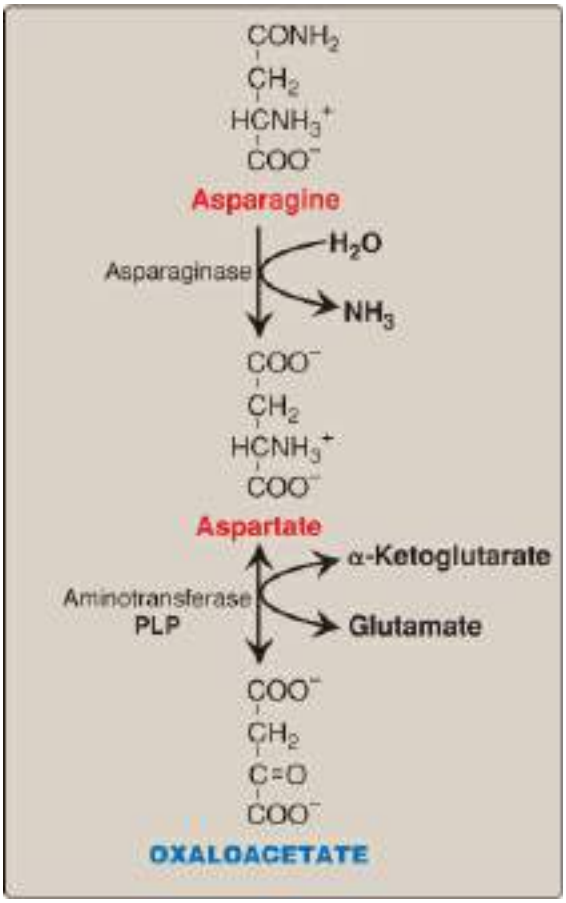


Figure 20.3
Metabolism of asparagine and aspartate. PLP = pyridoxal phosphate; NH_3 = ammonia.

4. Histidine: Histidine is oxidatively deaminated by histidase to urocanic acid, which subsequently forms N-formiminoglutamate ([FIGLU], Fig. 20.4). FIGLU donates its formimino group to tetrahydrofolate (THF), leaving glutamate, which is degraded as described above. A deficiency in histidase results in the relatively benign inborn error of metabolism histidinemia, characterized by elevated levels of histidine in blood and urine. (Note: Individuals deficient in folic acid excrete increased amounts of FIGLU in the urine, particularly after ingestion of a large dose of histidine. The FIGLU excretion test has been used in diagnosing a deficiency of folic acid. See p. 296 for a discussion of folic acid, THF, and one-carbon metabolism.)

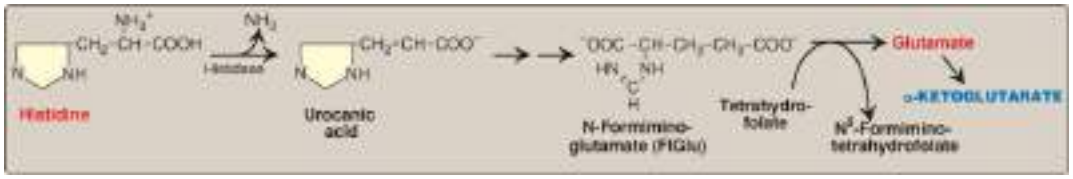


Figure 20.4
Degradation of histidine. NH_3 = ammonia.

C. Amino acids that form pyruvate

1. Alanine: This amino acid loses its amino group by transamination to form pyruvate (Fig. 20.5). (Note: Tryptophan catabolism produces alanine and, therefore, pyruvate [see Fig. 20.10 on p. 294].)

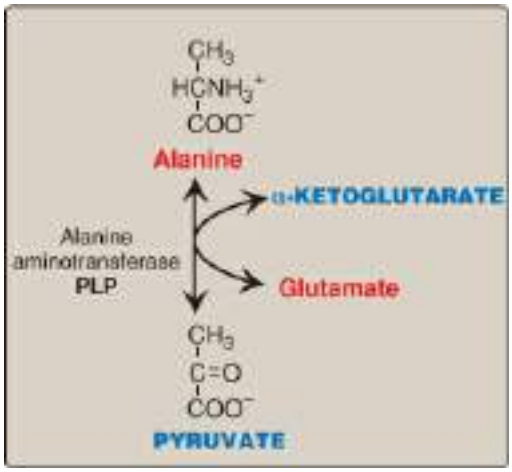


Figure 20.5
Transamination of alanine to pyruvate. PLP = pyridoxal phosphate.

2. Serine: This amino acid can be converted to glycine as THF becomes N^5,N^{10}-methylenetetrahydrofolate (N^5,N^{10}-MTHF), as shown in Figure 20.6A. Serine can also be converted to pyruvate (see Fig. 20.6B).

3. Glycine: This amino acid can be converted to serine by the reversible addition of a methylene group from N^5,N^{10}-MTHF (see Fig. 20.6A) or oxidized to CO_2 and ammonia by the glycine cleavage system. Glycine can be deaminated to glyoxylate (by a D-amino acid oxidase; see p. 279), which can be oxidized to oxalate or transaminated to glycine. Deficiency of the transaminase in liver peroxisomes causes overproduction of oxalate, the formation of oxalate stones, and kidney damage (primary oxaluria type 1).

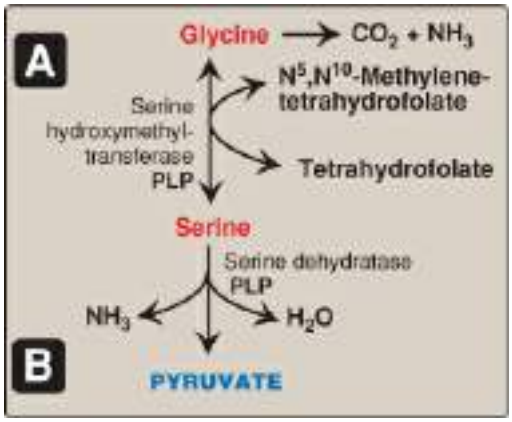


Figure 20.6
A: Interconversion of serine and glycine and oxidation of glycine. B: Dehydration of serine to pyruvate. PLP = pyridoxal phosphate; NH_3 = ammonia.

4. Cysteine: This sulfur-containing amino acid undergoes desulfurization to yield pyruvate. (Note: The sulfate released can be used to synthesize 3′-phosphoadenosine-5′-phosphosulfate [PAPS], an activated sulfate donor to a variety of acceptors.) Cysteine can also be oxidized to its disulfide derivative, cystine.

5. Threonine: This amino acid is converted to pyruvate in most organisms but is a minor pathway (at best) in humans.

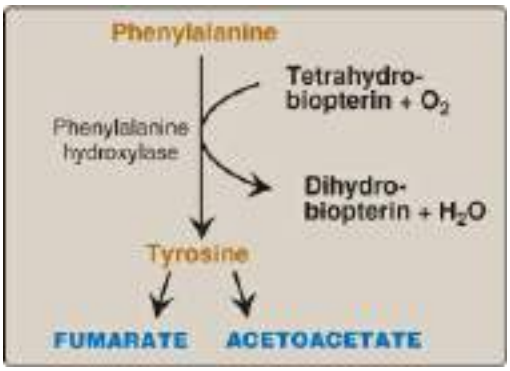


Figure 20.7
Degradation of phenylalanine.

D. Amino acids that form fumarate

1. Phenylalanine and tyrosine: Hydroxylation of phenylalanine produces tyrosine (Fig. 20.7). This irreversible reaction, catalyzed by tetrahydrobiopterin (BH_4)-requiring phenylalanine hydroxylase (PAH), initiates the catabolism of phenylalanine. Thus, phenylalanine metabolism and tyrosine metabolism merge, leading ultimately to fumarate and acetoacetate formation. Therefore, phenylalanine and tyrosine are both glucogenic and ketogenic.

2. Inherited deficiencies: Inherited deficiencies in the enzymes of phenylalanine and tyrosine metabolism lead to the diseases phenylketonuria (PKU) (see p. 298), tyrosinemia (see p. 303), and alkaptonuria (see p. 303) as well as the condition of albinism (see p. 303).

E. Amino acids that form succinyl CoA: methionine

Methionine is one of four amino acids that form succinyl CoA. This sulfur-containing amino acid deserves special attention because it is converted to S-adenosylmethionine (SAM), the major methyl group donor in one-carbon metabolism (Fig. 20.8). Methionine is also the source of homocysteine (Hcy), a metabolite associated with atherosclerotic vascular disease and thrombosis (see p. 294).

1. S-Adenosylmethionine synthesis: Methionine condenses with ATP, forming SAM, a high-energy compound that is unusual in that it contains no phosphate. The formation of SAM is driven by hydrolysis of all three phosphate bonds in ATP (see Fig. 20.8).

2. Activated methyl group: The methyl group attached to the sulfur in SAM is activated and can be transferred by methyltransferases to a variety of acceptors such as norepinephrine in the synthesis of epinephrine. The methyl group is usually transferred to nitrogen or oxygen atoms (as with epinephrine synthesis and degradation, respectively; see p. 318) and sometimes to carbon atoms (as with cytosine). The reaction product, *S*-adenosylhomocysteine (SAH), is a simple thioether, analogous to methionine. The resulting loss of free energy makes methyl transfer essentially irreversible.

3. S-Adenosylhomocysteine hydrolysis: After donation of the methyl group, SAH is hydrolyzed to Hcy and adenosine. Hcy has two fates. If there is a deficiency of methionine, Hcy may be remethylated to methionine (see Fig. 20.8). If methionine stores are adequate, Hcy may enter the transsulfuration pathway, where it is converted to cysteine.

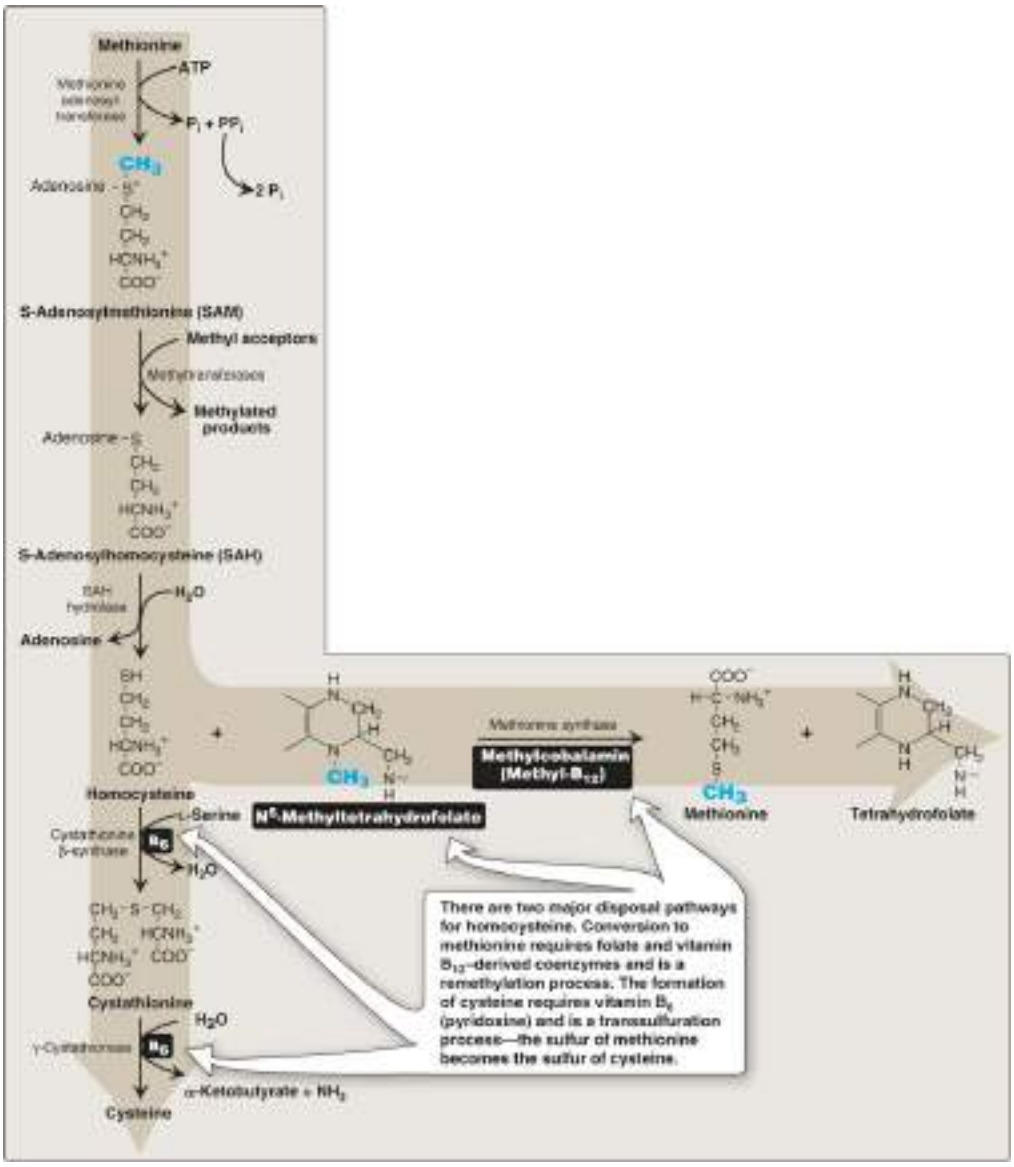


Figure 20.8
Degradation and resynthesis of methionine. (Note: The resynthesis of methionine from homocysteine is the only reaction in which tetrahydrofolate both carries and donates a methyl [$-CH_3$] group. In all other reactions, SAM is the methyl group carrier and donor.) PP_i = pyrophosphate; P_i = inorganic phosphate; NH_3 = ammonia.

a. Methionine resynthesis: Hcy accepts a methyl group from N^5-methyltetrahydrofolate (N^5-methyl-THF) in a reaction requiring methylcobalamin, a coenzyme derived from vitamin B_{12} (see p. 425). (Note: The methyl group is transferred by methionine synthase from the B_{12} derivative to Hcy, regenerating methionine. Cobalamin is remethylated from N^5-methyl-THF.)

b. Cysteine synthesis: Catalyzed by cystathionine β-synthase, Hcy condenses with serine, forming cystathionine, which is hydrolyzed to α-ketobutyrate and

cysteine (see Fig. 20.8). This vitamin B_6–requiring sequence has the net effect of converting serine to cysteine and Hcy to α-ketobutyrate, which is oxidatively decarboxylated to form propionyl CoA. Propionyl CoA is converted to succinyl CoA (see Fig. 16.20). Because Hcy is synthesized from the essential amino acid methionine, cysteine is not an essential amino acid as long as sufficient methionine is available.

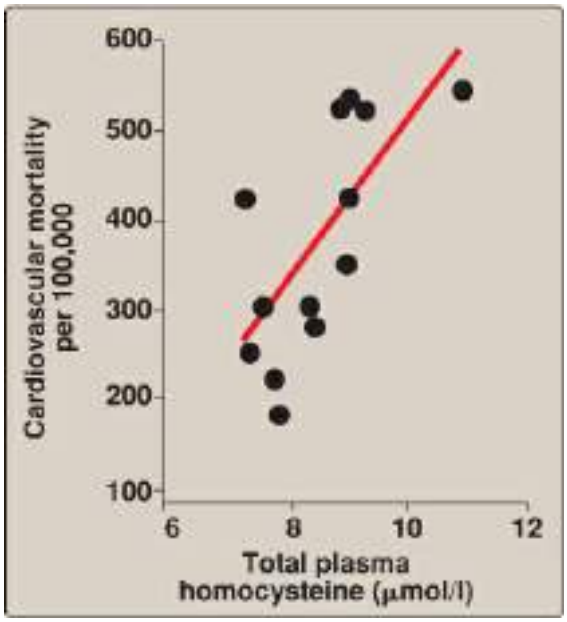


Figure 20.9
Association between cardiovascular disease mortality and total plasma homocysteine.

4. Relationship of homocysteine to vascular disease: Elevations in plasma Hcy levels promote oxidative damage, inflammation, and endothelial dysfunction and are an independent risk factor for occlusive vascular diseases such as cardiovascular disease (CVD) and stroke (Fig. 20.9). Mild elevations (hyperhomocysteinemia) are seen in ~7% of the population. Epidemiologic studies have shown that plasma Hcy levels are inversely related to plasma levels of folate, B_{12}, and B_6, the three vitamins involved in the conversion of Hcy to methionine and cysteine. Supplementation with these vitamins has been shown to reduce circulating levels of Hcy. However, in patients with established CVD, vitamin therapy does not decrease cardiovascular events or death. This raises the question as to whether Hcy is a cause of the vascular damage or merely a marker of such damage. (Note: Large elevations in plasma Hcy as a result of rare deficiencies in cystathionine β-synthase of the transsulfuration pathway are seen in patients with classic homocystinuria [resulting from severe hyperhomocysteinemia (>100 μmol/l), see p. 303].) Deficiencies in the remethylation reaction also result in a rise in Hcy.

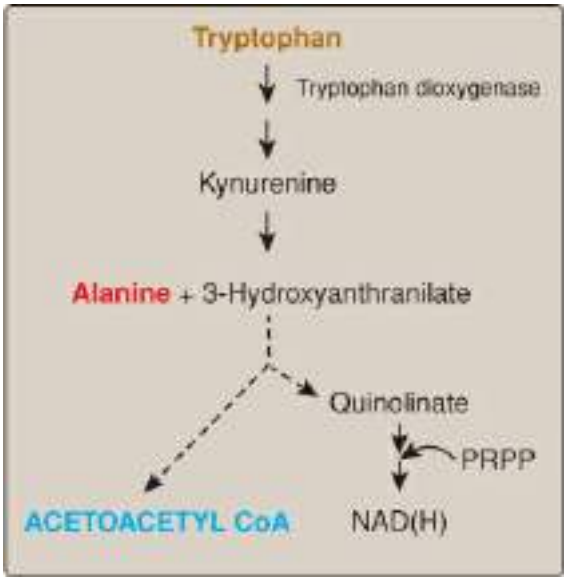


Figure 20.10
Metabolism of tryptophan by the kynurenine pathway (abbreviated). CoA = coenzyme A; PRPP = phosphoribosyl pyrophosphate; NAD(H) = nicotinamide adenine dinucleotide.

In pregnant women, elevated Hcy levels usually indicate a deficiency in folic acid, which is associated with an increased incidence of neural tube defects (improper closure, as in spina bifida) in the fetus (see p. 425). Periconceptual supplementation with folate reduces the risk of such defects.

F. Other amino acids that form succinyl CoA

Degradation of valine, isoleucine, and threonine also results in the production of succinyl CoA, a TCA cycle intermediate and gluconeogenic compound. (Note: It is metabolized to pyruvate.)

1. Valine and isoleucine: These amino acids are branched-chain amino acids (BCAAs) that generate propionyl CoA, which is converted to methylmalonyl CoA and then succinyl CoA by biotin- and vitamin B_{12}–requiring reactions.

2. Threonine: This amino acid is dehydrated to α-ketobutyrate, which is converted to propionyl CoA and then to succinyl CoA. Propionyl CoA, then, is generated by the catabolism of the amino acids methionine, valine, isoleucine, and threonine. (Note: Propionyl CoA also is generated by the oxidation of odd-numbered fatty acids [see p. 215].)

G. Amino acids that form acetyl CoA or acetoacetyl CoA

Tryptophan, leucine, isoleucine, and lysine form acetyl CoA or acetoacetyl CoA directly, without pyruvate serving as an intermediate. As noted earlier, phenylalanine and tyrosine also give rise to acetoacetate during their catabolism

(see Fig. 20.7). Therefore, there are a total of six partly or wholly ketogenic amino acids.

1. Tryptophan: This amino acid is both glucogenic and ketogenic, because its catabolism yields alanine and acetoacetyl CoA (Fig. 20.10). (Note: Quinolinate from tryptophan catabolism is used in the synthesis of nicotinamide adenine dinucleotide [(NAD), see p. 430].)

2. Leucine: This amino acid is exclusively ketogenic, because its catabolism yields acetyl CoA and acetoacetate (Fig. 20.11). The first two reactions in the catabolism of leucine and the other BCAAs, isoleucine and valine, are catalyzed by enzymes that use all three BCAAs (or their derivatives) as substrates (see H. below).

3. Isoleucine: This amino acid is both ketogenic and glucogenic, because its metabolism yields acetyl CoA and propionyl CoA.

4. Lysine: This amino acid is exclusively ketogenic and is unusual in that neither of its amino groups undergoes transamination as the first step in catabolism. Lysine is ultimately converted to acetoacetyl CoA.

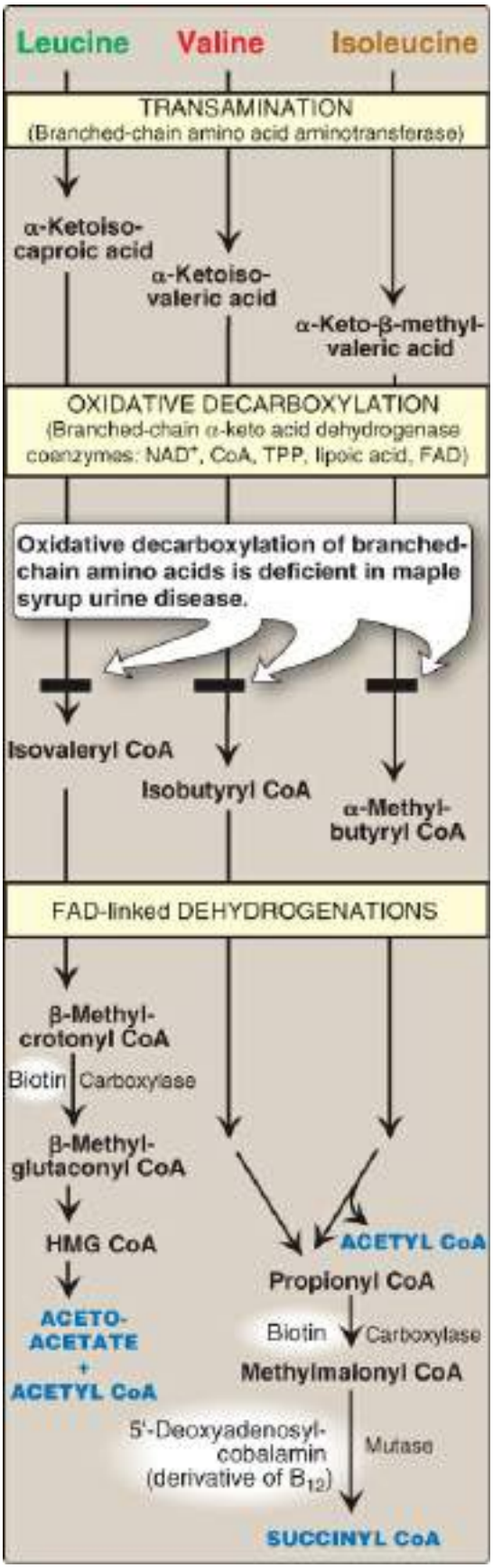


Figure 20.11
Degradation of leucine, valine, and isoleucine. (Note: β-Methylcrotonyl CoA carboxylase is one of four biotin-requiring carboxylases discussed in this book. The other three are pyruvate carboxylase, acetyl CoA carboxylase, and propionyl CoA carboxylase.) TPP = thiamine pyrophosphate; FAD = flavin adenine dinucleotide; CoA = coenzyme A; NAD = nicotinamide adenine dinucleotide; HMG = hydroxymethylglutarate.

H. Branched-chain amino acid degradation

The BCAAs, isoleucine, leucine, and valine are essential amino acids. In contrast to other amino acids, they are catabolized primarily by the peripheral tissues (particularly muscle), rather than by the liver. Because these three amino acids have a similar route of degradation, it is convenient to describe them as a group (see Fig. 20.11).

1. Transamination: Transfer of the amino groups of all three BCAAs to α-ketoglutarate is catalyzed by a single, vitamin B_6–requiring enzyme, branched-chain amino acid aminotransferase that is expressed primarily in skeletal muscle.

2. Oxidative decarboxylation: Removal of the carboxyl group of the α-keto acids derived from leucine, valine, and isoleucine is catalyzed by a single multienzyme complex, branched-chain α-keto acid dehydrogenase (BCKD) complex. An enzymatic deficiency in this complex results in maple syrup urine disease (MSUD) (see Fig. 20.11, and p. 302). This complex uses thiamine pyrophosphate, lipoic acid, oxidized flavin adenine dinucleotide (FAD), NAD^+, and CoA as its coenzymes and produces NADH. (Note: This reaction is similar to the conversion of pyruvate to acetyl CoA by the pyruvate dehydrogenase [PDH] complex [see p. 120] and α-ketoglutarate to succinyl CoA by the α-ketoglutarate dehydrogenase complex [see p. 123]. The dihydrolipoyl dehydrogenase [Enzyme 3, or E3] component is identical in all three complexes.)

3. Dehydrogenations: Oxidation of the products formed in the BCKD reaction produces α-β-unsaturated acyl CoA derivatives and $FADH_2$. These reactions are analogous to the FAD-linked dehydrogenation in the β-oxidation of fatty acids (see p. 212). (Note: Deficiency in the dehydrogenase specific for isovaleryl CoA causes neurologic problems and is associated with a “sweaty feet” odor in body fluids.)

4. End products: The catabolism of isoleucine ultimately yields acetyl CoA and succinyl CoA, rendering it both ketogenic and glucogenic. Valine yields succinyl CoA and is glucogenic. Leucine is ketogenic, being metabolized to acetoacetate and acetyl CoA. In addition, NADH and $FADH_2$ are produced in the decarboxylation and dehydrogenation reactions, respectively. (Note: BCAA catabolism also results in glutamine and alanine being synthesized and sent out into the blood from muscle [see p. 279].)

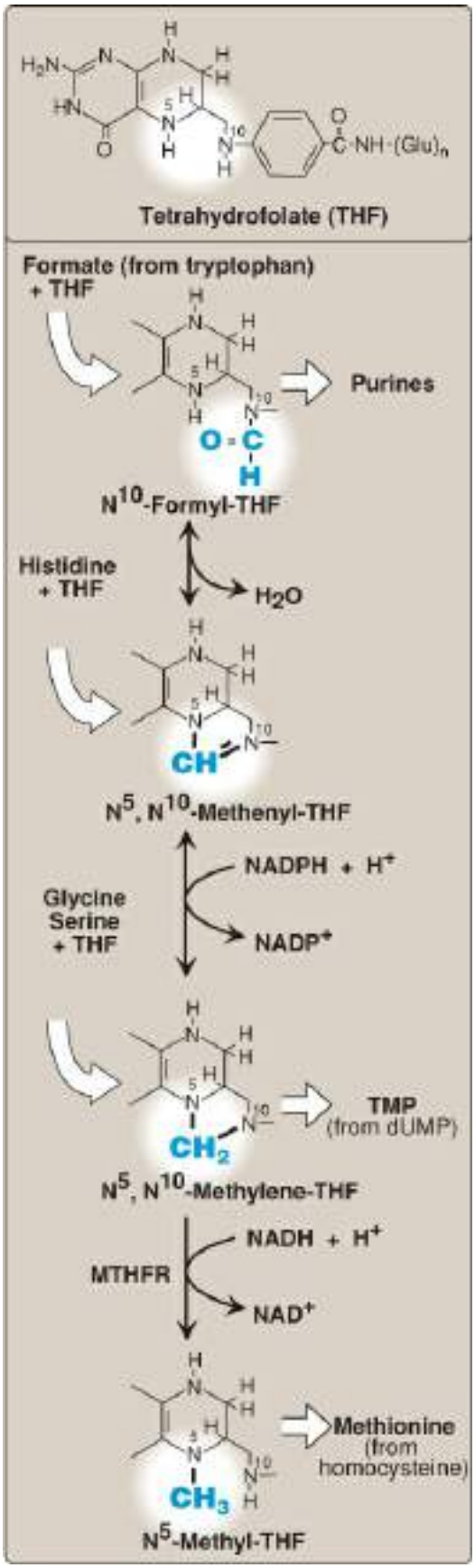


Figure 20.12

Summary of the interconversions and uses of THF. (Note: N^5,N^{10}-Methenyl-THF also arises from N^5-formimino-THF [see Fig. 20.4].) NADP(H) = nicotinamide adenine dinucleotide phosphate; NAD(H) = nicotinamide adenine dinucleotide; TMP = thymidine monophosphate; dUMP = deoxyuridine monophosphate; MTHFR = N^5,N^{10}-methylene-THF reductase.

IV. FOLIC ACID AND AMINO ACID METABOLISM

Some synthetic pathways require the addition of single-carbon groups that exist in a variety of oxidation states, including formyl, methenyl, methylene, and methyl. These single-carbon groups can be transferred from carrier compounds such as THF and SAM to specific structures that are being synthesized or modified. The "one-carbon pool" refers to the single-carbon units attached to this group of carriers. (Note: CO_2, coming from bicarbonate [HCO_3^-], is carried by the vitamin biotin [see p. 431], which is a prosthetic group for most carboxylation reactions but is not considered a member of the one-carbon pool. Defects in the ability to add or remove biotin from carboxylases result in multiple carboxylase deficiency. Treatment is supplementation with biotin.)

A. Folic acid and one-carbon metabolism

The active form of folic acid, THF, is produced from folate by dihydrofolate reductase in a two-step reaction requiring two nicotinamide adenine dinucleotide phosphate (NADPH). The one-carbon unit carried by THF is bound to N^5 or N^{10} or to both N^5 and N^{10}. Figure 20.12 shows the structures of the various members of the THF family and their interconversions and indicates the sources of the one-carbon units and the synthetic reactions in which the specific members participate. (Note: Folate deficiency presents as a megaloblastic anemia because of decreased availability of the purines and of the thymidine monophosphate needed for DNA synthesis [see p. 336].)

V. BIOSYNTHESIS OF NONESSENTIAL AMINO ACIDS

Nonessential amino acids are synthesized from intermediates of metabolism or, as in the case of tyrosine and cysteine, from the essential amino acids phenylalanine and methionine, respectively. The synthetic reactions for the nonessential amino acids are described below and are summarized in Figure 20.15. (Note: Some amino acids found in proteins, such as hydroxyproline and hydroxylysine [see p. 47], are produced by posttranslational modification [after incorporation into a protein] of their precursor [parent] amino acids.)

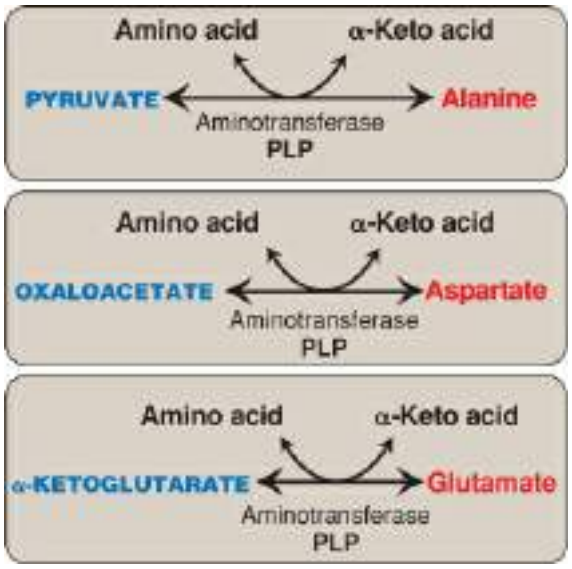


Figure 20.13
Formation of alanine, aspartate, and glutamate from the corresponding α-keto acids by transamination. PLP = pyridoxal phosphate.

A. Synthesis from α-keto acids

Alanine, aspartate, and glutamate are synthesized by transfer of an amino group to the α-keto acids pyruvate, oxaloacetate, and α-ketoglutarate, respectively. These transamination reactions (Fig. 20.13) are the most direct of the biosynthetic pathways. Glutamate is unusual in that it can also be synthesized by reversal of oxidative deamination, catalyzed by glutamate dehydrogenase, when ammonia levels are high (see Fig. 19.11).

B. Synthesis by amidation

1. Glutamine: This amino acid, which contains an amide linkage with ammonia at the γ-carboxyl, is formed from glutamate and ammonia by glutamine synthetase (see Fig. 19.18, p. 283). The reaction is driven by the hydrolysis of ATP. In addition to producing glutamine for protein synthesis, the reaction also serves as a major mechanism for the transport of ammonia in a nontoxic form. (See p. 283 for a discussion of ammonia metabolism.)

2. Asparagine: This amino acid, which contains an amide linkage with ammonia at the β-carboxyl, is formed from aspartate by asparagine synthetase, using glutamine as the amide donor. Like the synthesis of glutamine, the reaction requires ATP and has an equilibrium far in the direction of amide synthesis.

C. Proline

Glutamate via glutamate semialdehyde is converted to proline by cyclization and reduction reactions. (Note: The semialdehyde can also be transaminated to ornithine.)

D. Serine, glycine, and cysteine

The pathways of synthesis for these amino acids are interconnected.

1. Serine: This amino acid arises from 3-phosphoglycerate, a glycolytic intermediate (see Fig. 8.18), which is first oxidized to 3-phosphopyruvate and then transaminated to 3-phosphoserine. Serine is formed by hydrolysis of the phosphate ester. Serine can also be formed from glycine through transfer of a hydroxymethyl group by serine hydroxymethyltransferase using N^5,N^{10}-MTHF as the one-carbon donor (see Fig. 20.6A). (Note: Selenocysteine [Sec], the 21st genetically encoded amino acid, is synthesized from serine and selenium [see p. 454], while serine is attached to transfer RNA. Sec is found in ~25 human proteins including glutathione peroxidase [see p. 163] and thioredoxin reductase [see p. 330].)

2. Glycine: This amino acid is synthesized from serine by removal of a hydroxymethyl group, also by serine hydroxymethyltransferase (see Fig. 20.6A). THF is the one-carbon acceptor.

3. Cysteine: This amino acid is synthesized by two consecutive reactions in which Hcy combines with serine, forming cystathionine, which, in turn, is hydrolyzed to α-ketobutyrate and cysteine (see Fig. 20.8). (Note: Hcy is derived from methionine, as described on p. 293. Because methionine is an essential amino acid, cysteine synthesis requires adequate dietary intake of methionine.)

E. Tyrosine

Tyrosine is formed from phenylalanine by PAH (see p. 292). The reaction requires molecular oxygen and the coenzyme BH_4, which is synthesized from guanosine triphosphate. One atom of molecular oxygen becomes the hydroxyl group of tyrosine, and the other atom is reduced to water. During the reaction, BH_4 is oxidized to dihydrobiopterin (BH_2). BH_4 is regenerated from BH_2 by NADH-requiring dihydropteridine reductase. Tyrosine, like cysteine, is formed from an essential amino acid and is, therefore, nonessential only in the presence of adequate dietary phenylalanine.

VI. AMINO ACID METABOLISM DISORDERS

These single-gene disorders, a subset of the inborn errors of metabolism, are generally caused by loss-of-function mutations in enzymes involved in amino acid metabolism. The inherited defects may be expressed as a total loss of enzyme activity or, more frequently, as a partial deficiency in catalytic activity. Without treatment, the amino acid disorders almost invariably result in intellectual disability or other developmental abnormalities, as a consequence of harmful accumulation of metabolites. Although >50 of these disorders have been described, many are rare, occurring in <1 per 250,000 in most populations (Fig. 20.14). Collectively, however, they constitute a very significant

portion of pediatric genetic diseases (Fig. 20.15).

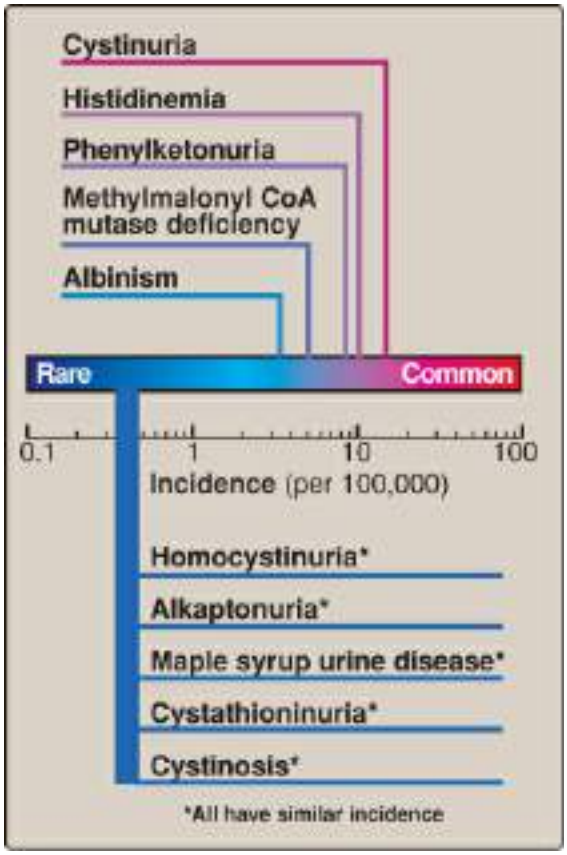


Figure 20.14
Incidence of inherited diseases of amino acid metabolism. (Note: Cystinuria is the most common inborn error of amino acid transport.)

A. Phenylketonuria

PKU is the most common clinically encountered inborn error of amino acid metabolism (incidence 1:15,000). Classical PKU is an autosomal recessive disorder resulting from loss of function mutations in the gene coding for PAH (Fig. 20.16). Biochemically, PKU is characterized by hyperphenylalaninemia. Phenylalanine is present in high concentrations (10 times normal) not only in plasma but also in urine and body tissues. Tyrosine, which normally is formed from phenylalanine by PAH, is deficient. Treatment includes dietary restriction of phenylalanine and supplementation with tyrosine. (Note: Hyperphenylalaninemia may also be caused by rare deficiencies in any of the several enzymes required to synthesize BH_4 or in dihydropteridine reductase, which regenerates BH_4 from BH_2 [Fig. 20.17]. Such deficiencies indirectly raise phenylalanine concentrations, because PAH requires BH_4 as a coenzyme. BH_4 is also required for tyrosine hydroxylase and tryptophan hydroxylase, which catalyze reactions leading to the synthesis of neurotransmitters, such as serotonin and the catecholamines. Simply restricting dietary phenylalanine does not reverse the central nervous system effects due to deficiencies in

neurotransmitters. Supplementation with BH_4 and replacement therapy with L-3,4-dihydroxyphenylalanine [L-DOPA, see p. 318] and 5-hydroxytryptophan [products of the affected tyrosine hydroxylase– and tryptophan hydroxylase–catalyzed reactions] improves the clinical outcome in these variant forms of hyperphenylalaninemia, although the response is unpredictable.)

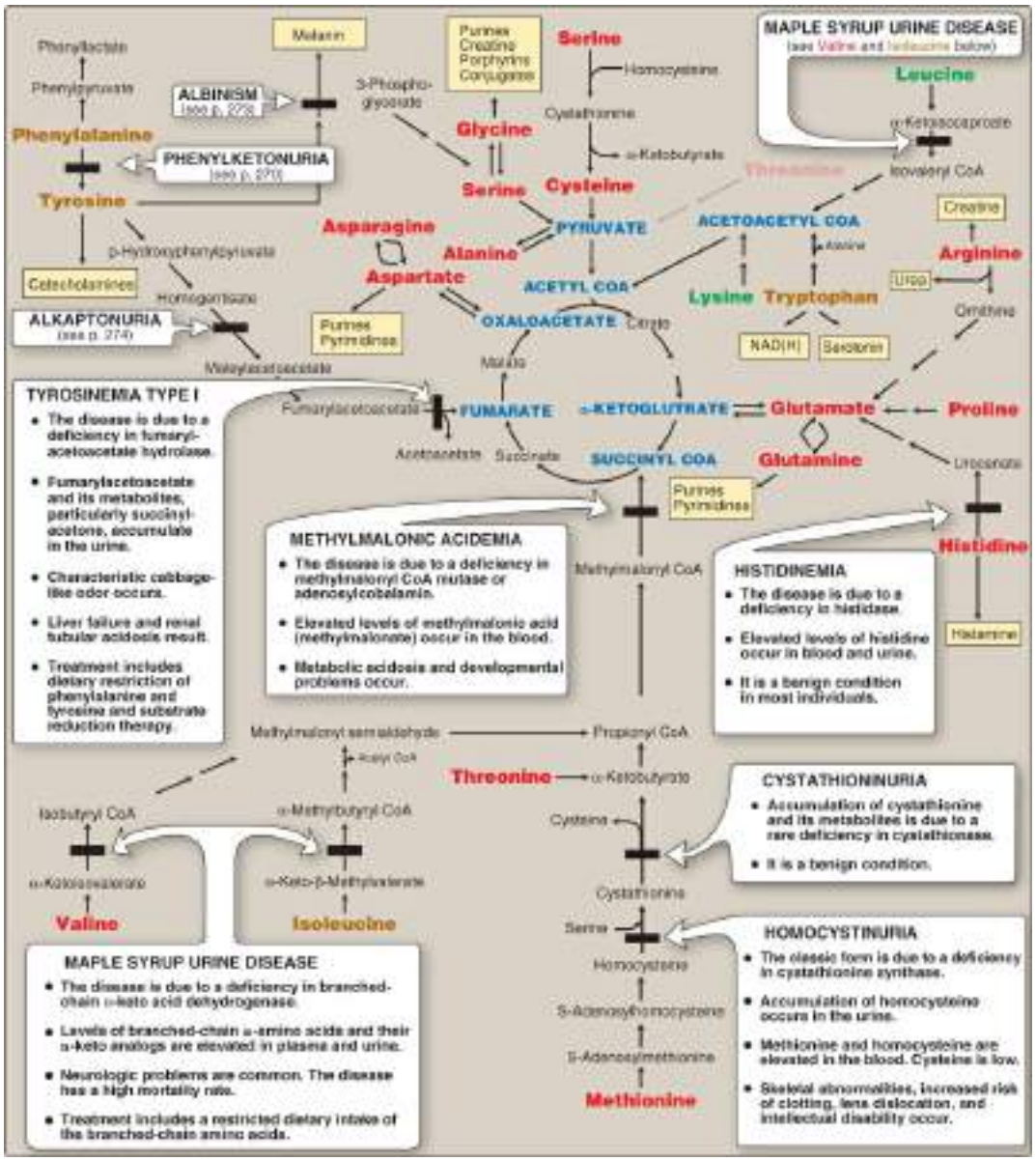


Figure 20.15
Summary of the metabolism of amino acids in humans. Genetically determined enzyme deficiencies are summarized in white boxes. Nitrogen-containing compounds derived from amino acids are shown in small, yellow boxes. Classification of amino acids is color coded: **Red** = glucogenic; **brown** = glucogenic and ketogenic; **green** = ketogenic. Compounds in **BLUE ALL CAPS** are the seven metabolites to which all amino acid metabolism converges. CoA = coenzyme A; NAD(H) = nicotinamide adenine dinucleotide.

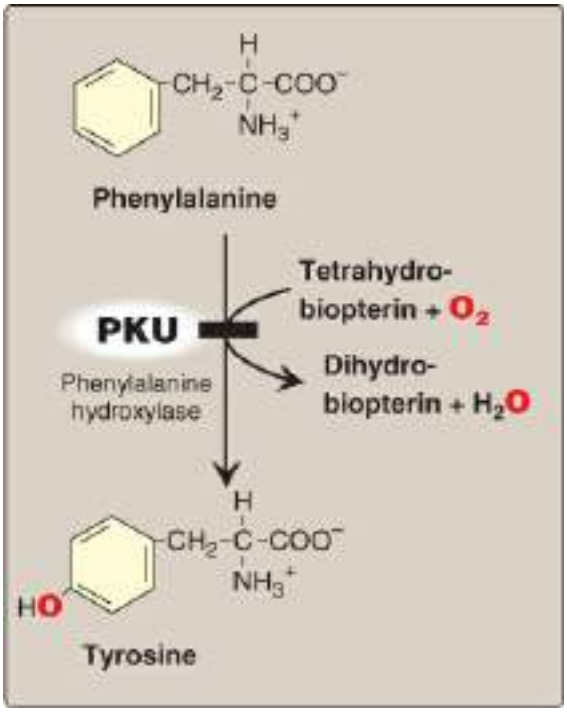


Figure 20.16
A deficiency in phenylalanine hydroxylase results in the disease phenylketonuria (PKU).

Screening of newborns for a number of treatable disorders, including inborn errors of amino acid metabolism, is done by tandem mass spectrometry of blood obtained from a heel prick. By law, all states must screen for >20 disorders, with some screening for >50. All states screen for PKU.

1. Additional characteristics: As the name suggests, PKU is also characterized by elevated levels of a phenylketone in the urine.

a. Elevated phenylalanine metabolites: Phenylpyruvate (a phenylketone), phenylacetate, and phenyllactate, which are not normally produced in significant amounts in the presence of functional PAH, are also elevated in PKU, in addition to phenylalanine (Fig. 20.18). These metabolites give urine a characteristic musty ("mousy") odor.

b. Central nervous system effects: Severe intellectual disability, developmental delay, microcephaly, and seizures are characteristic findings in untreated PKU. The affected individual typically shows symptoms of intellectual disability by age 1 year and rarely achieves an intelligence quotient (IQ) >50 (Fig. 20.19). (Note: These clinical manifestations are now rarely seen as a result of newborn screening programs, which allow early diagnosis and treatment.)

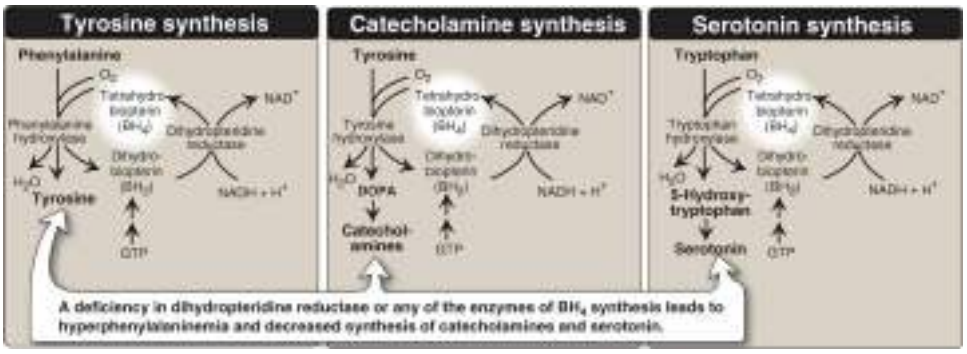


Figure 20.17
Biosynthetic reactions involving amino acids and tetrahydrobiopterin. (Note: Aromatic amino acid hydroxylases use BH_4 and not PLP [pyridoxal phosphate].) NAD(H) = nicotinamide adenine dinucleotide; GTP = guanosine triphosphate; DOPA = L-3,4-dihydroxyphenylalanine.

c. Hypopigmentation: Patients with untreated PKU may show a deficiency of pigmentation (fair hair, light skin color, and blue eyes). The hydroxylation of tyrosine by copper-requiring tyrosinase, which is the first step in the formation of the pigment melanin, is decreased in PKU because tyrosine is decreased.

2. Newborn screening and diagnosis: Early diagnosis of PKU is important because the disease is treatable by dietary means. Because of the lack of neonatal symptoms, laboratory testing for elevated blood levels of phenylalanine is mandatory for detection. However, the infant with PKU frequently has normal blood levels of phenylalanine at birth because the mother clears increased blood phenylalanine in her affected fetus through the placenta. Normal levels of phenylalanine may persist until the newborn is exposed to 24 to 48 hours of protein feeding. Thus, screening tests are typically done after this time to avoid false negatives. For newborns with a positive screening test, diagnosis is confirmed through quantitative determination of phenylalanine levels.

3. Prenatal diagnosis: Classic PKU is caused by any of 100 or more different mutations in the gene that encodes PAH. The frequency of any given mutation varies among populations, and the disease is often doubly heterozygous (i.e., the *PAH* gene has a different mutation in each allele). Despite this complexity, prenatal diagnosis is possible (see p. 544).

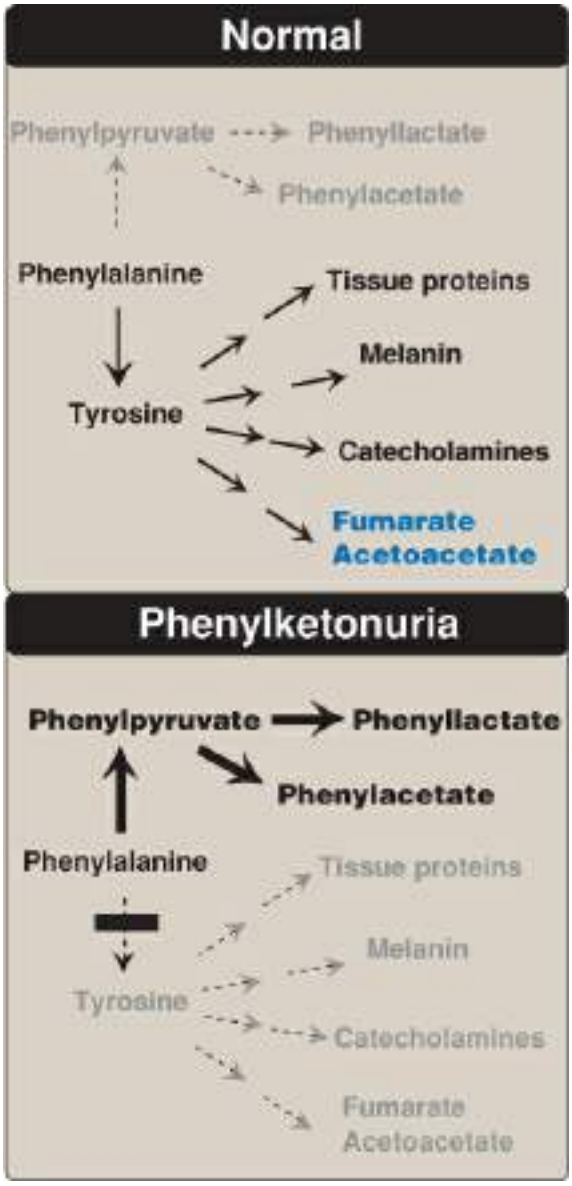


Figure 20.18
Pathways of phenylalanine metabolism in normal individuals and in patients with phenylketonuria.

4. Treatment: Because most natural protein contains phenylalanine, an essential amino acid, it is impossible to satisfy the body's protein requirement without exceeding the phenylalanine limit when ingesting a normal diet. Therefore, in PKU, blood phenylalanine level is maintained close to the normal range by feeding synthetic amino acid preparations free of phenylalanine, supplemented with some natural foods (such as fruits, vegetables, and certain cereals) selected for their low phenylalanine content. The amount is adjusted according to the tolerance of the individual as measured by blood phenylalanine levels. The earlier treatment is started, the more completely neurologic damage can be prevented. Individuals who are appropriately treated can have normal

intelligence. (Note: Treatment must begin during the first 7 to 10 days of life to prevent cognitive impairment.) Because phenylalanine is an essential amino acid, overzealous treatment that results in blood phenylalanine levels below normal is avoided. In patients with PKU, tyrosine cannot be synthesized from phenylalanine, and, therefore, it becomes an essential amino acid and must be supplied in the diet. Discontinuance of the phenylalanine-restricted diet in early childhood is associated with poor performance on IQ tests. Adult PKU patients show deterioration of IQ scores after discontinuation of the diet (Fig. 20.20). Therefore, lifelong restriction of dietary phenylalanine is recommended. (Note: Individuals with PKU are advised to avoid aspartame, an artificial sweetener that contains phenylalanine.)

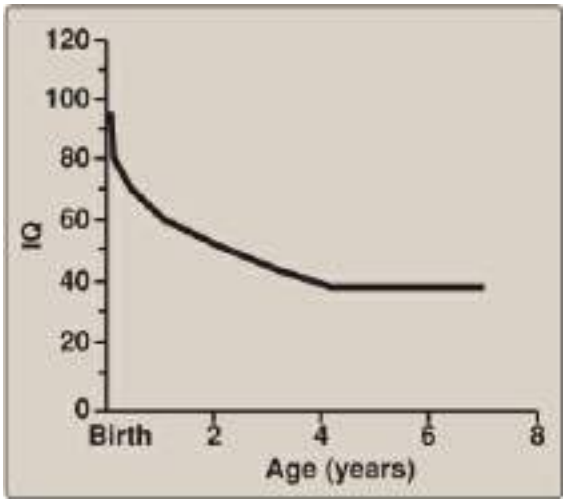


Figure 20.19
Typical intellectual ability in untreated patients of different ages with phenylketonuria. IQ = intelligence quotient.

5. Maternal phenylketonuria: If women with PKU who are not on a low-phenylalanine diet become pregnant, the offspring can still be affected with maternal PKU syndrome. Even if the fetus has not inherited the disease (i.e., the fetus is heterozygous for the *PAH* mutation), high blood phenylalanine in the mother has a teratogenic effect, causing microcephaly and congenital heart abnormalities in the fetus. Because these developmental responses to high phenylalanine occur during the first months of pregnancy, dietary control of blood phenylalanine must begin prior to conception and be maintained throughout the pregnancy.

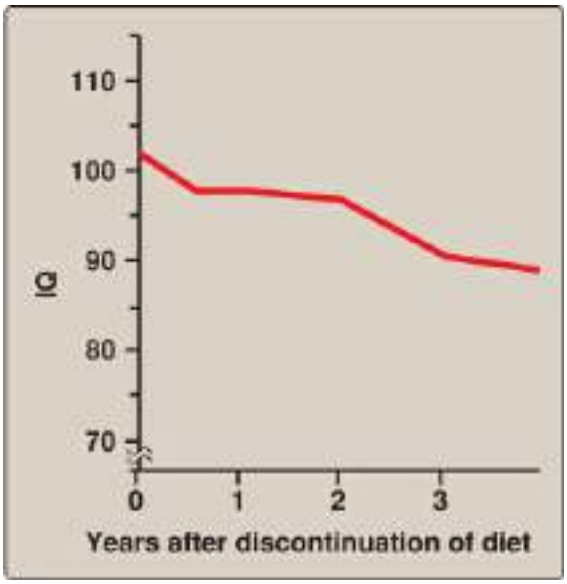


Figure 20.20
Changes in intelligence quotient (IQ) scores after discontinuation of low-phenylalanine diet in patients with phenylketonuria.

B. Maple syrup urine disease

MSUD is a rare (1:185,000), autosomal-recessive disorder in which there is a partial or complete deficiency in BCKD, the mitochondrial enzyme complex that oxidatively decarboxylates leucine, isoleucine, and valine (see Fig. 20.11). These BCAAs and their corresponding α-keto acids accumulate in the blood, causing a toxic effect that interferes with brain functions. The disease is characterized by feeding problems, vomiting, ketoacidosis, changes in muscle tone, neurologic problems that can result in coma (primarily because of the rise in leucine), and a characteristic maple syrup–like odor of the urine because of the rise in isoleucine. If untreated, the disease is fatal. If treatment is delayed, intellectual disability results.

1. Classification: MSUD includes a classic type and several variant forms. The classic, neonatal-onset form is the most common type of MSUD. Leukocytes or cultured skin fibroblasts from these patients show little or no BCKD activity. Infants with classic MSUD show symptoms within the first several days of life. If not diagnosed and treated, classic MSUD is lethal in the first weeks of life. Patients with intermediate forms have a higher level of enzyme activity (up to 30% of normal). The symptoms are milder and show an onset from infancy to adolescence. Patients with the rare thiamine-dependent variant of MSUD respond to large doses of this vitamin.

2. Screening and diagnosis: As with PKU, prenatal diagnosis and newborn screening are available, and most affected individuals are compound heterozygotes.

3. Treatment: MSUD is treated with a synthetic formula that is free of BCAA,

supplemented with limited amounts of leucine, isoleucine, and valine to allow for normal growth and development without producing toxic levels. (Note: Elevated leucine is the cause of the neurologic damage in MSUD, and its level is carefully monitored.) Early diagnosis and lifelong dietary treatment are essential if the child with MSUD is to develop normally. (Note: BCAAs are an important energy source in times of metabolic need, and individuals with MSUD are at risk of decompensation during periods of increased protein catabolism.)

C. Albinism

Albinism refers to a group of conditions in which a defect in tyrosine metabolism results in a deficiency in the production of melanin. These defects result in the partial or full absence of pigment from the skin, hair, and eyes. Albinism appears in different forms, and it may be inherited by one of several modes: autosomal recessive (primary mode), autosomal dominant, or X linked. Total absence of pigment from the hair, eyes, and skin (Fig. 20.21), tyrosinase-negative oculocutaneous albinism (type 1 albinism), results from an absent or defective copper-requiring tyrosinase, which catalyzes the first two steps in the synthesis of melanin from tyrosine. It is the most severe form of the condition. In addition to hypopigmentation, affected individuals have vision defects and photophobia (sunlight hurts their eyes). They also are at increased risk for skin cancer.

Figure 20.21
Patient with oculocutaneous albinism, showing white eyebrows and lashes and eyes that appear red in color.

D. Homocystinuria

The homocystinurias are a group of disorders involving defects in the metabolism of Hcy. These autosomal-recessive diseases are characterized by high urinary levels of Hcy, high plasma levels of Hcy and methionine, and low plasma levels of cysteine. The most common cause of homocystinuria is a defect in the enzyme cystathionine β-synthase, which converts Hcy to cystathionine (Fig. 20.22). Individuals homozygous for cystathionine β-synthase deficiency exhibit dislocation of the lens (ectopia lentis), skeletal anomalies (long limbs and fingers), intellectual

disability, and an increased risk for developing thrombi (blood clots). Thrombosis is the major cause of early death in these individuals. Treatment includes restriction of methionine and supplementation with vitamin B_{12} and folate. Cysteine becomes an essential amino acid, and must be supplemented. As glutathione is synthesized from cysteine (Fig. 13.6), adding cysteine to the diet is also helpful to reduce oxidative stress. Additionally, some patients are responsive to oral administration of pyridoxine (vitamin B_6), which is converted to pyridoxal phosphate, the coenzyme of cystathionine β-synthase. These patients usually have a milder and later onset of clinical symptoms compared with B_6-nonresponsive patients. (Note: Deficiencies in methylcobalamin [see Fig. 20.8] or N^5,N^{10}-MTHF reductase [(MTHFR), see Fig. 20.12] also result in elevated Hcy.)

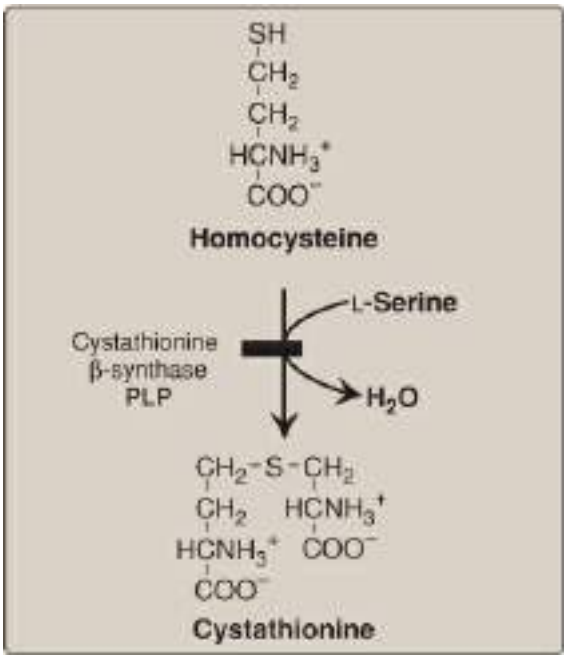


Figure 20.22
Enzyme deficiency in homocystinuria. PLP = pyridoxal phosphate.

E. Alkaptonuria

Alkaptonuria is a rare organic aciduria involving a deficiency in homogentisic acid oxidase, resulting in the accumulation of homogentisic acid (HA), an intermediate in the degradative pathway of tyrosine (see Fig. 20.15). The condition has three characteristic symptoms: homogentisic aciduria (the urine contains elevated levels of HA, which is oxidized to a dark pigment on standing, as shown in Fig. 20.23A), early onset of arthritis in the large joints, and deposition of black pigment (ochronosis) in cartilage and collagenous tissue (see Fig. 20.23B). Dark staining of diapers can indicate the disease in infants, but usually no symptoms are present until about age 40 years. Treatment includes dietary restriction of phenylalanine and tyrosine to reduce HA levels. Although alkaptonuria is not life threatening, the

associated arthritis may be severely crippling. (Note: Deficiencies in fumarylacetoacetate hydrolase, the terminal enzyme of tyrosine metabolism, result in tyrosinemia type I [see Fig. 20.15] and a characteristic cabbage-like odor to urine.)

F. Methylmalonic acidemia

Methylmalonic acidemia (MMA) is a rare (1:100,000) autosomal recessive disorder caused by a deficiency in methylmalonyl CoA mutase, which converts L-methylmalonyl CoA to succinyl CoA. Since the mutase requires vitamin B_{12}, the disease can also result from a severe B_{12} deficiency. The breakdown of odd-chain length fatty acids, valine, isoleucine, methionine, and threonine can all result in MMA, due to this enzyme deficiency. Elevation of methylmalonate in the blood and urine can result in a metabolic acidosis. There may also be an increase in propionyl-CoA, exacerbating the aciduria with an accumulation of additional propionic acid. Symptoms appear in early infancy, varying due to the degree of the enzyme deficiency, including failure to thrive, vomiting, dehydration, hypotonia, developmental delay, seizures, hepatomegaly, hyperammonemia, and a progressive encephalopathy. If severe and left untreated, it can lead to intellectual disability, chronic renal or hepatic damage, pancreatitis, and coma or death. Treatment includes a low-protein, high-calorie diet, and vitamin B_{12} supplementation. The diet limits the intake of isoleucine, threonine, methionine, and valine, as these amino acids can lead to the buildup of methylmalonic acid by the mutase deficiency.

Figure 20.23
Specimens from a patient with alkaptonuria. **A:** Urine. **B:** Vertebrae.

VII. Chapter Summary

- **Amino acids** whose catabolism yields **pyruvate** or an **intermediate** of the **TCA cycle** are termed **glucogenic** (Fig. 20.24). They can give rise to the net formation of **glucose** in the **liver** and **kidneys**. The solely glucogenic amino acids are glutamine, glutamate, proline, arginine, histidine, alanine, serine, glycine, cysteine, methionine, valine, threonine, aspartate, and asparagine.
- Amino acids whose catabolism yields either acetyl CoA (directly, without pyruvate serving as an intermediate) or acetoacetate (or its precursor acetoacetyl CoA) are termed **ketogenic**. Leucine and lysine are solely ketogenic.
- Tyrosine, phenylalanine, tryptophan, and isoleucine are both ketogenic and glucogenic.
- **Nonessential amino acids** can be synthesized from metabolic intermediates or from the carbon skeletons of essential amino acids.
- **Essential amino acids** need to be obtained from the **diet**. They include histidine, methionine, threonine, valine, isoleucine, phenylalanine, tryptophan, leucine, and lysine.
- **PKU** is caused by a **deficiency** of **PAH**, which converts phenylalanine to tyrosine. **Hyperphenylalaninemia** may also be caused by deficiencies in the enzymes that synthesize or regenerate the coenzyme for PAH, **BH_4**. Untreated individuals with PKU suffer from severe intellectual disability, developmental delay, microcephaly, seizures, and a characteristic musty (mousy) smell of the urine. Treatment involves controlling dietary phenylalanine. **Tyrosine** becomes an essential dietary component for people with PKU.
- **MSUD** is caused by a partial or complete deficiency in **BKCD**, the enzyme that decarboxylates the **BCAAs, leucine**, **isoleucine**, and **valine**. Symptoms include feeding problems, vomiting, ketoacidosis, changes in muscle tone, and a characteristic sweet smell of the urine. If untreated, the disease leads to neurologic problems that result in death. Treatment involves controlling BCAA intake.
- Other important genetic diseases associated with amino acid metabolism include **albinism**, **homocystinuria**, **MMA**, **alkaptonuria**, **histidinemia**, **tyrosinemia**, and **cystathioninuria**.

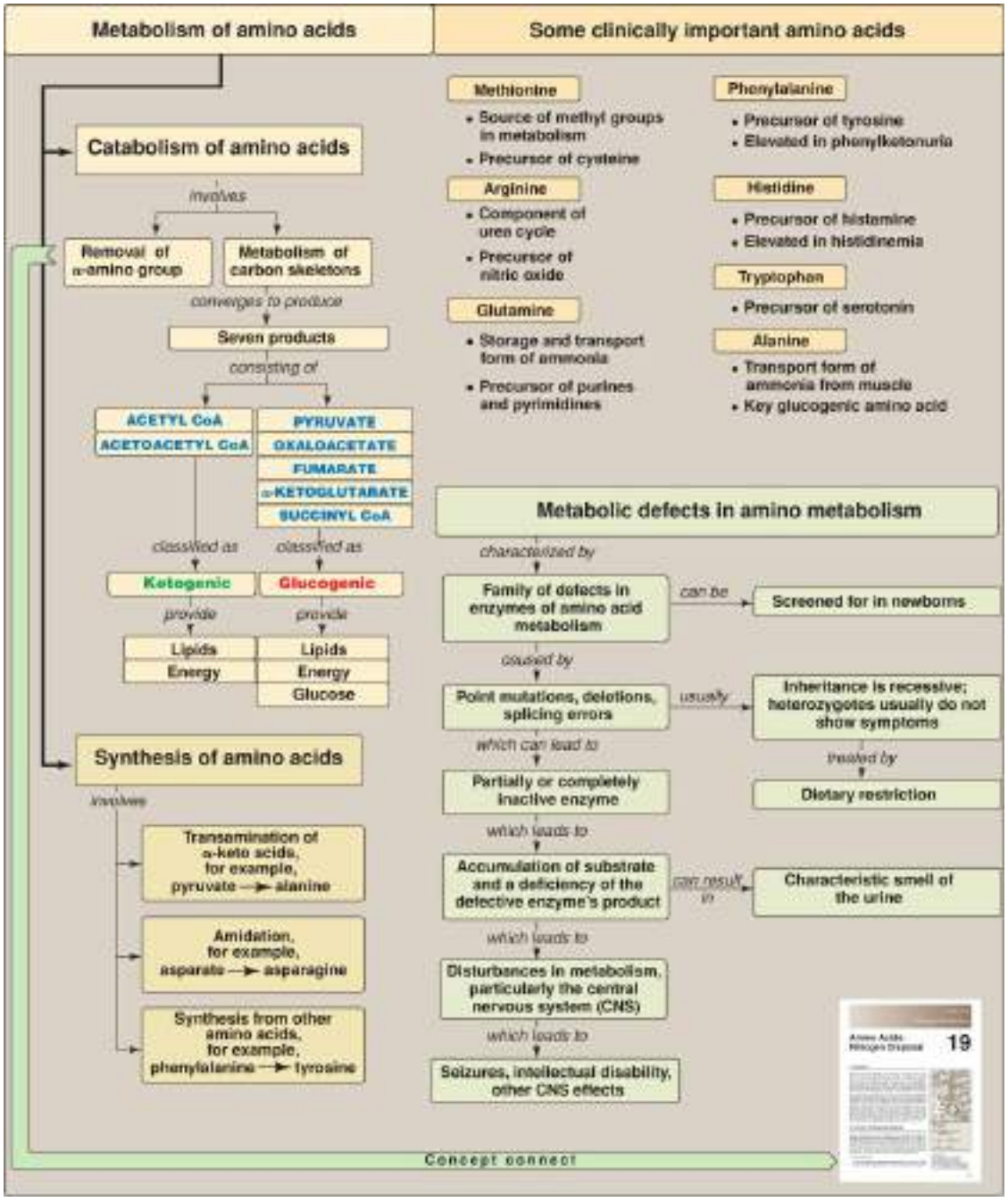


Figure 20.24
Key concept map for amino acid metabolism. CoA = coenzyme A.

Study Questions

Choose the ONE best answer.

For Questions 20.1 to 20.3, match the deficient enzyme with the associated clinical sign or laboratory finding in urine.

A. Black pigmentation of cartilage
B. Sweaty feet–like odor of fluids

C. Cystine crystals in urine
D. White hair, red eye color
E. Increased branched-chain amino acids
F. Increased homocysteine
G. Increased methionine
H. Increased phenylalanine

20.1 Cystathionine β-synthase

20.2 Homogentisic acid oxidase

20.3 Tyrosinase

Correct answers = F, A, D, respectively. A deficiency in cystathionine β-synthase of methionine degradation results in a rise in homocysteine. A deficiency in homogentisic acid oxidase of tyrosine degradation results in a rise in homogentisic acid, which forms a black pigment that is deposited in connective tissue (ochronosis). A deficiency in tyrosinase results in decreased formation of melanin from tyrosine in the skin, hair, and eyes. A sweaty feet–like odor is characteristic of isovaleryl coenzyme A dehydrogenase deficiency. Cystine crystals in urine are seen with cystinuria, a defect in intestinal and renal cystine absorption. Increased branched-chain amino acids are seen in maple syrup urine disease, increased methionine is seen in defects in homocysteine metabolism, and increased phenylalanine is seen in phenylketonuria.

20.4 A 1-week-old infant, who was born at home in a rural, medically underserved area, has undetected classic phenylketonuria. Which statement about this baby and/or her treatment is correct?

A. A diet devoid of phenylalanine should be initiated immediately.
B. Dietary treatment will be discontinued in adulthood.
C. Supplementation with vitamin B_6 is required.
D. Tyrosine is an essential amino acid.
E. Folic acid supplementation may increase PAH activity.

Correct answer = D. In patients with phenylketonuria, tyrosine cannot be synthesized from phenylalanine and, hence, becomes essential and must be supplied in the diet. Phenylalanine in the diet must be controlled but cannot be eliminated entirely because it is an essential amino acid. Dietary treatment must begin during the first 7 to 10 days of life to prevent intellectual disability, and lifelong restriction of phenylalanine is recommended to prevent cognitive decline. Additionally, elevated levels of phenylalanine are teratogenic to a developing fetus. The cofactor for PAH is tetrahydrobiopterin (BH_4). BH_4 supplementation may help reduced phenyalanine levels if the enzyme defect is in BH_4 production or its reduction from dihydrobiopterin.

20.5 Which one of the following statements concerning amino acids is correct?

A. Alanine is ketogenic.
B. Amino acids that are catabolized directly to acetyl coenzyme A (CoA) (without forming pyruvate as an intermediate) are glucogenic.
C. Branched-chain amino acids are catabolized primarily in the liver.
D. Cysteine is essential for individuals consuming a diet severely limited in methionine.
E. Alanine is an essential amino acid.

Correct answer = D. Methionine is the precursor of cysteine, which becomes essential if methionine is severely restricted. Alanine is a key glucogenic amino acid. Acetyl CoA cannot be used for the net synthesis of glucose. Amino acids catabolized to acetyl CoA, acetoacetate, and acetoacetyl CoA are ketogenic. Branched-chain amino acids are catabolized primarily in skeletal muscle. Alanine is a nonessential amino acid, synthesized from pyruvate by a transaminase.

20.6 In an individual with the dihydrolipoyl dehydrogenase (E3)-deficient form of maple syrup urine disease, why would lactic acidosis be an expected finding?

The three α-ketoacid dehydrogenase complexes (pyruvate dehydrogenase [PDH], α-ketoglutarate dehydrogenase, and branched-chain α-keto acid dehydrogenase [BCKD]) have Enzyme 3, or E3 in common. In E3-deficient maple syrup urine disease, in addition to the branched-chain amino acids and their α-keto acid derivatives accumulating as a result of decreased activity of BCKD, lactate will also be increased because of decreased activity of PDH.

20.7 In contrast to the vitamin B_6–derived pyridoxal phosphate required in most enzymic reactions involving amino acids, what coenzyme is required by the aromatic amino acid hydroxylases?

Tetrahydrobiopterin, made from guanosine triphosphate, is the required coenzyme.

Amino Acids: Conversion to Specialized Products 21

I. OVERVIEW

In addition to serving as building blocks for proteins, amino acids are precursors of many nitrogen (N)-containing compounds that have important physiologic functions (Fig. 21.1). These molecules include porphyrins, neurotransmitters, hormones, purines, and pyrimidines. (Note: See p. 166 for the synthesis of nitric oxide from arginine.)

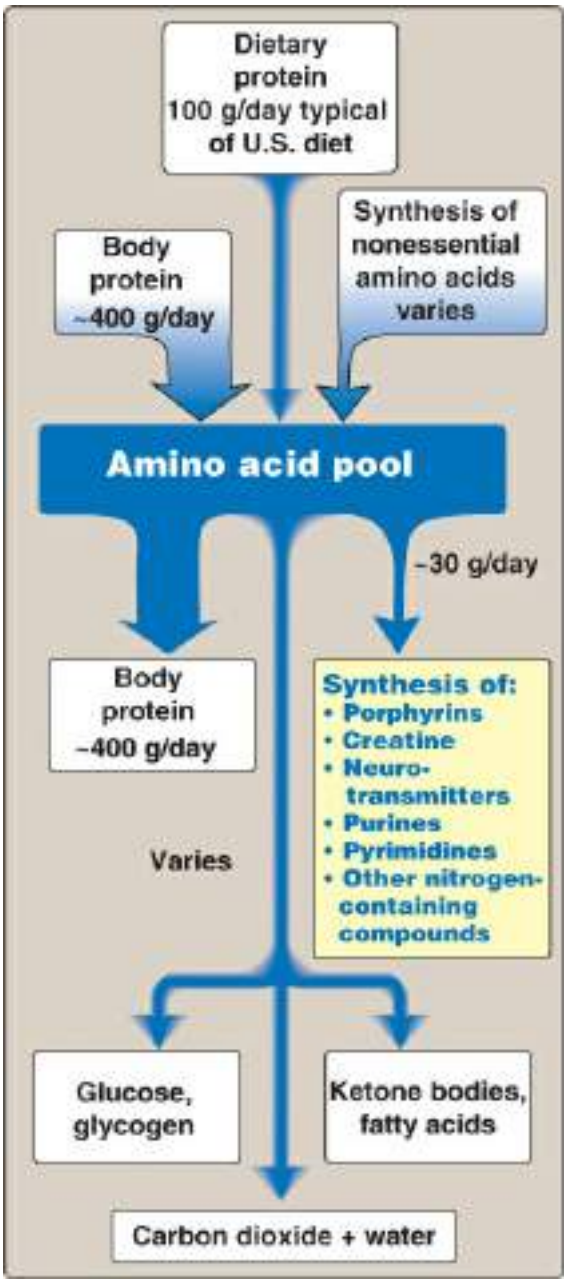


Figure 21.1
Amino acids as precursors of nitrogen-containing compounds.

II. PORPHYRIN METABOLISM

Porphyrins are cyclic compounds that readily bind metal ions, usually ferrous (Fe^{2+}) or ferric (Fe^{3+}) iron. The most prevalent metalloporphyrin in humans is heme, which consists of one Fe^{2+} coordinated in the center of the tetrapyrrole ring of protoporphyrin IX (see p. 310). Heme is the prosthetic group for hemoglobin (Hb), myoglobin, the cytochromes, including the cytochrome P450 (CYP) monooxygenase system, catalase, nitric oxide synthase, and peroxidase. These heme proteins are rapidly synthesized and

degraded. For example, 6 to 7 g of Hb is synthesized each day to replace heme lost through the normal turnover of erythrocytes. The synthesis and degradation of the associated porphyrins and recycling of the iron are coordinated with the turnover of heme proteins.

A. Structure

Porphyrins are cyclic planar molecules formed by the linkage of four pyrrole rings through methenyl bridges (Fig. 21.2). Three structural features of these molecules are relevant to understanding their medical significance.

1. Side chains: Different porphyrins vary in the nature of the side chains attached to each of the four pyrrole rings. Uroporphyrin contains acetate ($-CH_2-COO-$) and propionate ($-CH_2-CH_2-COO-$) side chains; coproporphyrin contains methyl ($-CH_3$) and propionate groups; and protoporphyrin IX (and heme b, the most common heme) contains vinyl ($-CH = CH_2$), methyl, and propionate groups. (Note: The methyl and vinyl groups are produced by decarboxylation of acetate and propionate side chains, respectively.)

Figure 21.2
Structures of uroporphyrin I and uroporphyrin III.

2. Side chain distribution: The side chains of porphyrins can be ordered around the tetrapyrrole nucleus in four different ways, designated by Roman numerals I to IV. Only type III porphyrins, which contain an asymmetric substitution on ring D (Fig. 21.2), are physiologically important in humans. (Note: Protoporphyrin IX is a member of the type III series.)

3. Porphyrinogens: These porphyrin precursors (e.g., uroporphyrinogen) exist in a chemically reduced, colorless form and serve as intermediates between porphobilinogen (PBG) and the oxidized, colored protoporphyrins in heme biosynthesis.

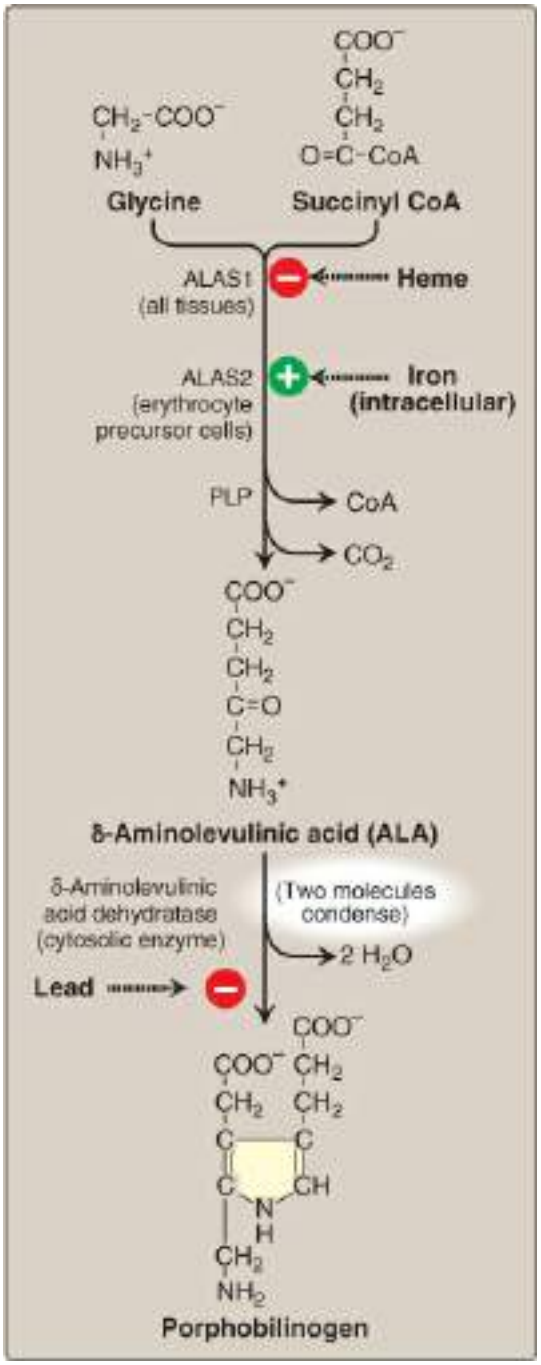


Figure 21.3
Pathway of porphyrin synthesis: Formation of porphobilinogen. (Note: ALAS1 is regulated by heme; ALAS2 is regulated by iron.) ALAS, δ-aminolevulinic acid synthase; CoA, coenzyme A; CO_2, carbon dioxide; PLP, pyridoxal phosphate. (Continued in Figs. 21.4 and 21.5.)

B. Heme biosynthesis

The major sites of heme biosynthesis are the liver and the erythrocyte-producing cells of the bone marrow. In the liver, which synthesizes a number of heme proteins (particularly the CYP proteins), the rate of heme synthesis is highly variable,

responding to alterations in the cellular heme pool caused by fluctuating demands for heme proteins. In contrast, heme synthesis in erythroid cells, which are active in Hb synthesis, is relatively constant and is matched to the rate of globin synthesis. (Note: Over 85% of all heme synthesis occurs in erythroid tissue. Mature red blood cells (RBCs) lack mitochondria and are unable to synthesize heme.) The initial reaction and the last three steps in the formation of porphyrins occur in mitochondria, whereas the intermediate steps of the biosynthetic pathway occur in the cytosol. (Note: Fig. 21.8 summarizes heme synthesis.)

1. δ-Aminolevulinic acid formation: All the carbon and nitrogen atoms of the porphyrin molecule are provided by glycine (a nonessential amino acid) and succinyl coenzyme A (a tricarboxylic acid cycle intermediate) that condense to form δ-aminolevulinic acid (ALA) in a reaction catalyzed by ALA synthase ([ALAS], Fig. 21.3). This reaction requires pyridoxal phosphate ([PLP], see p. 428) as a coenzyme and is the committed and rate-limiting step in porphyrin biosynthesis. (Note: There are two ALAS isoforms, each produced by different genes and controlled by different mechanisms. ALAS1 is found in all tissues, whereas ALAS2 is erythroid specific. Loss-of-function mutations in ALAS2 result in X-linked sideroblastic anemia and iron overload.)

   a. Heme (hemin) effects: When porphyrin production exceeds the availability of the apoproteins that require it, heme accumulates and is converted to hemin by the oxidation of Fe^{2+} to Fe^{3+}. Hemin decreases the amount (and, thus, the activity) of ALAS1 by repressing transcription of its gene, increasing degradation of its messenger RNA, and decreasing import of the enzyme into mitochondria. (Note: In erythroid cells, ALAS2 is controlled by the availability of intracellular iron [see p. 525].)

   b. Drug effects: Administration of any of a large number of drugs (and various environmental xenobiotic chemicals, present in certain foods, cosmetics, and commercial products) results in a significant increase in hepatic ALAS1 activity. These molecules are metabolized by the microsomal CYP monooxygenase system, a heme protein oxidase system found in the liver (see p. 164). In response to these drugs, the synthesis of CYP proteins increases, leading to an enhanced consumption of heme, a component of these proteins. This, in turn, causes a decrease in the concentration of free or unbound heme in liver cells. The lower intracellular concentration of unbound heme leads to an increase in the synthesis of ALAS1 and prompts a corresponding increase in the synthesis of ALA.

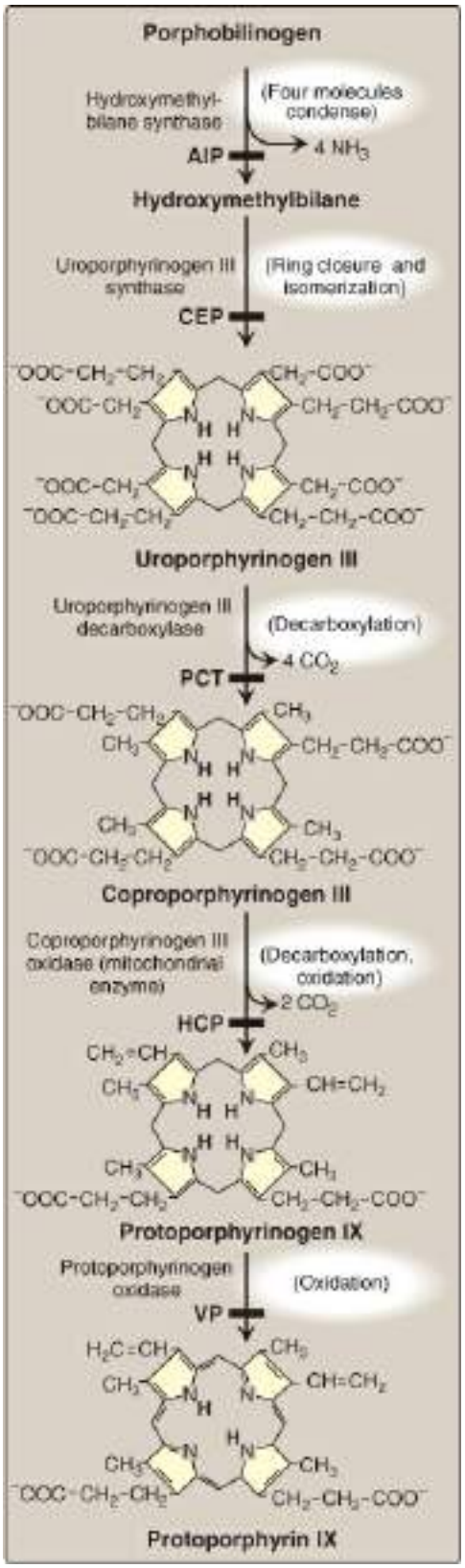


Figure 21.4
Pathway of porphyrin synthesis: formation of protoporphyrin IX. (Continued from Fig. 21.3.)

The prefixes uro- (urine) and copro- (feces) reflect initial sites of discovery. Enzyme deficiencies in porphyrias are indicated with black bars. AIP = acute intermittent porphyria; CEP = congenital erythropoietic porphyria; PCT = porphyria cutanea tarda; HCP = hereditary coproporphyria; VP = variegate porphyria. (Note: Deficiency in uroporphyrinogen III synthase prevents isomerization but not ring closure, resulting in production of type I porphyrins.)

2. Porphobilinogen formation: The cytosolic condensation of two ALA to form PBG by zinc-containing ALA dehydratase (PBG synthase) is extremely sensitive to inhibition by heavy metal ions (e.g., lead) that replace the zinc (Fig. 21.3). This inhibition is, in part, responsible for the elevation in ALA and the anemia caused by lead poisoning.

3. Uroporphyrinogen formation: Condensation of four PBG molecules, catalyzed by hydroxymethylbilane synthase, produces the linear tetrapyrrole hydroxymethylbilane. A deficiency in this enzyme results in acute intermittent porphyria (AIP, Fig. 21.4, also see p. 313 and 21.8 for more details for different forms of porphyrias). Uroporphyrinogen III synthase cyclizes and isomerizes hydroxymethylbilane to produce the asymmetric uroporphyrinogen III. A deficiency in this enzyme results in congenital erythropoietic porphyria (CEP). Uroporphyrinogen III undergoes decarboxylation of its acetate groups by uroporphyrinogen III decarboxylase (UROD), generating coproporphyrinogen III. A deficiency in this enzyme results in porphyria cutanea tarda (PCT). These three reactions occur in the cytosol.

4. Heme formation: Coproporphyrinogen III enters the mitochondrion, and two propionate side chains are decarboxylated by coproporphyrinogen III oxidase to vinyl groups generating protoporphyrinogen IX. A deficiency in this enzyme results in hereditary coproporphyria (HCP). Protoporphyrinogen IX is oxidized by protoporphyrinogen oxidase to protoporphyrin IX. A deficiency in this enzyme results in variegate porphyria (VP). The introduction of iron (as Fe^{2+}) into protoporphyrin IX produces heme. This step can occur spontaneously, but the rate is enhanced by ferrochelatase, an enzyme that, like ALA dehydratase, is inhibited by lead (Fig. 21.5). A deficiency in this enzyme results in erythropoietic protoporphyria (EPP).

Clinical Application 21.1: Lead Poisoning

Lead poisoning is a buildup of lead in the body over a period of months to years. Common sources for lead include exposure to lead-based paints and paint dust or flakes common in older buildings; lead in household plumbing pipes may also contaminate drinking water. Exposure can occur through inhalation, contact with the skin or mucous membranes, or ingestion. Lead has a sweet taste, and ingestion exposure is of special concern for infants and toddlers. Symptoms of lead poisoning may include developmental delays, learning disabilities and low IQ, abdominal pain, constipation, neurologic changes, and irritability. Very high lead levels can be fatal. Lead inhibits ALA dehydratase and ferrochelatase, both enzymes involved in the synthesis of heme, and therefore causes a decrease in heme synthesis. Further, high levels of lead impair iron utilization. This results in increased use of zinc (instead of iron) as substrate for chelation to protoporphyrin IX by ferrochelatase. Consequently, patients with lead poisoning may present with anemia and elevated levels of zinc protoporphyrin. The increase in ALA can be toxic to neurons. Lead can also cross the blood–brain barrier and is neurotoxic. The usual treatment is to remove

the source of exposure to the lead contaminant, but in cases of severe lead poisoning (greater than 45 µg/dl measured in the serum), divalent chelators such as succimer (DMSA, 2,3-dimercaptosuccinic acid), calcium disodium ethylenediaminetetraacetic acid (EDTA) or others may be used to remove excess lead ions from the blood.

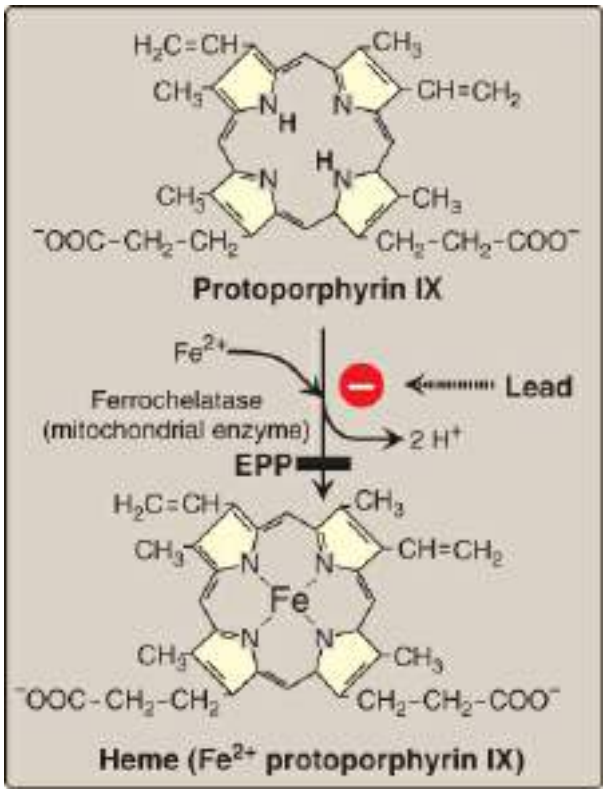


Figure 21.5
Pathway of porphyrin synthesis: formation of heme b. (Continued from Figs. 21.3 and 21.4.) Fe^{2+} = ferrous iron. Enzyme deficiency in porphyria is indicated with *black bar*; EPP = erythropoietic protoporphyria.

C. Porphyrias

Porphyrias are rare, inherited (or sometimes acquired) defects in heme synthesis, resulting in the accumulation and increased excretion of porphyrins or porphyrin precursors (Fig. 21.8). (Note: Inherited porphyrias are autosomal-dominant (AD) or autosomal-recessive (AR) disorders.) Each porphyria results in the accumulation of a unique pattern of intermediates caused by the deficiency of an enzyme in the heme synthetic pathway. (Note: Porphyria, derived from the Greek for purple, refers to the red-blue color caused by pigment-like porphyrins in the urine of some patients with defects in heme synthesis.)

1. Clinical manifestations: The porphyrias are classified as erythropoietic or hepatic, depending on whether the enzyme deficiency occurs in the erythropoietic cells of the bone marrow or in the liver. Hepatic porphyrias can be further classified as chronic or acute. In general, individuals with an enzyme defect prior to the synthesis of the tetrapyrroles manifest abdominal and

neuropsychiatric signs, whereas those with enzyme defects leading to the accumulation of tetrapyrrole intermediates show photosensitivity (i.e., their skin itches and burns [pruritus] when exposed to sunlight). (Note: Photosensitivity is a result of the oxidation of colorless porphyrinogens to colored porphyrins, which are photosensitizing molecules thought to participate in the formation of superoxide radicals from oxygen. These radicals can oxidatively damage membranes and cause the release of destructive enzymes from lysosomes.)

a. Chronic hepatic porphyria: PCT, the most common porphyria, is a chronic disease of the liver. The disease is associated with severe deficiency of UROD, but clinical expression of the deficiency is influenced by various factors, such as hepatic iron overload, exposure to sunlight, alcohol ingestion, estrogen therapy, and the presence of hepatitis B or C or HIV infections. (Note: Mutations to UROD are found in only 20% of affected individuals. Inheritance is AD.) Clinical onset is typically during the fourth or fifth decade of life. Porphyrin accumulation leads to cutaneous symptoms (Fig. 21.6) as well as urine that is red to brown in natural light (Fig. 21.7) and pink to red in fluorescent light.

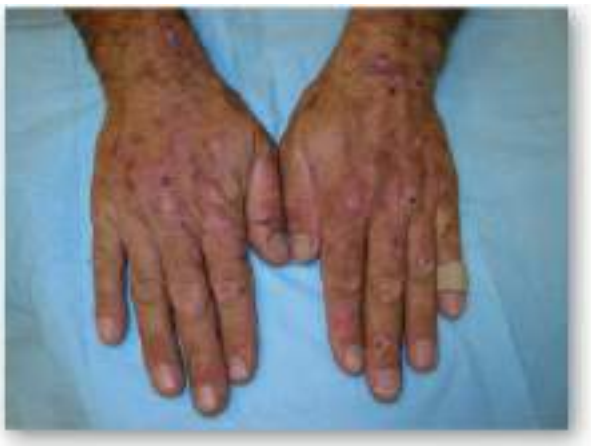


Figure 21.6
Skin eruptions in a patient with porphyria cutanea tarda.

b. Acute hepatic porphyrias: Acute hepatic porphyrias (ALA dehydratase–deficiency porphyria, AIP, HCP, and VP) are characterized by acute attacks of gastrointestinal (GI), neuropsychiatric, and motor symptoms that may be accompanied by photosensitivity (Fig. 21.8). Porphyrias leading to accumulation of ALA and PBG, such as AIP, cause abdominal pain and neuropsychiatric disturbances, ranging from anxiety to delirium. Symptoms of the acute hepatic porphyrias are often precipitated by use of drugs, such as barbiturates and ethanol, which induce the synthesis of the heme-containing CYP microsomal drug-oxidation system. This further decreases the amount of available heme, which, in turn, promotes increased synthesis of ALAS1.

c. Erythropoietic porphyrias: The chronic erythropoietic porphyrias (CEP and EPP) cause photosensitivity characterized by skin rashes and blisters that appear in early childhood (Fig. 21.8).

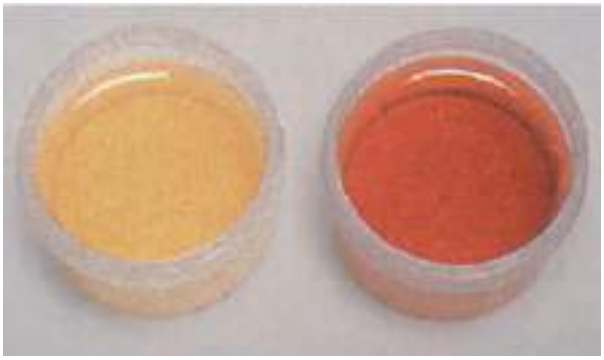


Figure 21.7
Urine from a patient with porphyria cutanea tarda (right) and from a patient with normal porphyrin excretion (left).

2. Increased δ-aminolevulinic acid synthase activity: One common feature of the hepatic porphyrias is decreased synthesis of heme. In the liver, heme normally functions as a repressor of the ALAS1 gene. Therefore, the absence of this end product results in an increase in the synthesis of ALAS1 (derepression/activation). This causes an increased synthesis of intermediates that occur prior to the genetic block. The accumulation of these toxic intermediates is the major pathophysiology of the porphyrias.

3. Treatment: During acute porphyria attacks, patients require medical support, particularly treatment for pain and vomiting. The severity of acute symptoms of the porphyrias can be diminished by intravenous injection of hemin and glucose. Hemin consists of a protoporphyrin structure with a ferric iron (Fe^{3+}) coordinated with a chloride ion. Hemin administration reduces the deficit of porphyrins. This in turn decreases the synthesis of ALAS1 and minimizes the production of toxic porphyrin intermediates. High doses of glucose can also decrease porphyrin biosynthesis in the liver. These treatments are particularly effective to treat AIP and other acute porphyrias. Protection from sunlight, ingestion of β-carotene (provitamin A; see p. 432) that scavenges free radicals, and phlebotomy (removes porphyrins) are helpful in porphyrias with photosensitivity.

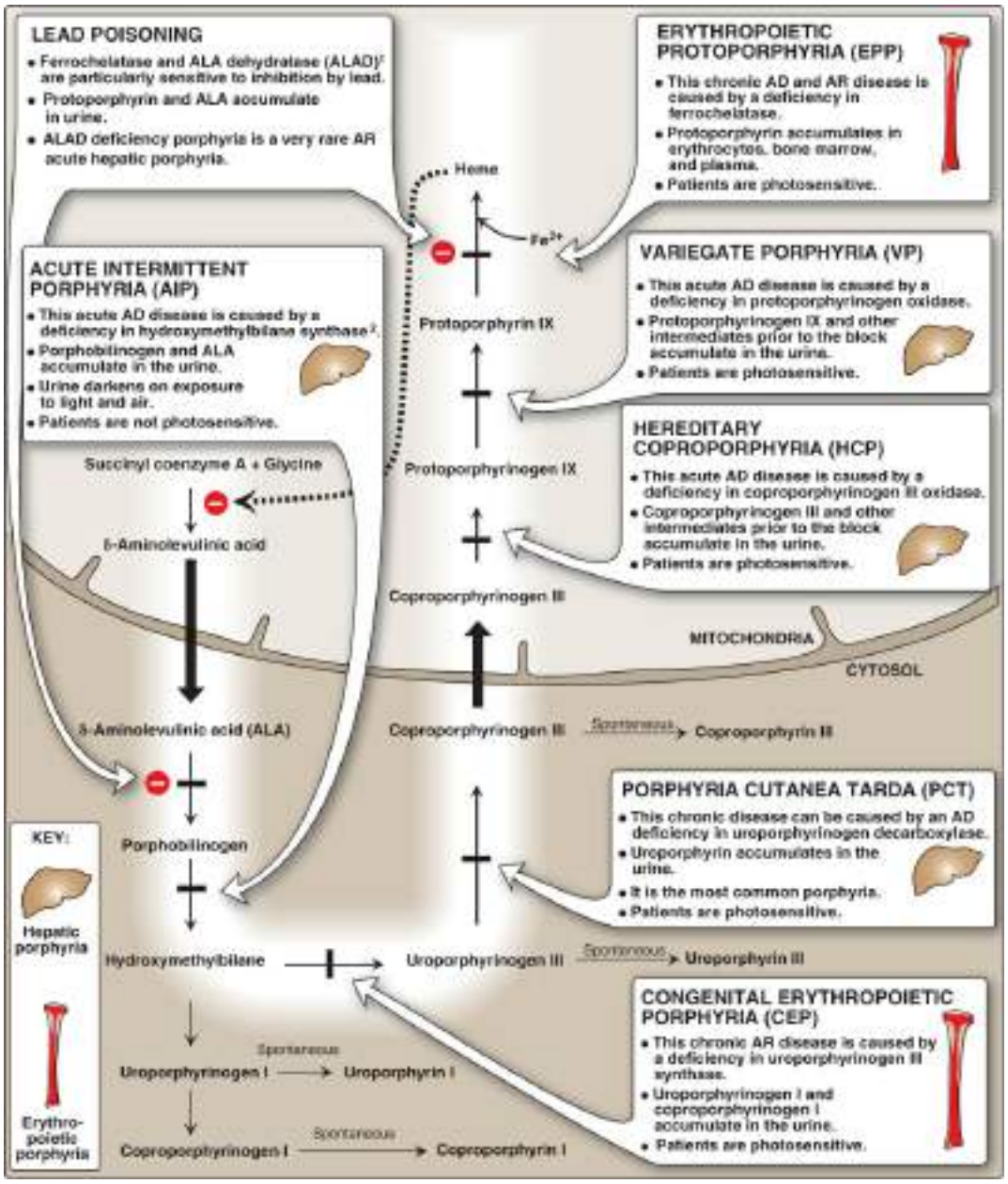


Figure 21.8

Summary of heme synthesis. [1]Also referred to as porphobilinogen synthase. [2]Also referred to as porphobilinogen deaminase. (Note: Symptomatic deficiencies in ALA synthase-1 [ALAS1] are unknown. Deficiencies in X-linked ALAS2 result in an anemia.) ALA = δ-aminolevulinic acid; AD = autosomal dominant; AR = autosomal recessive; Fe = iron.

D. Heme degradation

After ~120 days in the circulation, RBCs are taken up and degraded by the mononuclear phagocyte system (MPS), particularly in the liver and spleen (Fig. 21.9). Approximately 85% of heme destined for degradation comes from senescent RBCs (Fig. 21.10). The remainder is from the degradation of heme proteins other than Hb.

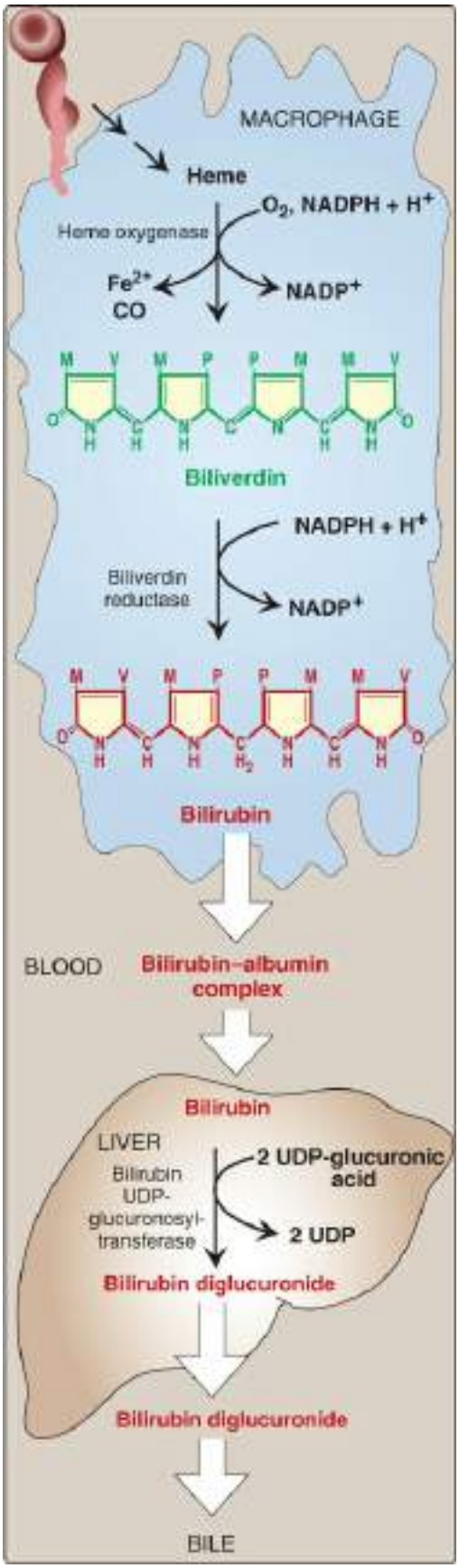


Figure 21.9
Formation of bilirubin from heme and its conversion to bilirubin diglucuronide. UDP = uridine diphosphate; Fe = iron; CO = carbon monoxide; NADP(H) = nicotinamide adenine dinucleotide phosphate.

1. Bilirubin formation: The first step in the degradation of heme is catalyzed by microsomal heme oxygenase in macrophages of the MPS. In the presence of nicotinamide adenine dinucleotide phosphate and oxygen, the enzyme catalyzes three successive oxygenations that result in opening of the porphyrin ring (converting cyclic heme to linear biliverdin), production of carbon monoxide (CO), and release of Fe^{2+} (Fig. 21.9). (Note: The CO has biologic function, acting as a signaling molecule and anti-inflammatory. Iron is discussed in Chapter 29.) Biliverdin, a green pigment, is reduced, forming the red-orange bilirubin. Bilirubin and its derivatives are collectively termed bile pigments. (Note: The changing colors of a bruise reflect the varying pattern of intermediates that occurs during heme degradation.)

> Bilirubin, unique to mammals, appears to function at low levels as an antioxidant. In this role, it is oxidized to biliverdin, which is then reduced by biliverdin reductase, regenerating bilirubin.

2. Bilirubin uptake by the liver: Because bilirubin is only slightly soluble in plasma, it is transported through blood to the liver by binding noncovalently to albumin. (Note: Certain anionic drugs, such as salicylates and sulfonamides, can displace bilirubin from albumin, permitting bilirubin to enter the central nervous system [CNS]. This causes the potential for neural damage in infants [see p. 317].) Bilirubin dissociates from the carrier albumin molecule, enters a hepatocyte via facilitated diffusion, and binds to intracellular proteins, particularly the protein ligandin.

3. Bilirubin diglucuronide formation: In the hepatocyte, bilirubin solubility is increased by the sequential addition of two molecules of glucuronic acid in a process called conjugation. The reactions are catalyzed by microsomal bilirubin UDP-glucuronosyltransferase (bilirubin UGT) using uridine diphosphate (UDP)-glucuronic acid as the glucuronate donor. The bilirubin diglucuronide product is referred to as conjugated bilirubin (CB). (Note: Varying degrees of deficiency of bilirubin UGT result in Crigler–Najjar I and II and Gilbert syndrome, with Crigler–Najjar I being the most severe.)

4. Bilirubin secretion into bile: CB is actively transported against a concentration gradient into the bile canaliculi and then into the bile. This energy-dependent, rate-limiting step is susceptible to impairment in liver disease. (Note: A rare deficiency in the protein required for transport of CB out of the liver results in Dubin–Johnson syndrome.) Unconjugated bilirubin (UCB) is normally not secreted into bile.

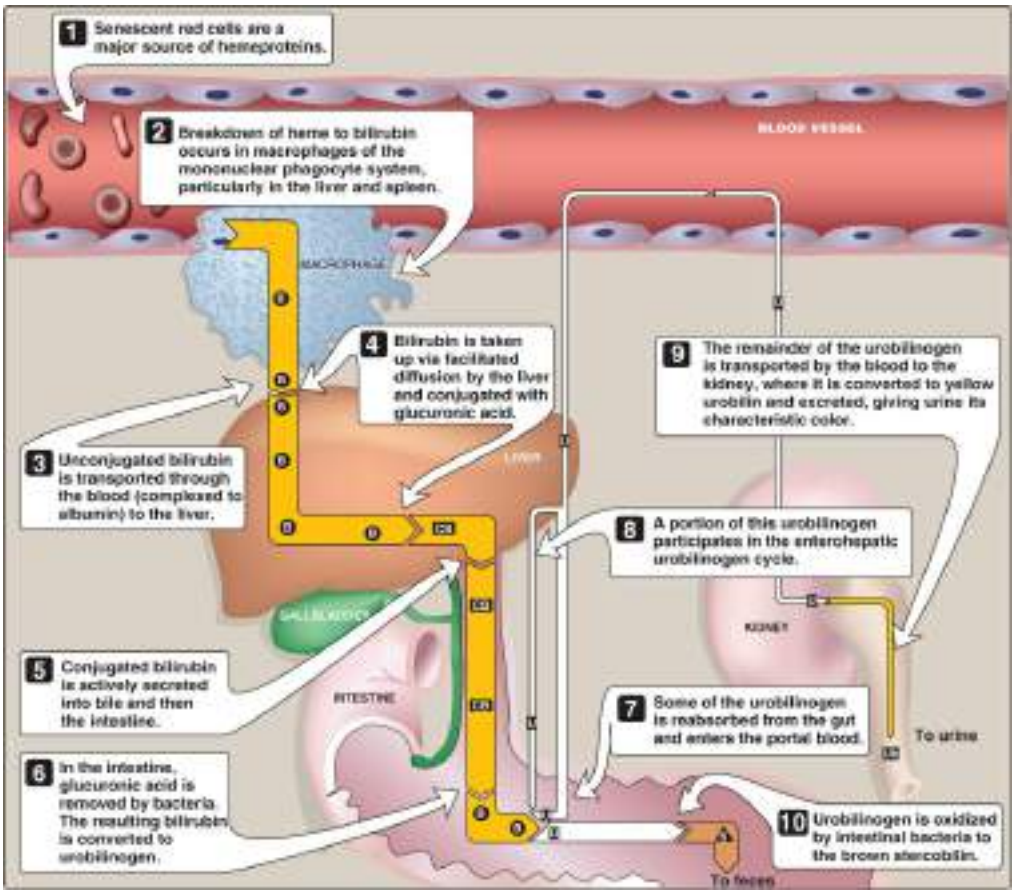


Figure 21.10
Catabolism of heme. B = bilirubin; CB = conjugated bilirubin; U = urobilinogen; UB = urobilin; S = stercobilin.

5. Urobilin formation in the intestine: CB is hydrolyzed and reduced by gut bacteria to yield urobilinogen, a colorless compound. Most of the urobilinogen is further oxidized by bacteria to stercobilin, which gives feces the characteristic brown color. However, some is reabsorbed from the gut and enters the portal blood. A portion of this urobilinogen participates in the enterohepatic urobilinogen cycle in which it is taken up by the liver and then resecreted into the bile. The remainder of the urobilinogen is transported by the blood to the kidney, where it is converted to yellow urobilin and excreted, giving urine its characteristic color. The metabolism of bilirubin is summarized in Figure 21.10.

E. Jaundice

Jaundice (or, icterus) refers to the yellow color of skin, nail beds, and sclerae (whites of the eyes) caused by bilirubin deposition, secondary to increased bilirubin levels in the blood (hyperbilirubinemia) as shown in Figure 21.11. Although not a disease, jaundice is usually a symptom of an underlying disorder. (Note: Blood bilirubin levels are normally ≤1 mg/dl. Jaundice is seen at 2 to 3 mg/dl.)

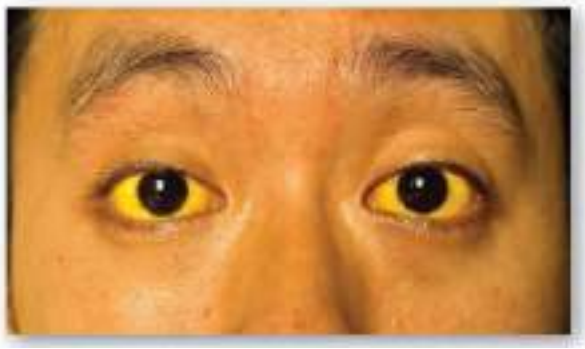


Figure 21.11
Jaundiced patient with the sclerae of his eyes appearing yellow.

1. Types: Jaundice can be classified into three major types described below. However, in clinical practice, jaundice is often more complex than indicated in this simple classification. For example, the accumulation of bilirubin may be a result of defects at more than one step in its metabolism.

a. Hemolytic (prehepatic): The liver has the capacity to conjugate and excrete >3,000 mg of bilirubin/day, whereas the normal production of bilirubin is only 300 mg/day. This excess capacity allows the liver to respond to increased heme degradation with a corresponding increase in conjugation and secretion of CB. However, extensive hemolysis (e.g., in patients with sickle cell anemia or deficiency of pyruvate kinase or glucose 6-phosphate dehydrogenase) may produce bilirubin faster than it can be conjugated. UCB levels in the blood become elevated (unconjugated hyperbilirubinemia), causing jaundice (Fig. 21.12A). Due to hemolysis, CB levels may be greatly elevated to the upper most range of normal hepatic capacity and excreted into the bile. The amount of urobilinogen entering the enterohepatic circulation is also increased, as well as urinary urobilinogen. Still, CB, urobilinogen, stercobilin, and urobilin levels are seen at the higher side of their normal ranges. In hemolytic jaundice, only UCB levels are abnormally high in the blood.

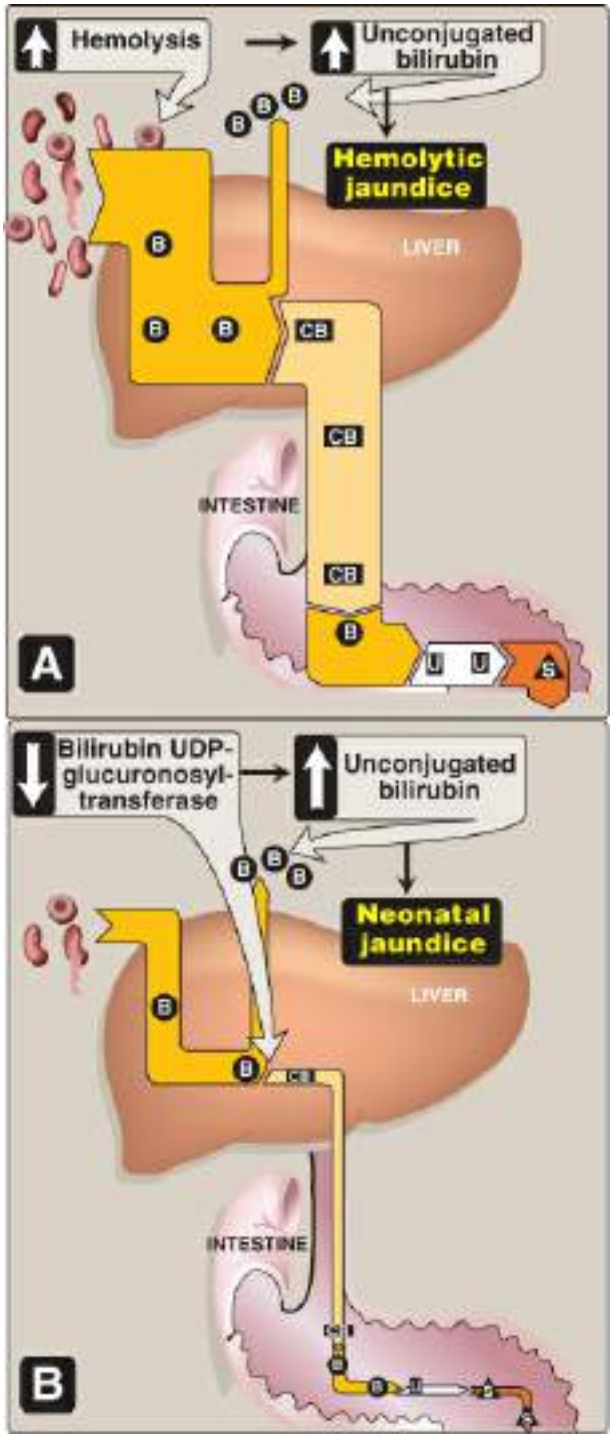


Figure 21.12
Alterations in the metabolism of heme. A: Hemolytic jaundice. B: Neonatal jaundice. CB = conjugated bilirubin; B = bilirubin; U = urobilinogen; S = stercobilin; UDP = uridine diphosphate.

b. Hepatocellular (hepatic): Damage to liver cells (e.g., in patients with cirrhosis or hepatitis) can cause unconjugated hyperbilirubinemia as a result of decreased conjugation. Urobilinogen is increased in the urine because

hepatic damage decreases the enterohepatic circulation of this compound, allowing more to enter the blood, from which it is filtered into the urine. The urine consequently darkens, whereas stools may be a pale, clay color. Plasma levels of alanine and aspartate transaminases (ALT and AST, respectively; see p. 276) are elevated. If CB is made but is not efficiently secreted from the liver into bile (intrahepatic cholestasis), it can leak into the blood (regurgitation), causing a conjugated hyperbilirubinemia. In hepatic jaundice, both UCB and CB levels are abnormally elevated in the blood.

c. Obstructive (posthepatic): In this instance, jaundice is not caused by overproduction of bilirubin or decreased conjugation but, instead, results from obstruction of the common bile duct (extrahepatic cholestasis). For example, the presence of a tumor or bile stones may block the duct, preventing passage of CB into the intestine. Patients with obstructive jaundice experience GI pain and nausea and produce stools that are a pale, clay color. The CB regurgitates into the blood (conjugated hyperbilirubinemia). The CB is eventually excreted in the urine (which darkens over time) and is referred to as urinary bilirubin. Urinary urobilinogen is absent.

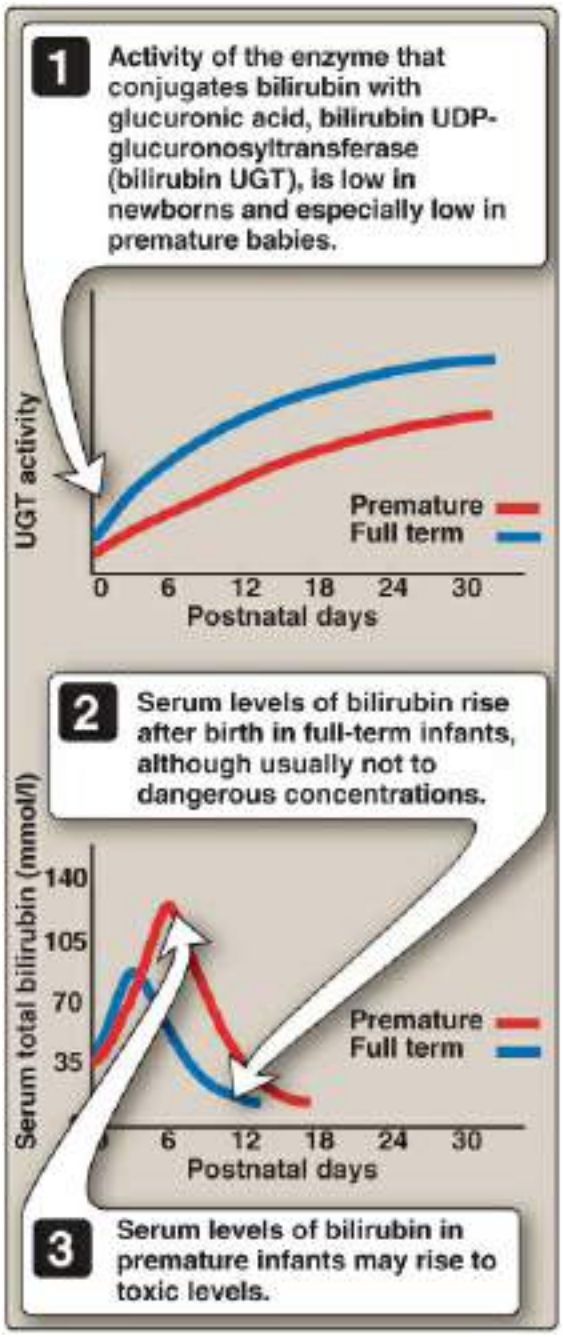


Figure 21.13
Neonatal jaundice. UDP = uridine diphosphate.

2. Jaundice in newborns: Most newborn infants (60% of full term and 80% of preterm) show a rise in UCB in the first postnatal week (and a transient, physiologic jaundice) because the activity of hepatic bilirubin UGT is low at birth (it reaches adult levels in about 4 weeks), as shown in Figures 21.12B and Figure 21.13. Elevated UCB, in excess of the binding capacity of albumin (20 to 25 mg/dl), can diffuse into the basal ganglia, causing toxic encephalopathy (kernicterus) and a pathologic jaundice. Therefore, newborns with significantly

elevated bilirubin levels are treated with blue fluorescent light (phototherapy), as shown in Figure 21.14, which converts bilirubin to more polar and, therefore, water-soluble isomers. These photoisomers can be excreted into the bile without conjugation to glucuronic acid. (Note: Because of solubility differences, only UCB crosses the blood–brain barrier, and only CB appears in urine.)

3. Bilirubin measurement: Bilirubin is commonly measured by the van den Bergh reaction, in which diazotized sulfanilic acid reacts with bilirubin to form red azodipyrroles that are measured colorimetrically. In aqueous solution, the water-soluble CB reacts rapidly with the reagent (within 1 minute) and is said to be direct reacting. The UCB, which is much less soluble in aqueous solution, reacts more slowly. However, when the reaction is carried out in methanol, both CB and UCB are soluble and react with the reagent, providing the total bilirubin value. The indirect-reacting bilirubin, which corresponds to the UCB, is obtained by subtracting the direct-reacting bilirubin from the total bilirubin. (Note: In normal plasma, only ~4% of the total bilirubin is conjugated, or direct reacting, because most is secreted into bile.)

III. OTHER NITROGEN-CONTAINING COMPOUNDS

A. Catecholamines

Dopamine, norepinephrine (NE), and epinephrine (or, adrenaline) are biologically active (biogenic) amines that are collectively termed catecholamines. Dopamine and NE are synthesized in the brain and function as neurotransmitters. Epinephrine is synthesized from NE in the adrenal medulla.

Figure 21.14
Phototherapy in neonatal jaundice.

1. Function: Outside the CNS, NE and its methylated derivative, epinephrine, are hormone regulators of carbohydrate and lipid metabolism. NE and epinephrine are released from storage vesicles in the adrenal medulla in response to fright, exercise, cold, and low levels of blood glucose. They increase the degradation of glycogen and triacylglycerol as well as increase blood pressure and the output of the heart. These effects are part of a coordinated response to prepare the individual for stress and are often called the “fight-or-flight” reactions.

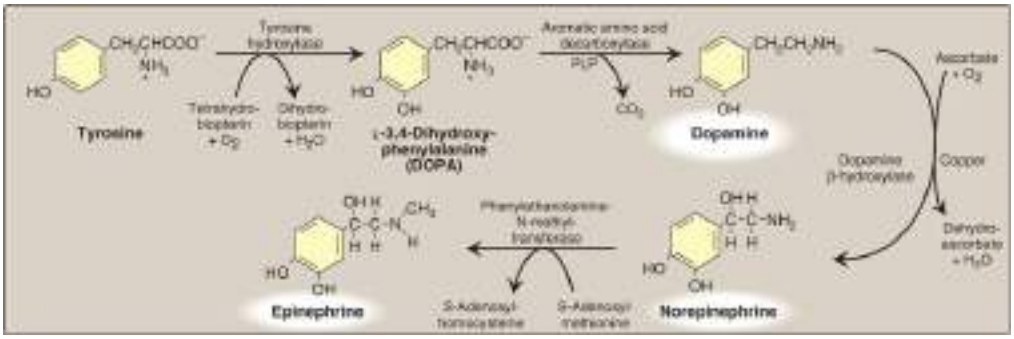


Figure 21.15
Synthesis of catecholamines. (Note: Catechols have two adjacent hydroxyl groups.) PLP = pyridoxal phosphate.

2. Synthesis: The catecholamines are synthesized from tyrosine, as shown in Figure 21.15. Tyrosine is first hydroxylated by tyrosine hydroxylase to form L-3,4-dihydroxyphenylalanine (DOPA, a catechol) in a reaction analogous to that described for the hydroxylation of phenylalanine (see p. 292). The tetrahydrobiopterin (BH_4)-requiring enzyme is abundant in the CNS, the sympathetic ganglia, and the adrenal medulla, and it catalyzes the rate-limiting step of the pathway. DOPA is then decarboxylated in a reaction catalyzed by DOPA decarboxylase (DDC) and requiring PLP to form dopamine (the first catecholamine in the pathway). Dopamine is next hydroxylated by dopamine β-hydroxylase to yield NE in a reaction that requires ascorbic acid (vitamin C) and copper. Epinephrine is formed from NE by an N-methylation reaction using S-adenosylmethionine (SAM) as the methyl donor (see p. 293).

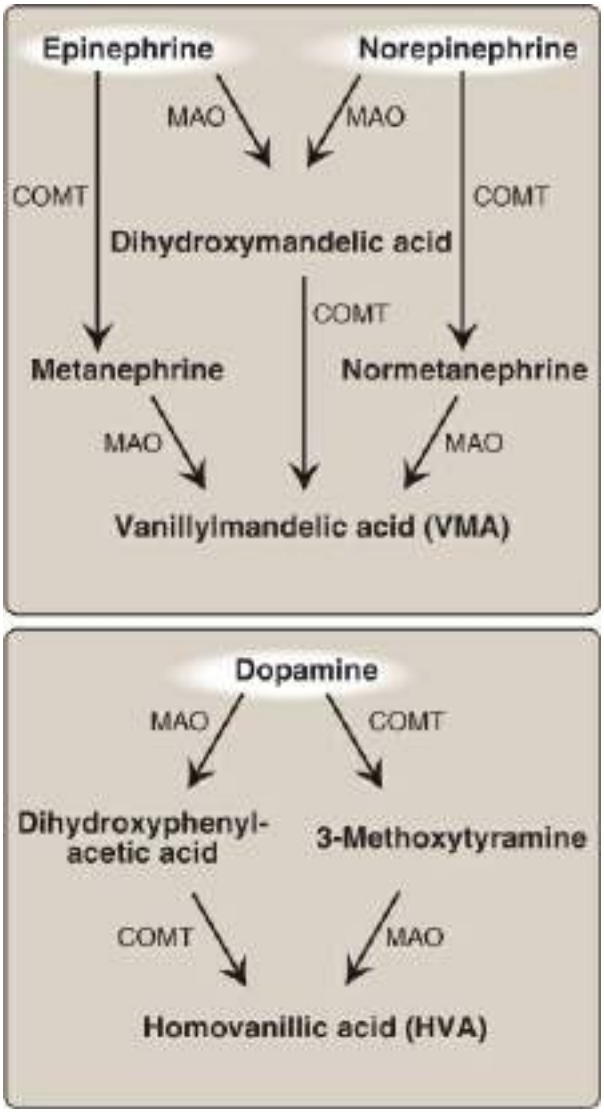


Figure 21.16
Metabolism of the catecholamines by catechol-O-methyltranferase (COMT) and monoamine oxidase (MAO). (Note: COMT requires S-adenosylmethionine.)

3. Degradation: The catecholamines are inactivated by oxidative deamination catalyzed by monoamine oxidase (MAO) and by O-methylation catalyzed by catechol-O-methyltransferase (COMT) using SAM as the methyl donor (Fig. 21.16). The reactions can occur in either order. The aldehyde products of the MAO reaction are oxidized to the corresponding acids. The products of these reactions are excreted in the urine as vanillylmandelic acid (VMA) from epinephrine and NE and homovanillic acid (HVA) from dopamine. (Note: VMA and the metanephrines are increased with pheochromocytomas, rare tumors of the adrenal gland characterized by excessive production of catecholamines.)

Clinical Applicaton 21.2: Parkinson Disease

Parkinson disease, a neurodegenerative movement disorder, is due to insufficient dopamine production as a result of the idiopathic loss of dopamine-producing cells in the brain. Administration of levodopa (L-DOPA) is the most common treatment, because dopamine cannot cross the blood–brain barrier. Carbidopa is a drug that inhibits DDC activity, preventing the conversion of L-DOPA to dopamine in the peripheral nervous system. Since carbidopa cannot cross the blood–brain barrier, when used in tandem with L-DOPA, it allows more peripheral L-DOPA to cross the blood–brain barrier, to reach a more therapeutic range in the CNS. In the case of a BH_4-deficiency, L-DOPA may be given as a neurotransmitter supplement to produce dopamine, NE, and epinephrine.

4. Monoamine oxidase inhibitors: MAO is found in neural and other tissues, such as the intestine and liver. In the neuron, this enzyme oxidatively deaminates and inactivates any excess neurotransmitter molecules (NE, dopamine, or serotonin) that may leak out of synaptic vesicles when the neuron is at rest. MAO inhibitors (MAOI) may irreversibly or reversibly inactivate the enzyme, permitting neurotransmitter molecules to escape degradation and, therefore, both to accumulate within the presynaptic neuron and to leak into the synaptic space. This causes activation of NE and serotonin receptors and may be responsible for the antidepressant action of MAOI. (Note: The interaction of MAOI with tyramine-containing foods is discussed in p. 418.)

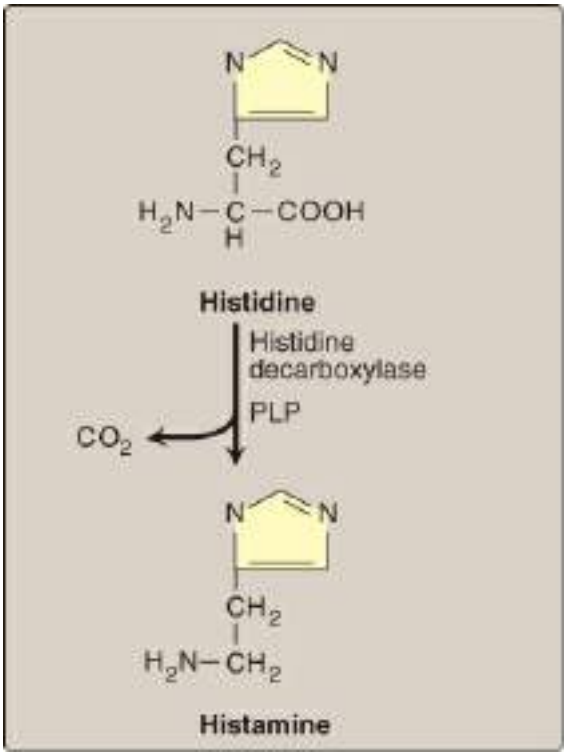


Figure 21.17
Biosynthesis of histamine. PLP = pyridoxal phosphate.

B. Histamine

Histamine is a chemical messenger that mediates a wide range of cellular responses, including allergic and inflammatory reactions and gastric acid secretion.

A powerful vasodilator, histamine is formed by decarboxylation of histidine in a reaction catalyzed by histidine decarboxylase and requiring PLP as a cofactor (Fig. 21.17). It is secreted by mast cells as a result of allergic reactions or trauma. Histamine has no clinical applications, but antihistamines that interfere with the action of histamine have important therapeutic applications. Antihistamines are generally histamine analogs that block histamine binding to its receptors to reduce histamine responses.

C. Serotonin

Serotonin, also called 5-hydroxytryptamine (5-HT), is synthesized and/or stored at several sites in the body (Fig. 21.18). The largest amount by far is found in the intestinal mucosa. Smaller amounts occur in the CNS, where it functions as a neurotransmitter, and in platelets (see online Chapter 35). Serotonin is synthesized from tryptophan, which is hydroxylated in a BH_4-requiring reaction analogous to that catalyzed by phenylalanine hydroxylase. The product, 5-hydroxytryptophan, is decarboxylated to 5-HT. In the case of a BH_4-deficiency, 5-hydroxytryptophan may be given as a neurotransmitter supplement to produce serotonin. Serotonin has multiple physiologic roles including pain perception and regulation of sleep, appetite, temperature, blood pressure, cognitive functions, and mood (causes a feeling of well-being). (Note: Selective serotonin reuptake inhibitors [SSRIs] maintain serotonin levels, thereby functioning as antidepressants.) Serotonin is degraded by MAO to 5-hydroxy-3-indoleacetic acid (5-HIAA).

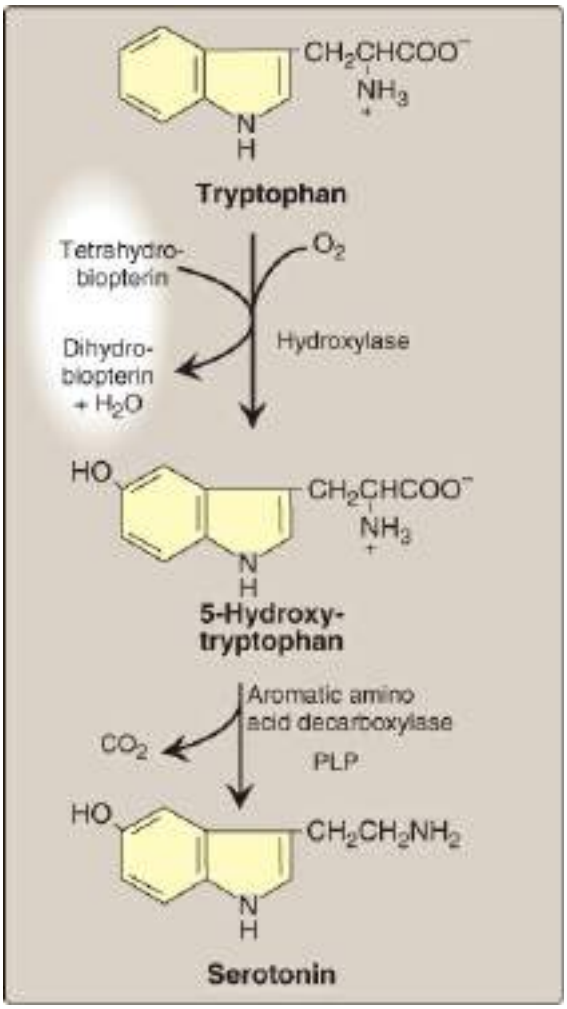


Figure 21.18
Synthesis of serotonin. (Note: Serotonin is converted to melatonin, a regulator of circadian rhythm, in the pineal gland.) PLP = pyridoxal phosphate; CO_2 = carbon dioxide.

D. Creatine

Creatine phosphate (also called phosphocreatine), the phosphorylated derivative of creatine found in muscle, is a high-energy compound that provides a small but rapidly mobilized reserve of high-energy phosphates that can be reversibly transferred to adenosine diphosphate (Fig. 21.19) to maintain the intracellular level of ATP during the first few minutes of intense muscular contraction. (Note: The amount of creatine phosphate in the body is proportional to the muscle mass.)

1. Synthesis: Creatine is synthesized in the liver and kidneys from glycine and the guanidino group of arginine, plus a methyl group from SAM (Fig. 21.19). Animal products are dietary sources. Creatine is reversibly phosphorylated to creatine phosphate by creatine kinase, using ATP as the phosphate donor. (Note: The presence of creatine kinase [MB isozyme] in the plasma is indicative of heart damage and is used in the diagnosis of myocardial infarction [see p. 70].)

2. Degradation: Creatine and creatine phosphate spontaneously cyclize at a slow but constant rate to form creatinine, which is excreted in the urine. The amount excreted is proportional to the total creatine phosphate content of the body and, therefore, can be used to estimate muscle mass. When muscle mass decreases for any reason (e.g., from paralysis or muscular dystrophy), the creatinine content of the urine falls. In addition, a rise in blood creatinine is a sensitive indicator of kidney malfunction, because creatinine normally is rapidly cleared from the blood and excreted. A typical adult male excretes ~1 to 2 g of creatinine/day.

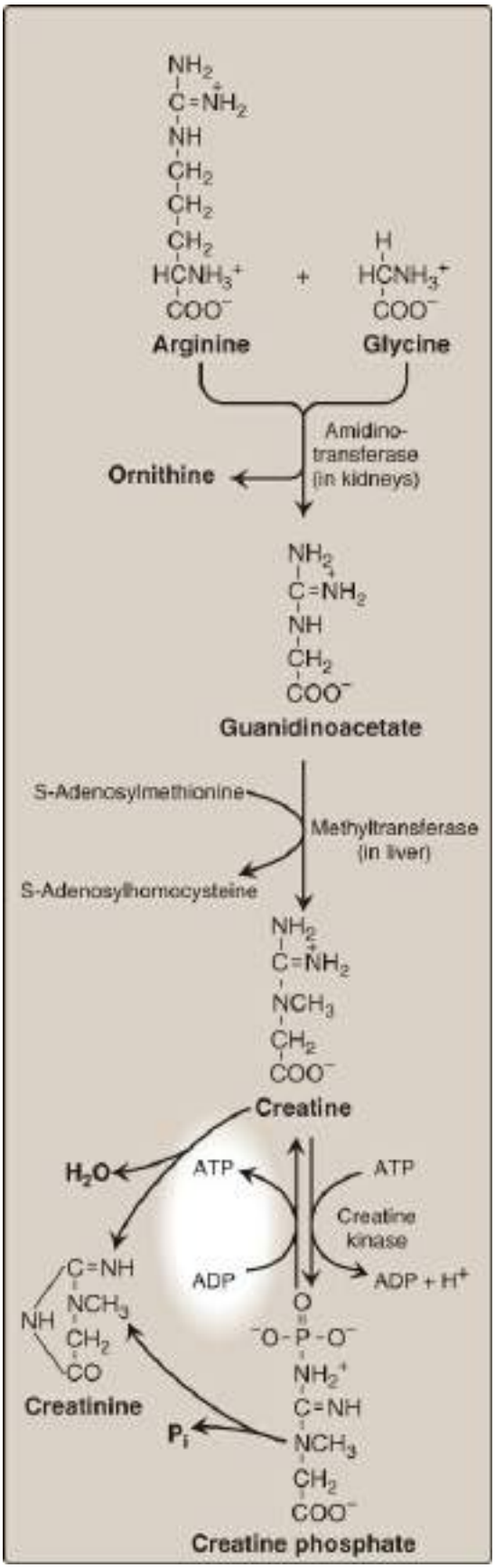


Figure 21.19
Synthesis of creatine. ADP = adenosine diphosphate; P_i = inorganic phosphate.

E. Melanin

Melanin is a pigment that occurs in several tissues, particularly the eye, hair, and skin. It is synthesized from tyrosine in melanocytes (pigment-forming cells) of the epidermis. It functions to protect underlying cells from the harmful effects of sunlight. A defect in melanin production results in oculocutaneous albinism, the most common type being due to defects in copper-containing tyrosinase (see p. 303).

IV. Chapter Summary

- **Amino acids** are **precursors** of many N-containing compounds including **porphyrins**, which, in combination with **Fe^{2+} iron**, form **heme** (Fig. 21.20).
- The major sites of **heme biosynthesis** are the **liver** and the **erythrocyte-producing cells** of the bone marrow. In the liver, the rate of heme synthesis is highly variable, responding to alterations in the cellular heme pool caused by fluctuating demands for heme proteins (particularly **CYP enzymes**). In contrast, heme synthesis in erythroid cells is relatively constant and is matched to the rate of Hb synthesis.
- Heme synthesis starts with **glycine** and **succinyl coenzyme A**. The **committed step** is the formation of **δ-ALA**. This mitochondrial reaction is catalyzed by **ALAS1** in the liver (inhibited by **hemin**, the oxidized form of heme that accumulates when heme is being underutilized) and **ALAS2** in erythroid tissues (regulated by iron).
- **Porphyrias** are caused by inherited or acquired (**lead poisoning**) defects in heme synthesis, resulting in the accumulation and increased excretion of porphyrins or porphyrin precursors. Enzyme defects early in the pathway cause **abdominal pain** and **neuropsychiatric symptoms**, whereas later defects cause **photosensitivity**.
- **Degradation** of heme occurs in the **MPS**, particularly in the **liver** and **spleen**. The first step is the production by **heme oxygenase** of **biliverdin**, which is subsequently reduced to **bilirubin**. Bilirubin is transported by **albumin** to the liver, where its solubility is increased by the addition of two molecules of **glucuronic acid** by **bilirubin UGT**. **Bilirubin diglucuronide** (**CB**) is transported into the **bile canaliculi**, where it is first hydrolyzed and reduced by gut bacteria to yield **urobilinogen**, which is further oxidized by bacteria to **stercobilin**.
- **Jaundice** (**icterus**) refers to the yellow color of the skin and sclerae that is caused by deposition of bilirubin, secondary to increased bilirubin levels in the blood. Three commonly encountered types of jaundice are **hemolytic** (**prehepatic**), **obstructive** (**posthepatic**), and **hepatocellular** (**hepatic**) (see Fig. 21.20).
- Other important N-containing compounds derived from amino acids include the **catecholamines** (**dopamine**, **NE**, and **epinephrine**), **creatine**, **histamine**, **serotonin**, **melanin**, and **nitric oxide**.

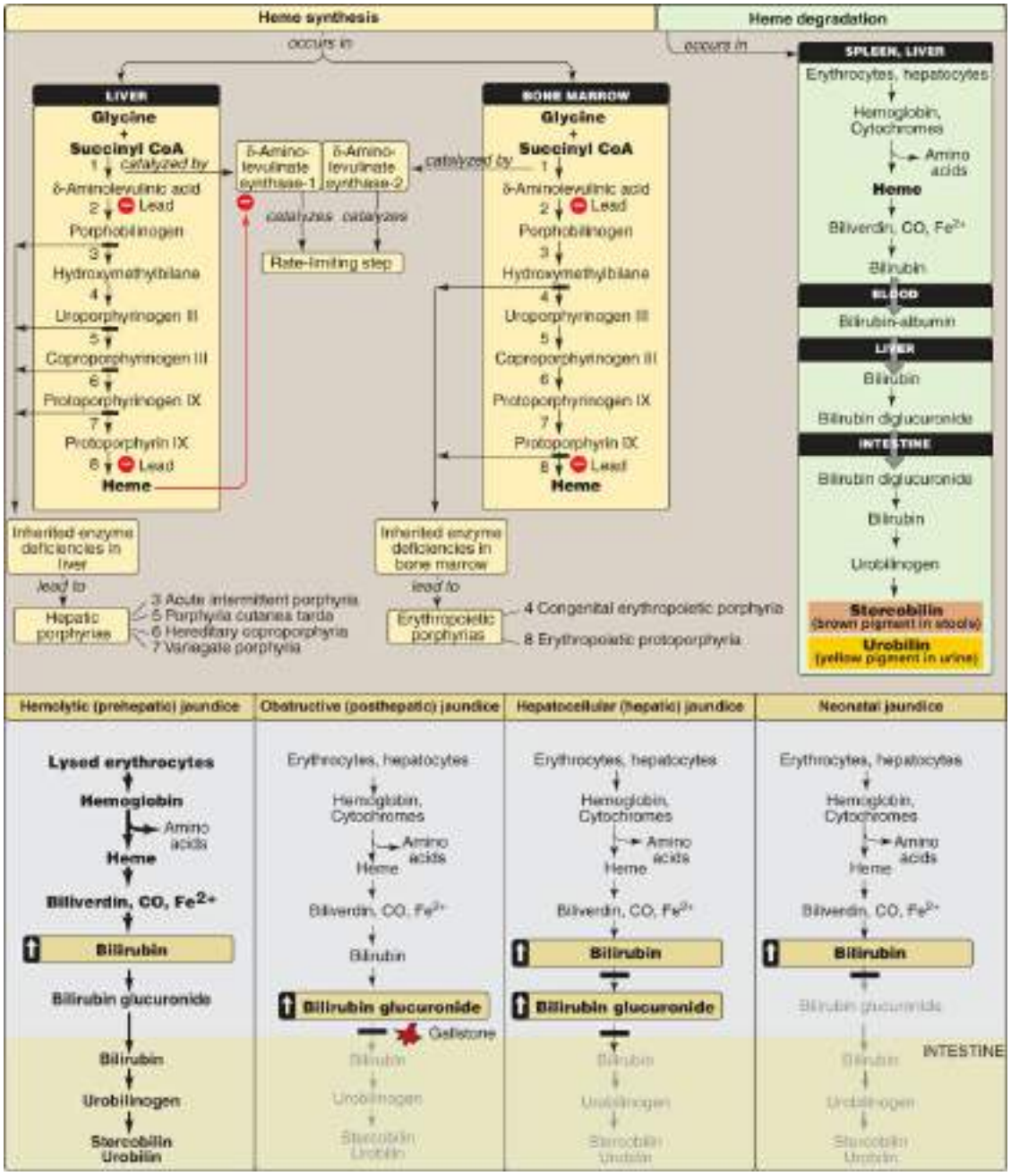


Figure 21.20
Key concept map for heme metabolism. ■ = Block in the pathway. (Note: Hepatocellular jaundice can be caused by decreased conjugation of bilirubin or decreased secretion of conjugated bilirubin from the liver into bile.) CoA = coenzyme A; CO = carbon monoxide; Fe = iron.

Study Questions

Choose the ONE best answer.

21.1 δ-Aminolevulinic acid synthase activity:

A. Catalyzes the committed step in porphyrin biosynthesis.
B. Is decreased by iron in erythrocytes.

C. Is decreased in the liver in individuals treated with certain drugs such as the barbiturate phenobarbital.
D. Occurs in the cytosol.
E. Requires tetrahydrobiopterin as a coenzyme.

Correct answer = A. δ-Aminolevulinic acid synthase is mitochondrial and catalyzes the rate-limiting and regulated step of porphyrin synthesis. It requires pyridoxal phosphate as a coenzyme. Iron increases production of the erythroid isozyme. The hepatic isozyme is increased in patients treated with certain drugs.

21.2 A 50-year-old male presented with painful blisters on the backs of his hands. He was a golf instructor and indicated that the blisters had erupted shortly after the golfing season began. He did not have recent exposure to common skin irritants. He had partial complex seizure disorder that had begun ~3 years earlier after a head injury. The patient had been taking phenytoin (his only medication) since the onset of the seizure disorder. He admitted to an average weekly ethanol intake of ~18 12-oz cans of beer. The patient's urine was reddish orange. Cultures obtained from skin lesions failed to grow organisms. A 24-hour urine collection showed elevated uroporphyrin (1,000 mg; normal, <27 mg). The most likely diagnosis is:

A. acute intermittent porphyria.
B. congenital erythropoietic porphyria.
C. erythropoietic protoporphyria.
D. hereditary coproporphyria.
E. porphyria cutanea tarda.

Correct answer = E. The disease is associated with a deficiency in uroporphyrinogen III decarboxylase (UROD), but clinical expression of the enzyme deficiency is influenced by hepatic injury caused by environmental (e.g., ethanol) and infectious (e.g., hepatitis B virus) agents. Exposure to sunlight can also be a precipitating factor. Clinical onset is typically during the fourth or fifth decade of life. Porphyrin accumulation leads to cutaneous symptoms and urine that is red to brown. Treatment of the patient's seizure disorder with phenytoin caused increased synthesis of δ-aminolevulinic acid synthase and, therefore, of uroporphyrinogen, the substrate of the deficient UROD. The laboratory and clinical findings are inconsistent with other porphyrias.

21.3 A patient presents with jaundice, abdominal pain, and nausea. Clinical laboratory results are shown below:

Plasma bilirubin	Urine urobilinogen	Urinary bilirubin
Increase in conjugated bilirubin (CB)	Not present	Present

What is the most likely cause of the jaundice?

A. Decreased hepatic conjugation of bilirubin
B. Decreased hepatic uptake of bilirubin
C. Decreased secretion of bile into the intestine
D. Increased hemolysis

Correct answer = C. The data are consistent with an obstructive jaundice, in which a block in the common bile duct decreases the secretion of bile-containing CB into the intestine (stool will be pale in color). The CB regurgitates into the blood (conjugated hyperbilirubinemia). The CB is excreted in the urine (which darkens) and is referred to as urinary bilirubin. Urinary urobilinogen is not present because its source is intestinal urobilinogen, which is low. The other choices do not match the data.

21.4 A 2-year-old child was brought to his pediatrician for evaluation of gastrointestinal problems. The parents report that the boy has been listless for the last few weeks. Lab tests reveal a microcytic, hypochromic anemia. Blood lead levels are elevated. Which of the enzymes listed below is most likely to have higher-than-normal activity in the liver of this child?

A. δ-Aminolevulinic acid synthase
B. Bilirubin UDP glucuronosyltransferase
C. Ferrochelatase

D. Heme oxygenase
E. Porphobilinogen synthase

Correct answer = A. This child has the acquired porphyria of lead poisoning. Lead inhibits both δ-aminolevulinic acid dehydratase and ferrochelatase and, consequently, heme synthesis. The decrease in heme derepresses δ-aminolevulinic acid synthase-1 (the hepatic isozyme), resulting in an increase in its activity. The decrease in heme also results in decreased hemoglobin synthesis, and anemia is seen. Ferrochelatase is directly inhibited by lead. The other choices are enzymes of heme degradation.

21.5 A 50-year-old male presents with hand tremors, a slow unsteady gait, and stiffness. After neurologic scans and additional testing, the patient is diagnosed with Parkinson disease. Which one of the following treatments listed below would be most effective in this patient?

A. Biopterin
B. β-Carotene
C. Hemin
D. Levodopa–carbidopa
E. Serotonin reuptake inhibitors

Correct answer = D. Levodopa (L-DOPA) can cross the blood–brain barrier to be used as a substrate for DOPA decarboxylase to increase dopamine levels in the central nervous system. Carbidopa cannot cross the blood–brain barrier and inhibits peripheral DOPA decarboxylase. This provides higher therapeutic L-DOPA levels for the central nervous system. Biopterin can be provided as a useful therapeutic agent for aromatic amino acid hydroxylase reactions when the cofactor is deficient. β-Carotene is an antioxidant, which can scavenge free radicals. Along with phlebotomy, it can help with photosensitivity in acute porphyria cases. Hemin reduces the deficit of porphyrins. This in turn decreases the synthesis of ALAS1 and minimizes the production of toxic porphyrin intermediates. Serotonin reuptake inhibitors help maintain serotonin levels, and function as antidepressants.

21.6 Kidney malfunction in a patient may be indicated by which one of the following lab tests?

A. Increased blood creatine kinase MB isoenzyme levels
B. Increased urine vanillylmandelic acid and metanephrine levels
C. Increased blood bilirubin diglucuronide levels
D. Decreased urine creatinine levels
E. Increased blood creatinine levels

Correct answer = E. Creatinine is normally very rapidly cleared from the blood by the kidneys and excreted in the urine. An increase in blood creatinine concentration levels indicates renal malfunction. Increased blood creatine kinase MB isoenzyme levels would be indicative of heart damage and/or myocardial infarction. Increased urine vanillylmandelic acid and metanephrine levels would be indicative of tumors of the adrenal gland, characterized by increased production of catecholamines. Increased blood bilirubin diglucuronide levels would be indicative of obstructive jaundice. Decreased urine creatinine levels would be indicative of decreased muscle mass, such as muscle atrophy from paralysis or muscular dystrophy.

Nucleotide Metabolism 22

I. OVERVIEW

Ribonucleoside and deoxyribonucleoside phosphates (nucleotides) are essential for all cells. Without them, neither ribonucleic acid (RNA) nor deoxyribonucleic acid (DNA) can be produced, and, therefore, proteins cannot be synthesized nor can cells proliferate. Nucleotides also serve as carriers of activated intermediates in the synthesis of some carbohydrates, lipids, and conjugated proteins (e.g., uridine diphosphate [UDP]-glucose and cytidine diphosphate [CDP]-choline) and are structural components of several essential coenzymes, such as coenzyme A, flavin adenine dinucleotide (FAD[H_2]), nicotinamide adenine dinucleotide (NAD[H]), and nicotinamide adenine dinucleotide phosphate (NADP[H]). Nucleotides, such as cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), serve as second messengers in signal transduction pathways. In addition, nucleotides play an important role as energy sources in the cell. Finally, nucleotides are important regulatory compounds for many of the pathways of intermediary metabolism, inhibiting or activating key enzymes. The purine and pyrimidine bases found in nucleotides can be synthesized *de novo* or can be obtained through salvage pathways that allow the reuse of the preformed bases resulting from normal cell turnover. (Note: Few of the purines and pyrimidines supplied by diet are utilized; instead, nearly all of the nucleic acids that enter the gastrointestinal [GI] tract are degraded.)

II. STRUCTURE

Nucleotides are composed of a nitrogenous base; a pentose monosaccharide; and one, two, or three phosphate groups. The nitrogen-containing bases belong to two families of compounds: the purines and the pyrimidines.

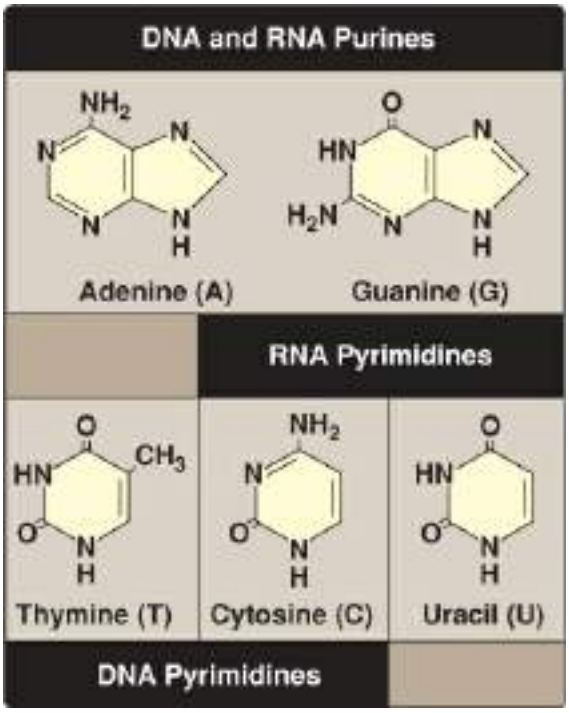


Figure 22.1
Purines and pyrimidines commonly found in DNA and RNA.

A. Purine and pyrimidine bases

Purines are double-ringed structures, whereas pyrimidines have a single ring. Both DNA and RNA contain the same purine bases: adenine (A) and guanine (G). Both DNA and RNA contain the pyrimidine cytosine (C), but they differ in their second pyrimidine base: DNA contains thymine (T), whereas RNA contains uracil (U). T and U differ in that only T has a methyl group (Fig. 22.1). Unusual (modified) bases are occasionally found in some species of DNA (e.g., in some viral DNA) and RNA (e.g., in transfer RNA [tRNA]). Base modifications include methylation, glycosylation, acetylation, and reduction. Some examples of unusual bases are shown in Figure 22.2. (Note: The presence of an unusual base in a nucleotide sequence may aid in its recognition by specific enzymes or protect it from being degraded by nucleases.)

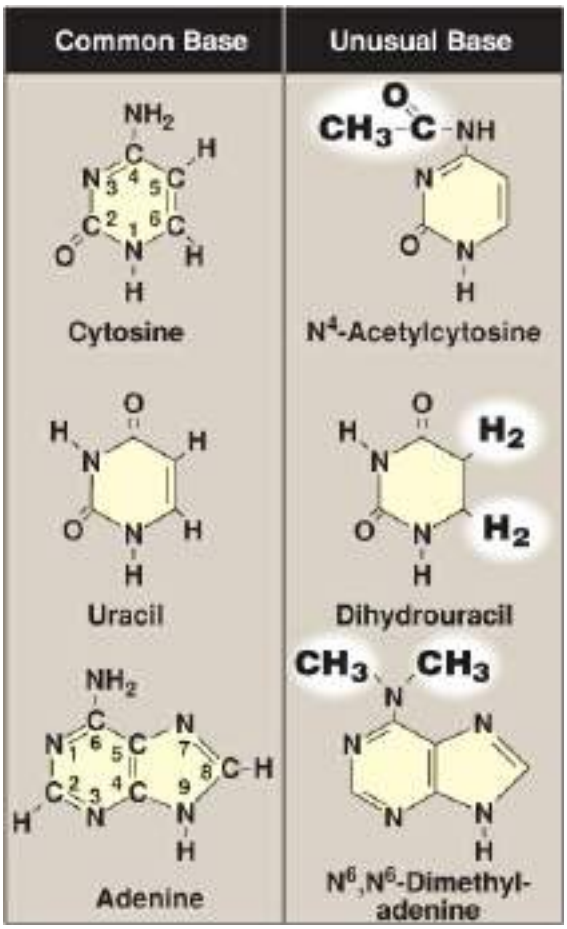


Figure 22.2
Examples of unusual bases.

B. Nucleosides

The addition of a pentose sugar to a base through an N-glycosidic bond (see p. 94) produces a nucleoside. If the sugar is ribose, a ribonucleoside is produced, and if the sugar is 2-deoxyribose, a deoxyribonucleoside is produced (Fig. 22.3A). The ribonucleosides of A, G, C, and U are named adenosine, guanosine, cytidine, and uridine, respectively. The deoxyribonucleosides of A, G, C, and T have the added prefix deoxy- (e.g., deoxyadenosine). (Note: The compound deoxythymidine is often simply called thymidine, with the deoxy- prefix being understood, because it is incorporated into DNA only.) The carbon and nitrogen atoms in the rings of the base and the sugar are numbered separately (see Fig. 22.3B). (Note: Carbons in the pentose are numbered 1′ to 5′. Thus, when the 5′-carbon of a nucleoside [or nucleotide] is referred to, a carbon atom in the pentose, rather than an atom in the base, is being specified.)

C. Nucleotides

The addition of one or more phosphate groups to a nucleoside produces a

nucleotide. The first phosphate group is attached by an ester linkage to the 5′-OH of the pentose, forming a nucleoside 5′-phosphate or a 5′-nucleotide. The type of pentose is denoted by the prefix in the names 5′-ribonucleotide and 5′-deoxyribonucleotide. If one phosphate group is attached to the 5′-carbon of the pentose, the structure is a nucleoside monophosphate, like adenosine monophosphate (AMP, or adenylate). If a second or third phosphate is added to the nucleoside, a nucleoside diphosphate (e.g., adenosine diphosphate [ADP] or triphosphate, e.g., ATP) results (Fig. 22.4). The second and third phosphates are each connected to the nucleotide by a “high-energy bond” (a bond with a large, negative change in free energy [−ΔG, see p. 78] of hydrolysis). (Note: The phosphate groups are responsible for the negative charges associated with nucleotides and cause DNA and RNA to be referred to as nucleic acids.)

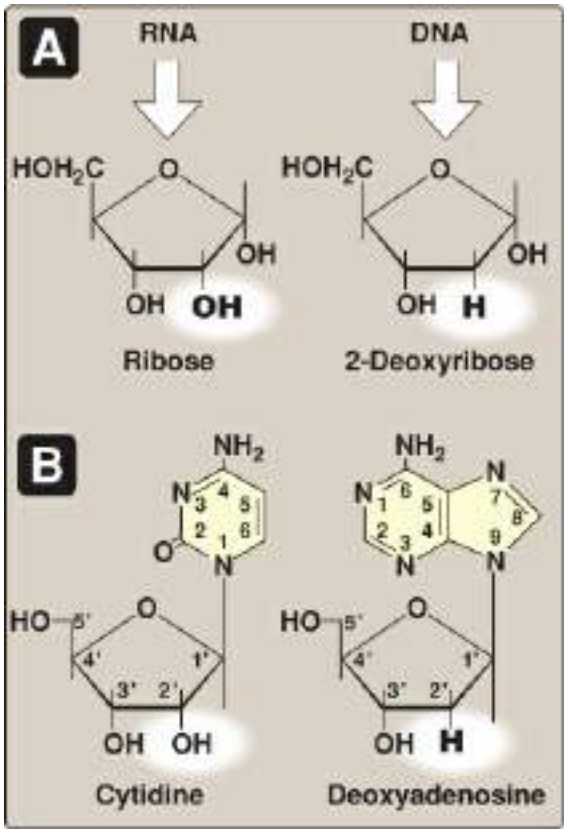


Figure 22.3
A: Pentoses found in nucleic acids. **B:** Examples of the numbering systems for purine- and pyrimidine-containing nucleosides.

III. PURINE NUCLEOTIDE SYNTHESIS

The atoms of the purine ring are contributed by a number of compounds, including amino acids (aspartate, glycine, and glutamine), carbon dioxide (CO_2), and N^{10}-formyltetrahydrofolate (N^{10}-formyl-THF), as shown in Figure 22.5. The purine ring is constructed primarily in the liver by a series of reactions that add the donated carbons

and nitrogens to a preformed ribose 5-phosphate. (Note: Synthesis of ribose 5-phosphate from glucose 6-phosphate by the pentose phosphate pathway is discussed on p. 162.)

A. 5-Phosphoribosyl-1-pyrophosphate synthesis

5-Phosphoribosyl-1-pyrophosphate (PRPP) is an activated pentose that participates in the synthesis and salvage of purines and pyrimidines. Synthesis of PRPP from ATP and ribose 5-phosphate is catalyzed by PRPP synthetase (Fig. 22.6). This X-linked enzyme is activated by inorganic phosphate and inhibited by purine nucleotides (end product inhibition). (Note: Because the sugar moiety of PRPP is ribose, ribonucleotides are the end products of *de novo* purine synthesis. When deoxyribonucleotides are required for DNA synthesis, the ribose sugar moiety is reduced [see p. 330].)

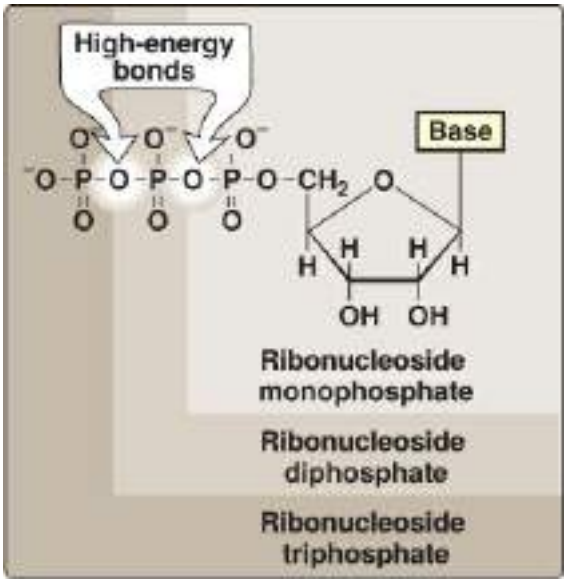


Figure 22.4
Ribonucleoside monophosphate, diphosphate, and triphosphate.

B. 5-Phosphoribosylamine synthesis

Synthesis of 5-phosphoribosylamine from PRPP and glutamine is shown in Figure 22.7. The amide group of glutamine replaces the pyrophosphate group attached to carbon 1 of PRPP. This is the committed step in purine nucleotide biosynthesis. The enzyme that catalyzes the reaction, glutamine:phosphoribosylpyrophosphate amidotransferase (GPAT), is inhibited by the purine 5′-nucleotides AMP and guanosine monophosphate (GMP, or guanylate), the end products of the pathway. The rate of the reaction is also controlled by the intracellular concentration of PRPP. (Note: The concentration of PRPP is normally far below the Michaelis constant [K_m] for the GPAT. Therefore, any small change in the PRPP concentration causes a proportional change in rate of the reaction [see p. 63].)

C. Inosine monophosphate synthesis

The next nine steps in purine nucleotide biosynthesis leading to the synthesis of inosine monophosphate ([IMP] whose base is hypoxanthine) are illustrated in Figure 22.7. IMP is the parent purine nucleotide for AMP and GMP. Four steps in this pathway require ATP as an energy source, and two steps in the pathway require N^{10}-formyl-THF as a one-carbon donor (see p. 296). (Note: Hypoxanthine is found in tRNA [see Fig. 32.9 on p. 503].)

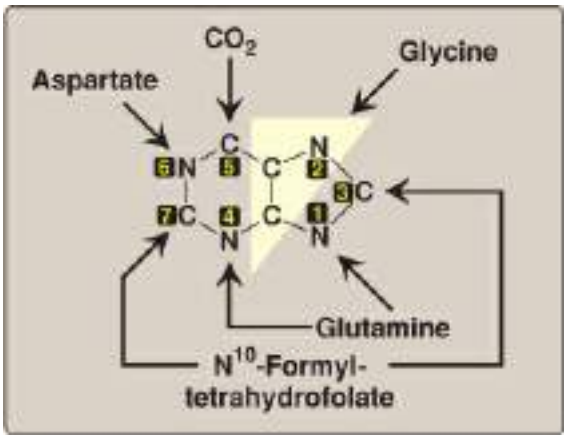


Figure 22.5
Sources of the individual atoms in the purine ring. The order in which the atoms are added is shown by the numbers in the black boxes (see Fig. 22.7). CO_2 = carbon dioxide.

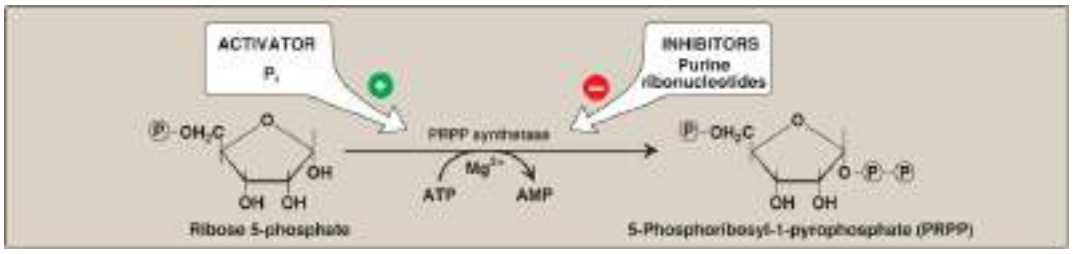


Figure 22.6
Synthesis of PRPP, showing the activator and inhibitors of the reaction. (Note: This is not the committed step of purine synthesis because PRPP is used in other pathways such as salvage [see p. 329].) Ⓟ = phosphate; P_i = inorganic phosphate; AMP = adenosine monophosphate; Mg = magnesium.

D. Synthetic inhibitors

Some synthetic inhibitors of purine synthesis (e.g., the sulfonamides) are designed to inhibit the growth of rapidly dividing microorganisms without interfering with human cell functions (see Fig. 22.7). Other purine synthesis inhibitors, such as structural analogs of folic acid (e.g., methotrexate), are used pharmacologically to control the spread of cancer by interfering with the synthesis of nucleotides and, therefore, of DNA and RNA (see Fig. 22.7).

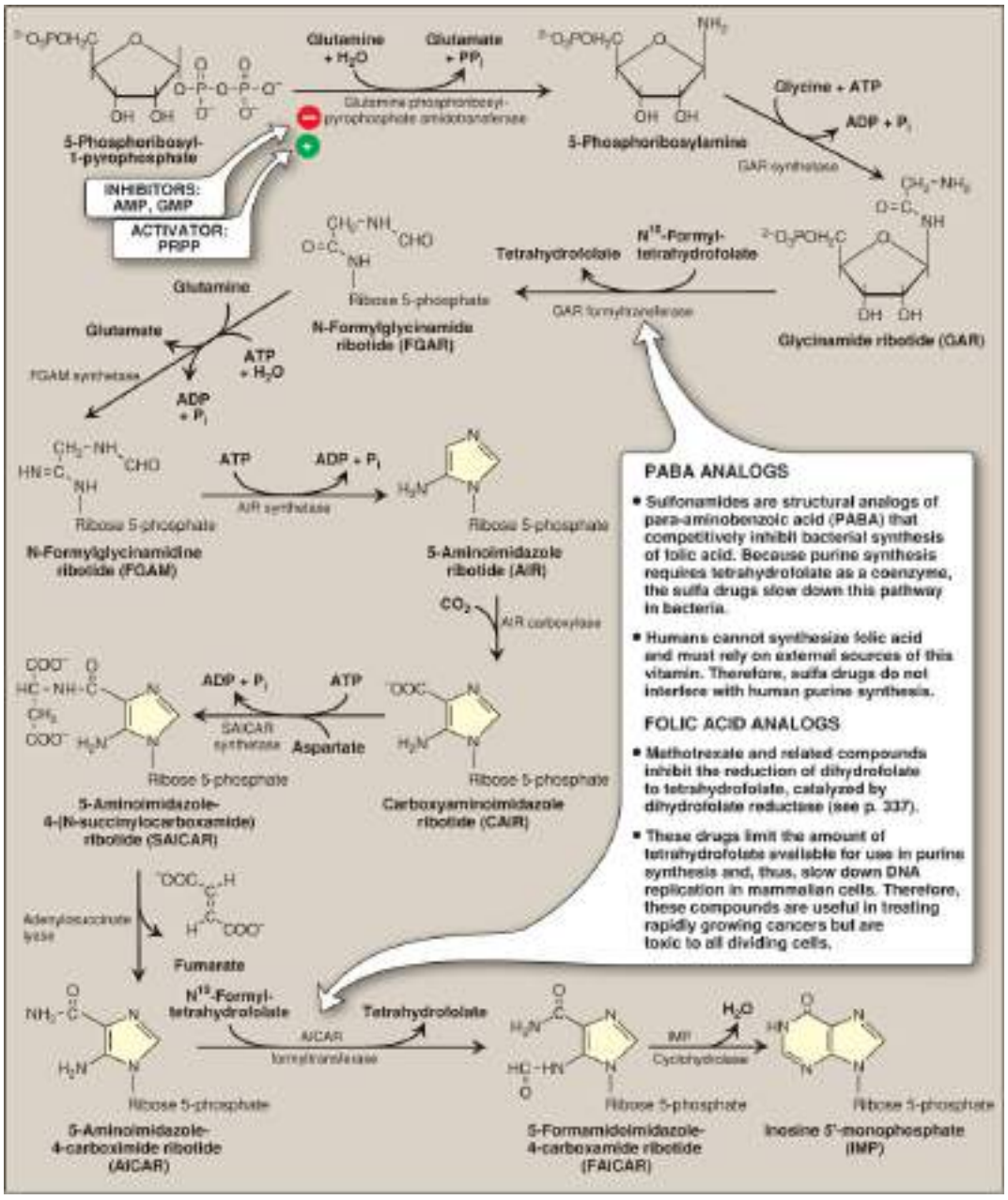


Figure 22.7
De novo synthesis of purine nucleotides, showing the inhibitory effect of some structural analogs. AMP and ADP = adenosine mono- and diphosphates; GMP = guanosine monophosphate; PRPP = 5-phosphoribosyl-1-pyrophosphate; P_i = inorganic phosphate; PP_i = pyrophosphate; CO_2 = carbon dioxide.

Inhibitors of human purine synthesis are extremely toxic to tissues, especially to developing structures such as those in a fetus, or to cell types that normally replicate rapidly, including those of bone marrow, skin, GI tract, immune system, or hair follicles. As a result, individuals taking such anticancer drugs can experience adverse effects, including anemia, scaly skin, GI tract disturbance, immunodeficiency, and hair loss.

E. Adenosine and guanosine monophosphate synthesis

The conversion of IMP to either AMP or GMP uses a two-step, energy- and nitrogen-requiring pathway (Fig. 22.8). (Note: AMP synthesis requires guanosine triphosphate [GTP] as an energy source and aspartate as a nitrogen source, whereas GMP synthesis requires ATP and glutamine.) Also, the first reaction in each pathway is inhibited by the end product of that pathway. This provides a mechanism for diverting IMP to the synthesis of the purine present in lesser amounts. If both AMP and GMP are present in adequate amounts, the *de novo* pathway of purine nucleotide synthesis is inhibited at the GPAT step.

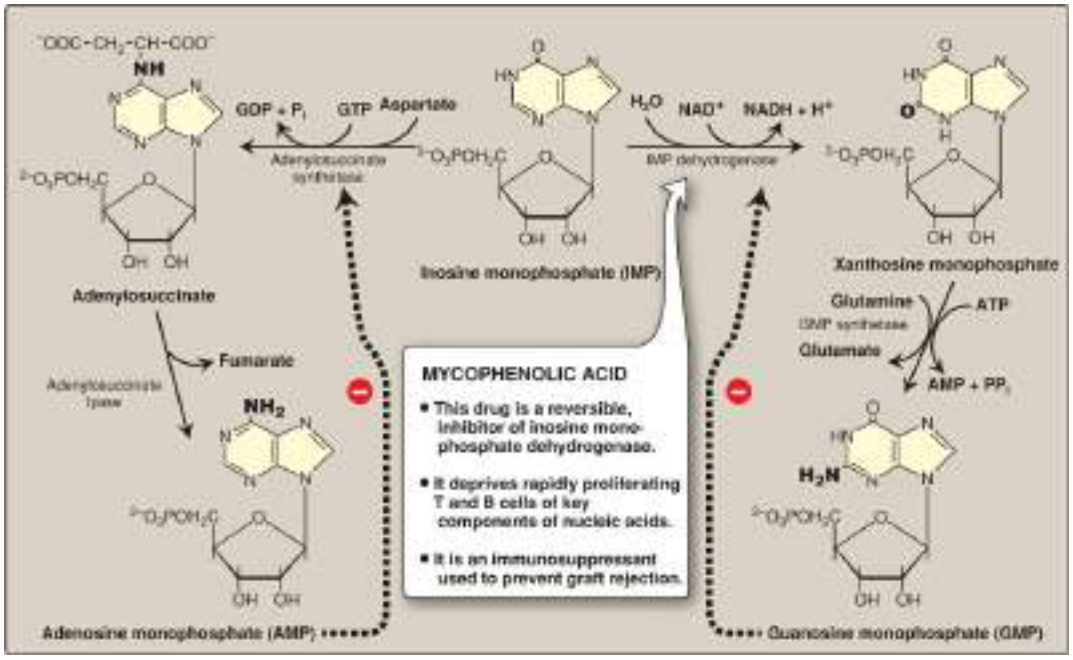


Figure 22.8
Conversion of IMP to AMP (or, adenylate) and GMP (or, guanylate) showing feedback inhibition. NAD(H) = nicotinamide adenine dinucleotide; GDP and GTP = guanosine di- and triphosphates; P_i = inorganic phosphate; PP_i = pyrophosphate.

> Mycophenolic acid is a reversible inhibitor of IMP dehydrogenase, the enzyme used to generate GMP. Proliferating T and B lymphocytes are highly susceptible to low levels of this key purine nucleotide, so mycophenolic acid is an effective immunosuppressant agent to prevent organ transplant rejection (kidney, heart, and liver), as well as to treat certain immune disorders such as lupus or Crohn disease.

F. Nucleoside di- and triphosphate synthesis

Nucleoside diphosphates are synthesized from the corresponding nucleoside monophosphates by base-specific nucleoside monophosphate kinases (Fig. 22.9). (Note: These kinases do not discriminate between ribose and deoxyribose in the substrate.) ATP is generally the source of the transferred phosphate because it is

present in higher concentrations than the other nucleoside triphosphates. Adenylate kinase is particularly active in the liver and in muscle, where the turnover of energy from ATP is high. Its function is to maintain equilibrium among the adenine nucleotides (AMP, ADP, and ATP). Nucleoside diphosphates and triphosphates are interconverted by nucleoside diphosphate kinase, an enzyme that, unlike the monophosphate kinases, has broad substrate specificity.

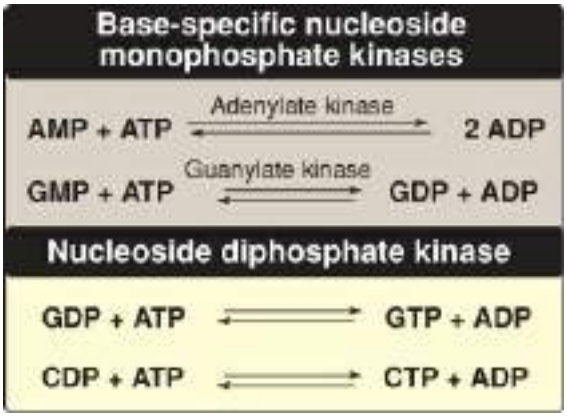


Figure 22.9
Conversion of nucleoside monophosphates to di- and triphosphates. AMP and ADP = adenosine mono- and diphosphates; GMP, GDP, and GTP = guanosine mono-, di-, and triphosphates; CDP and CTP = cytidine di- and triphosphates.

G. Purine salvage pathway

Purines that result from the normal turnover of cellular nucleic acids, or the small amount that is obtained from the diet and not degraded, can be converted to nucleoside triphosphates and used by the body. This is referred to as the salvage pathway for purines. (Note: Salvage is particularly important in the brain.)

1. Purine base salvage to nucleotides: Two enzymes are involved: adenine phosphoribosyltransferase (APRT) and X-linked hypoxanthine–guanine phosphoribosyltransferase (HGPRT). Both use PRPP as the source of the ribose 5-phosphate group (Fig. 22.10). The release of pyrophosphate and its subsequent hydrolysis by pyrophosphatase makes these reactions irreversible. (Note: Adenosine is the only purine nucleoside to be salvaged. It is phosphorylated to AMP by adenosine kinase.)

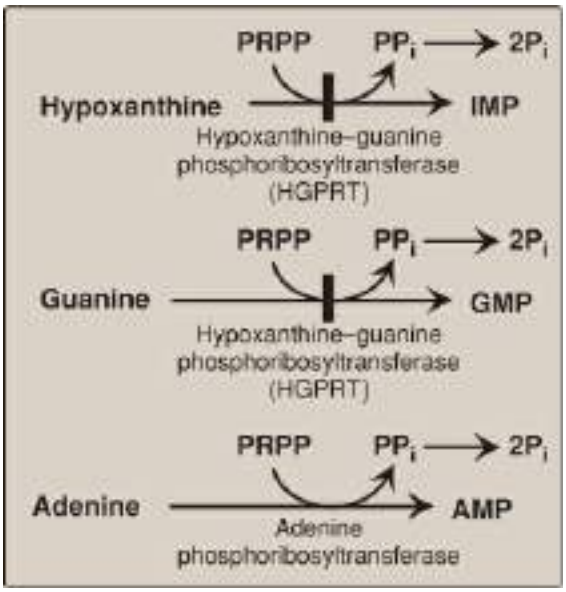


Figure 22.10
Salvage pathways of purine nucleotide synthesis. (Note: Virtually complete deficiency of HGPRT results in Lesch–Nyhan syndrome. Partial deficiencies of HGPRT are known. As the amount of functional enzyme increases, the severity of the symptoms decreases.) IMP, GMP, and AMP = inosine, guanosine, and adenosine monophosphates; PRPP = 5-phosphoribosyl-1-pyrophosphate; PP_i = pyrophosphate.

2. Lesch–Nyhan syndrome: This is a rare, X-linked recessive disorder associated with a virtually complete deficiency of HGPRT. The deficiency results in an inability to salvage hypoxanthine or G, from which excessive amounts of uric acid, the end product of purine degradation, are then produced (see p. 331). In addition, the lack of this salvage pathway causes increased PRPP levels and decreased IMP and GMP levels. As a result, GPAT (the regulated step in purine synthesis) has excess substrate and decreased inhibitors available, and *de novo* purine synthesis is increased. The combination of decreased purine reutilization and increased purine synthesis results in increased degradation of purines and the production of large amounts of uric acid, making HGPRT deficiency an inherited cause of hyperuricemia. In patients with Lesch–Nyhan syndrome, the hyperuricemia frequently results in the formation of uric acid stones in the kidneys (urolithiasis) and the deposition of urate crystals in the joints (gouty arthritis) and soft tissues. In addition, the syndrome is characterized by motor dysfunction, cognitive deficits, and behavioral disturbances that include self-mutilation (e.g., biting of lips and fingers), as shown in Figure 22.11.

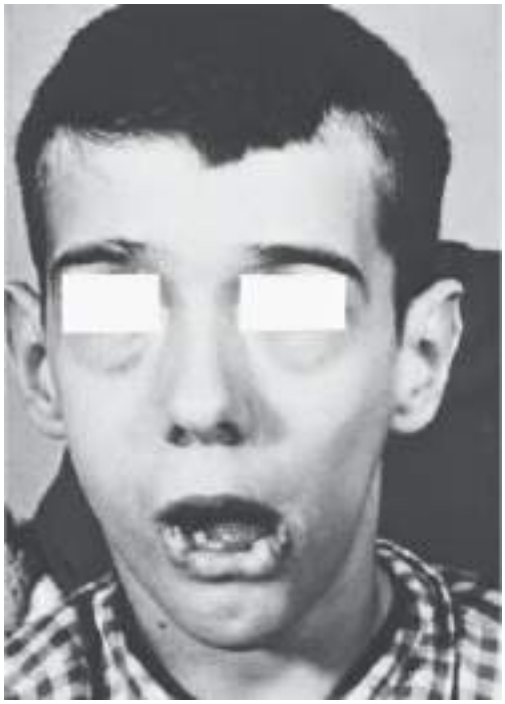


Figure 22.11
Lesions on the lips of a patient with Lesch–Nyhan syndrome.

IV. DEOXYRIBONUCLEOTIDE SYNTHESIS

The nucleotides described thus far all contain ribose (ribonucleotides). DNA synthesis, however, requires 2′-deoxyribonucleotides, which are produced from ribonucleoside diphosphates by the enzyme ribonucleotide reductase during the S-phase of the cell cycle (see p. 471). (Note: The same enzyme acts on pyrimidine ribonucleotides.)

A. Ribonucleotide reductase

Ribonucleotide reductase (ribonucleoside diphosphate reductase) is a dimer composed of two nonidentical subunits, R1 (or, α) and the smaller R2 (or, β), and is specific for the reduction of purine nucleoside diphosphates (ADP and GDP) and pyrimidine nucleoside diphosphates (CDP and UDP) to their deoxy forms (dADP, dGDP, dCDP, and dUDP). The immediate donors of the hydrogen atoms needed for the reduction of the 2′-hydroxyl group are two sulfhydryl (–SH) groups on the enzyme itself (R1 subunit), which form a disulfide bond during the reaction (see p. 19). (Note: R2 contains the stable tyrosyl radical required for catalysis at R1.)

1. Reduced enzyme regeneration: In order for ribonucleotide reductase to continue to produce deoxyribonucleotides at R1, the disulfide bond created during the production of the 2′-deoxy carbon must be reduced. The source of the reducing equivalents is thioredoxin, a protein coenzyme of ribonucleotide reductase. Thioredoxin contains two cysteine residues separated by two amino acids in the peptide chain. The two –SH groups of thioredoxin donate their hydrogen atoms

to ribonucleotide reductase, forming a disulfide bond in the process (Fig. 22.12).

2. Reduced thioredoxin regeneration: Thioredoxin must be converted back to its reduced form in order to continue performing its function. The reducing equivalents are provided by NADPH + H^+, and the reaction is catalyzed by thioredoxin reductase, a selenoprotein (see p. 297).

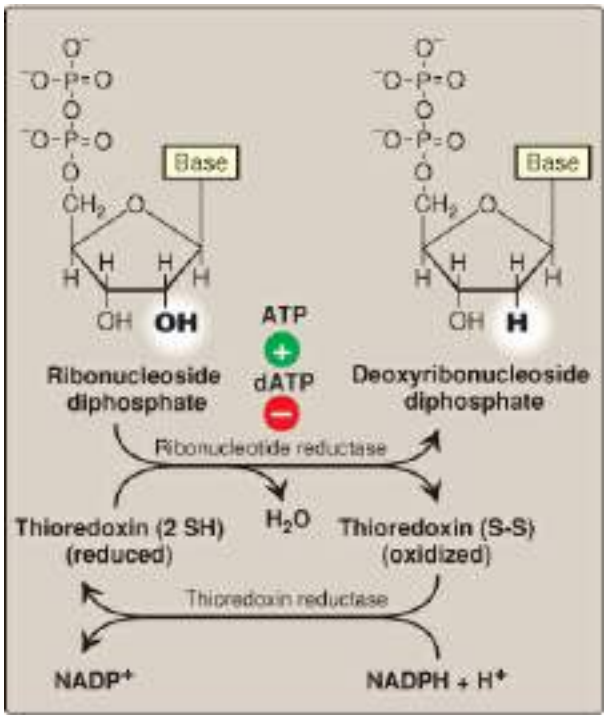


Figure 22.12
Conversion of ribonucleotides to deoxyribonucleotides. NADP(H) = nicotinamide adenine dinucleotide phosphate; dATP = deoxyadenosine triphosphate.

B. Deoxyribonucleotide synthesis regulation

Ribonucleotide reductase is responsible for maintaining a balanced supply of the deoxyribonucleotides required for DNA synthesis. Consequently, the regulation of the enzyme is complex. In addition to the catalytic site, R1 contains two distinct allosteric sites involved in regulating enzymic activity (Fig. 22.13).

Clinical Application 22.1: Hydroxyurea

The drug hydroxyurea (hydroxycarbamide) inhibits ribonucleotide reductase, thereby inhibiting the generation of substrates for DNA synthesis. The drug is an antineoplastic agent and is used in the treatment of cancers such as melanoma. Hydroxyurea is also used in the treatment of sickle cell anemia (see p. 38). However, the increase in fetal hemoglobin seen with hydroxyurea is because of changes in gene expression and not to ribonucleotide reductase inhibition.

1. Activity sites: The binding of deoxyadenosine triphosphate (dATP) to allosteric

sites (known as activity sites) on R1 inhibits the overall catalytic activity of the enzyme and, therefore, prevents the reduction of any of the four nucleoside diphosphates. This effectively prevents DNA synthesis and explains the toxicity of increased levels of dATP seen in conditions such as adenosine deaminase (ADA) deficiency (see p. 334). In contrast, ATP bound to these sites activates the enzyme.

2. Substrate specificity sites: The binding of nucleoside triphosphates to additional allosteric sites (known as substrate specificity sites) on R1 regulates substrate specificity, causing an increase in the conversion of different species of ribonucleotides to deoxyribonucleotides as they are required for DNA synthesis. For example, deoxythymidine triphosphate binding at the specificity site causes a conformational change that allows reduction of GDP to dGDP at the catalytic site when ATP is at the activity site.

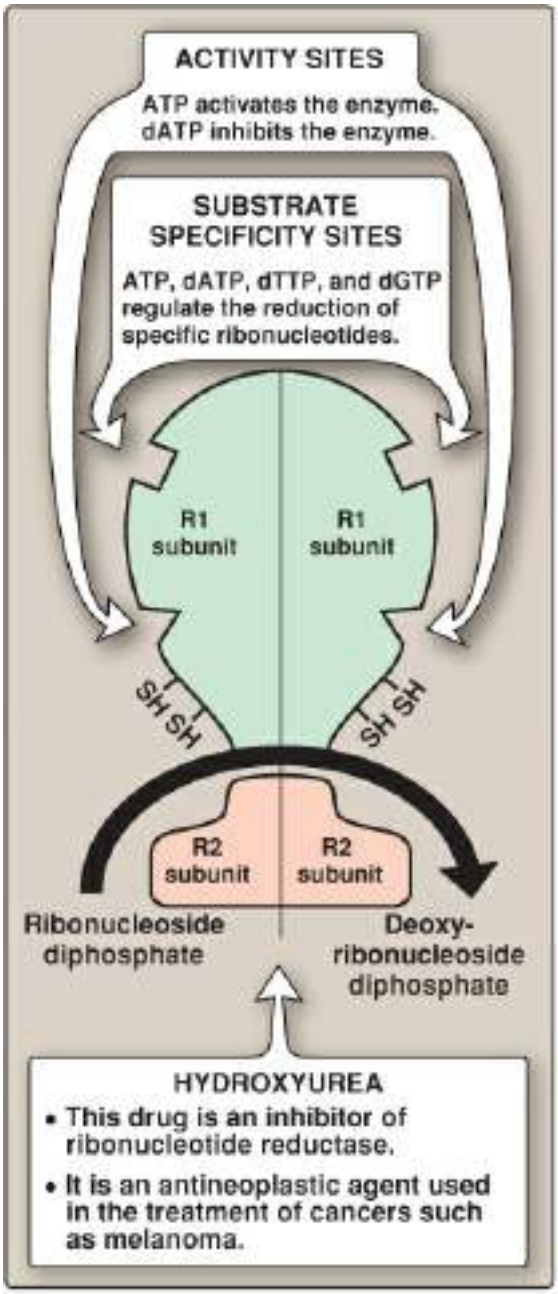


Figure 22.13
Regulation of ribonucleotide reductase. (Note: The R1 subunit is also referred to as α and the R2 as β.) dATP, dTTP, and dGTP = deoxyadenosine, deoxythymidine, and deoxyguanosine triphosphates.

V. PURINE NUCLEOTIDE DEGRADATION

Degradation of dietary nucleic acids occurs in the small intestine, where pancreatic nucleases hydrolyze them to nucleotides. The nucleotides are sequentially degraded by intestinal enzymes to nucleosides, phosphorylated sugars, and free bases. Uric acid is the end product of intestinal purine degradation. (Note: Purine nucleotides from *de novo* synthesis are degraded in the liver primarily. The free bases are sent out from the liver

and salvaged by peripheral tissues.)

A. Degradation in the small intestine

Ribonucleases and deoxyribonucleases, secreted by the pancreas, hydrolyze dietary RNA and DNA to oligonucleotides that are further hydrolyzed by pancreatic phosphodiesterases, producing a mixture of 3′- and 5′-mononucleotides. At the intestinal mucosal surface, nucleotidases remove the phosphate groups hydrolytically, releasing nucleosides that are taken into enterocytes by sodium-dependent transporters and degraded by nucleosidases (nucleoside phosphorylases) to free bases plus (deoxy) ribose 1-phosphate. Dietary purine bases are not used to any appreciable extent for the synthesis of tissue nucleic acids. Instead, they are degraded to uric acid in the enterocytes. Most of the uric acid enters the blood and is eventually excreted in the urine. A summary of this pathway is shown in Figure 22.14. (Note: Mammals other than primates express urate oxidase [uricase], which cleaves the purine ring, generating allantoin. Modified recombinant urate oxidase is now used clinically to lower urate levels.)

B. Uric acid formation

A summary of the steps in the production of uric acid and the genetic diseases associated with deficiencies of specific degradative enzymes are shown in Figure 22.15. (Note: The bracketed numbers refer to specific reactions in the figure.)

1. An amino group is removed from AMP to produce IMP by AMP (adenylate) deaminase or from adenosine to produce inosine (hypoxanthine-ribose) by ADA.
2. IMP and GMP are converted into their respective nucleoside forms, inosine and guanosine, by the action of 5′-nucleotidase.
3. Purine nucleoside phosphorylase converts inosine and guanosine into their respective purine bases, hypoxanthine and G. (Note: A mutase interconverts ribose 1- and ribose 5-phosphate.)
4. G is deaminated to form xanthine.
5. Hypoxanthine is oxidized by molybdenum-containing xanthine oxidase (XO) to xanthine, which is further oxidized by XO to uric acid, the final product of human purine degradation. Uric acid is excreted primarily in the urine.

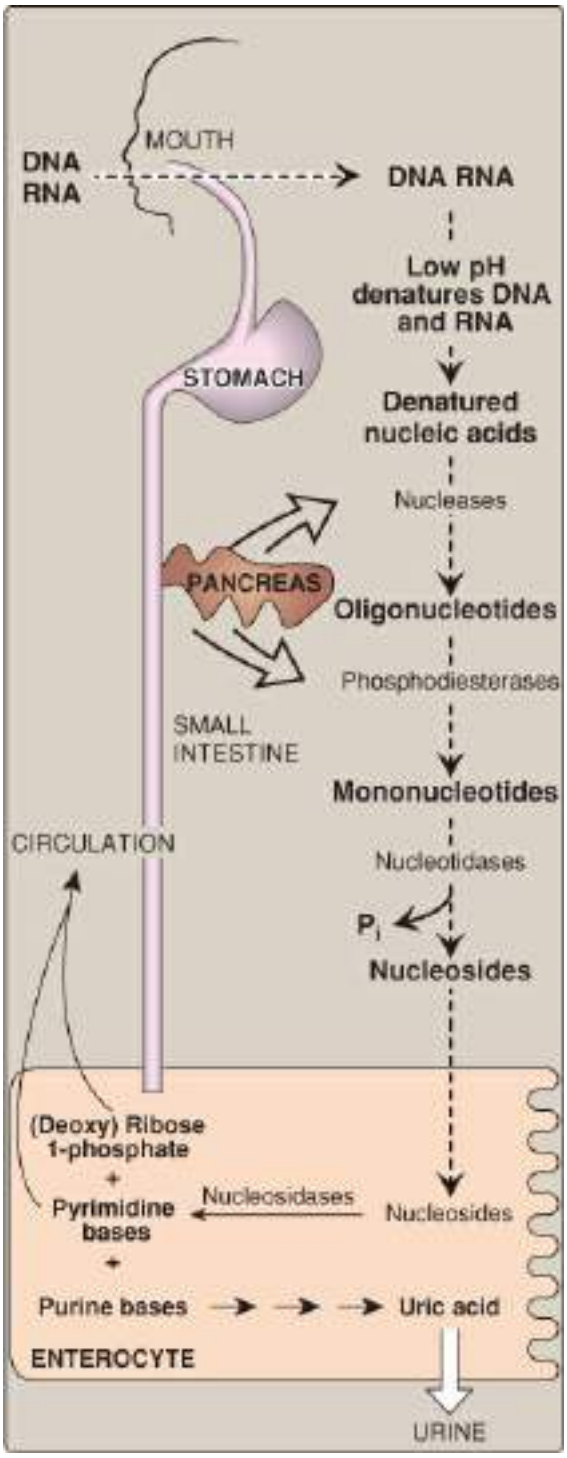


Figure 22.14
Digestion of dietary nucleic acids. P_i = inorganic phosphate.

C. Diseases associated with purine degradation

1. Gout: Gout is a disorder initiated by high levels of uric acid (the end product of purine catabolism) in blood (hyperuricemia), as a result of either the

overproduction or underexcretion of uric acid. The hyperuricemia can lead to the deposition of monosodium urate (MSU) crystals in the joints and an inflammatory response to the crystals, causing first acute and then progressing to chronic gouty arthritis. Nodular masses of MSU crystals (tophi) may be deposited in the soft tissues, resulting in chronic tophaceous gout (Fig. 22.16). Formation of uric acid stones in the kidney (urolithiasis) may also be seen. (Note: Hyperuricemia is not sufficient to cause gout, but gout is always preceded by hyperuricemia. Hyperuricemia is typically asymptomatic but may be indicative of comorbid conditions such as hypertension.) The definitive diagnosis of gout requires aspiration and examination of synovial fluid (Fig. 22.17) from an affected joint (or material from a tophus) using polarized light microscopy to confirm the presence of needle-shaped MSU crystals (Fig. 22.18).

a. Uric acid underexcretion: In >90% of individuals with hyperuricemia, the cause is underexcretion of uric acid. Underexcretion can be primary, because of as-yet-unidentified inherent excretory defects, or secondary to known disease processes that affect how the kidney handles urate (e.g., in lactic acidosis, lactate increases renal urate reabsorption, thereby decreasing its excretion) and to environmental factors such as the use of drugs (e.g., thiazide diuretics) or exposure to lead (saturnine gout).

b. Uric acid overproduction: A less common cause of hyperuricemia is from the overproduction of uric acid. Primary hyperuricemia is, for the most part, idiopathic (having no known cause). However, several identified mutations in the gene for X-linked PRPP synthetase result in the enzyme having an increased maximal velocity ([V_{max}], see p. 61) for the production of PRPP, a lower K_m (see p. 63) for ribose 5-phosphate, or a decreased sensitivity to purine nucleotides, its allosteric inhibitors (see p. 66). In each case, increased availability of PRPP increases purine production, resulting in elevated levels of plasma uric acid. Lesch–Nyhan syndrome (see p. 329) also causes hyperuricemia as a result of the decreased salvage of hypoxanthine and G and the subsequent increased availability of PRPP. Secondary hyperuricemia is typically the consequence of increased availability of purines (e.g., in patients with myeloproliferative disorders or who are undergoing chemotherapy and so have a high rate of cell turnover). Hyperuricemia can also be the result of seemingly unrelated metabolic diseases, such as von Gierke disease (see Fig. 11.8 on p. 141) or hereditary fructose intolerance (see p. 152).

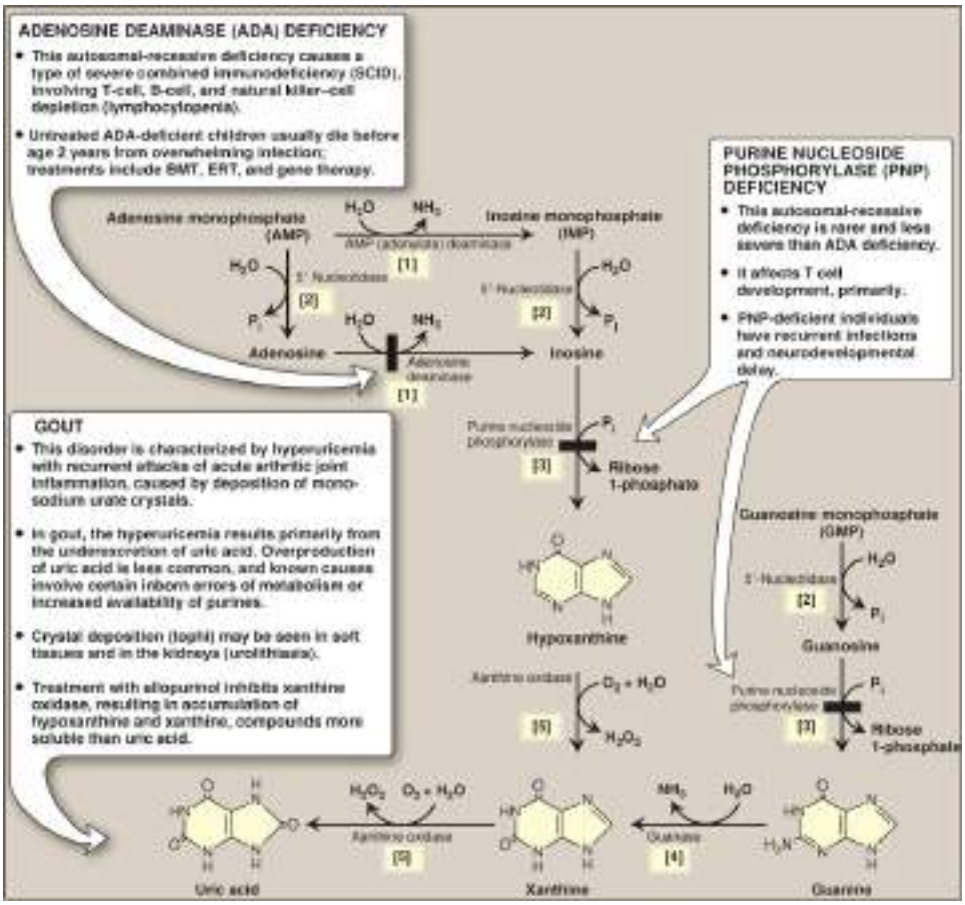


Figure 22.15
The degradation of purine nucleotides to uric acid, illustrating some of the genetic diseases associated with this pathway. (Note: The numbers in brackets refer to the corresponding numbered citations in the text.) BMT = bone marrow transplantation; ERT = enzyme replacement therapy; P_i = inorganic phosphate; H_2O_2 = hydrogen peroxide; NH_3 = ammonia.

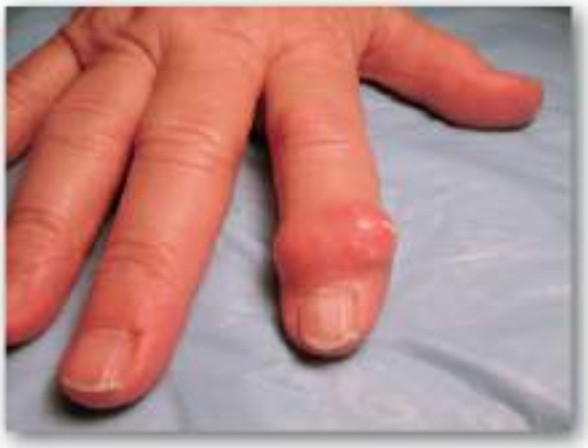


Figure 22.16
Tophaceous gout.

A diet rich in meat, seafood (particularly shellfish), and ethanol is associated with increased risk of gout, whereas a diet rich in low-fat dairy products is associated with a decreased risk.

c. Treatment: Acute attacks of gout are treated with anti-inflammatory agents. Colchicine, steroidal drugs such as prednisone, and nonsteroidal drugs such as indomethacin are used. (Note: Colchicine prevents formation of microtubules, thereby decreasing the movement of neutrophils into the affected area. Like the other anti-inflammatory drugs, it has no effect on uric acid levels.) Long-term therapeutic strategies for gout involve lowering the uric acid level below its saturation point (6.5 mg/dl), thereby preventing the deposition of MSU crystals. Uricosuric agents, such as probenecid or sulfinpyrazone, that increase renal excretion of uric acid, are used in patients who are underexcretors of uric acid. Allopurinol, a structural analog of hypoxanthine, inhibits uric acid synthesis and is used in patients who are overproducers of uric acid. Allopurinol is oxidized to oxypurinol, a long-lived inhibitor of XO. This results in an accumulation of hypoxanthine and xanthine (see Fig. 22.15), compounds more soluble than uric acid and, therefore, less likely to initiate an inflammatory response. In patients with normal levels of HGPRT, the hypoxanthine can be salvaged, reducing the levels of PRPP and, therefore, *de novo* purine synthesis. Febuxostat, a nonpurine inhibitor of XO, is also available. (Note: Uric acid levels in the blood normally are close to the saturation point. One reason for this may be the strong antioxidant effects of uric acid.)

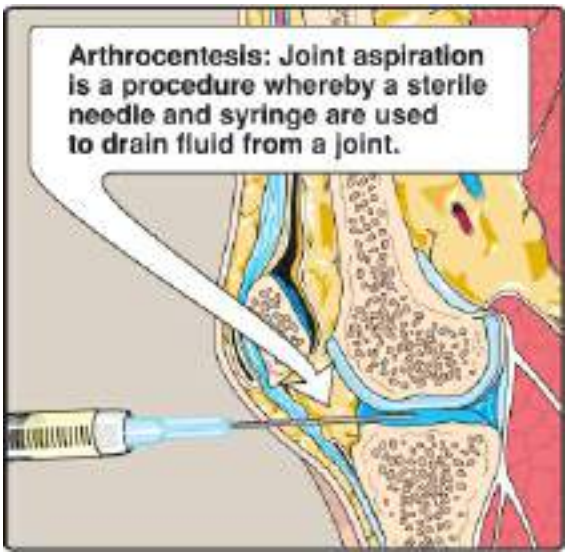


Figure 22.17
Analysis of joint fluid can help to define causes of joint swelling and arthritis, such as infection, gout, and rheumatoid disease.

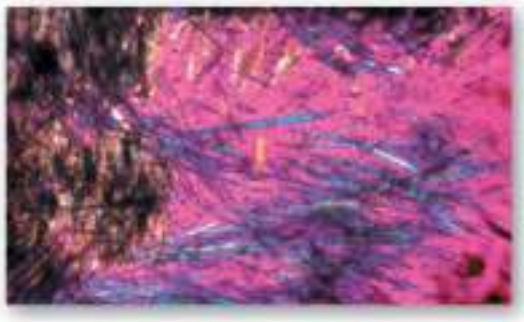


Figure 22.18
Gout can be diagnosed by the presence of negatively birefringent monosodium urate crystals in aspirated synovial fluid examined by polarized light microscopy. Here, crystals are seen within polymorphonuclear leukocytes.

2. Adenosine deaminase deficiency: ADA is expressed in a variety of tissues, but, in humans, lymphocytes have the highest activity of this cytoplasmic enzyme. A deficiency of ADA results in an accumulation of adenosine, which is converted to its ribonucleotide or deoxyribonucleotide forms by cellular kinases. As dATP levels rise, ribonucleotide reductase is inhibited, thereby preventing the production of all deoxyribose-containing nucleotides (see p. 330). Consequently, cells cannot make DNA and divide. (Note: The dATP and adenosine that accumulate in ADA deficiency lead to developmental arrest and apoptosis of lymphocytes.) In its most severe form, this autosomal-recessive disorder causes a type of severe combined immunodeficiency disease (SCID), involving a decrease in T cells, B cells, and natural killer cells. ADA deficiency accounts for ~14% of cases of SCID in the United States. Treatments include bone marrow transplantation, enzyme replacement therapy, and gene therapy (see p. 552). Without appropriate treatment, children with this disorder usually die from infection by age 2 years. (Note: Purine nucleoside phosphorylase deficiency results in a less severe immunodeficiency primarily involving T cells.)

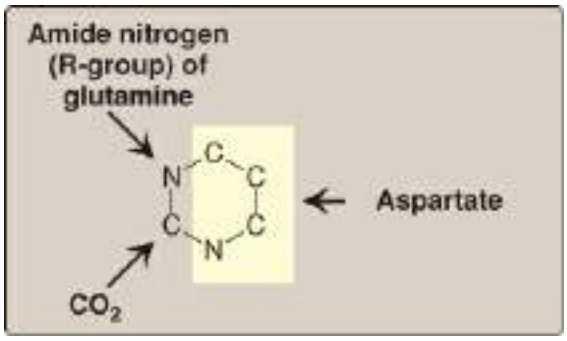


Figure 22.19
Sources of the individual atoms in the pyrimidine ring. CO_2 = carbon dioxide.

VI. PYRIMIDINE SYNTHESIS AND DEGRADATION

Unlike the synthesis of the purine ring, which is constructed on a pre-existing ribose 5-phosphate, the pyrimidine ring is synthesized before being attached to ribose 5-phosphate, which is donated by PRPP. The sources of the atoms in the pyrimidine ring are glutamine, CO_2, and aspartate (Fig. 22.19).

A. Carbamoyl phosphate synthesis

The regulated step of this pathway in mammalian cells is the synthesis of carbamoyl phosphate from glutamine and CO_2, catalyzed by carbamoyl phosphate synthetase (CPS) II. CPS II is inhibited by uridine triphosphate (UTP, the end product of this pathway, which can be converted into the other pyrimidine nucleotides) and is activated by PRPP. (Note: Carbamoyl phosphate, synthesized by CPS I, is also a precursor of urea [see p. 281]. Defects in ornithine transcarbamylase (OTC) of the urea cycle promote pyrimidine synthesis because of increased availability of carbamoyl phosphate. A comparison of the two enzymes is presented in Figure 22.20.)

Variable	CPS I	CPS II
Cellular location	Mitochondria	Cytosol
Pathway involved	Urea cycle	Pyrimidine synthesis
Source of nitrogen	Ammonia	γ-Amide group of glutamine
Regulators	Activator: N-acetyl-glutamate	Activator: PRPP Inhibitor: UTP

Figure 22.20
Summary of the differences between carbamoyl phosphate synthetase (CPS) I and II. PRPP = 5-phosphoribosyl-1-pyrophosphate; UTP = uridine triphosphate.

B. Orotic acid synthesis

The second step in pyrimidine synthesis is the formation of carbamoylaspartate, catalyzed by aspartate transcarbamoylase. The pyrimidine ring is then closed by dihydroorotase. The resulting dihydroorotate is oxidized to produce orotic acid (orotate), as shown in Figure 22.21. Flavin mononucleotide (FMN) is reduced in this reaction.

C. Pyrimidine nucleotide synthesis

The completed pyrimidine ring is converted to the nucleotide orotidine monophosphate (OMP) in the second stage of pyrimidine nucleotide synthesis (see Fig. 22.21). As seen with the purines, PRPP is the ribose 5-phosphate donor. The enzyme orotate phosphoribosyltransferase produces OMP and releases pyrophosphate, thereby making the reaction biologically irreversible. (Note: Both purine and pyrimidine synthesis require glutamine, aspartic acid, and PRPP as essential precursors.) OMP (orotidylate) is decarboxylated to uridine monophosphate (UMP) by orotidylate decarboxylase. The phosphoribosyltransferase and decarboxylase activities are separate catalytic domains of a single polypeptide called UMP synthase. Hereditary orotic aciduria (a very rare disorder) may be caused by a deficiency of one or both activities of this bifunctional enzyme, resulting in orotic acid in the urine (see Fig. 22.21). Since the first reaction of pyrimidine biosynthesis is feedback inhibited by UTP, hereditary orotic aciduria and its associated anemia is treated with uridine. Recall that a deficiency of OTC in the urea cycle would present with elevated urinary levels of orotate (see p. 535). This is because the carbamoyl phosphate substrate of OTC is funneled instead into pyrimidine synthesis. UMP is sequentially phosphorylated to UDP and UTP. (Note: The UDP is a substrate for ribonucleotide reductase, which generates dUDP. The dUDP is phosphorylated to dUTP, which is rapidly hydrolyzed to dUMP by UTP diphosphatase [dUTPase]. Thus, dUTPase plays an important role in reducing availability of dUTP as a substrate for DNA synthesis, thereby preventing erroneous incorporation of U into DNA.)

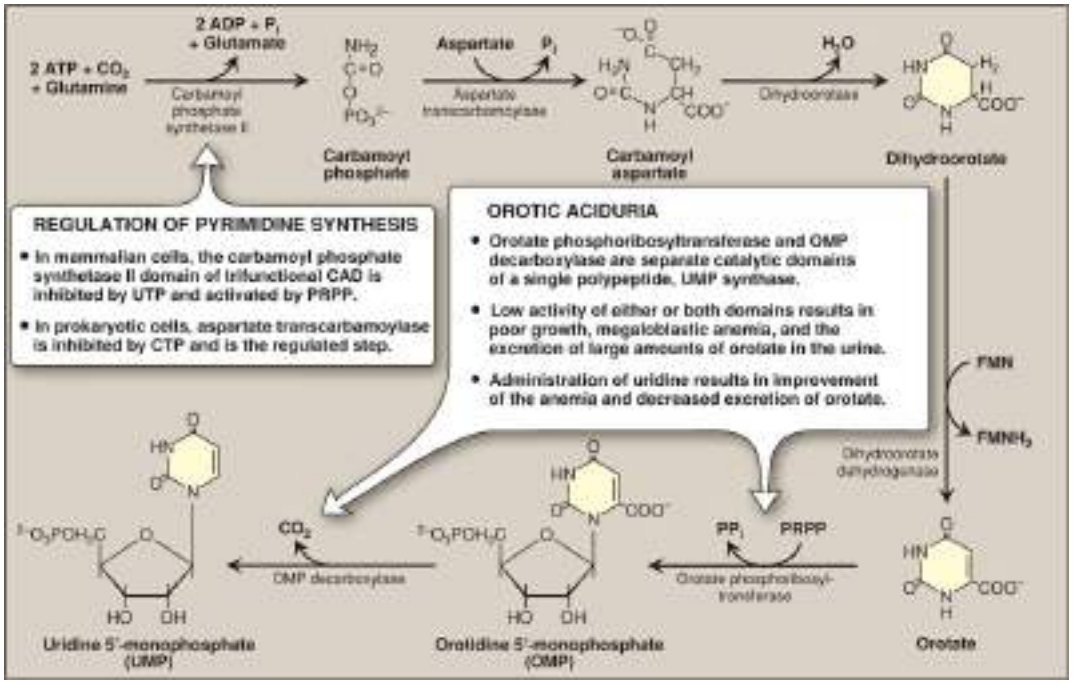


Figure 22.21
De novo pyrimidine synthesis. ADP = adenosine diphosphate; P_i = inorganic phosphate; FMN(H_2) = flavin mononucleotide; CTP = cytidine triphosphate; PRPP = 5-phosphoribosyl-1-pyrophosphate; PP_i = pyrophosphate.

D. Cytidine triphosphate synthesis

Cytidine triphosphate (CTP) is produced by amination of UTP by CTP synthetase (Fig. 22.22), with glutamine providing the nitrogen. Some of this CTP is dephosphorylated to CDP, which is a substrate for ribonucleotide reductase. The dCDP product can be phosphorylated to dCTP for DNA synthesis or dephosphorylated to dCMP that is deaminated to dUMP.

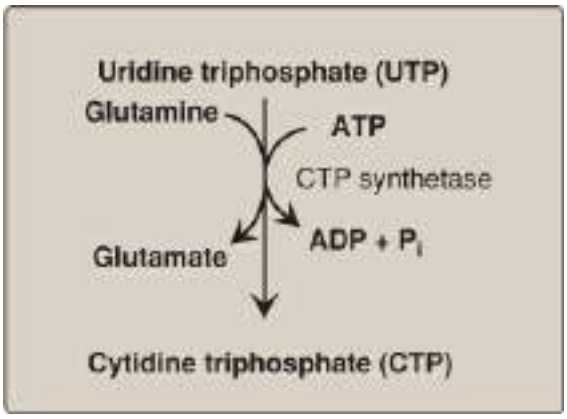


Figure 22.22
Synthesis of CTP from UTP. (Note: CTP, required for RNA synthesis, is converted to dCTP for DNA synthesis.) ADP = adenosine diphosphate; P_i = inorganic phosphate.

E. Deoxythymidine monophosphate synthesis

dUMP is converted to deoxythymidine monophosphate (dTMP) by thymidylate synthase, which uses N^5,N^{10}-methylene-THF as the source of the methyl group (see p. 296). This is an unusual reaction in that THF contributes not only a one-carbon unit but also two hydrogen atoms from the pteridine ring, resulting in the oxidation of THF to dihydrofolate ([DHF], Fig. 22.23). Inhibitors of thymidylate synthase include T analogs such as 5-fluorouracil, which serve as antitumor agents. 5-Fluorouracil is metabolically converted to 5-fluorodeoxyuridine monophosphate (5-FdUMP), which becomes permanently bound to the inactivated thymidylate synthase, making the drug a suicide inhibitor (see p. 64). DHF can be reduced to THF by DHF reductase (see Fig. 28.2, p. 424), an enzyme that is inhibited by folate analogs such as methotrexate. By decreasing the supply of THF, these drugs not only inhibit purine synthesis (see Fig. 22.7), but, by preventing methylation of dUMP to dTMP, they also decrease the availability of this essential component of DNA. DNA synthesis is inhibited and cell growth slowed. Thus, these drugs are used to treat cancer. (Note: Acyclovir [a purine analog] and 3′-azido-3′-deoxythymidine [AZT, a pyrimidine analog] are used to treat infections of herpes simplex virus and human immunodeficiency virus, respectively. Each inhibits the viral DNA polymerase.)

F. Pyrimidine salvage and degradation

Unlike the purine ring, which is not cleaved in humans and is excreted as poorly soluble uric acid, the pyrimidine ring is opened and degraded to highly soluble products, β-alanine (from the degradation of CMP and UMP) and β-aminoisobutyrate (from TMP degradation), with the production of ammonia and CO_2. Pyrimidine bases can be salvaged to nucleosides, which are phosphorylated to nucleotides. However, their high solubility makes pyrimidine salvage less significant clinically than purine salvage. (Note: The salvage of pyrimidine nucleosides is the basis for using uridine in the treatment of hereditary orotic aciduria [see p. 336].)

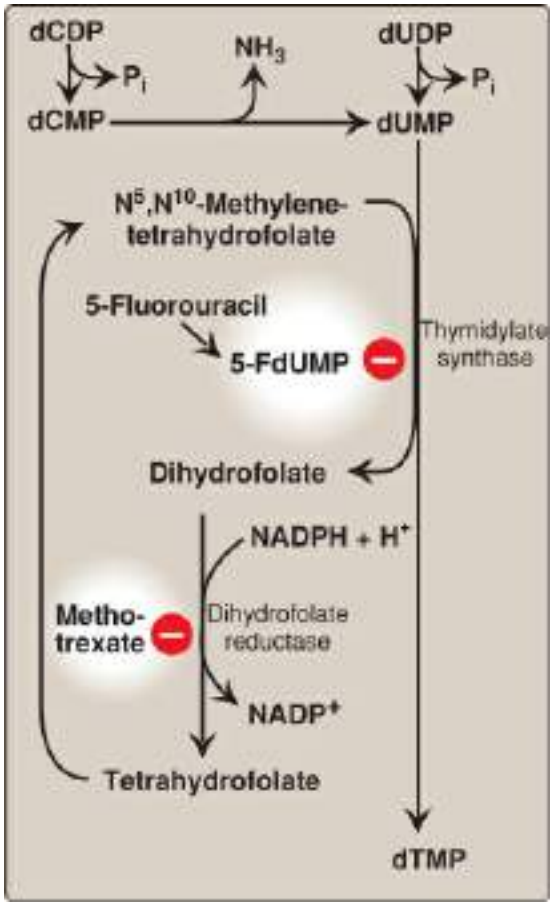


Figure 22.23
Synthesis of dTMP from dUMP, illustrating sites of action of antineoplastic drugs.

VII. Chapter Summary

- **Nucleotides** are composed of a **nitrogenous base** (**A**, **G**, **C**, **U**, and **T**); a **pentose sugar**; and one, two, or three **phosphate groups** (Fig. 22.24).
- A and G are **purines**, and C, U, and T are **pyrimidines**.
- If the sugar is **ribose**, the nucleotide is a **ribonucleoside phosphate** (e.g., AMP), and it can have several functions in the cell, including being a component of **RNA**. If the sugar is **deoxyribose**, the nucleotide is a **deoxyribonucleoside phosphate** (e.g., deoxyAMP) and will be found almost exclusively as a component of **DNA**.
- The **committed step** in **purine synthesis** uses **PRPP** (an activated pentose that provides the **ribose 5-phosphate** for *de novo* purine and pyrimidine synthesis and salvage) and nitrogen from **glutamine** to produce phosphoribosylamine. The enzyme is **GPAT** and is inhibited by AMP and GMP (the end products of the pathway) and activated by PRPP.
- Purine nucleotides can also be produced from preformed purine bases by using **salvage reactions** catalyzed by **APRT** and **HGPRT**. A near-total deficiency of HGPRT causes **Lesch–Nyhan syndrome**, a severe, inherited form of hyperuricemia accompanied by compulsive self-mutilation.
- All deoxyribonucleotides are synthesized from ribonucleotides by the enzyme **ribonucleotide reductase**. This enzyme is highly regulated (e.g., it is strongly inhibited by **dATP**, a compound that is overproduced in bone marrow cells in individuals with **ADA deficiency**). ADA deficiency causes **SCID**.
- The end product of purine degradation is **uric acid**, a compound of low solubility whose overproduction or undersecretion causes **hyperuricemia** that, if accompanied by the deposition of **MSU crystals** in joints and soft tissues and an inflammatory response to those crystals, results in **gout**.
- The first step in **pyrimidine synthesis**, the production of carbamoyl phosphate by **CPS II**, is the **regulated** step in this pathway (it is inhibited by **UTP** and activated by PRPP). The UTP produced by this pathway can be converted to CTP.
- **Deoxyuridine monophosphate** can be converted to dTMP by **thymidylate synthase**, an enzyme targeted by anticancer drugs such as **5-fluorouracil**.
- The regeneration of **tetrahydrofolate** from DHF produced in the thymidylate synthase reaction requires **dihydrofolate reductase**, an enzyme targeted by the drug **methotrexate**.

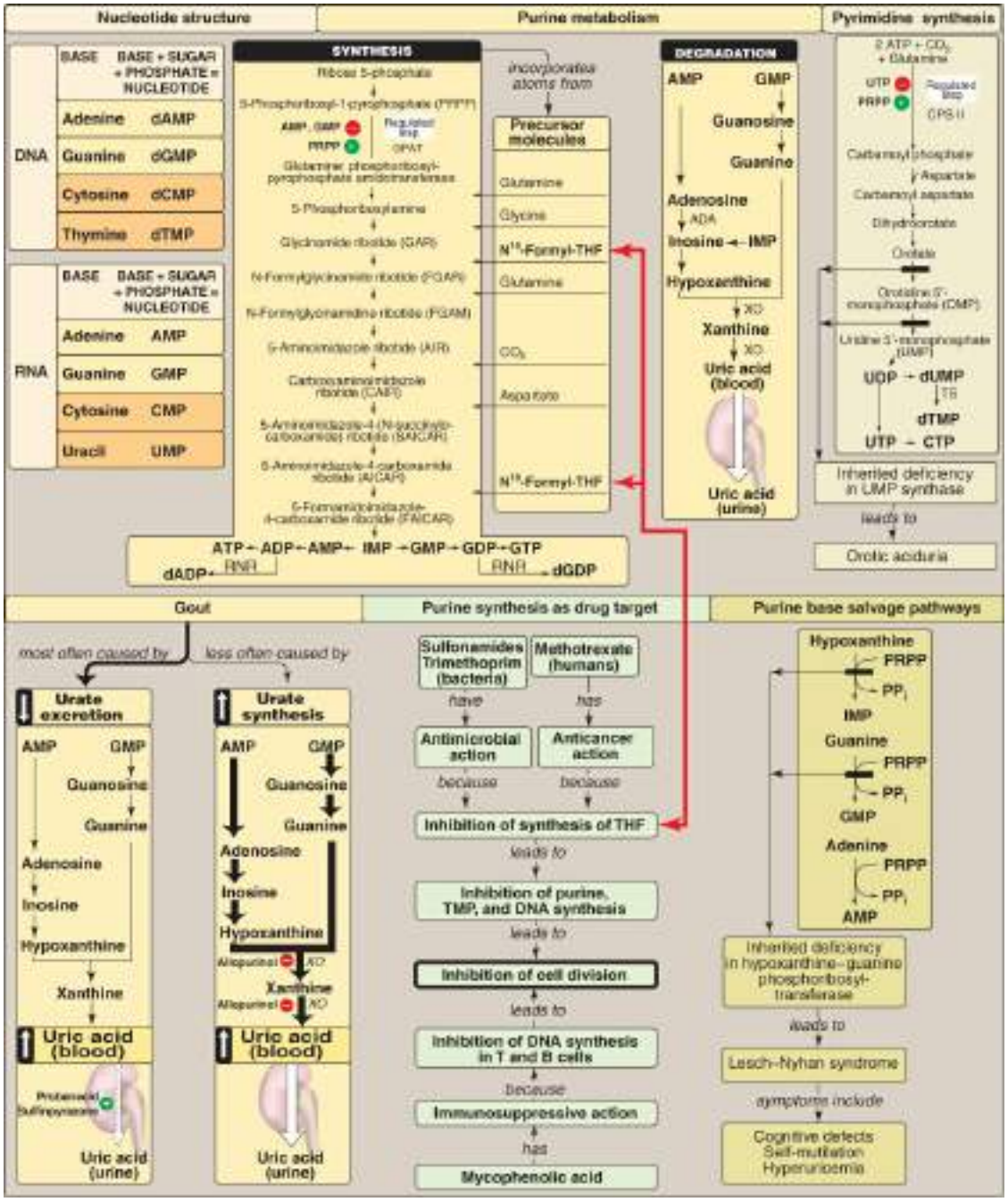


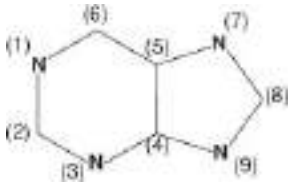
Figure 22.24
Key concept map for nucleotide metabolism. THF = tetrahydrofolate; GPAT = glutamine:phosphoribosylpyrophosphate amidotransferase; ADA = adenosine deaminase; XO = xanthine oxidase; TS = thymidylate synthase; RNR = ribonucleotide reductase; CPS II = carbamoyl phosphate synthetase II; AMP, GMP, CMP, TMP, and IMP = adenosine, guanosine, cytidine, thymidine, and inosine monophosphates; d = deoxy; PP_i = pyrophosphate; PRPP = 5-phosphoribosyl-1-pyrophosphate.

Study Questions

Choose the ONE best answer.

22.1 Azaserine, a drug with research applications, inhibits glutamine-dependent enzymes. Incorporation of which of the ring nitrogens (N) in the generic purine structure shown would most likely be affected by azaserine?

A. 1
B. 3
C. 7
D. 9



Correct answer = D. The N at position 9 is supplied by glutamine in the first step of purine *de novo* synthesis, and its incorporation would be affected by azaserine. The N at position 1 is supplied by aspartate and at position 7 by glycine. The N at position 3 is also supplied by glutamine, but azaserine would have inhibited purine synthesis prior to this step.

22.2 A 42-year-old male undergoing radiation therapy for prostate cancer develops severe pain in the metatarsal phalangeal joint of his right big toe. Monosodium urate crystals are detected by polarized light microscopy in fluid obtained from this joint by arthrocentesis. This patient's pain is directly caused by the overproduction of the end product of which of the following metabolic pathways?

A. *De novo* pyrimidine biosynthesis
B. Pyrimidine degradation
C. *De novo* purine biosynthesis
D. Purine salvage
E. Purine degradation

Correct answer = E. The patient's pain is caused by gout, resulting from an inflammatory response to the crystallization of excess urate (as monosodium urate) in his joints. Radiation therapy caused cell death, with degradation of nucleic acids and their constituent purines. Uric acid, the end product of purine degradation, is a relatively insoluble compound that can cause gout (and kidney stones). Pyrimidine metabolism is not associated with uric acid production. Overproduction of purines can indirectly result in hyperuricemia. Purine salvage decreases uric acid production.

22.3 Which one of the following enzymes of nucleotide metabolism is correctly paired with its pharmacologic inhibitor?

A. Dihydrofolate reductase—methotrexate
B. Inosine monophosphate dehydrogenase—hydroxyurea
C. Ribonucleotide reductase—5-fluorouracil
D. Thymidylate synthase—allopurinol
E. Xanthine oxidase—probenecid

Correct answer = A. Methotrexate interferes with folate metabolism by acting as a competitive inhibitor of the enzyme dihydrofolate reductase. This starves cells for tetrahydrofolate and makes them unable to synthesize purines and thymidine monophosphate. Inosine monophosphate dehydrogenase is inhibited by mycophenolic acid. Ribonucleotide reductase is inhibited by hydroxyurea. Thymidylate synthase is inhibited by 5-fluorouracil. Xanthine oxidase is inhibited by allopurinol. Probenecid increases renal excretion of urate but does not inhibit its production.

22.4 A 1-year-old female patient is lethargic, weak, and anemic. Her height and weight are low for her age. Her urine contains an elevated level of orotic acid. Activity of uridine monophosphate synthase is low. Administration of

which of the following is most likely to alleviate her symptoms?

A. Adenine
B. Guanine
C. Hypoxanthine
D. Thymidine
E. Uridine

Correct answer = E. The elevated excretion of orotic acid and low activity of uridine monophosphate (UMP) synthase indicate that the patient has orotic aciduria, a very rare genetic disorder affecting *de novo* pyrimidine synthesis. Deficiencies in one or both catalytic domains of UMP synthase leave the patient unable to synthesize pyrimidines. Uridine, a pyrimidine nucleoside, is a useful treatment because it bypasses the missing activities and can be salvaged to UMP, which can be converted to all the other pyrimidines. Although thymidine is a pyrimidine nucleoside, it cannot be converted to other pyrimidines. Hypoxanthine, guanine, and adenine are all purine bases and cannot be converted to pyrimidines.

22.5 What laboratory test would help in distinguishing an orotic aciduria caused by ornithine transcarbamylase deficiency from that caused by uridine monophosphate synthase deficiency?

In both orotic aciduria and ornithine transcarbamylase deficiency, there are elevated urinary orotate levels. Blood ammonia level would be expected to be elevated in ornithine transcarbamylase deficiency that affects the urea cycle, but not in uridine monophosphate synthase deficiency.

UNIT V:
Integration of Metabolism

Metabolic Effects of Insulin and Glucagon 23

I. OVERVIEW

Four major tissues play a dominant role in fuel metabolism: liver, adipose, muscle, and brain. These tissues contain unique sets of enzymes, such that each tissue is specialized for the storage, use, or generation of specific fuels. These tissues do not function in isolation but rather form part of a network in which one tissue may provide substrates to another or process compounds produced by other tissues. Communication between tissues is mediated by the nervous system, by the availability of circulating substrates, and by variation in the levels of plasma hormones (Fig. 23.1). The integration of energy metabolism is controlled primarily by the actions of two peptide hormones, insulin and glucagon (secreted in response to changing substrate levels in the blood), with the catecholamines epinephrine and norepinephrine (secreted in response to neural signals) playing a supporting role. Changes in the circulating levels of these hormones allow the body to store energy when food is abundant or to make stored energy available such as during fasting or survival crises (e.g., famine, severe injury, and “fight-or-flight” situations). This chapter describes the structure, secretion, and metabolic effects of the two hormones that most profoundly affect energy metabolism.

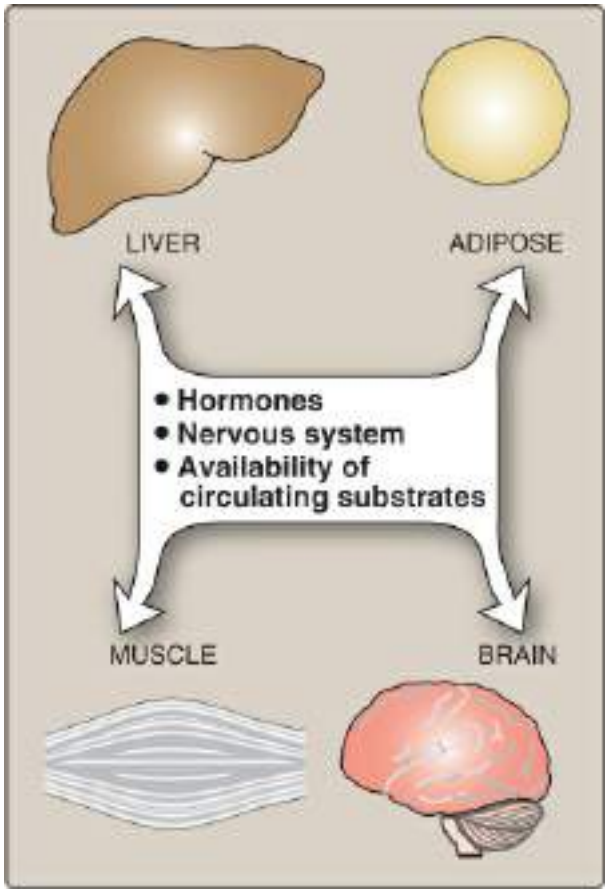


Figure 23.1
Mechanisms of communication between four major tissues.

II. INSULIN

Insulin is a peptide hormone produced by the β cells of the islets of Langerhans, which are clusters of cells embedded in the endocrine portion of the pancreas (Fig. 23.2). (Note: “Insulin” is from the Latin for island.) The islets make up only about 1% to 2% of the total cells of the pancreas. Insulin is the most important hormone coordinating the use of fuels by tissues. Its metabolic effects are anabolic, favoring, for example, synthesis of glycogen, triacylglycerol (TAG), and protein.

A. Structure

Insulin is composed of 51 amino acids arranged in two polypeptide chains, designated A (21 amino acids) and B, which are linked together by two disulfide bonds (Fig. 23.3A). The insulin molecule also contains an intramolecular disulfide bond between cysteine residues of the A chain. (Note: Insulin was the first peptide for which the primary structure was determined and the first therapeutic molecule made by recombinant DNA technology [see p. 537].)

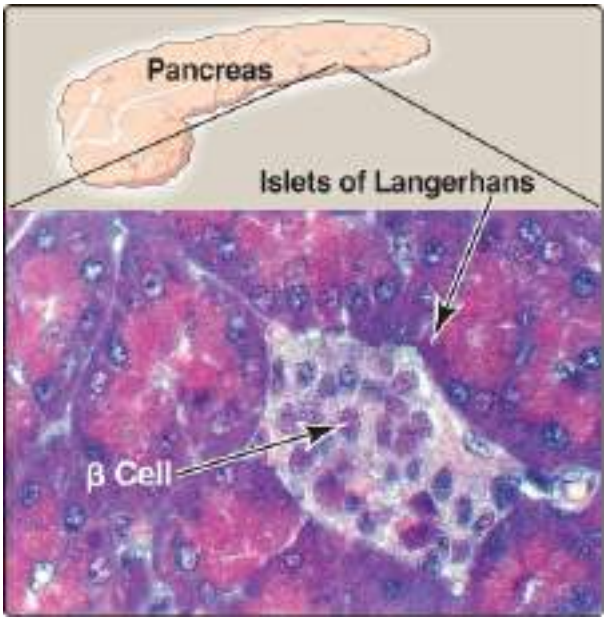


Figure 23.2
Islets of Langerhans.

B. Synthesis and degradation

The processing and transport of intermediates that occur during the synthesis of insulin are shown in Figures 23.3B and 23.4. Biosynthesis involves production of two inactive precursors, preproinsulin and proinsulin, which contain the A and B chains as a single peptide. These precursors are sequentially cleaved to form the active two-chain hormone plus a connecting or C-peptide in a 1:1 ratio. (Note: The C-peptide is essential for proper insulin folding. Also, because its half-life in plasma is longer than that of insulin, the C-peptide level is a good indicator of insulin production and secretion.) Insulin is stored in cytosolic granules that, given the proper stimulus (see C.1. below), are released by exocytosis. (See p. 505 for a discussion of the synthesis of secreted proteins.) Insulin is degraded by insulin-degrading enzyme, which is present in the liver and, to a lesser extent, in the kidneys. Insulin has a plasma half-life of ~6 minutes. This short duration of action permits rapid changes in circulating levels of the hormone.

C. Secretion regulation

Secretion of insulin is regulated by blood-borne fuels and hormones.

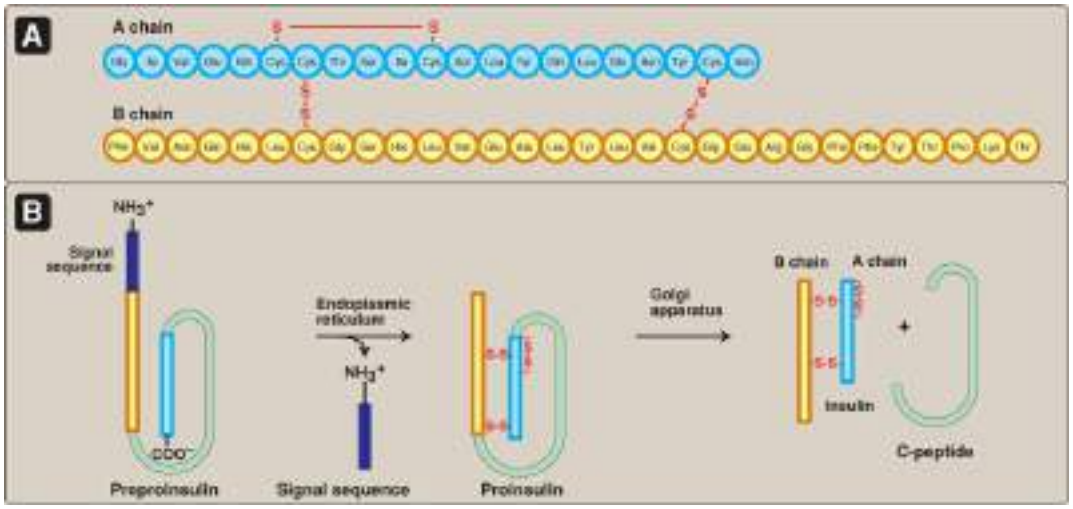


Figure 23.3
A: Structure of insulin. **B:** Formation of human insulin from preproinsulin. S-S = disulfide bond.

1. Increased secretion: Insulin secretion by the pancreatic β cells is closely coordinated with the secretion of glucagon by pancreatic α cells (Fig. 23.5). The relative amounts of glucagon and insulin released are normally regulated such that the rate of hepatic glucose production is kept equal to the use of glucose by peripheral tissues. This maintains blood glucose between 70 and 140 mg/dl (3.9 and 7.8 mmol/l, respectively). In view of its coordinating role, it is not surprising that the β cell responds to a variety of stimuli. In particular, insulin secretion is increased by glucose, amino acids, and gastrointestinal peptide hormones.

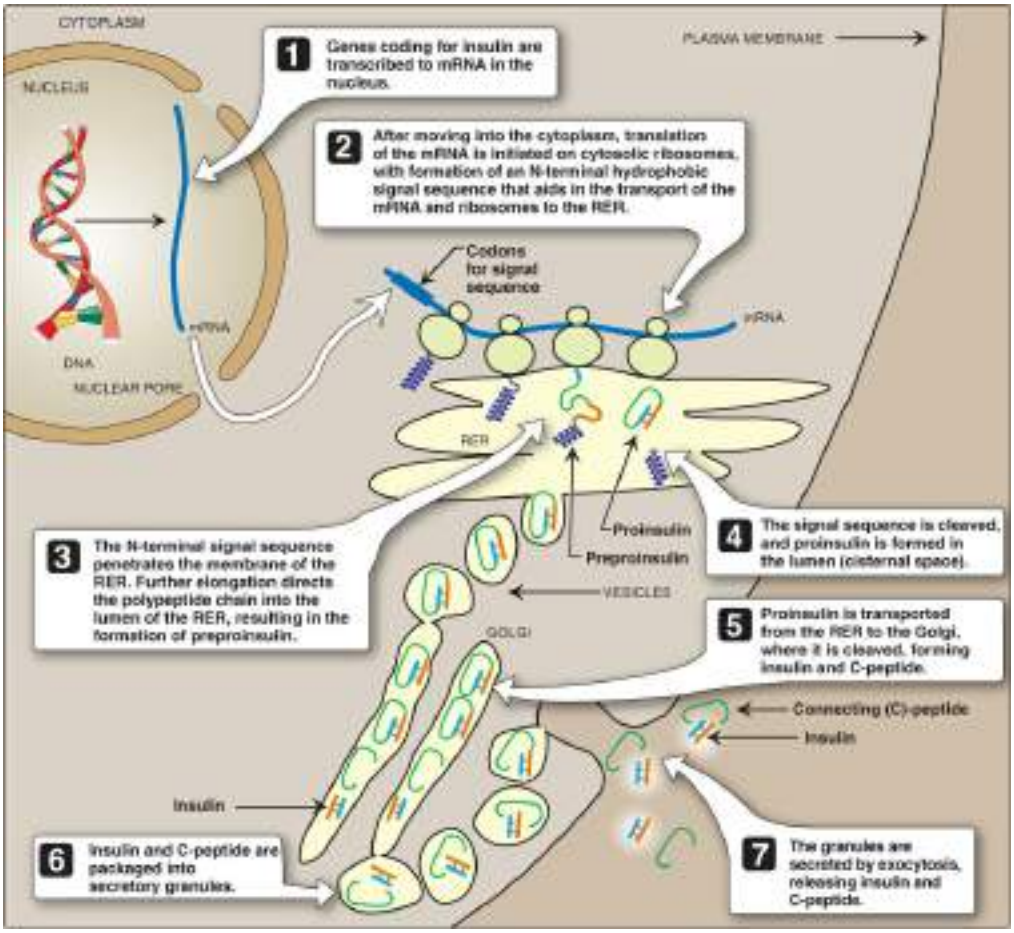


Figure 23.4
Intracellular movements of insulin and its precursors. mRNA = messenger RNA; RER = rough endoplasmic reticulum.

a. Glucose: Ingestion of a carbohydrate-rich meal leads to a rise in blood glucose, the primary stimulus for insulin secretion (Fig. 23.5). The β cells are the most important glucose-sensing cells in the body. Like the liver, β cells contain GLUT-2 transporters and express glucokinase (hexokinase IV; see p. 108). At blood glucose levels >45 mg/dl, glucokinase phosphorylates glucose in amounts proportional to the glucose concentration. Proportionality results from the lack of direct inhibition of glucokinase by glucose 6-phosphate, its product. Additionally, the sigmoidal relationship between the velocity of the reaction and substrate concentration maximizes the enzyme's responsiveness to changes in blood glucose level. The subsequent metabolism of glucose 6-phosphate in glycolysis generates ATP, leading to insulin secretion (see box below).

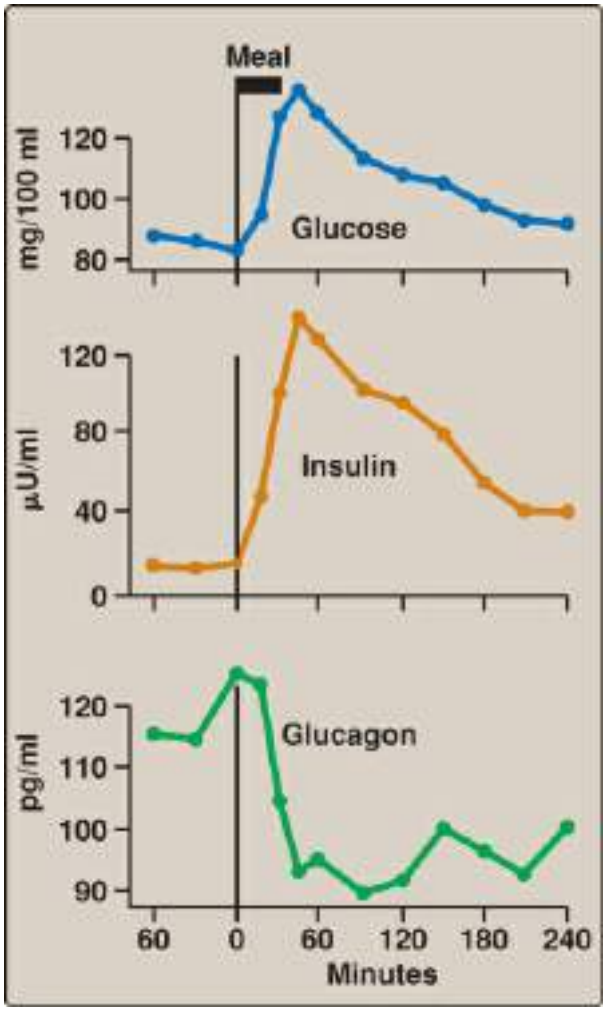


Figure 23.5
Changes in blood levels of glucose, insulin, and glucagon after ingestion of a carbohydrate-rich meal.

b. Amino acids: Ingestion of protein causes a transient rise in plasma amino acid levels (e.g., arginine) that enhances the glucose-stimulated secretion of insulin from endocrine pancreatic β cells. (Note: Fatty acids have a similar effect.)

c. Gastrointestinal peptide hormones: The intestinal peptides glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide ([GIP] also called glucose-dependent insulinotropic peptide) increase the sensitivity of β cells to glucose. They are released from the small intestine after the ingestion of food, causing an anticipatory rise in insulin levels and, thus, are referred to as incretins. Their action may account for the fact that the same amount of glucose given orally induces a much greater secretion of insulin than if given intravenously (IV). Antihyperglycemic drugs of the incretin mimetic class, used to treat type 2 diabetes function to increase glucose sensitivity of the β cells, thereby increasing insulin secretion.

Glucose-dependent release of insulin into blood is mediated through an intracellular rise in calcium (Ca^{2+}) concentration in β cells. Glucose taken into β cells by GLUT-2 is phosphorylated and metabolized, with a subsequent rise in intracellular ATP levels. ATP-sensitive potassium (K^+) channels close in response to the rise in ATP levels, causing depolarization of the plasma membrane. Depolarization mediates the opening of voltage-gated Ca^{2+} channels in the plasma membrane, and an influx of Ca^{2+}. An increase in cytosolic Ca^{2+} signals exocytosis of insulin-containing vesicles from β cells. Sulfonylureas function to increase insulin secretion by closing the ATP-sensitive K^+ channels. They are referred to as insulin secretagogues and are oral agents used to treat hyperglycemia in type 2 diabetes. Meglitinides function similar to sulfonylureas, but have a weaker binding affinity and higher dissociation for the ATP-sensitive K^+ channels, and therefore are shorter-acting insulin secretagogues. Conversely, diazoxides open the ATP-sensitive K^+ channel, leading to decreased insulin secretion. Diazoxides are used to treat hypoglycemia caused by congenital hyperinsulinism or in insulinomas (insulin-producing tumors).

2. Decreased secretion: The synthesis and release of insulin are decreased when there is a scarcity of dietary fuels and also during periods of physiologic stress (e.g., infection, hypoxia, and vigorous exercise), thereby preventing hypoglycemia. These effects are mediated primarily by the catecholamines norepinephrine and epinephrine, which are made from tyrosine in the sympathetic nervous system (SNS) and the adrenal medulla and then secreted. Secretion is largely controlled by neural signals. The catecholamines (primarily epinephrine) have a direct effect on energy metabolism, causing a rapid mobilization of energy-yielding fuels, including glucose from the liver (produced by glycogenolysis or gluconeogenesis; see p. 131) and fatty acids (FA) from adipose tissue (produced by lipolysis; see p. 209). In addition, these biogenic amines can override the normal glucose-stimulated release of insulin. Thus, in emergency situations, the SNS largely replaces the plasma glucose concentration as the controlling influence over β-cell secretion. The regulation of insulin secretion is summarized in Figure 23.6.

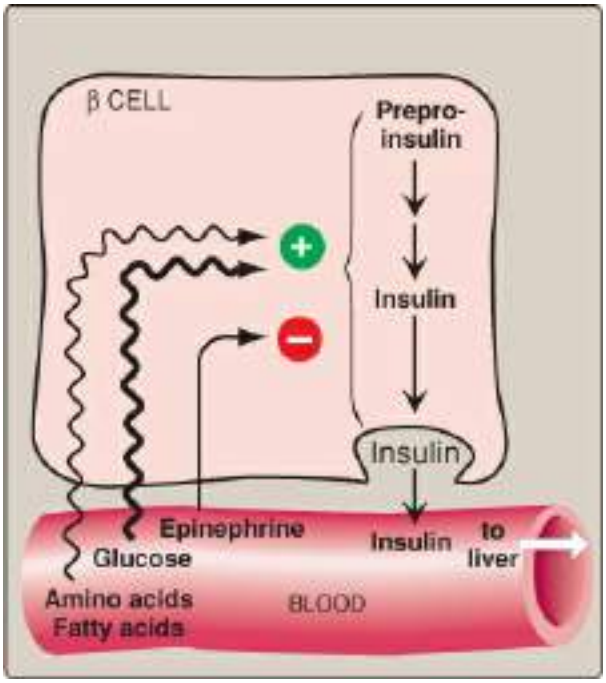


Figure 23.6
Regulation of insulin release from pancreatic β cells. (Note: Gastrointestinal peptide hormones also stimulate insulin release.)

D. Metabolic effects

Insulin promotes the cellular uptake of nutrients (primarily glucose); it also promotes the storage of nutrients, including glycogen, TAG, and protein, and inhibits their mobilization.

1. Effects on carbohydrate metabolism: The effects of insulin on glucose metabolism promote its storage and are most prominent in three tissues: liver, muscle, and adipose. In liver and muscle, insulin increases glycogen synthesis. In muscle and adipose, insulin increases glucose uptake by increasing the number of glucose transporters (GLUT-4; see p. 106) in the cell membrane. Thus, the IV administration of insulin causes an immediate decrease in blood glucose level. In the liver, insulin decreases the production of glucose through the inhibition of glycogenolysis and gluconeogenesis. (Note: The effects of insulin are due not just to changes in enzyme activity but also in enzyme amount insofar as insulin signaling alters gene transcription.)

2. Effects on lipid metabolism: A rise in insulin rapidly causes a significant reduction in the release of FA from adipose tissue by inhibiting the activity of hormone-sensitive lipase, a key enzyme of TAG degradation in adipocytes. Insulin acts by promoting the dephosphorylation and, hence, inactivation of the enzyme (see p. 210). Insulin also increases the transport and metabolism of glucose into adipocytes, providing the glycerol 3-phosphate substrate for TAG synthesis (see p. 208). Expression of the gene for lipoprotein lipase (LL), which

degrades TAG in circulating chylomicrons and very–low-density lipoproteins ([VLDL]; see p. 256), is increased by insulin in adipose, thereby providing FA for esterification to the glycerol. (Note: Insulin also promotes the conversion of glucose to TAG in the liver. The TAGs are secreted in VLDL.)

3. Effects on protein synthesis: In most tissues, insulin stimulates both the entry of amino acids into cells and protein synthesis (translation). (Note: Insulin stimulates protein synthesis through covalent activation of factors required for translation initiation.)

E. Mechanism

Insulin binds to specific, high-affinity receptors in the cell membrane of most tissues, including liver, muscle, and adipose. This is the first step in a cascade of reactions ultimately leading to a diverse array of biologic actions (Fig. 23.7).

1. Insulin receptor: The insulin receptor is synthesized as a single polypeptide that is glycosylated and cleaved into α and β subunits, which are then assembled into a tetramer linked by disulfide bonds (Fig. 23.7). The extracellular α subunits contain the insulin-binding site. A hydrophobic domain in each β subunit spans the plasma membrane. The cytosolic domain of the β subunit is a tyrosine kinase, which is activated by insulin. As a result, the insulin receptor is classified as a receptor tyrosine kinase.

2. Signal transduction: The binding of insulin to the α subunits of the insulin receptor induces conformational changes that are transmitted to the β subunits. This promotes a rapid autophosphorylation of specific tyrosine residues on each β subunit (Fig. 23.7). Autophosphorylation initiates a cascade of cell-signaling responses, including phosphorylation of a family of proteins called insulin receptor substrates (IRSs). At least four IRSs have been identified that show similar structures but different tissue distributions. Phosphorylated IRS proteins interact with other signaling molecules through specific domains (known as SH2), activating a number of pathways that affect gene expression, cell metabolism, and growth. The actions of insulin are terminated by dephosphorylation of the receptor.

3. Membrane effects: Glucose transport into certain tissues, including muscle and adipose, increases in the presence of insulin (Fig. 23.8). Insulin promotes movement of insulin-sensitive glucose transporters (GLUT-4) from a pool located in intracellular vesicles to the cell membrane. (Note: Movement is the result of a signaling cascade in which an IRS binds to and activates a kinase [phosphoinositide 3-kinase], leading to phosphorylation of the membrane phospholipid phosphatidylinositol 4,5-bisphosphate [PIP_2] to the 3,4,5-trisphosphate form [PIP_3] that binds to and activates phosphoinositide-dependent kinase 1. This kinase, in turn, activates Akt [or protein kinase B], resulting in GLUT-4 movement.) In contrast, other tissues have insulin-

insensitive systems for glucose transport (Fig. 23.9). For example, hepatocytes, erythrocytes, and cells of the nervous system, intestinal mucosa, renal tubules, and cornea do not require insulin for glucose uptake.

4. Receptor regulation: Binding of insulin is followed by internalization of the hormone–receptor complex. Once inside the cell, insulin is degraded in the lysosomes. The receptors may be degraded, but most are recycled to the cell surface. (Note: Elevated levels of insulin promote the degradation of receptors, thereby decreasing the number of surface receptors. This is one type of downregulation.)

5. Time course: The binding of insulin provokes a wide range of actions. The most immediate response is an increase in glucose transport into adipocytes and skeletal and cardiac muscle cells that occurs within seconds of insulin binding to its membrane receptor. Insulin-induced changes in enzymic activity in many cell types occur over minutes to hours and reflect changes in the phosphorylation states of existing proteins. Insulin-induced increase in the amount of many enzymes, such as glucokinase, liver pyruvate kinase, acetyl coenzyme A (CoA) carboxylase (ACC), and fatty acid synthase, requires hours to days. These changes reflect an increase in gene expression through increased transcription (mediated by sterol regulatory element–binding protein-1c; see p. 204) and translation.

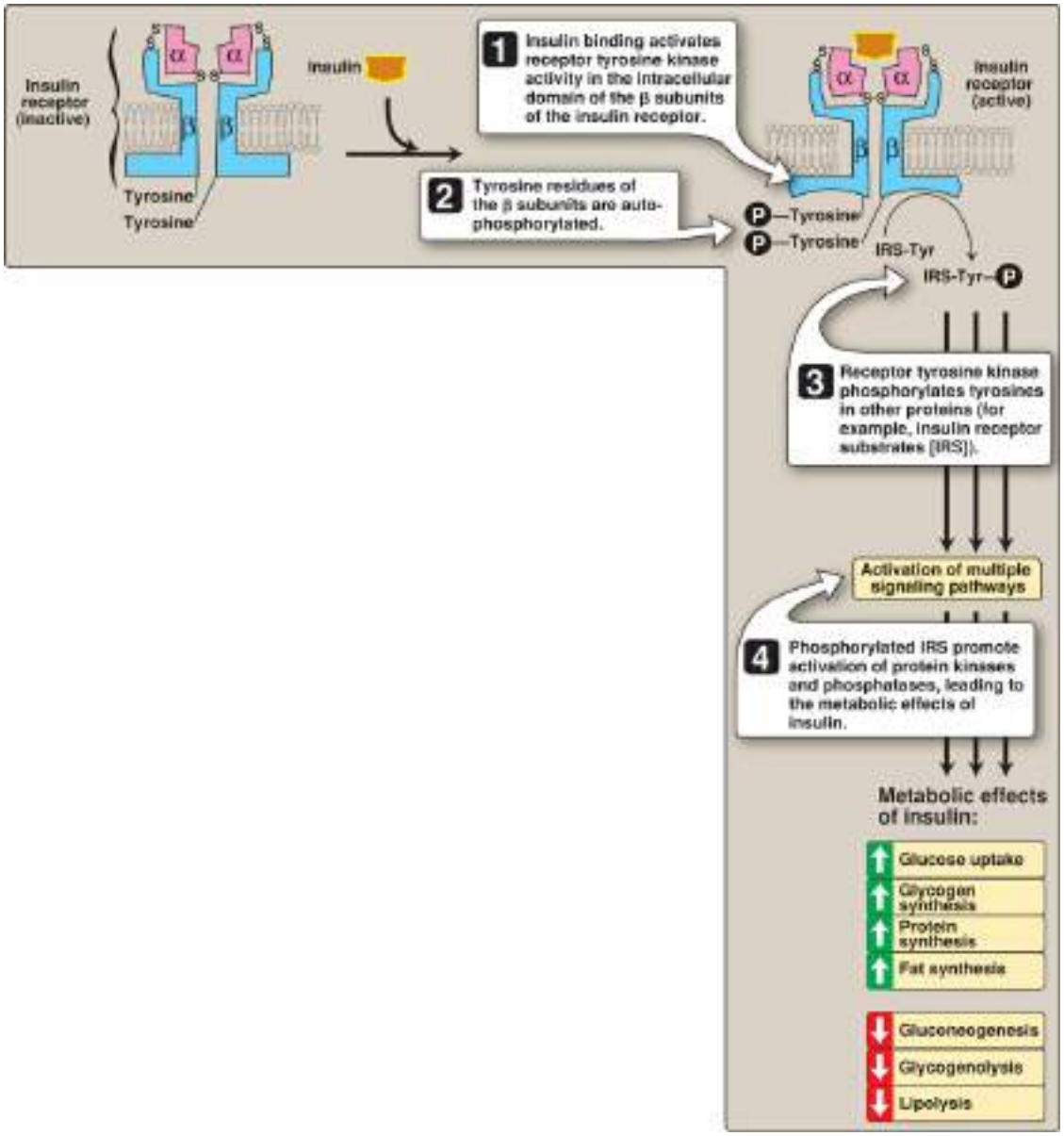


Figure 23.7
Mechanism of action of insulin. (P) = phosphate; Tyr = tyrosine; S-S = disulfide bond.

III. GLUCAGON

Glucagon is a peptide hormone secreted by the α cells of the pancreatic islets of Langerhans. Glucagon, along with epinephrine, norepinephrine, cortisol, and growth hormone (the counterregulatory hormones), opposes many of the actions of insulin (Fig. 23.10). Most importantly, glucagon acts to maintain blood glucose levels by activation of hepatic glycogenolysis and gluconeogenesis. Glucagon is composed of 29 amino acids arranged in a single polypeptide chain. (Note: Unlike insulin, the amino acid sequence of glucagon is the same in all mammalian species examined to date.) Glucagon is synthesized as a large precursor molecule (preproglucagon) that is converted to

glucagon through a series of selective proteolytic cleavages, similar to those described for insulin biosynthesis (see Fig. 23.3). In contrast to insulin, preproglucagon is processed to different products in different tissues, for example, GLP-1 in intestinal L cells. Like insulin, glucagon has a short half-life.

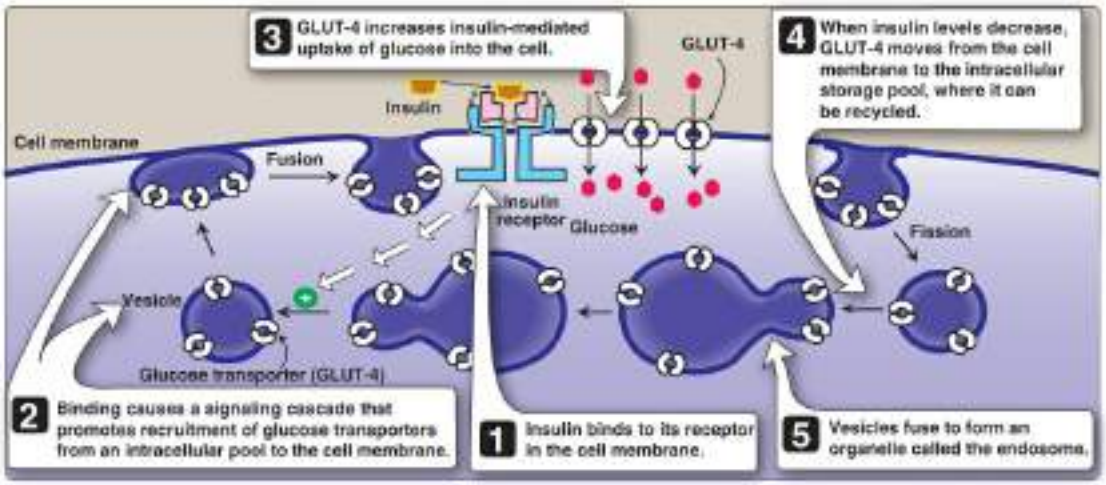


Figure 23.8
Insulin-mediated recruitment of GLUT-4 from intracellular stores to the cell membrane in skeletal and cardiac muscle and adipose tissue. S-S = disulfide bond.

A. Increased secretion

The α cell is responsive to a variety of stimuli that signal actual or potential hypoglycemia (Fig. 23.11). Specifically, glucagon secretion is increased by low blood glucose, amino acids, and catecholamines.

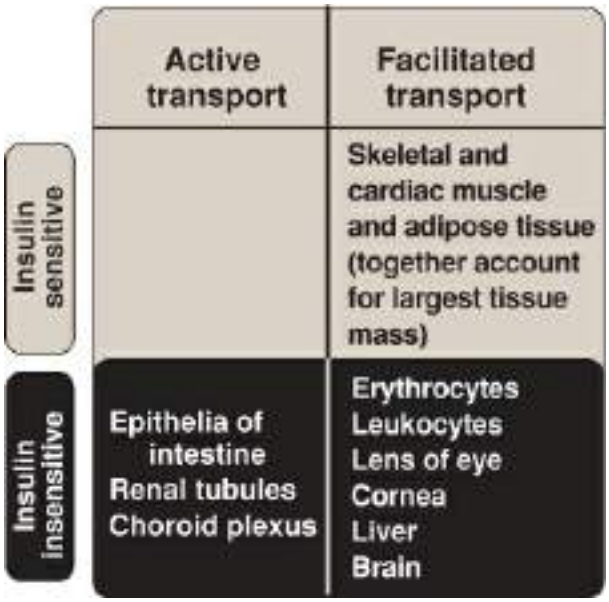


Figure 23.9
Characteristics of glucose transport in various tissues.

1. Low blood glucose: A decrease in plasma glucose concentration is the primary stimulus for glucagon release. During an overnight or prolonged fast, elevated glucagon levels prevent hypoglycemia (see Section IV below for a discussion of hypoglycemia).

2. Amino acids: Amino acids (e.g., arginine) derived from a meal containing protein stimulate the release of glucagon. The glucagon effectively prevents the hypoglycemia that would otherwise occur as a result of the increased insulin secretion that also occurs after a protein meal.

3. Catecholamines: Elevated levels of circulating epinephrine (from the adrenal medulla), norepinephrine (from sympathetic innervation of the pancreas), or both stimulate the release of glucagon. Thus, during periods of physiologic stress, the elevated catecholamine levels can override the effect on the α cell of circulating substrates. In these situations, regardless of the concentration of blood glucose, glucagon levels are elevated in anticipation of increased glucose use. In contrast, insulin levels are depressed.

B. Decreased secretion

Glucagon secretion is significantly decreased by elevated blood glucose and by insulin. Both substances are increased following ingestion of glucose or a carbohydrate-rich meal (Fig. 23.5). The regulation of glucagon secretion is summarized in Figure 23.11.

C. Metabolic effects

Glucagon is a catabolic hormone that promotes the maintenance of blood glucose levels. Its primary target is the liver. Glucagon also has an effect in the mobilization and utilization of FA in adipose and muscle tissues.

1. Effects on carbohydrate metabolism: The IV administration of glucagon leads to an immediate rise in blood glucose. This results from an increase in the degradation of hepatic glycogen stores (see p. 132) and an increase in gluconeogenesis (see p. 122). During the daytime, postprandial blood glucose levels are maintained primarily by hepatic glycogenolysis, with additional blood glucose provided by gluconeogenesis. Since the interprandial period is longer as we sleep and glycogen stores become more limited, gluconeogenesis increases providing more of the blood glucose source as the night progresses. Glucagon also inhibits glycolysis by decreasing levels of the PFK-1 allosteric activator, fructose 2,6-bisphosphate (see p. 122).

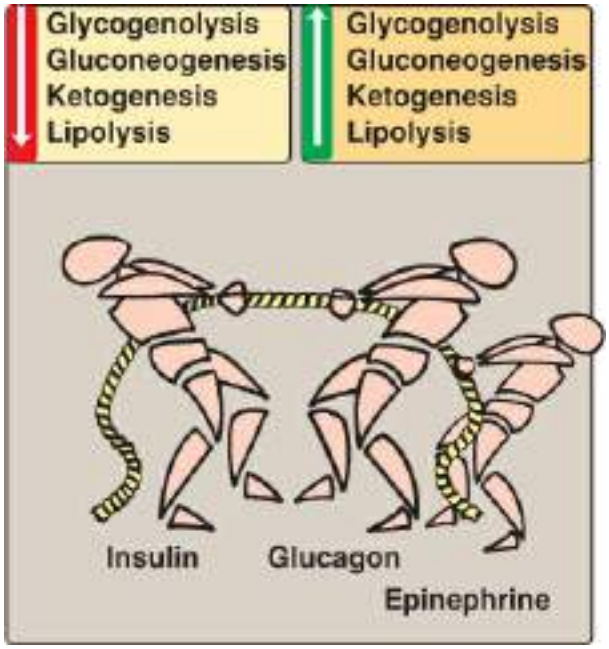


Figure 23.10
Opposing actions of insulin and glucagon plus epinephrine.

2. Effects on lipid metabolism: The primary effect of glucagon on lipid metabolism is inhibition of FA synthesis through phosphorylation and subsequent inactivation of ACC by adenosine monophosphate (AMP)–activated protein kinase (see p. 204). The resulting decrease in malonyl CoA production removes the inhibition on carnitine palmitoyltransferase-1, required for transport of long-chain FA into the mitochondrial matrix for β oxidation (see p. 210). Glucagon also plays a role in lipolysis in adipocytes, but the major activators of hormone-sensitive lipase (via phosphorylation by protein kinase A) are the catecholamines. The free FA mobilized by adipocytes are taken up by liver and muscle tissues and oxidized to acetyl CoA. The liver uses the acetyl CoA in ketone body synthesis. Muscle cells will use the acetyl CoA for energy. Glucagon and catecholamines also activate LL in cardiac and skeletal muscle tissues, to allow uptake of FA from VLDL complexes in the fasting state. Considering that glucagon stimulates GLUT-4 intracellular sequestration, it makes sense that muscle tissues would increase utilization of FA as an energy source.

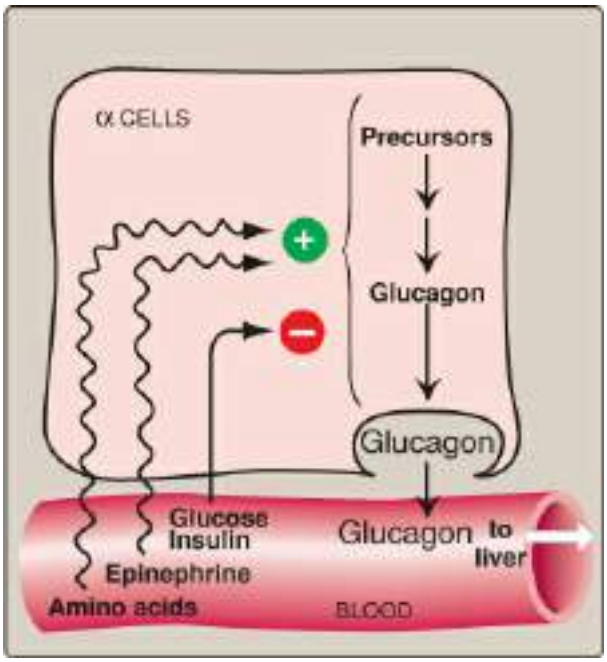


Figure 23.11
Regulation of glucagon release from pancreatic α cells. (Note: Amino acids increase release of insulin and glucagon, whereas glucose increases release of insulin and decreases release of glucagon.)

3. Effects on protein metabolism: Glucagon increases uptake by the liver of amino acids supplied by muscle, resulting in increased availability of carbon skeletons for gluconeogenesis. As a consequence, plasma levels of amino acids are decreased.

D. Mechanism

Glucagon binds to high-affinity G protein–coupled receptors (GPCRs) on the cell membrane of hepatocytes. The GPCR for glucagon is distinct from the GPCR that bind epinephrine. (Note: Epinephrine, not glucagon, receptors are found on skeletal muscle.) Glucagon binding results in activation of adenylyl cyclase in the plasma membrane (Fig. 23.12; see p. 103). This causes a rise in cyclic AMP (cAMP), which, in turn, activates cAMP-dependent protein kinase A and increases the phosphorylation of specific enzymes or other proteins. This cascade of increasing enzymic activities results in the phosphorylation-mediated activation or inhibition of key regulatory enzymes involved in carbohydrate and lipid metabolism. An example of such a cascade in glycogen degradation is shown in Figure 11.9 on p. 144. (Note: Glucagon, like insulin, affects gene transcription; e.g., glucagon induces expression of phosphoenolpyruvate carboxykinase [see p. 133].)

IV. HYPOGLYCEMIA

Hypoglycemia is characterized by (1) central nervous system (CNS) symptoms,

including confusion, aberrant behavior, or coma; (2) a simultaneous blood glucose level ≤50 mg/dl; and (3) symptoms being resolved within minutes following glucose administration (Fig. 23.13). Hypoglycemia is a medical emergency because the CNS has an absolute requirement for a continuous supply of blood-borne glucose to serve as a metabolic fuel. Transient hypoglycemia can cause cerebral dysfunction, whereas severe, prolonged hypoglycemia causes brain damage. Therefore, it is not surprising that the body has multiple overlapping mechanisms to prevent or correct hypoglycemia. The most important hormone changes in combating hypoglycemia are increased secretion of glucagon and the catecholamines, combined with decreased insulin secretion.

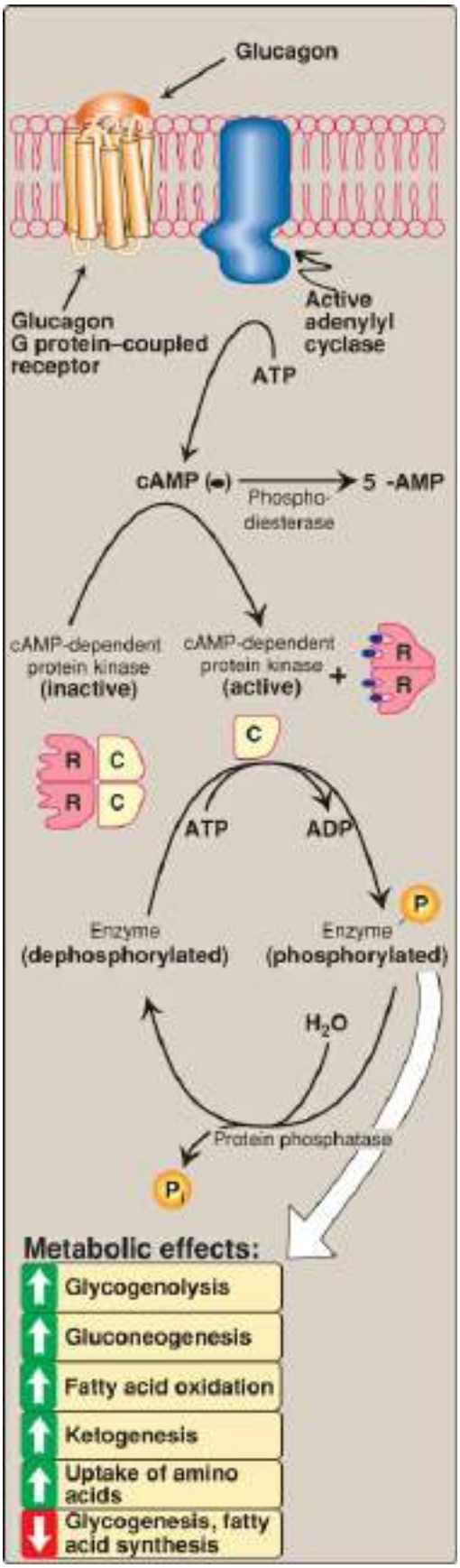


Figure 23.12
Mechanism of action of glucagon. (Note: For clarity, G-protein activation of adenylyl cyclase has been omitted.) R = regulatory subunit; C = catalytic subunit; cAMP = cyclic adenosine monophosphate; ADP = adenosine diphosphate; P = phosphate.

A. Symptoms

The symptoms of hypoglycemia can be divided into two categories. Adrenergic (neurogenic, autonomic) symptoms, such as anxiety, palpitation, tremor, and sweating, are mediated by catecholamine release (primarily epinephrine) regulated by the hypothalamus in response to hypoglycemia. Adrenergic symptoms typically occur when blood glucose levels fall abruptly. The second category of hypoglycemic symptoms is neuroglycopenic. The impaired delivery of glucose to the brain (neuroglycopenia) results in impairment of brain function, causing headache, confusion, slurred speech, seizures, coma, and death. Neuroglycopenic symptoms often result from a gradual decline in blood glucose, often to levels <50 mg/dl. The slow decline in glucose deprives the CNS of fuel but fails to trigger an adequate adrenergic response.

B. Glucoregulatory systems

Humans have two overlapping glucose-regulating systems that are activated by hypoglycemia: (1) the pancreatic α cells, which release glucagon, and (2) receptors in the hypothalamus, which respond to abnormally low concentrations of blood glucose. The hypothalamic glucoreceptors can trigger both the secretion of catecholamines (mediated by the sympathetic division of the autonomic nervous system) and release of adrenocorticotropic hormone (ACTH) and growth hormone by the anterior pituitary (see Fig. 23.13). (Note: ACTH increases cortisol synthesis and secretion in the adrenal cortex [see p. 264].) Glucagon, the catecholamines, cortisol, and growth hormone are sometimes called the counterregulatory hormones because each opposes the action of insulin on glucose use.

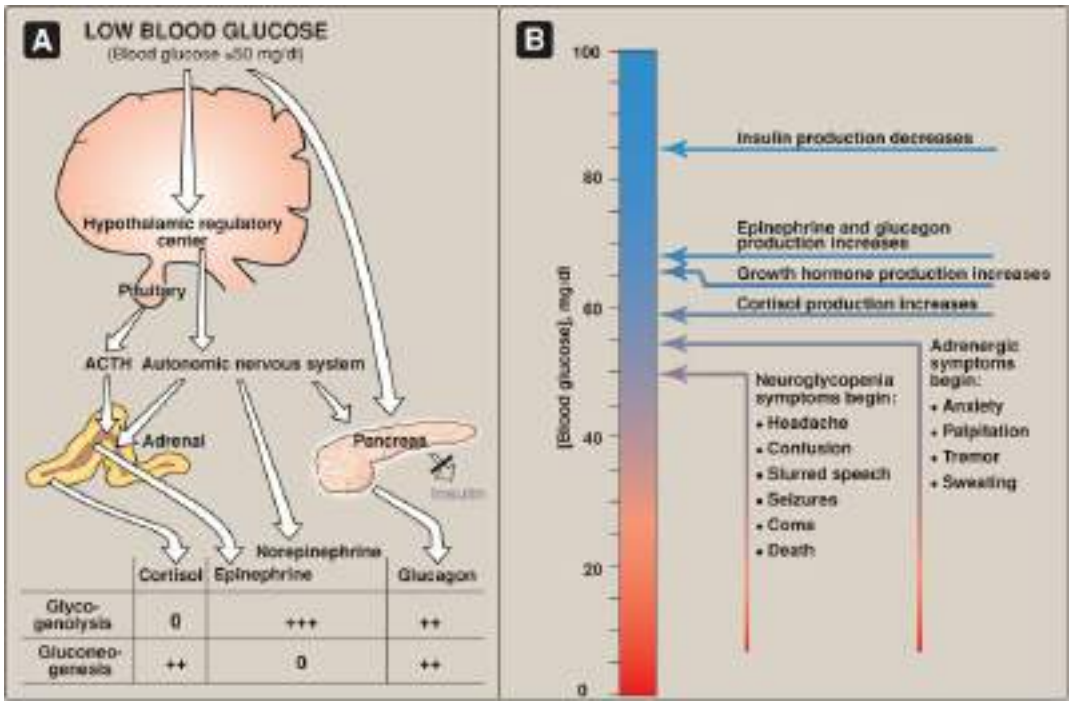


Figure 23.13
A: Actions of some of the glucoregulatory hormones in response to low blood glucose. **B:** Glycemic thresholds for the various responses to hypoglycemia. (Note: Normal fasted blood glucose is 70 to 99 mg/dl.) + = weak stimulation; ++ = moderate stimulation; +++ = strong stimulation; 0 = no effect; ACTH = adrenocorticotropic hormone.

1. Glucagon and epinephrine: Secretion of these counterregulatory hormones is most important in the acute, short-term regulation of blood glucose levels. Glucagon stimulates hepatic glycogenolysis and gluconeogenesis. Epinephrine promotes glycogenolysis and lipolysis. It inhibits insulin secretion, thereby preventing GLUT-4–mediated uptake of glucose by muscle and adipose tissues. Epinephrine assumes a critical role in hypoglycemia when glucagon secretion is deficient, for example, in the late stages of type 1 diabetes mellitus (see p. 379). The prevention or correction of hypoglycemia fails when the secretion of both glucagon and epinephrine is deficient.

2. Cortisol and growth hormone: These counterregulatory hormones are less important in the short-term maintenance of blood glucose concentrations. They do, however, play a role in the long-term (transcriptional) management of glucose metabolism.

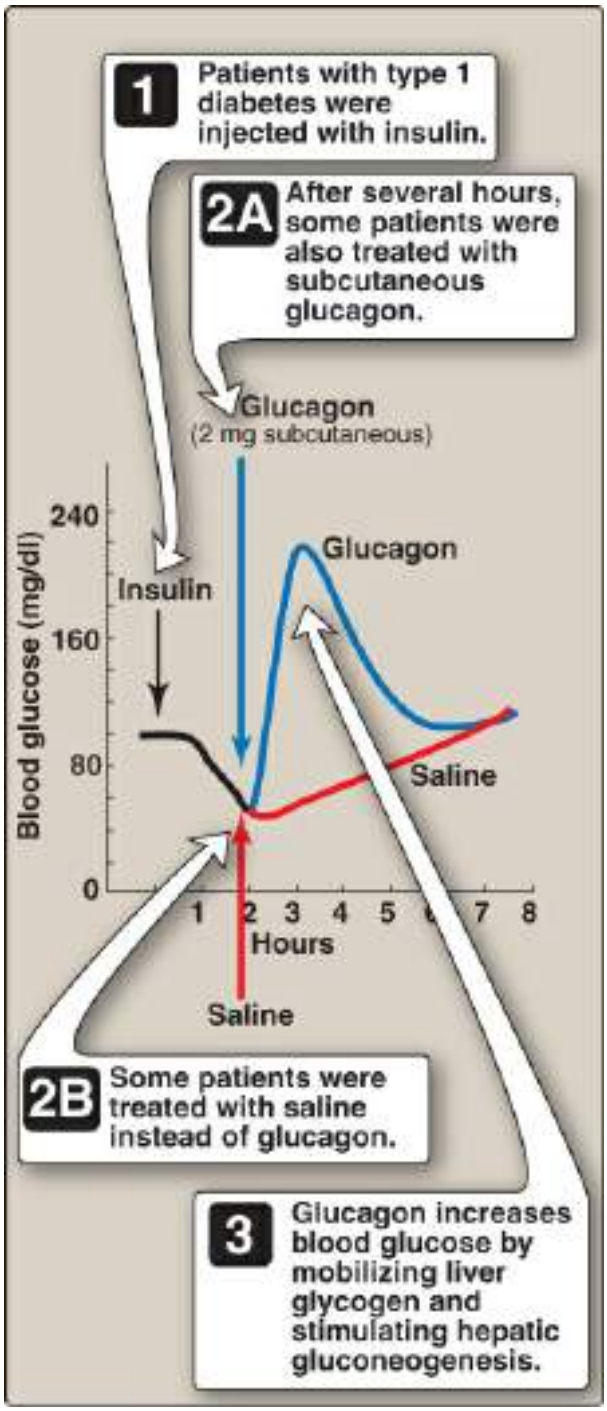


Figure 23.14
Reversal of insulin-induced hypoglycemia by administration of subcutaneous glucagon.

C. Types

Hypoglycemia may be divided into four types: (1) insulin induced, (2) postprandial (sometimes called reactive hypoglycemia), (3) fasting hypoglycemia, and (4) alcohol related.

1. Insulin-induced hypoglycemia: Hypoglycemia occurs frequently in patients with diabetes who are receiving insulin treatment, particularly those striving to achieve tight control of blood glucose levels. Mild hypoglycemia in fully conscious patients is treated by oral administration of carbohydrate. Unconscious patients are typically given glucagon subcutaneously or intramuscularly (Fig. 23.14).

2. Postprandial hypoglycemia: This is the second most common form of hypoglycemia. It is caused by an exaggerated insulin release following a meal, prompting transient hypoglycemia with mild adrenergic symptoms. The plasma glucose level returns to normal even if the patient is not fed. The only treatment usually required is that the patient eats frequent small meals rather than the usual three large meals.

3. Fasting hypoglycemia: Low blood glucose during fasting is rare but is more likely to present as a serious medical problem. Fasting hypoglycemia, which tends to produce neuroglycopenic symptoms, may result from a reduction in the rate of glucose production by hepatic glycogenolysis or gluconeogenesis. Thus, low blood glucose levels are often seen in patients with hepatocellular damage or adrenal insufficiency or in fasting individuals who have consumed large quantities of ethanol (see 4. below). Alternately, fasting hypoglycemia may be the result of an increased rate of glucose use by the peripheral tissues because of overproduction of insulin by rare pancreatic tumors. If left untreated, a patient with fasting hypoglycemia may lose consciousness and experience convulsions and coma. (Note: Certain inborn errors of metabolism, e.g., defects in FA oxidation, result in fasting hypoglycemia.)

4. Alcohol-related hypoglycemia: Alcohol (ethanol) is metabolized in the liver by two oxidation reactions (Fig. 23.15). Ethanol is first converted to acetaldehyde by zinc-containing alcohol dehydrogenase. Acetaldehyde is subsequently oxidized to acetate by aldehyde dehydrogenase (ALDH). (Note: ALDH is inhibited by disulfiram, a drug that is used in the treatment of chronic alcoholism. The resulting rise in acetaldehyde results in flushing, tachycardia, hyperventilation, and nausea.) In each reaction, electrons are transferred to oxidized nicotinamide adenine dinucleotide (NAD^+), resulting in an increase in the ratio of the reduced form (NADH) to NAD^+. The abundance of NADH favors the reduction of pyruvate to lactate and of oxaloacetate (OAA) to malate. Recall from p. 129 that pyruvate and OAA are substrates in the synthesis of glucose. Thus, the ethanol-mediated increase in NADH causes these gluconeogenic precursors to be diverted into alternate pathways, resulting in the decreased synthesis of glucose. This can precipitate hypoglycemia, particularly in individuals who have depleted their stores of liver glycogen. (Note: Decreased availability of OAA allows acetyl CoA to be diverted to ketone body synthesis in the liver [see p. 215] and can result in alcoholic ketosis that may result in ketoacidosis.) Hypoglycemia can produce many of the behaviors associated with alcohol intoxication, such as agitation, impaired judgment, and

combativeness. Therefore, alcohol consumption in vulnerable individuals (such as those who are fasted or have engaged in prolonged, strenuous exercise) can produce hypoglycemia that may contribute to the behavioral effects of alcohol. Because alcohol consumption can also increase the risk for hypoglycemia in patients using insulin, those in an intensive insulin treatment protocol (see p. 379) are counseled about the increased risk of hypoglycemia that generally occurs many hours after alcohol ingestion.

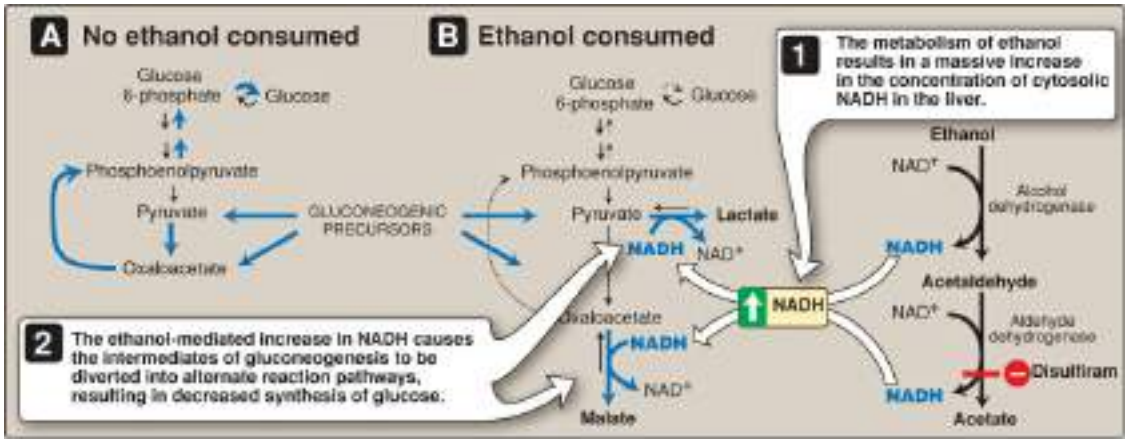


Figure 23.15
A: Normal gluconeogenesis in the absence of ethanol consumption. **B:** Inhibition of gluconeogenesis resulting from hepatic metabolism of ethanol. NAD(H) = nicotinamide adenine dinucleotide.

Chronic alcohol consumption can also result in alcoholic fatty liver because of increased hepatic synthesis of TAG coupled with impaired formation or release of VLDL. This occurs as a result of decreased FA oxidation because of a fall in the NAD^+/NADH ratio and increased lipogenesis because of the increased availability of FA (decreased catabolism) and of glyceraldehyde 3-phosphate (the dehydrogenase is inhibited by the low NAD^+/NADH ratio; see p. 111). With continued alcohol consumption, alcoholic fatty liver can progress first to alcoholic hepatitis and then to alcoholic cirrhosis.

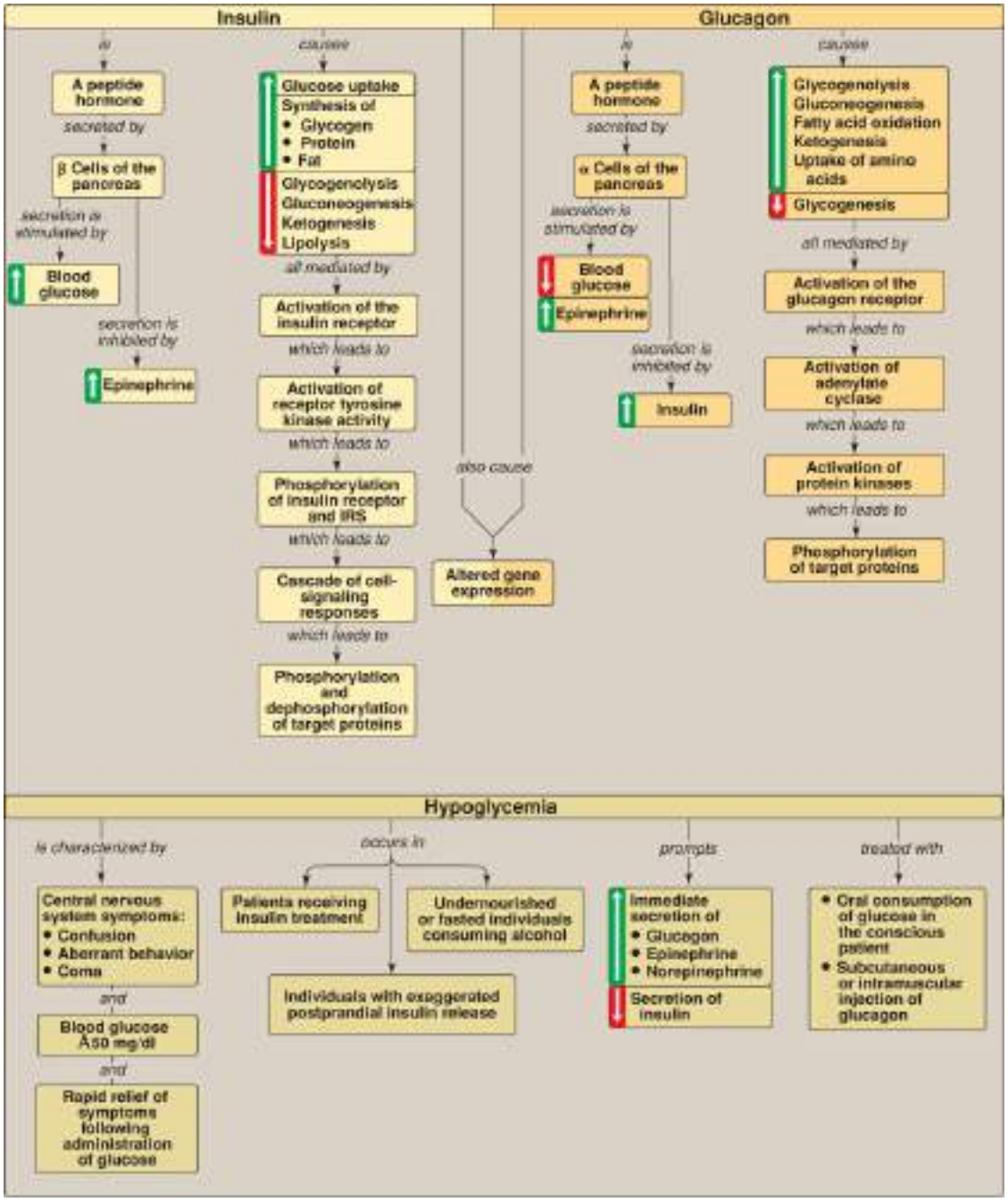


Figure 23.16
Key concept map for the metabolic effects of insulin and glucagon as well as hypoglycemia. IRS = insulin receptor substrates.

V. Chapter Summary

- The integration of **energy metabolism** is controlled primarily by **insulin** and the opposing actions of **glucagon** and the catecholamines, particularly **epinephrine** (Fig. 23.16). Changes in the circulating levels of these hormones allow the body to store energy when food is abundant or to make stored energy available in times of **physiologic stress** (e.g., during survival crises, such as famine).
- **Insulin** is a peptide hormone produced by the β **cells** of the **islets of Langerhans** of the **pancreas**. It consists of disulfide-linked A and B chains. A rise in blood glucose is the most important signal for insulin **secretion**. The **catecholamines**, secreted in response to stress, trauma, or extreme exercise, inhibit insulin secretion.
- Insulin increases glucose uptake (by glucose transporters [**GLUT-4**] in muscle and adipose tissue) and the synthesis of **glycogen**, **protein**, and **TAG**: It is an **anabolic** hormone. These actions are mediated by binding to its membrane **tyrosine kinase receptor**. Binding initiates a cascade of cell-signaling responses, including phosphorylation of a family of proteins called **IRS proteins**.
- **Glucagon** is a monomeric peptide hormone produced by the α **cells** of the pancreatic islets (both insulin and glucagon synthesis involve formation of inactive precursors that are cleaved to form the active hormones). Glucagon, along with epinephrine, norepinephrine, cortisol, and growth hormone (the **counterregulatory hormones**), opposes many of the actions of insulin.
- Glucagon acts to maintain blood glucose during periods of potential hypoglycemia. Glucagon increases **glycogenolysis**, **gluconeogenesis**, **fatty acid oxidation**, **ketogenesis**, and **amino acid uptake**: It is a **catabolic** hormone. Glucagon secretion is stimulated by low blood glucose, amino acids, and the catecholamines. Its secretion is inhibited by elevated blood glucose and by insulin.
- Glucagon binds to high-affinity **GPCRs** on the cell membrane of hepatocytes. Binding results in the activation of **adenylyl cyclase**, which produces the second messenger **cAMP**. Subsequent activation of **cAMP-dependent protein kinase A** results in the **phosphorylation**-mediated activation or inhibition of key regulatory enzymes involved in carbohydrate and lipid metabolism. Both insulin and glucagon affect **gene transcription**.
- **Hypoglycemia** is characterized by low blood glucose accompanied by **adrenergic** and **neuroglycopenic symptoms** that are rapidly resolved by the administration of glucose. Insulin-induced, postprandial, and fasting hypoglycemia result in release of glucagon and epinephrine. The rise in the reduced form of **nicotinamide adenine dinucleotide** (**NADH**) that accompanies **ethanol** metabolism inhibits gluconeogenesis, leading to hypoglycemia in individuals with depleted stores. Alcohol consumption also increases the risk for hypoglycemia in patients using insulin. Chronic alcohol consumption can cause **fatty liver disease**.

Study Questions

Choose the ONE best answer.

23.1 Which of the following statements is true for insulin but not for glucagon?

A. It is a peptide hormone secreted by pancreatic cells.
B. Its actions are mediated by binding to a receptor found on the cell membrane of liver cells.
C. Its effects include alterations in gene expression.
D. Its secretion is decreased by the catecholamines.
E. Its secretion is increased by amino acids.
F. Its synthesis involves a nonfunctional precursor that gets cleaved to yield a functional molecule.

Correct answer = D. Secretion of insulin by pancreatic β cells is inhibited by the catecholamines, whereas glucagon secretion by the α cells is stimulated by them. All of the other statements are true for both insulin and glucagon.

23.2 In which one of the following tissues is glucose transport into the cell insulin dependent?

A. Adipose
B. Brain
C. Liver
D. Red blood cells
E. Pancreas

Correct answer = A. The glucose transporter (GLUT-4) in adipose (and muscle) tissue is dependent on insulin. Insulin results in movement of GLUT-4 from intracellular vesicles to the cell membrane. The other tissues in the list contain GLUT that are independent of insulin because they are always located on the cell membrane.

23.3 A 39-year-old female is brought to the emergency room complaining of weakness and dizziness. She recalls getting up early that morning to do her weekly errands and had skipped breakfast. She drank a cup of coffee for lunch and had nothing to eat during the day. She met with friends at 8 PM and had a few drinks. As the evening progressed, she soon became weak and dizzy and was taken to the hospital. Laboratory tests revealed her blood glucose to be 45 mg/dl (normal = 70 to 99). She was given orange juice and immediately felt better. The biochemical basis of her alcohol-induced hypoglycemia is an increase in:

A. fatty acid oxidation.
B. the ratio of the reduced oxidized forms of nicotinamide adenine dinucleotide.
C. oxaloacetate and pyruvate.
D. use of acetyl coenzyme A in fatty acid synthesis.
E. glycogen synthesis

Correct answer = B. The oxidation of ethanol to acetate by dehydrogenases is accompanied by the reduction of nicotinamide adenine dinucleotide (NAD^+) to NADH. The rise in the NADH/NAD^+ ratio shifts pyruvate to lactate and oxaloacetate (OAA) to malate, decreasing the availability of substrates for gluconeogenesis and resulting in hypoglycemia. The rise in NADH also reduces the NAD^+ needed for fatty acid (FA) oxidation. The decrease in OAA shunts any acetyl coenzyme A produced to ketogenesis. Note that the inhibition of FA degradation results in their reesterification into triacylglycerol that can result in fatty liver. Glycogen would not be synthesized under hypoglycemic conditions.

23.4 A patient is diagnosed with an insulinoma, a rare neuroendocrine tumor, the cells of which are derived primarily from pancreatic β cells. Which of the following would logically be characteristic of an insulinoma?

A. Decreased body weight
B. Decreased connecting peptide in the blood
C. Decreased glucose in the blood
D. Decreased insulin in the blood
E. Decreased GLUT-4 activity

Correct answer = C. Insulinomas are characterized by constant production of insulin (and, therefore, of C-peptide) by the tumor cells. The increase in insulin drives glucose uptake by tissues such as muscle and adipose that have insulin-dependent GLUT-4 glucose transporters, resulting in hypoglycemia. However, the hypoglycemia is insufficient to suppress insulin production and secretion. Insulinomas, then, are characterized by increased blood insulin and decreased blood glucose. Insulin, as an anabolic hormone, results in weight gain.

23.5 In a patient with an even rarer glucagon-secreting tumor derived from the α cells of the pancreas, how would the presentation be expected to differ relative to the patient in Question 23.4?

A glucagon-secreting tumor of the pancreas (glucagonoma) would result in hyperglycemia, not hypoglycemia. The constant production of glucagon would result in constant gluconeogenesis, using amino acids from proteolysis as substrates. This results in loss of body weight.

The Feed–Fast Cycle 24

I. OVERVIEW OF THE ABSORPTIVE STATE

The absorptive (well-fed) state is the 2- to 4-hour period after ingestion of a normal meal. During this interval, transient increases in plasma glucose, amino acids, and triacylglycerols (TAGs) occur, the latter primarily as components of chylomicrons synthesized and secreted by the intestinal mucosal cells (see p. 254). Islet tissue of the pancreas responds to gastrointestinal incretin secretion (see p. 344) and the elevated level of glucose with increased secretion of insulin and decreased secretion of glucagon. The elevated insulin/glucagon ratio and the ready availability of circulating substrates make the absorptive state an anabolic period characterized by increased synthesis and storage of TAG and glycogen to replenish fuel stores as well as increased synthesis of protein. During this absorptive period, virtually all tissues use glucose as a fuel, and the metabolic response of the body is dominated by alterations in the metabolism of liver, adipose tissue, skeletal muscle, and brain. In this chapter, an “organ map” is introduced that traces the movement of metabolites between tissues. The goal is to create an expanded and clinically useful vision of whole-body metabolism.

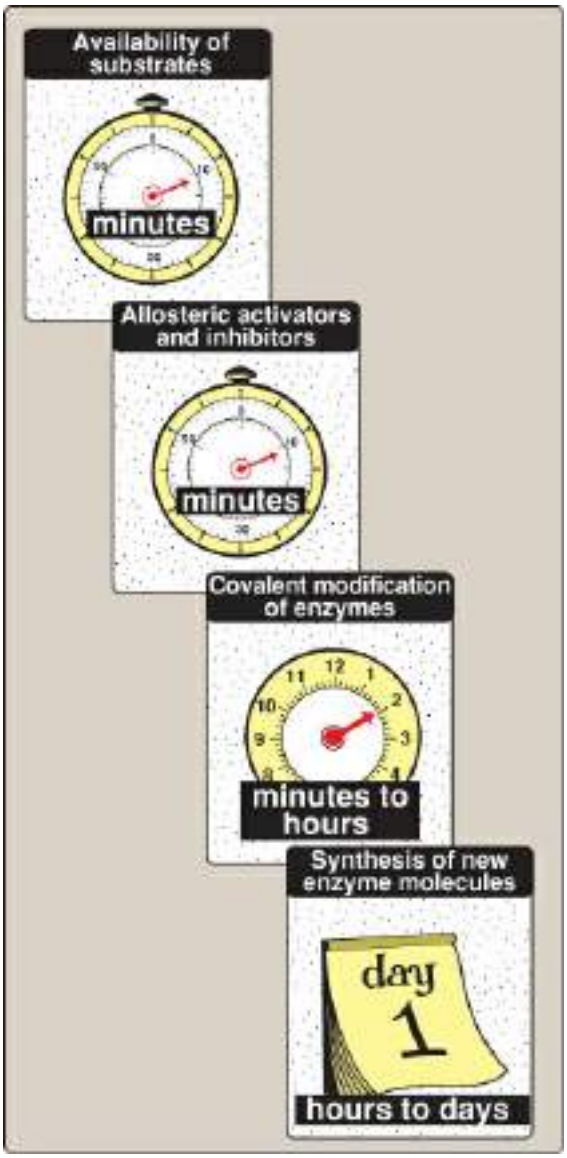


Figure 24.1
Control mechanisms of metabolism and some typical response times. (Note: Response times may vary according to the nature of the stimulus and from tissue to tissue.)

II. REGULATORY MECHANISMS

The flow of intermediates through metabolic pathways is controlled by four mechanisms: (1) the availability of substrates, (2) allosteric regulation of enzymes, (3) covalent modification of enzymes, and (4) induction–repression of enzyme synthesis, primarily through regulation of transcription. Although this scheme may at first seem redundant, each mechanism operates on a different timescale (Fig. 24.1) and allows the body to adapt to a wide variety of physiologic situations. In the absorptive state, these regulatory mechanisms ensure that available nutrients are captured as glycogen, TAG, and protein.

A. Availability of substrates

In the absorptive phase, glucose is the predominant energy substrate for virtually all cell types. The GLUT transporter facilitates glucose uptake, and hexokinase traps glucose in the cell by phosphorylation, according to the relative kinetics (K_m [Michaelis constant]) of each enzyme. With high blood glucose levels of the absorptive phase, the increased substrate concentration guarantees that there is even sufficient glucose for the hepatic isoforms, GLUT-2 and glucokinase (hexokinase IV), which possess higher K_m values (lower affinity) as compared to skeletal muscle isoforms, GLUT-4 and hexokinase I, respectively.

B. Allosteric effectors

Allosteric changes usually involve rate-determining reactions. For example, glycolysis in the liver is stimulated following a meal by an increase in fructose 2,6-bisphosphate, an allosteric activator of phosphofructokinase-1 ([PFK-1], see p. 109). In contrast, gluconeogenesis is decreased by fructose 2,6-bisphosphate, an allosteric inhibitor of fructose 1,6-bisphosphatase (see p. 133).

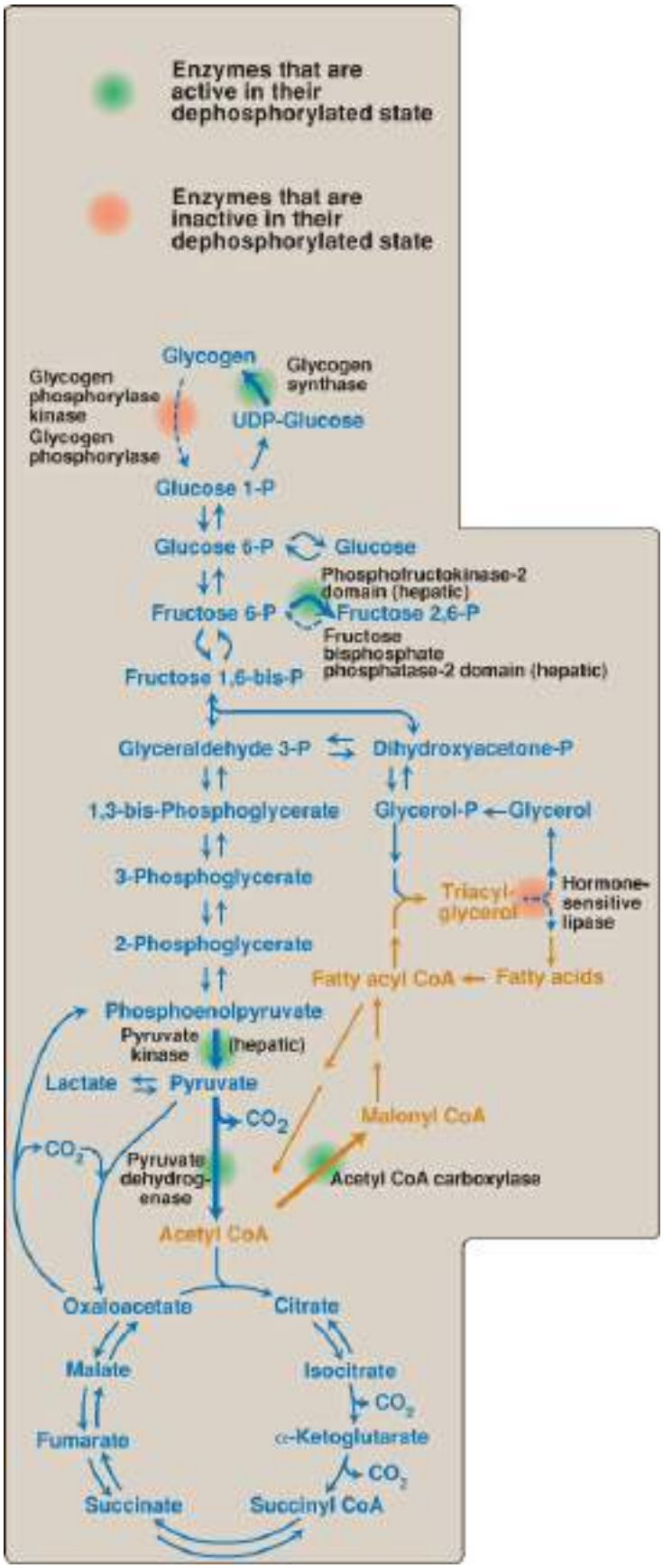


Figure 24.2
Important reactions of intermediary metabolism regulated by enzyme phosphorylation. **Blue text** = intermediates of carbohydrate metabolism; **brown text** = intermediates of lipid metabolism; P = phosphate; CoA = coenzyme A; CO_2 = carbon dioxide.

C. Covalent modification

The activity of many enzymes is regulated by the addition (via kinases, such as cyclic adenosine monophosphate [cAMP]–dependent protein kinase A [PKA] and adenosine monophosphate–activated protein kinase [AMPK]) or removal (via phosphatases) of phosphate groups from specific serine, threonine, or tyrosine residues of the protein. In the absorptive state, most of the covalently regulated enzymes are in the dephosphorylated form and are active (Fig. 24.2). Three exceptions are glycogen phosphorylase kinase (see p. 144), glycogen phosphorylase (see p. 144), and hormone-sensitive lipase (HSL) (see p. 209), which are inactive in their dephosphorylated form. (Note: In the liver, the dephosphorylated form of the bifunctional phosphofructokinase-2 [PFK-2] domain is active, increasing production of the allosteric activator fructose 2,6-bisphosphate [see p. 110]. The other domain, fructose 2,6-bisphosphatase, is also inactive in the dephosphorylated form.)

D. Induction and repression of enzyme synthesis

Increased (induction of) or decreased (repression of) enzyme synthesis leads to changes in the number of enzyme molecules, rather than changing the activity of existing enzyme molecules. Enzymes subject to synthesis regulation are often those that are needed under specific physiologic conditions. For example, in the well-fed state, elevated insulin levels result in an increase in the synthesis of key enzymes, such as acetyl coenzyme A (CoA) carboxylase (ACC) and fatty acid (FA) synthase (see p. 354), involved in anabolic metabolism. In the fasted state, glucagon induces expression of phosphoenolpyruvate carboxykinase (PEPCK) of gluconeogenesis (see p. 370). (Note: Both hormones affect gene transcription.)

III. LIVER: NUTRIENT DISTRIBUTION CENTER

The liver is uniquely situated to process and distribute dietary nutrients because the venous drainage of the gut and pancreas passes through the hepatic portal vein before entry into the general circulation. Thus, after a meal, the liver is bathed in blood containing absorbed nutrients and elevated levels of insulin secreted by the pancreas. During the absorptive period, the liver takes up carbohydrates, lipids, and most amino acids. These nutrients are then metabolized, stored, or routed to other tissues. In this way, the liver smooths out potentially broad fluctuations in the availability of nutrients for the peripheral tissues.

A. Carbohydrate metabolism

The liver is normally a glucose-producing rather than a glucose-using organ. However, after a meal containing carbohydrate, the liver becomes a net consumer, retaining roughly 60 g of every 100 g of glucose presented by the portal system. This increased use reflects increased glucose uptake by the hepatocytes. Their insulin-independent glucose transporter (GLUT-2) has a high K_m for glucose and, therefore, takes up glucose only when blood glucose is high (see p. 108). Processes that are upregulated when hepatic glucose is increased include the following.

1. Increased glucose phosphorylation: The elevated levels of glucose within the hepatocyte (as a result of elevated extracellular levels) allow glucokinase to phosphorylate glucose to glucose 6-phosphate (Fig. 24.3). (Note: Glucokinase has a high K_m for glucose, is not subject to direct product inhibition, and has a sigmoidal reaction curve [see p. 108].)

2. Increased glycogenesis: The conversion of glucose 6-phosphate to glycogen is favored by the activation of glycogen synthase, both by dephosphorylation and by increased availability of glucose 6-phosphate, its positive allosteric effector (Fig. 24.3).

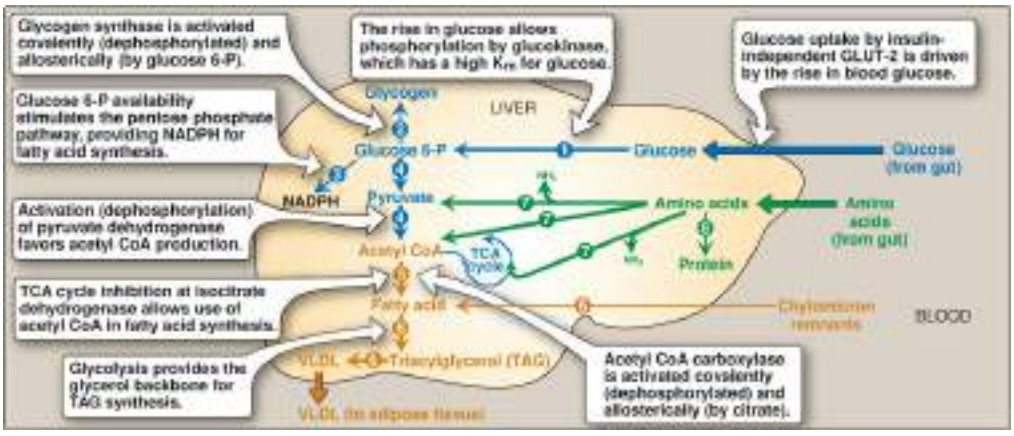


Figure 24.3
Major metabolic pathways in the liver in the absorptive state. (Note: The acetyl coenzyme A [CoA] is also used for cholesterol synthesis.) The *numbers in circles*, which appear both in the figure and in the text, indicate important pathways for carbohydrate, fat, or protein metabolism. Blue text = intermediates of carbohydrate metabolism; brown text = intermediates of lipid metabolism; green text = intermediates of protein metabolism; P = phosphate; TCA = tricarboxylic acid; VLDL = very–low-density lipoprotein; GLUT = glucose transporter; NADPH = nicotinamide adenine dinucleotide phosphate; NH_3 = ammonia.

3. Increased pentose phosphate pathway activity: The increased availability of glucose 6-phosphate, combined with the active use of nicotinamide adenine dinucleotide phosphate (NADPH) in hepatic lipogenesis, stimulates the pentose phosphate pathway (see p. 161). This pathway typically accounts for 5% to 10% of the glucose metabolized by the liver (Fig. 24.3).

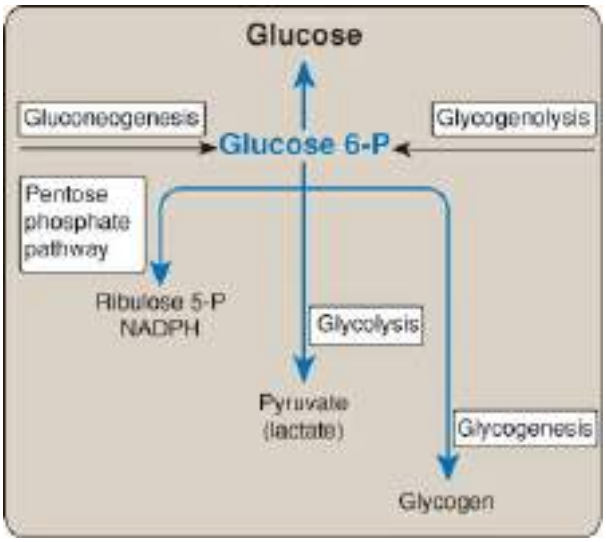


Figure 24.4
Central role of glucose 6-phosphate in metabolism. (Note: The presence of glucose 6-phosphatase in the liver allows the production of free glucose from the glucose 6-phosphate produced in glycogenolysis and gluconeogenesis.) NADPH = nicotinamide adenine dinucleotide phosphate; P = phosphate.

4. Increased glycolysis: In the liver, glycolysis is significant only during the absorptive period following a carbohydrate-rich meal. The conversion of glucose to pyruvate is stimulated by the elevated insulin/glucagon ratio that results in increased amounts of the regulated enzymes of glycolysis: glucokinase, PFK-1, and pyruvate kinase ([PK], see p. 115). Additionally, PFK-1 is allosterically activated by fructose 2,6-bisphosphate generated by the active (dephosphorylated) kinase domain of bifunctional PFK-2. PK is dephosphorylated and active. Pyruvate dehydrogenase (PDH), which converts pyruvate to acetyl CoA, is active (dephosphorylated) because pyruvate inhibits PDH kinase (Fig. 24.3). The acetyl CoA either is used as a substrate for FA synthesis or is oxidized for energy in the tricarboxylic acid (TCA) cycle (Fig. 24.4 for the central role of glucose 6-phosphate.)

5. Decreased glucose production: While glycolysis and glycogenesis (pathways that promote glucose storage) are being stimulated in the liver in the absorptive state, gluconeogenesis and glycogenolysis (pathways that generate glucose) are being inhibited. Pyruvate carboxylase (PC), which catalyzes the first step in gluconeogenesis, is largely inactive because of low levels of acetyl CoA, its allosteric activator (see p. 130). (Note: The acetyl CoA is being used for FA synthesis.) The high insulin/glucagon ratio also favors inactivation of other gluconeogenic enzymes such as fructose 1,6-bisphosphatase by the allosteric inhibitor fructose 2,6-bisphosphate (Fig. 8.17, p. 110). Glycogenolysis is inhibited by dephosphorylation of glycogen phosphorylase and phosphorylase kinase. (Note: The increased uptake and decreased hepatic production of blood glucose in the absorptive period prevents hyperglycemia.)

B. Fat metabolism

1. Increased FA synthesis: Liver is the primary site of *de novo* synthesis of FA (Fig. 24.3). FA synthesis, a cytosolic process, is favored in the absorptive period by availability of the substrates acetyl CoA (from glucose and amino acid metabolism) and NADPH (from glucose metabolism in the pentose phosphate pathway) and by the activation of ACC, both by dephosphorylation and by the presence of its allosteric activator, citrate. (Note: Inactivity of AMPK favors dephosphorylation.) ACC catalyzes the formation of malonyl CoA from acetyl CoA, the rate-limiting reaction for FA synthesis (see p. 203). (Note: Malonyl CoA also inhibits carnitine palmitoyltransferase-I [CPT-I] of FA oxidation [see p. 211]. Thus, citrate directly activates FA synthesis and indirectly inhibits FA degradation.)

    a. Source of cytosolic acetyl CoA: Pyruvate from aerobic glycolysis enters the mitochondria and is decarboxylated by PDH. The acetyl CoA product is combined with oxaloacetate (OAA) to form citrate via citrate synthase of the TCA cycle. When the TCA cycle is very active, ATP levels rise. ATP inhibits isocitrate dehydrogenase, leading to a buildup of citrate. Citrate leaves the mitochondria and enters the cytosol. Citrate is cleaved by ATP citrate lyase (induced by insulin), producing the acetyl CoA substrate of ACC plus OAA.

    b. Additional source of NADPH: The OAA is reduced to malate, which is oxidatively decarboxylated to pyruvate by malic enzyme as NADPH is formed (Fig. 16.11 on p. 207).

2. Increased triacylglycerol synthesis: TAG synthesis is favored because fatty acyl CoAs are available both from *de novo* synthesis and from hydrolysis of the TAG component of chylomicron remnants removed from the blood by hepatocytes (see p. 196). Glycerol 3-phosphate, the backbone for TAG synthesis, is provided by glycolysis (see p. 209). The liver packages these endogenous TAG into very–low-density lipoprotein (VLDL) particles that are secreted into the blood for use by extrahepatic tissues, particularly adipose and muscle tissues (Fig. 24.3).

C. Amino acid metabolism

1. Increased amino acid degradation: In the absorptive period, more amino acids are present than the liver can use in the synthesis of proteins and other nitrogen-containing molecules. The surplus amino acids are not stored but are either released into the blood for other tissues to use in protein synthesis or deaminated, with the resulting carbon skeletons being degraded by the liver to pyruvate, acetyl CoA, or TCA cycle intermediates. These metabolites can be oxidized for energy or used in FA synthesis (Fig. 24.3). The liver has limited capacity to initiate degradation of the branched-chain amino acids (BCAAs)—leucine, isoleucine, and valine. They pass through the liver essentially unchanged and are metabolized in muscle (see p. 295).

2. Increased protein synthesis: The body does not store protein for energy in the same way that it maintains glycogen or TAG reserves. However, a transient increase in the synthesis of hepatic proteins does occur in the absorptive state, resulting in replacement of any proteins that may have been degraded during the previous period of fasting (Fig. 24.3).

IV. ADIPOSE TISSUE: ENERGY STORAGE DEPOT

Adipose tissue is second only to the liver in its ability to distribute fuel molecules. In a 70-kg man, white adipose tissue (WAT) weighs ~14 kg, or about half as much as the total muscle mass. Nearly the entire volume of each adipocyte in WAT can be occupied by a droplet of anhydrous, calorically dense TAG (Fig. 24.5).

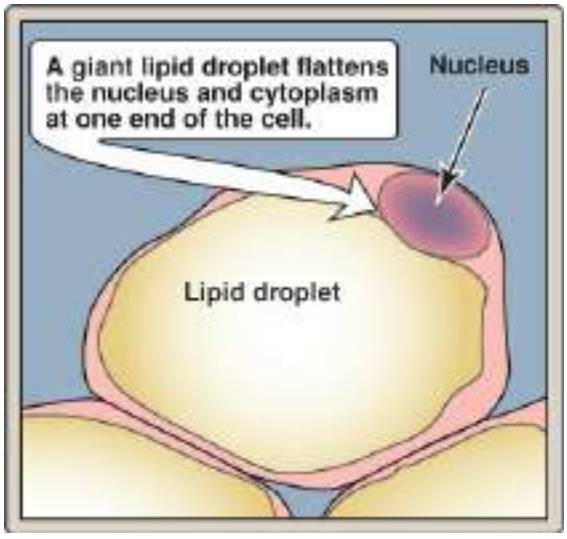


Figure 24.5
Colorized transmission electron micrograph of adipocytes.

A. Carbohydrate metabolism

1. Increased glucose transport: Circulating insulin levels are elevated in the absorptive state, resulting in an influx of glucose into adipocytes via insulin-sensitive GLUT-4 recruited to the cell surface from intracellular vesicles (Fig. 24.6). The glucose is phosphorylated by hexokinase.

2. Increased glycolysis: The increased intracellular availability of glucose results in an enhanced rate of glycolysis (Fig. 24.6). In adipose tissue, glycolysis serves a synthetic function by supplying glycerol 3-phosphate for TAG synthesis (see p. 208). (Note: Adipose tissue lacks glycerol kinase.)

3. Increased pentose phosphate pathway activity: Adipose tissue can metabolize glucose by means of the pentose phosphate pathway, thereby producing NADPH, which is essential for FA synthesis (Fig. 24.6). However, in humans, *de novo* synthesis is not a major source of FA in adipose tissue, except when

refeeding a previously fasted individual (Fig. 24.6).

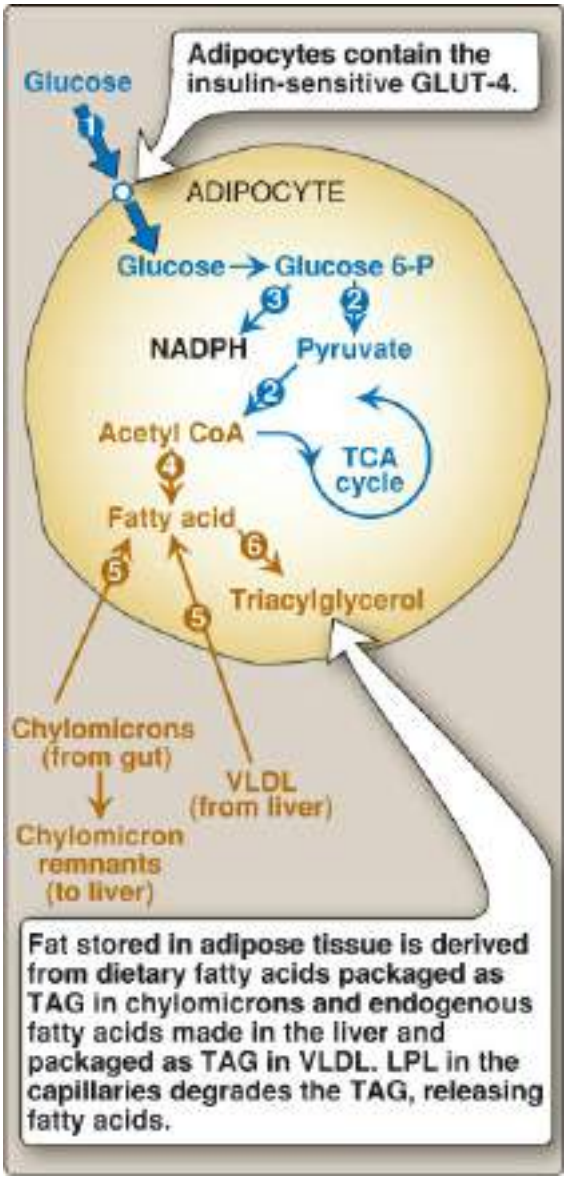


Figure 24.6
Major metabolic pathways in adipose tissue in the absorptive state. (Note: The numbers in the *circles*, which appear both in the figure and in the corresponding text, indicate important pathways for adipose tissue metabolism.) GLUT = glucose transporter; P = phosphate; NADPH = nicotinamide adenine dinucleotide; CoA = coenzyme A; TCA = tricarboxylic acid; TAG = triacylglycerol; VLDL = very–low-density lipoprotein; LPL = lipoprotein lipase.

B. Fat metabolism

Most of the FA added to the TAG stores of adipocytes after consumption of a lipid-containing meal are provided by the degradation of exogenous (dietary) TAG in chylomicrons sent out by the intestine and endogenous TAG in VLDL sent out by the liver (Fig. 24.6). The FA are released from the lipoproteins by lipoprotein lipase

(LPL), an extracellular enzyme attached to the endothelial cells of capillary walls in many tissues, particularly adipose and muscle (see p. 254). In adipose tissue, LPL is upregulated by insulin. Thus, in the fed state, elevated levels of glucose and insulin favor storage of TAG (Fig. 24.6), all the carbons of which are supplied by glucose. (Note: Elevated insulin favors the dephosphorylated [inactive] form of HSL [see p. 209], thereby inhibiting lipolysis of the stored TAG in the well-fed state.)

V. RESTING SKELETAL MUSCLE

Skeletal muscle accounts for ~40% of the body mass in individuals of healthy weight, and it can use glucose, amino acids, FA, and ketone bodies as fuel. In the well-fed state, muscle takes up glucose via GLUT-4 (for energy and glycogen synthesis) and amino acids (for energy and protein synthesis). In contrast to liver, there is no covalent regulation of PFK-2 in skeletal muscle. However, in the cardiac isozyme, the kinase domain is activated by epinephrine-mediated phosphorylation (see p. 110).

Skeletal muscle is unique in being able to respond to substantial changes in the demand for ATP that accompanies contraction. At rest, muscle accounts for ~25% of the oxygen (O_2) consumption of the body, whereas during vigorous exercise, it is responsible for up to 90%. This underscores the fact that skeletal muscle, despite its potential for transient periods of anaerobic glycolysis, is an oxidative tissue.

A. Carbohydrate metabolism

1. Increased glucose transport: The transient increase in plasma glucose and insulin after a carbohydrate-rich meal leads to an increase in glucose transport into muscle cells (myocytes) by GLUT-4 (Fig. 24.7), thereby reducing blood glucose. Glucose is phosphorylated to glucose 6-phosphate by hexokinase and metabolized to meet the energy needs of myocytes.

2. Increased glycogenesis: The increased insulin/glucagon ratio and the availability of glucose 6-phosphate favor glycogen synthesis, particularly if glycogen stores have been depleted as a result of exercise (Fig. 24.7).

B. Fat metabolism

FA are released from chylomicrons and VLDL by the action of LPL (see pp. 254 and 257). However, FA are of secondary importance as a fuel for resting muscle during the well-fed state, in which glucose is the primary source of energy. As a result, skeletal muscle secretes a basal level of LPL in the absorptive phase. (Note: Cardiac muscle will always secrete LPL at a higher basal level compared to skeletal muscle. Cardiac muscle may obtain 50% to 60% of its energy from FA in the absorptive phase. In the fasting state, this can increase to 90%.)

C. Amino acid metabolism

1. Increased protein synthesis: An increase in amino acid uptake and protein synthesis occurs in the absorptive period after ingestion of a meal containing protein (Fig. 24.7). This synthesis replaces protein degraded since the previous meal.

2. Increased branched-chain amino acid uptake: Muscle is the principal site for degradation of the BCAA because it contains the required transaminase (see p. 295). The dietary BCAAs escape metabolism by the liver and are taken up by muscle, where they are used for protein synthesis (Fig. 24.7) and as energy sources.

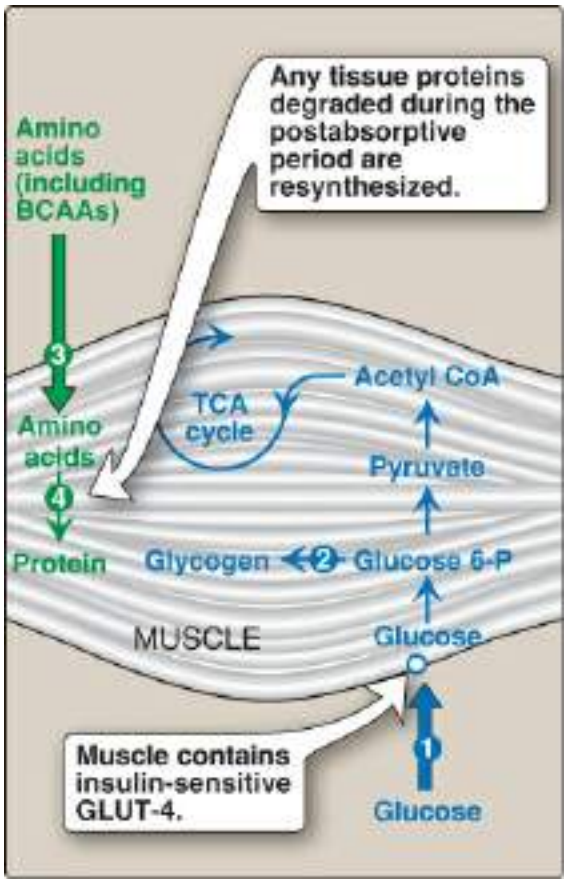


Figure 24.7
Major metabolic pathways in skeletal muscle in the absorptive state. (Note: The *numbers in circles*, which appear both in the figure and in the text, indicate important pathways for carbohydrate or protein metabolism.) CoA = coenzyme A; P = phosphate; GLUT = glucose transporter; BCAAs = branched-chain amino acids; TCA = tricarboxylic acid.

VI. BRAIN

Although contributing only 2% of the adult weight, the brain accounts for a consistent 20% of the basal O_2 consumption of the body at rest. Because the brain is vital to the proper functioning of all organs of the body, special priority is given to its fuel needs. To

provide energy, substrates must be able to cross the endothelial cells that line the blood vessels in the brain (the blood–brain barrier [BBB]). In the fed state, the brain exclusively uses glucose as a fuel (GLUT-1 of the BBB is insulin independent with a low K_m [1 to 2 mM]), completely oxidizing ~140 g/day to carbon dioxide and water. Because the brain contains no significant stores of glycogen, it is completely dependent on the availability of blood glucose (Fig. 24.8). (Note: If blood glucose levels fall to <50 mg/dl [normal fasted blood glucose is 70 to 99 mg/dl], cerebral function is impaired [Fig. 23.13 and p. 350].) The brain also lacks significant stores of TAG and the FA circulating in the blood make little contribution to energy production for reasons that are unclear. The intertissue exchanges characteristic of the absorptive period are summarized in Figure 24.9.

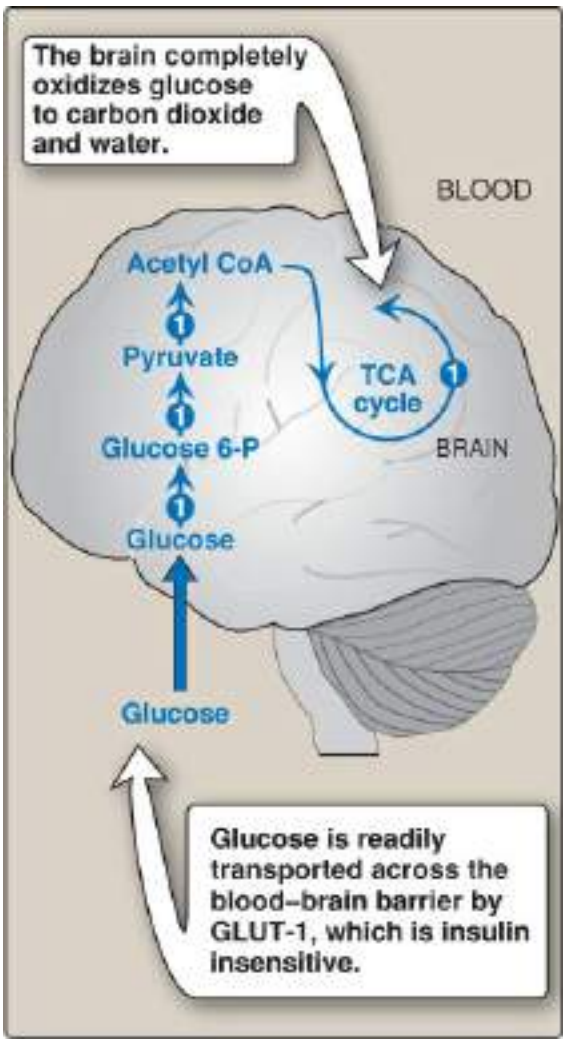


Figure 24.8
Major metabolic pathways in the brain in the absorptive state. (Note: The *numbers in circles*, which appear both in the figure and in the text, indicate important pathways for carbohydrate metabolism.) CoA = coenzyme A; TCA = tricarboxylic acid; P = phosphate; GLUT = glucose transporter.

VII. OVERVIEW OF THE FASTED STATE

Fasting begins if no food is ingested after the absorptive period. It may result from an inability to obtain food, the desire to lose weight rapidly, or clinical situations in which an individual cannot eat (e.g., because of trauma, surgery, cancer, or burns). In the absence of food, plasma levels of glucose, amino acids, and TAG fall, triggering a decline in insulin secretion and an increase in glucagon, epinephrine, and cortisol secretion. The decreased insulin/counterregulatory hormone ratio and the decreased availability of circulating substrates make the postabsorptive period of nutrient deprivation a catabolic period characterized by degradation of TAG, glycogen, and protein. This sets into motion an exchange of substrates among the liver, adipose tissue, skeletal muscle, and brain that is guided by two priorities: (1) the need to maintain adequate plasma levels of glucose to sustain energy metabolism in the brain, red blood cells, and other glucose-requiring tissues and (2) the need to mobilize FA from TAG in WAT for the synthesis and release of ketone bodies by the liver to supply energy to other tissues and spare body protein. As a result, blood glucose levels are maintained within a narrow range in fasting, while FA and ketone body levels increase. (Note: Maintaining glucose requires that the substrates for gluconeogenesis [such as pyruvate, alanine, and glycerol] be available.)

A. Fuel stores

The metabolic fuels available in a normal 70-kg man at the beginning of a fast are shown in Figure 24.10. Observe the enormous caloric stores available in the form of TAG compared with those contained in glycogen. (Note: Although protein is listed as an energy source, each protein also has a function unrelated to energy metabolism [e.g., as a structural component of the body or as an enzyme]. Therefore, only about one-third of the body's protein can be used for energy production without fatally compromising vital functions.)

B. Enzymic changes

In fasting (as in the well-fed state), the flow of intermediates through the pathways of energy metabolism is controlled by four mechanisms: (1) the availability of substrates, (2) allosteric regulation of enzymes, (3) covalent modification of enzymes, and (4) induction–repression of enzyme synthesis. The metabolic changes observed in fasting are generally opposite those described for the absorptive state (Fig. 24.9). For example, although most of the enzymes regulated by covalent modification are dephosphorylated and active in the well-fed state, they are phosphorylated and inactive in the fasted state. Three exceptions are glycogen phosphorylase (see p. 144), glycogen phosphorylase kinase (see p. 144), and HSL (see p. 209), which are active in their phosphorylated states. In fasting, substrates are not provided by the diet but are available from the breakdown of stores and/or tissues, such as glycogenolysis with release of glucose from the liver, lipolysis with release of FA and glycerol from TAG in adipose tissue, and proteolysis with release of amino acids from muscle. Recognition that the changes in fasting are the

reciprocal of those in the fed state is helpful in understanding the ebb and flow of metabolism.

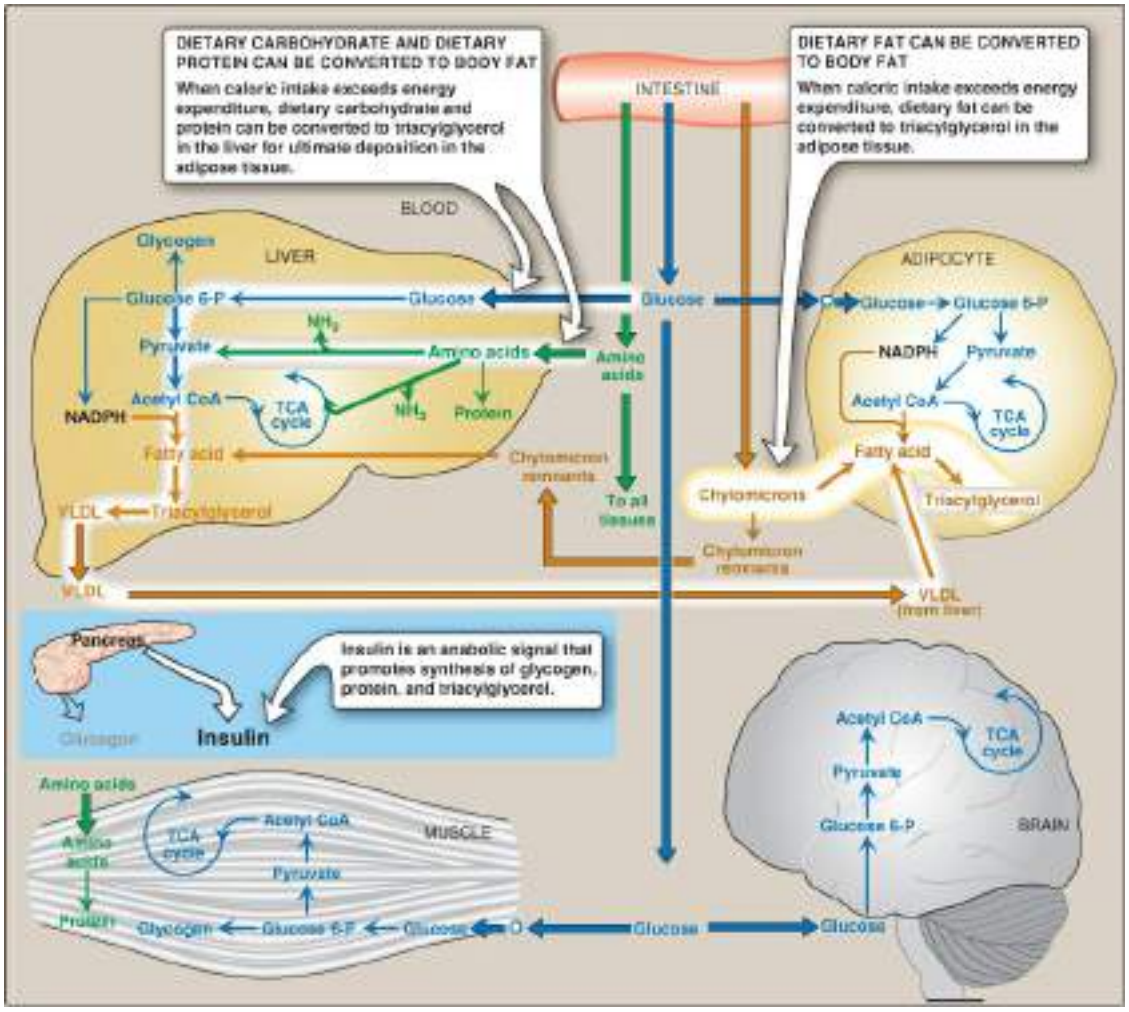


Figure 24.9
Intertissue relationships in the absorptive state and the hormonal signals that promote them. (Note: *Small circles* on the perimeter of muscle and the adipocyte indicate insulin-dependent glucose transporters.) P = phosphate; CoA = coenzyme A; NADPH = nicotinamide adenine dinucleotide phosphate; TCA = tricarboxylic acid; VLDL = very–low-density lipoprotein.

VIII. LIVER IN FASTING

The primary role of the liver in fasting is maintenance of blood glucose through the production of glucose (from glycogenolysis and gluconeogenesis) for glucose-requiring tissues and the synthesis and distribution of ketone bodies for use by other tissues. Therefore, hepatic metabolism is distinguished from peripheral (or extrahepatic) metabolism.

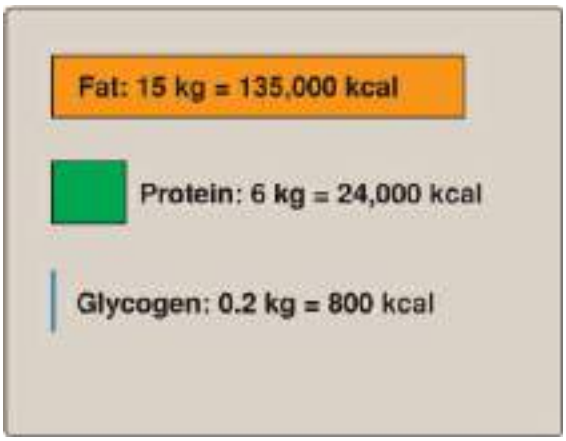


Figure 24.10
Metabolic fuels present in a 70-kg man at the beginning of a fast. The fat stores are sufficient to meet energy needs for ~80 days.

A. Carbohydrate metabolism

The liver first uses glycogen degradation and then gluconeogenesis to maintain blood glucose levels to sustain energy metabolism of the brain and other glucose-requiring tissues in the fasted state. (Note: Recall that the presence of glucose 6-phosphatase in the liver allows the production of free glucose both from glycogenolysis and from gluconeogenesis [Fig. 24.4].)

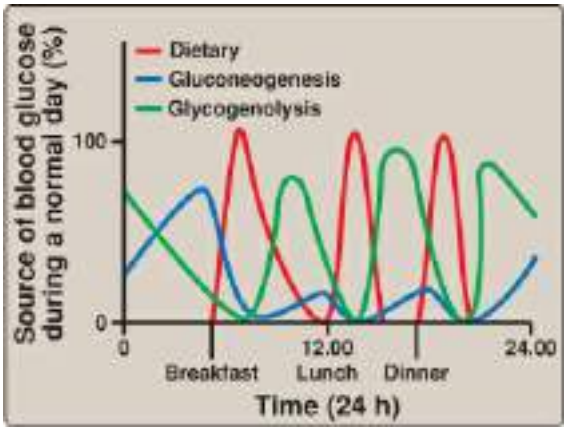


Figure 24.11
Sources of blood glucose during a typical three-meal day.

1. Increased glycogenolysis: Figure 24.11 shows the sources of blood glucose during a typical three-meal day. After breakfast, lunch, and dinner, the primary source for blood glucose levels in the absorptive phase is from the diet, which peaks within the first two hours. During this time, hepatic glycogen stores are replenished (Fig 24.3). As dietary blood glucose levels decline, there is increased secretion of glucagon and decreased secretion of insulin. The increased glucagon/insulin ratio causes a rapid mobilization of hepatic glycogen stores (~80 g of glycogen from the fed state) because of PKA-mediated

phosphorylation (and activation) of glycogen phosphorylase kinase that phosphorylates (and activates) glycogen phosphorylase (see p. 144). Gluconeogenesis contributes only a small percentage to blood glucose levels after breakfast and lunch, since the glycogen stores are sufficient during these shorter interprandial periods. Hepatic glycogen stores are barely sufficient to maintain blood glucose levels during the longer period of time (~12 hours) after dinner and fasting while we sleep. Late in the night or in early morning, as the major fraction of hepatic glycogen becomes depleted, gluconeogenesis becomes the primary source of blood glucose. If an individual continues fasting the following day, gluconeogenesis remains the main source for maintaining blood glucose levels. Figure 24.12 shows glycogen degradation as part of the overall metabolic response of the liver during fasting. (Note: Phosphorylation of glycogen synthase simultaneously inhibits glycogenesis.)

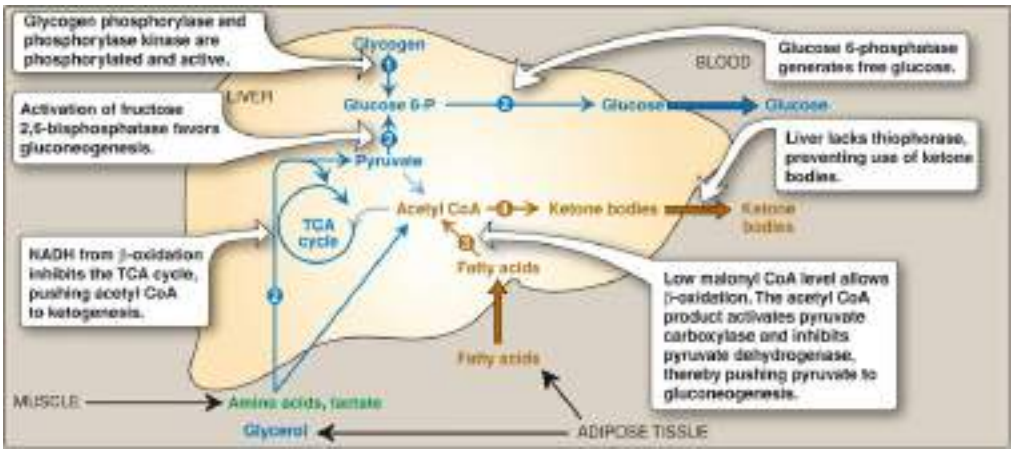


Figure 24.12
Major metabolic pathways in the liver during fasting. (Note: The *numbers in circles*, which appear both in the figure and in the corresponding citation in the text, indicate important metabolic pathways for carbohydrate or fat.) P = phosphate; CoA = coenzyme A; TCA = tricarboxylic acid; NADH = nicotinamide adenine dinucleotide.

2. Increased gluconeogenesis: The synthesis of glucose and its release into the circulation are vital hepatic functions during short- and long-term fasting (Fig. 24.12). The carbon skeletons for gluconeogenesis are derived primarily from glucogenic amino acids and lactate from muscle and glycerol from adipose tissue. Gluconeogenesis, favored by activation of fructose 1,6-bisphosphatase (because of decreased availability of its inhibitor fructose 2,6-bisphosphate; see p. 131) and by induction of PEPCK by glucagon (see p. 132), begins 4 to 6 hours after the last meal and becomes fully active as stores of liver glycogen are depleted (Fig. 24.11). (Note: The decrease in fructose 2,6-bisphosphate simultaneously inhibits glycolysis at PFK-1 [see p. 109s].)

B. Fat metabolism

1. Increased FA oxidation: The oxidation of FA obtained from TAG hydrolysis in

adipose tissue is the major source of energy in hepatic tissue in the fasted state (Fig. 24.12). The fall in malonyl CoA because of phosphorylation (inactivation) of ACC by AMPK removes the brake on CPT-I, allowing β-oxidation to occur (see p. 211). FA oxidation generates NADH, flavin adenine dinucleotide ($FADH_2$), and acetyl CoA. The NADH inhibits the TCA cycle and shifts OAA to malate. This results in acetyl CoA being available for ketogenesis. The acetyl CoA is also an allosteric activator of PC and an allosteric inhibitor of PDH, thereby favoring use of pyruvate in gluconeogenesis (Fig. 10.9, p. 134). (Note: Acetyl CoA cannot be used as a substrate for gluconeogenesis, in part because the PDH reaction is irreversible.) Oxidation of NADH and $FADH_2$ coupled with oxidative phosphorylation supplies the energy required by the PC and PEPCK reactions of gluconeogenesis.

2. Increased ketogenesis: The liver is unique in being able to synthesize and release ketone bodies, primarily 3-hydroxybutyrate but also acetoacetate, for use as fuel by peripheral tissues but not by the liver itself because liver lacks thiophorase (see p. 217). Ketogenesis, which starts during the first days of fasting (Fig. 24.13), is favored when the concentration of acetyl CoA from FA oxidation exceeds the oxidative capacity of the TCA cycle. (Note: Ketogenesis releases CoA, ensuring its availability for continued FA oxidation.) The availability of circulating water-soluble ketone bodies is important in fasting because they can be used for fuel by most tissues, including the brain, once their blood level is high enough. Ketone body concentration in blood increases from ~50 μM to ~6 mM in fasting. This reduces the need for gluconeogenesis from amino acid carbon skeletons, thus preserving essential protein (Fig. 24.11). Ketogenesis as part of the overall hepatic response to fasting is shown in Figure 24.12. (Note: Ketone bodies are organic acids and, when present at high concentrations, can cause ketoacidosis.)

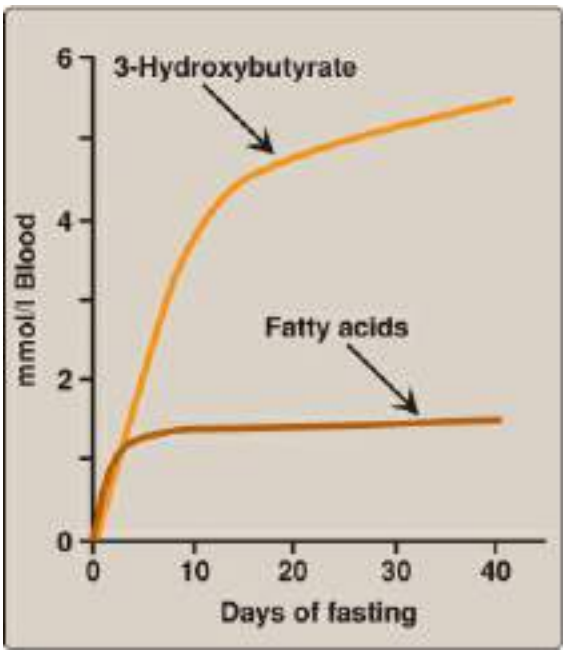


Figure 24.13
Concentrations of fatty acids and 3-hydroxybutyrate in the blood during fasting. (Note: 3-Hydroxybutyrate is made from the NADH-requiring reduction of acetoacetate.)

IX. ADIPOSE TISSUE IN FASTING

A. Carbohydrate metabolism

Glucose transport by insulin-sensitive GLUT-4 into the adipocyte and its subsequent metabolism are decreased because of low levels of circulating insulin. This results in decreased TAG synthesis.

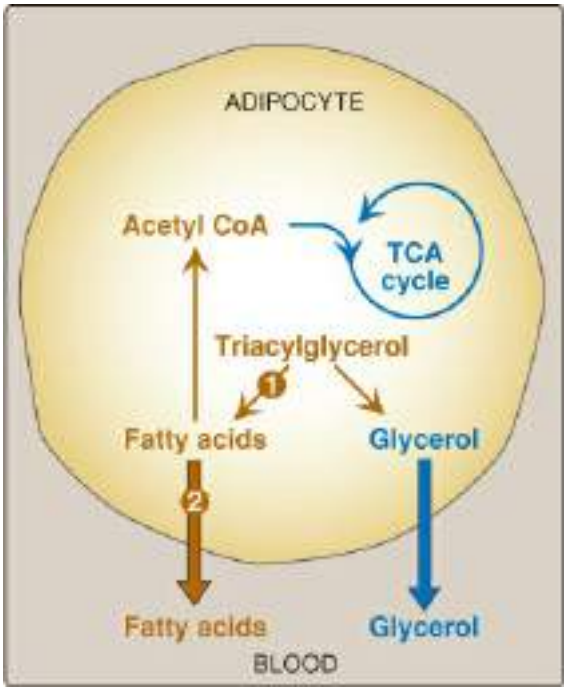


Figure 24.14
Major metabolic pathways in adipose tissue during fasting. (Note: The *numbers in the circles*, which appear both in the figure and in the corresponding citation in the text, indicate important pathways for fat metabolism.) CoA = coenzyme A; TCA = tricarboxylic acid.

B. Fat metabolism

1. Increased fat degradation: The PKA-mediated phosphorylation and activation of HSL (see p. 209) and subsequent hydrolysis of stored fat (TAG) are enhanced by the elevated catecholamines norepinephrine and epinephrine. These hormones, which are secreted from the sympathetic nerve endings in adipose tissue and/or from the adrenal medulla, are physiologically important activators of HSL (Fig. 24.14).

2. Increased FA release: FA obtained from hydrolysis of TAG stored in adipocytes are primarily released into the blood (Fig. 24.14). Bound to albumin, they are transported to a variety of tissues for use as fuel. The glycerol produced from TAG degradation is used as a gluconeogenic precursor by the liver, which contains glycerol kinase. (Note: FA can also be oxidized to acetyl CoA, which can enter the TCA cycle, thereby producing energy for the adipocyte.)

> FA can become reesterified to glycerol 3-phosphate in adipocytes from glyceroneogenesis (see p. 210). Thiazolidinedione antihyperglycemic drugs function to increase transcription of PEPCK in adipose tissues, increasing glyceroneogenesis and TAG reesterification. The reduction of plasma FA concentration improves insulin sensitivity, as muscle and other tissue types become more dependent on glucose oxidation for energy.

3. Decreased FA uptake: In fasting, LPL activity of adipose tissue is low. Consequently, FA in circulating TAG of lipoproteins are less available to adipose tissue than to muscle.

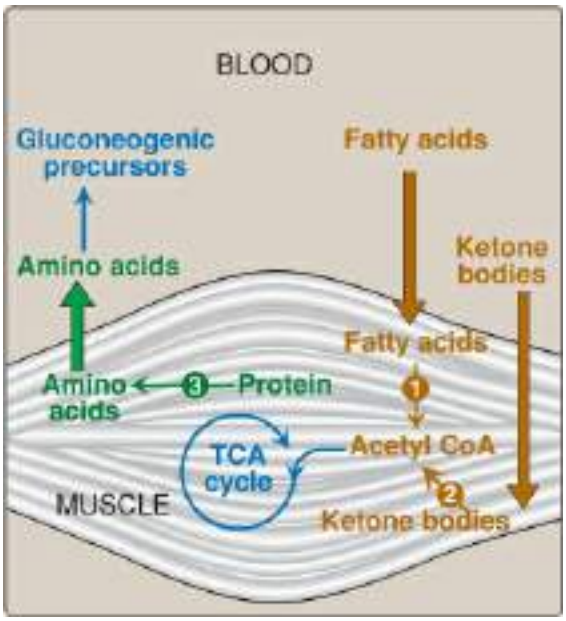


Figure 24.15
Major metabolic pathways in skeletal muscle during fasting. (Note: The *numbers in the circles*, which appear both in the figure and in the corresponding citation in the text, indicate important pathways for fat or protein metabolism.) CoA = coenzyme A; TCA = tricarboxylic acid.

X. RESTING SKELETAL MUSCLE IN FASTING

Resting muscle switches from glucose to FA as its major fuel source in fasting. (Note: By contrast, exercising muscle initially uses creatine phosphate and its glycogen stores. During intense exercise, glucose 6-phosphate from glycogenolysis is converted to lactate by anaerobic glycolysis [see p. 129]. The lactate is used by the liver for gluconeogenesis [Cori cycle; see p. 129]. As these glycogen reserves are depleted, free FA provided by the degradation of TAG in adipose tissue become the dominant energy source. The contraction-based rise in AMP activates AMPK that phosphorylates and inactivates the muscle isozyme of ACC, decreasing malonyl CoA and allowing FA oxidation [see p. 203].) (Note: Since muscle cells do not possess glucose 6-phosphatase, glucose 6-phosphate produced by muscle glycogenolysis in the fasting state cannot be dephosphorylated or contribute to maintaining blood glucose levels.)

A. Carbohydrate metabolism

Glucose transport into skeletal myocytes via insulin-sensitive GLUT-4 (see p. 106) and subsequent glucose metabolism are decreased because circulating insulin levels are low. Therefore, the glucose from hepatic gluconeogenesis is unavailable to muscle and adipose.

B. Lipid metabolism

Early in fasting, muscle uses FA from adipose tissue and ketone bodies from the liver as fuels (Fig. 24.15). In prolonged fasting, muscle decreases its use of ketone bodies (thus sparing them for the brain) and oxidizes FA almost exclusively. Epinephrine signaling increases LPL expression in muscle cells, allowing cells to take up additional FA from VLDL triglycerides in the fasting state. (Note: The acetyl CoA from FA oxidation indirectly inhibits PDH [by activation of PDH kinase]. Pyruvate is transaminated to alanine and used by the liver for gluconeogenesis [glucose–alanine cycle; see Fig. 19.13].)

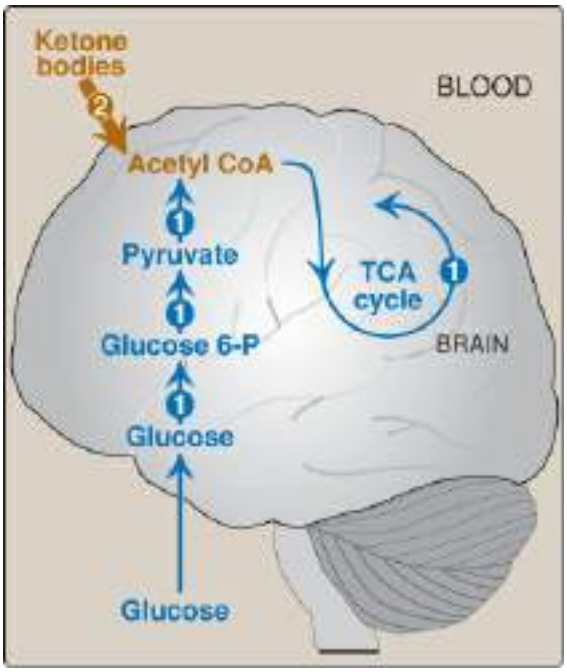


Figure 24.16
Major metabolic pathways in the brain during fasting. (Note: The *numbers in the circles*, which appear both in the figure and in the corresponding citation in the text, indicate important pathways for metabolism of fat or carbohydrates.) CoA = coenzyme A; TCA = tricarboxylic acid; P = phosphate.

C. Protein metabolism

During the first few days of fasting, there is a rapid breakdown of muscle protein (e.g., glycolytic enzymes), providing amino acids that are used by the liver for gluconeogenesis (Fig. 24.15). Because muscle does not have glucagon receptors, muscle proteolysis is initiated by a fall in insulin and sustained by a rise in glucocorticoids. (Note: Alanine and glutamine are quantitatively the most important glucogenic amino acids released from muscle. They are produced by the catabolism of BCAA [Fig. 19.13]. The glutamine is used as a fuel by enterocytes, for example, which send out alanine that is used in hepatic gluconeogenesis [glucose–alanine cycle]). In the second week of fasting, the rate of muscle proteolysis decreases, paralleling a decline in the need for glucose as a fuel for the brain, which has begun

using ketone bodies as a source of energy.

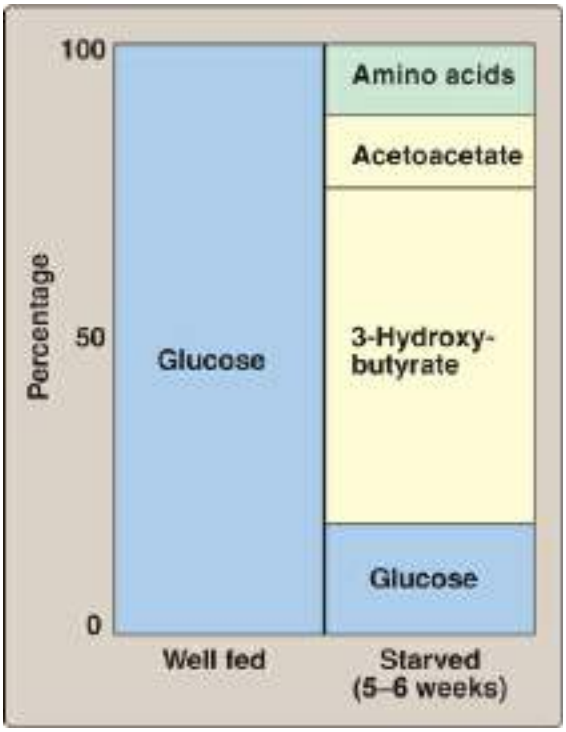


Figure 24.17
Fuel sources used by the brain to meet energy needs in the well-fed and starved states.

XI. BRAIN IN FASTING

During the early days of fasting, the brain continues to use only glucose as a fuel (Fig. 24.16). Blood glucose is maintained by hepatic gluconeogenesis from glucogenic precursors, such as amino acids from proteolysis and glycerol from lipolysis. In prolonged fasting (beyond 2 to 3 weeks), plasma ketone bodies (Fig. 24.12) reach significantly elevated levels and replace glucose as the primary fuel for the brain (see Figs. 24.16 and 24.17). This reduces the need for protein catabolism for gluconeogenesis: Ketone bodies spare glucose and, thus, muscle protein. (Note: As the duration of a fast extends from overnight to days to weeks, blood glucose levels initially drop and then are maintained at the lower level [65 to 70 mg/dl].) The metabolic changes that occur during fasting ensure that all tissues have an adequate supply of fuel molecules. The response of the major tissues involved in energy metabolism during fasting is summarized in Figure 24.18.

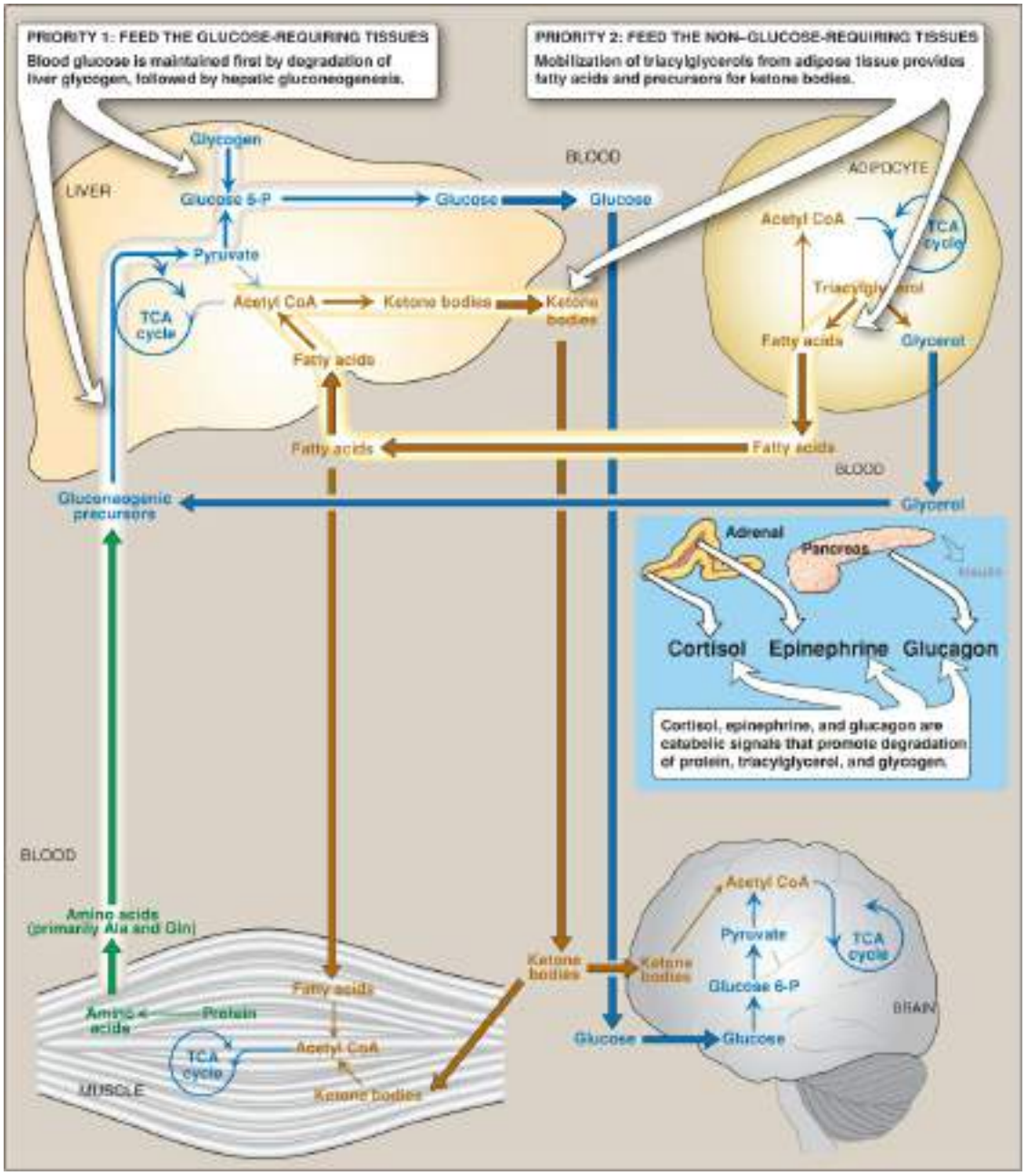


Figure 24.18
Intertissue relationships during fasting and the hormonal signals that promote them. P = phosphate; TCA = tricarboxylic acid; CoA = coenzyme A; Ala = alanine; Gln = glutamine.

XII. KIDNEY IN LONG-TERM FASTING

As fasting continues into early starvation and beyond, the kidney plays important roles. The renal cortex expresses the enzymes of gluconeogenesis, including glucose 6-phosphatase, and, in late fasting, ~50% of gluconeogenesis occurs here. (Note: A portion of this glucose is used by the kidney itself.) The kidney also provides compensation for the acidosis that accompanies the increased production of ketone

bodies (organic acids). The glutamine released from the muscle's metabolism of BCAA is taken up by the kidney and acted upon by renal glutaminase and glutamate dehydrogenase (see p. 315), producing α-ketoglutarate, which can be used as a substrate for gluconeogenesis, plus ammonia (NH_3). The NH_3 picks up protons from ketone body dissociation and is excreted in the urine as ammonium (NH_4^+), thereby decreasing the acid load in the body (Fig. 24.19). Therefore, in long-term fasting, there is a switch from nitrogen disposal in the form of urea to disposal in the form of NH_4^+. (Note: As ketone body concentration rises, enterocytes, typically consumers of glutamine, become consumers of ketone bodies. This allows more glutamine to be available to the kidney.)

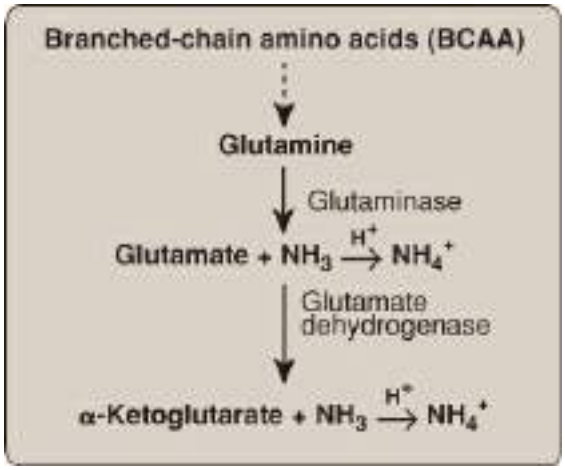


Figure 24.19
Use of glutamine from BCAA catabolism in muscle to generate ammonia (NH_3) used for the excretion of protons (H^+) as ammonium (NH_4^+) in the kidneys.

XIII. Chapter Summary

- The flow of intermediates through metabolic pathways is controlled by **four regulatory mechanisms**: (1) the availability of substrates, (2) allosteric activation and inhibition of enzymes, (3) covalent modification of enzymes, and (4) induction–repression of enzyme synthesis.
- In the **absorptive state**, the 2- to 4-hour period after ingestion of a meal, these mechanisms ensure that available nutrients are captured as **glycogen**, **TAG**, and **protein** (Fig. 24.20). During this interval, transient increases in plasma glucose, amino acids, and TAG occur, the last primarily as components of **chylomicrons** synthesized by the intestinal mucosal cells.
- The **pancreas** responds to the elevated levels of glucose with an increased secretion of insulin and a decreased secretion of glucagon. The elevated **insulin/glucagon ratio** and the ready availability of circulating substrates make the absorptive state an **anabolic period** during which virtually all tissues use **glucose** as a fuel.
- In the absorptive phase, the **liver** replenishes its glycogen stores, replaces any needed hepatic proteins, and increases TAG synthesis. The latter are packaged in **VLDLs**, which are exported to the peripheral tissues.
- In the absorptive phase, the **adipose tissue** increases TAG synthesis and storage, whereas **muscle** increases protein synthesis to replace protein degraded since the previous meal. The **brain** uses glucose exclusively as a fuel.
- In **fasting**, plasma levels of glucose, amino acids, and TAG fall, triggering a decline in insulin secretion and an increase in glucagon and **epinephrine** secretion. The decreased **insulin/counterregulatory hormone ratio** and the decreased availability of circulating substrates make the fasting state a **catabolic period**.
- Fasting sets into motion an **exchange of substrates** among the liver, adipose tissue, skeletal muscle, and brain that is guided by two priorities: (1) the need to maintain adequate plasma levels of glucose to sustain energy metabolism of the brain and other glucose-requiring tissues and (2) the need to mobilize FA from adipose tissue and release **ketone bodies** from liver to supply energy to other tissues.
- In fasting, the liver degrades glycogen and initiates **gluconeogenesis**, using increased **FA oxidation** to supply the energy and reducing equivalents needed for gluconeogenesis and the acetyl CoA building blocks for **ketogenesis**.
- In fasting, the adipose tissue degrades stored TAG, thus providing FA and **glycerol** to the liver. The muscle can also use FA as fuel as well as ketone bodies supplied by the liver. The liver uses the glycerol for gluconeogenesis.
- In fasting, **muscle protein** is degraded to supply amino acids for the liver to use in gluconeogenesis but decreases as ketone bodies increase. The brain can use both glucose and ketone bodies as fuels.
- From late fasting into starvation, the **kidneys** play important roles by synthesizing glucose and excreting the **protons** from ketone body dissociation as **NH_4^+**.

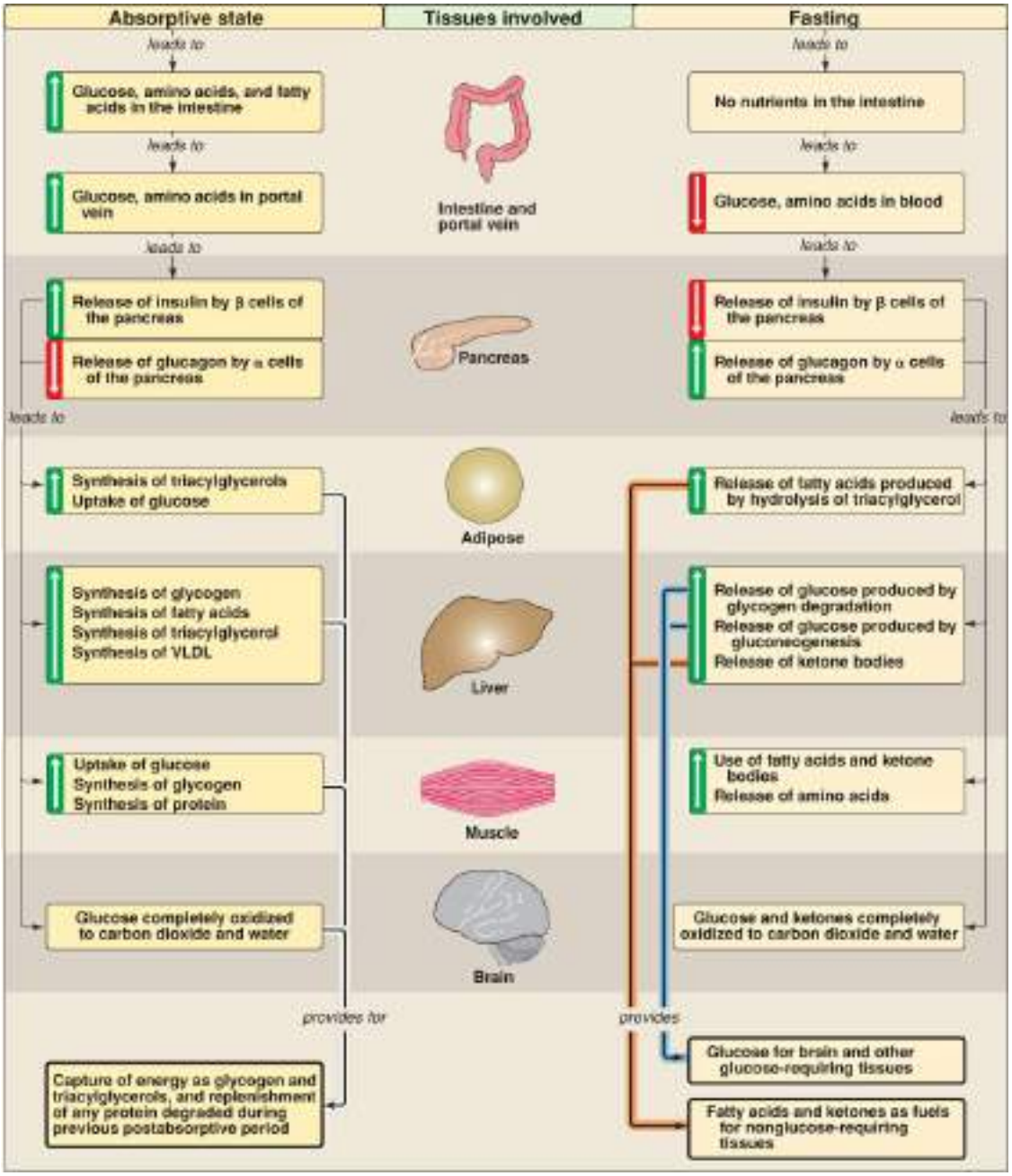


Figure 24.20
Key concept map for the feed–fast cycle. VLDL = very–low-density lipoprotein.

Study Questions

Choose the ONE best answer.

24.1 Which one of the following is elevated in plasma during the absorptive (well-fed) state as compared with the postabsorptive (fasted) state?

A. Acetoacetate
B. Chylomicrons
C. Free fatty acids

D. Glucagon
E. Glycerol

Correct answer = B. Triacylglycerol-rich chylomicrons are synthesized in (and released from) the intestine following ingestion of a meal. Acetoacetate, free fatty acids, glucagon, and glycerol are elevated in the fasted state, not the absorptive state.

24.2 Which one of the following statements concerning liver in the absorptive state is correct?

A. Fructose 2,6-bisphosphate is elevated.
B. Insulin stimulates the uptake of glucose.
C. Most covalently modified enzymes are in the phosphorylated state.
D. The oxidation of acetyl coenzyme A is increased.
E. The synthesis of glucokinase is repressed.

Correct answer = A. The increased insulin and decreased glucagon levels characteristic of the absorptive state promote the synthesis of fructose 2,6-bisphosphate, which allosterically activates phosphofructokinase-1 of glycolysis, while inhibiting fructose 1,6-bisphosphatase of gluconeogenesis. Most covalently modified enzymes are in the dephosphorylated state and are active, with the exception of glycogen phosphorylase kinase, glycogen phosphorylase, and hormone-sensitive lipase. Most of the acetyl coenzyme A is not oxidized in the well-fed state because it is being used in fatty acid synthesis. Uptake of glucose (by glucose transporter-2) into the liver is insulin independent. Synthesis of glucokinase is induced by insulin in the well-fed state.

24.3 Which one of the following enzymes is phosphorylated and active in an individual who has been fasting for 12 hours?

A. Arginase
B. Carnitine palmitoyltransferase-I
C. Fatty acid synthase
D. Glycogen synthase
E. Hormone-sensitive lipase
F. Phosphofructokinase-1
G. Pyruvate dehydrogenase

Correct answer = E. Hormone-sensitive lipase of adipocytes is phosphorylated and activated by protein kinase A in response to epinephrine. Choices A, B, C, and F are not regulated covalently. Choices D and G are regulated covalently but are inactive if phosphorylated.

24.4 For a 70-kg male, in which one of the periods listed below do ketone bodies supply the major portion of the caloric needs of brain?

A. Absorptive period
B. Overnight fast
C. Three-day fast
D. Four-week fast
E. Five-month fast

Correct answer = D. Ketone bodies, made from the acetyl coenzyme A product of fatty acid oxidation, increase in the blood in fasting but must reach a critical level to cross the blood–brain barrier. Typically, this occurs in the second to third week of a fast. Fat stores in a 70-kg (~154-lb) man would not be able to supply his energy needs for 5 months.

24.5 In prolonged starvation, the kidney excretes ammonium (NH_4^+), in addition to urea, to remove excess nitrogen groups. Which one of the following is also a main benefit of this NH_4^+ excretion?

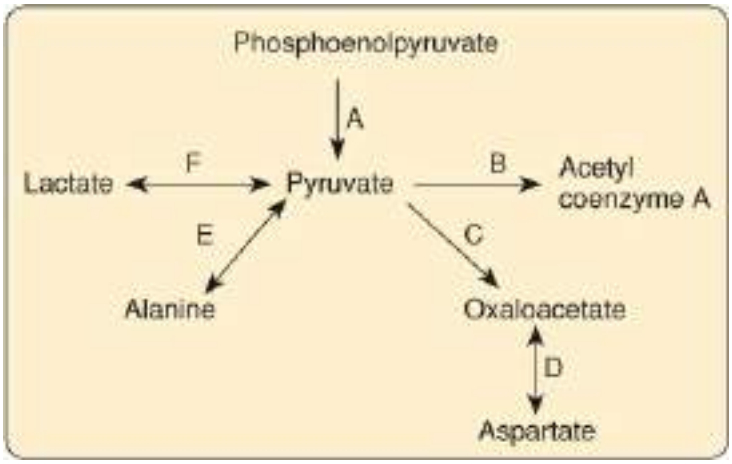
A. Decreases renal glutamine uptake

B. Decreases ketoacidosis
C. Increases enterocyte glutamine consumption
D. Increases muscle alanine transamination
E. Increases renal urea cycle capacity

Correct answer = B. In prolonged starvation, there is less proteolysis of muscle proteins for hepatic gluconeogenesis (via muscle alanine), and a corresponding increase of ketone body production. The kidney provides compensation for the acidosis that accompanies the increased ketone body production and dissociation. Glutamine consumption in intestinal enterocytes decreases and renal uptake of glutamine increases. Glutamine is converted by renal tissue to α-ketoglutarate, which can be used as a substrate for gluconeogenesis, plus ammonia (NH_3). The NH_3 picks up protons from ketone body dissociation and is excreted in the urine as ammonium (NH_4^+), thereby decreasing the acid load in the body (Fig. 24.19). In long-term fasting, there is a decrease in nitrogen disposal by the urea cycle, and an increase in the excretion of NH_4^+.

24.6 The diagram below shows inputs to and outputs from pyruvate, a central molecule in energy metabolism.

Which letter on the diagram represents a reaction that requires biotin and is activated by acetyl coenzyme A?



Correct answer = C. Pyruvate carboxylase, a mitochondrial enzyme of gluconeogenesis, requires biotin (and ATP) and is allosterically activated by acetyl coenzyme A from fatty acid oxidation. None of the other choices meets these criteria. A = pyruvate kinase; B = pyruvate dehydrogenase complex; D = aspartate aminotransferase; E = alanine aminotransferase; F = lactate dehydrogenase.

Diabetes Mellitus 25

I. OVERVIEW

Diabetes mellitus (diabetes) is not one disease but rather is a heterogeneous group of multifactorial, primarily polygenic syndromes characterized by elevated blood glucose caused by a relative or absolute deficiency in the hormone insulin. Over 30 million people in the United States (~9.4% of the population) have diabetes. Of this number, it is estimated that ~8 million are as yet undiagnosed. In addition, more than a third of U.S. adults are considered to have prediabetes, with the majority being unaware of their health status. Diabetes is the leading cause of adult blindness and amputation and a major cause of renal failure, nerve damage, heart attacks, and strokes. Diabetes is the 7th leading cause of death in the United States. Most cases of diabetes mellitus can be separated into two groups (Fig. 25.1): type 1 ([T1D], colloquially known as juvenile or juvenile-onset diabetes) and type 2 ([T2D], colloquially known as adult-onset diabetes). Both types of diabetes can affect adults and children, but the incidence of disease onset at younger versus older ages is reflected in the colloquial terminology. The incidence and prevalence of T2D is also increasing because of the aging of the U.S. population and the increasing prevalence of obesity and sedentary lifestyles (see p. 390). The increase in children with T2D is particularly disturbing. At current rates, the number of individuals with T2D under the age of 20 years could increase 49% by 2050, and if the rates increase, T2D cases in this age group could quadruple.

Characteristics	Type 1 Diabetes	Type 2 Diabetes
AGE OF ONSET	Usually during childhood or puberty; symptoms develop rapidly	Frequently after age 35 years; symptoms develop gradually
NUTRITIONAL STATUS AT TIME OF DISEASE ONSET	Frequently undernourished	Obesity usually present
PREVALENCE	<10% of diagnosed diabetics	>90% of diagnosed diabetics
GENETIC PREDISPOSITION	Moderate	Very strong
DEFECT OR DEFICIENCY	β-Cell destruction, eliminating production of insulin	Insulin resistance combined with inability of β cells to produce appropriate quantities of insulin
FREQUENCY OF KETOSIS	Common	Rare
PLASMA INSULIN	Low to absent	High early in disease; low to absent in disease of long duration
ACUTE COMPLICATIONS	Ketoacidosis	Hyperosmolar hyperglycemic state
RESPONSE TO ORAL HYPOGLYCEMIC DRUGS	Unresponsive	Responsive
TREATMENT	Insulin always necessary	Diet, exercise, oral hypoglycemic drugs, insulin (may or may not be necessary); reduction of risk factors (weight reduction, smoking cessation, blood pressure control, treatment of dyslipidemia) essential to therapy

Figure 25.1

Comparison of type 1 and type 2 diabetes mellitus. (Note: The name of the disease reflects the clinical presentation of copious amounts of glucose-containing urine and is derived from the Greek word for siphon [diabetes] and the Latin word for honey sweet [mellitus].)

II. TYPE 1 DIABETES

T1D constitutes <10% of the ~30 million known cases of diabetes in the United States. The disease is characterized by an absolute deficiency of insulin caused by an autoimmune attack on the islet β cells of the pancreas. In T1D, the islets of Langerhans become infiltrated with activated T lymphocytes, leading to a condition called insulitis. Over a period of years, this autoimmune attack leads to gradual depletion of the β-cell population (Fig. 25.2). However, symptoms appear abruptly only after 80% to 90% of the β cells have been destroyed. At this point, the pancreas fails to respond adequately to ingestion of glucose, and insulin therapy is required to restore metabolic control and prevent life-threatening ketoacidosis. β-cell destruction requires both a stimulus from the environment (such as a viral infection) and a genetic determinant that causes the β cells to be mistakenly identified as “nonself.” (Note: Among monozygotic [identical] twins, if one sibling develops T1D, the other twin has only a 30% to 50% chance of developing the disease. This contrasts with T2D [see p. 380], in which the genetic influence is stronger with eventual development of the disease for virtually all monozygotic twinships.) T1D can vary in incidence by ethnicity. In the United States, T1D is most common among non–Hispanic Caucasian/European American, followed by African American and Hispanic American populations. It is less common among Asian American populations, with Native Americans populations having the lowest incidence rates for T1D.

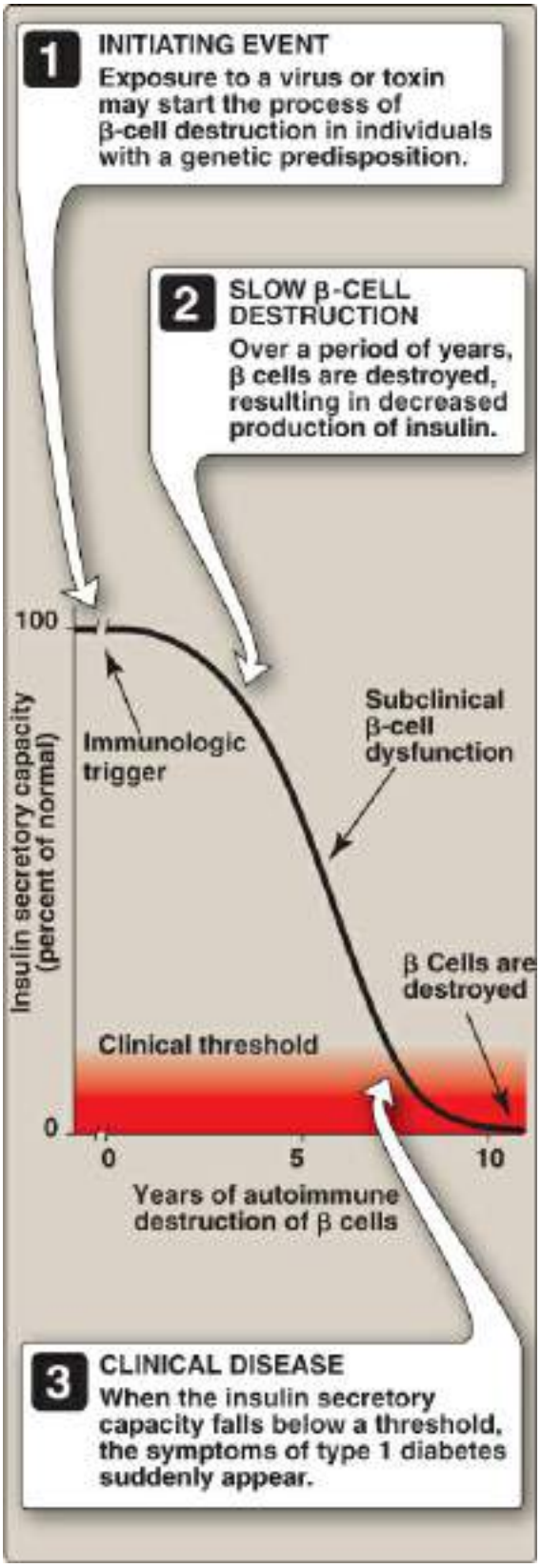


Figure 25.2
Insulin secretory capacity during onset of type 1 diabetes. (Note: Rate of autoimmune destruction of β

cells may be faster or slower than shown.)

A. Diagnosis

The onset of T1D is sudden, occurring typically during childhood or puberty, hence the classification of "juvenile-onset diabetes." Although in recent years with an increase in children diagnosed with T2D, this classification is less meaningful. Individuals with T1D can usually be recognized by the abrupt appearance of polyuria (frequent urination), polydipsia (excessive thirst), and polyphagia (excessive hunger), often triggered by physiologic stress such as an infection. These symptoms are usually accompanied by fatigue and weight loss. A clinical diagnosis of diabetes is confirmed by a fasting blood glucose (FBG) level >125 mg/dl (normal is 70 to 99). (Note: Fasting is defined as no caloric intake for at least 8 hours.) An FBG of 100 to 125 mg/dl is categorized as an impaired FBG, or impaired glucose tolerance. Individuals with impaired FBG have a blood glucose above normal, but not high enough to diagnose as diabetes. Such individuals are considered prediabetic and are at increased risk for developing T2D. Measurement of the percent of glycated hemoglobin (HbA_{1c}, see p. 33) in the blood can both diagnose diabetes and assess overall glycemic control of patients with diabetes. The rate of formation of HbA_{1c} is proportional to the average blood glucose concentration over the previous 2 to 3 months. Normal HbA_{1c} is <5.7%; impaired glucose tolerance or prediabetes range is 5.7% to 6.4%; diabetic diagnosis requires HbA_{1c} levels ≥6.5%. An HbA_{1c} test does not require fasting. Diagnosis can also be made on the basis of a nonfasting (random) blood glucose level >200 mg/dl with consistent clinical symptoms, although a FBG or HbA_{1c} test would likely be ordered to confirm the diagnosis of a nonfasting blood glucose test. The oral glucose tolerance test, in which blood glucose is measured 2 hours after ingestion of a solution containing 75 g of glucose, is also used but is less convenient. It is most typically used to screen pregnant women for gestational diabetes early in the third trimester (see p. 381).

> When blood glucose is >180 mg/dl, the ability of renal sodium–dependent glucose transporters (SGLTs) to reclaim glucose is impaired, and glucose "spills" into urine. The loss of glucose is accompanied by the loss of water, resulting in the characteristic polyuria (with dehydration) and polydipsia of diabetes.

B. Metabolic changes

The metabolic abnormalities of T1D, mainly hyperglycemia, ketonemia, and hypertriacylglycerolemia, result from an absolute deficiency of insulin that profoundly affects metabolism in three tissues: liver, skeletal muscle, and white adipose (Fig. 25.3).

1. Hyperglycemia and ketonemia: Elevated levels of blood glucose and ketone

bodies are the hallmarks of untreated T1D (Fig. 25.3, see Appendix Integrative Case 3, p. 578). Hyperglycemia is caused by increased hepatic production of glucose via gluconeogenesis and glycogenolysis, combined with diminished peripheral utilization of glucose (muscle and adipose tissue have the insulin-regulated glucose transporter GLUT-4; see p. 106). Ketonemia results from increased mobilization of fatty acids (FA) from adipose tissue triacylglycerol (TAG), combined with accelerated hepatic FA β oxidation and synthesis of 3-hydroxybutyrate and acetoacetate (ketone bodies; see p. 216). (Note: Acetyl coenzyme A from β oxidation is the substrate for ketogenesis and the allosteric activator of pyruvate carboxylase, a gluconeogenic enzyme.) Diabetic ketoacidosis (DKA), a type of metabolic acidosis caused by an imbalance between ketone body production and use, occurs in 25% to 40% of those newly diagnosed with T1D and may recur if the patient becomes ill (most commonly with an infection) or does not comply with therapy. DKA is treated by replacing fluid and electrolytes and administering short-acting insulin to gradually correct hyperglycemia without precipitating hypoglycemia.

2. Hypertriacylglycerolemia: Not all of the FA flooding the liver can be disposed of through oxidation and ketone body synthesis. These excess FA are converted to TAG, which are packaged and secreted into the bloodstream in very–low-density lipoproteins ([VLDLs], see p. 255). Chylomicrons rich in dietary TAGs are packaged by intestinal mucosal cells and secreted into the bloodstream following a meal (see p. 253). Because lipoprotein TAG degradation catalyzed by lipoprotein lipase in the capillary beds of adipose tissue (see p. 254) is low under diabetic conditions (synthesis of the enzyme is decreased when insulin levels are low), the blood plasma chylomicron and VLDL levels are elevated, resulting in hypertriacylglycerolemia (see Fig. 25.3).

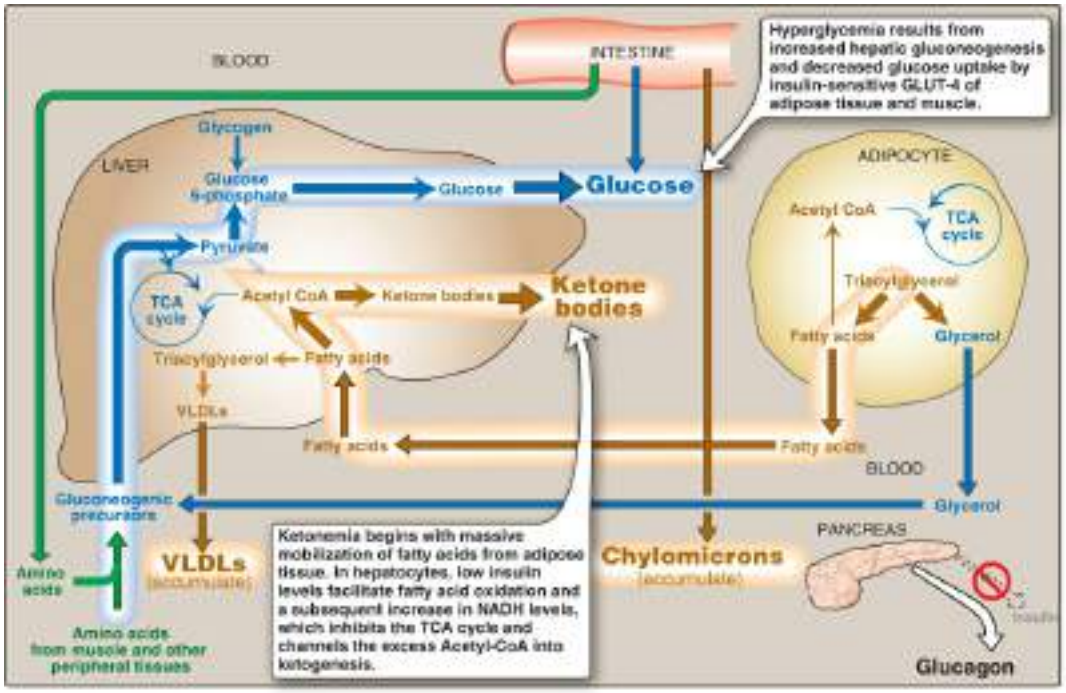


Figure 25.3
Intertissue relationships in type 1 diabetes. TCA = tricarboxylic acid; CoA = coenzyme A; VLDLs = very–low-density lipoproteins; GLUT = glucose transporter.

C. Treatment

Individuals with T1D must rely on exogenous insulin delivered subcutaneously (subq) either by periodic injection or by continuous pump-assisted infusion to control the hyperglycemia and ketonemia. Two types of therapeutic injection regimens are currently used, standard and intensive. (Note: Pump delivery is also considered intensive therapy.)

1. Standard versus intensive treatment: Standard treatment is typically two to three daily injections of recombinant human insulin. Mean blood glucose levels achieved by such treatment are typically 225 to 275 mg/dl, with a HbA_{1c} level of 8% to 9% of the total hemoglobin (blue arrow in Fig. 25.4). In contrast to standard therapy, intensive treatment seeks to more closely normalize blood glucose through more frequent monitoring and subsequent injections of insulin, typically ≥four times a day. Mean blood glucose levels of 150 mg/dl can be achieved, with HbA_{1c} ~7% of the total hemoglobin (see red arrow in Fig. 25.4). (Note: Normal mean blood glucose is ~100 mg/dl, and HbA_{1c} is ≤6% [see black arrow in Fig. 25.4].) Therefore, normalization of glucose values (euglycemia) is not achieved even in intensively treated patients. Nonetheless, patients on intensive therapy show a ≥50% reduction in the long-term microvascular complications of diabetes (i.e., retinopathy, nephropathy, and neuropathy) compared with patients receiving standard care. This confirms that the complications of diabetes are related to an elevation of plasma glucose.

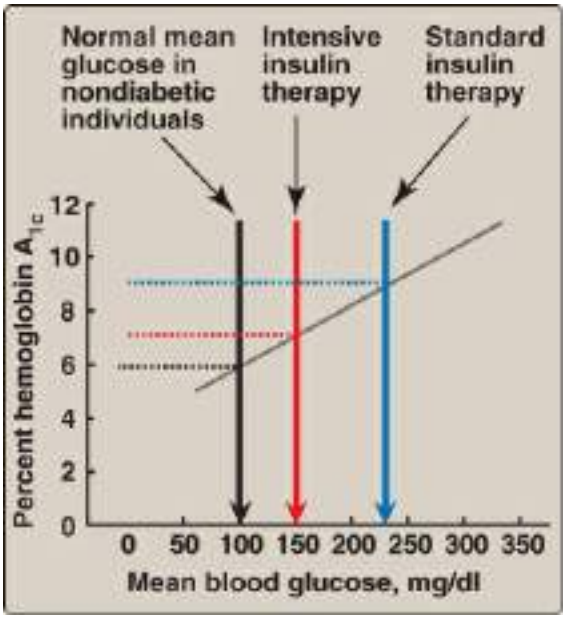


Figure 25.4
Correlation between mean blood glucose and percent hemoglobin A_{1c} in patients with type 1 diabetes receiving intensive or standard insulin therapy. (Note: Nondiabetic individuals are included for comparison.)

2. Complication of insulin therapy Hypoglycemia: One of the therapeutic goals in cases of diabetes is to decrease blood glucose levels in an effort to minimize the development of long-term complications of the disease (see p. 384 for a discussion of the chronic complications of diabetes). However, the appropriate dosage of insulin is difficult to achieve. Hypoglycemia caused by excess insulin is the most common complication of insulin therapy, occurring in >90% of patients. The frequency of hypoglycemic episodes, seizures, and coma is particularly high with intensive treatment regimens designed to achieve tight control of blood glucose (Fig. 25.5). In normal individuals, hypoglycemia triggers a compensatory secretion of counterregulatory hormones, most notably glucagon and epinephrine, which promote hepatic production of glucose (see p. 350). However, patients with T1D also develop a deficiency of glucagon secretion. This defect occurs early in the disease and is almost universally present 4 years after diagnosis. Therefore, these patients rely on epinephrine secretion to prevent severe hypoglycemia. However, as the disease progresses, T1D patients show diabetic autonomic neuropathy and impaired ability to secrete epinephrine in response to hypoglycemia. The combined deficiency of glucagon and epinephrine secretion creates a symptom-free condition sometimes called “hypoglycemia unawareness.” Thus, patients with long-standing T1D are particularly vulnerable to hypoglycemia. Hypoglycemia can also be caused by strenuous exercise. Because exercise promotes glucose uptake into muscle and decreases the need for exogenous insulin, patients are advised to check blood glucose levels before or after intensive exercise to prevent or abort hypoglycemia.

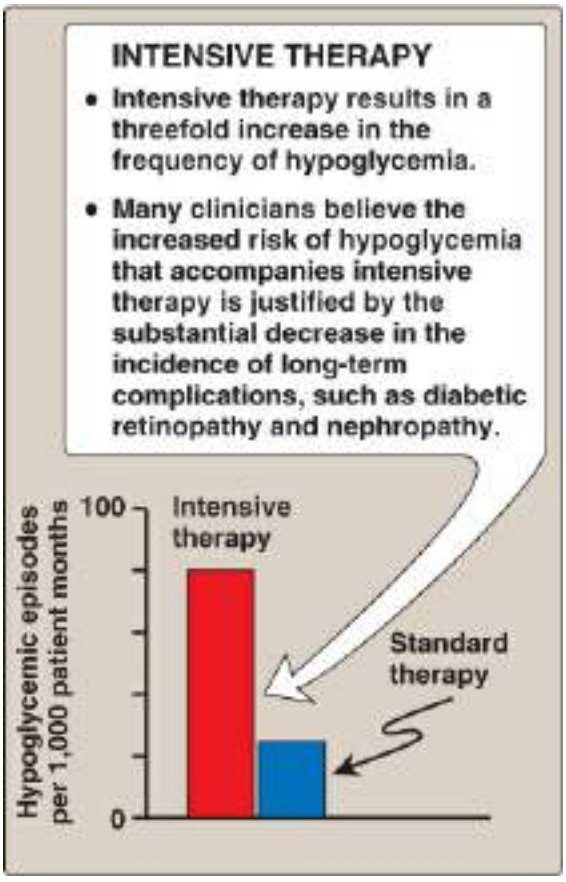


Figure 25.5
Effect of intensive therapy or standard therapy on hypoglycemic episodes in patient populations.

3. Contraindications for intensive therapy (tight control): Children are not put on a program of tight control of blood glucose before age 8 years because of the risk that episodes of hypoglycemia may adversely affect brain development. Elderly people typically do not go on tight control because hypoglycemia can cause strokes and heart attacks in this population. Also, the major goal of tight control is to prevent complications many years later. Tight control, then, is most worthwhile for otherwise healthy people who can expect to live at least 10 more years. (Note: For most nonpregnant adults with diabetes, the individual treatment strategies and goals are based on the duration of diabetes, age/life expectancy, and known comorbid conditions.)

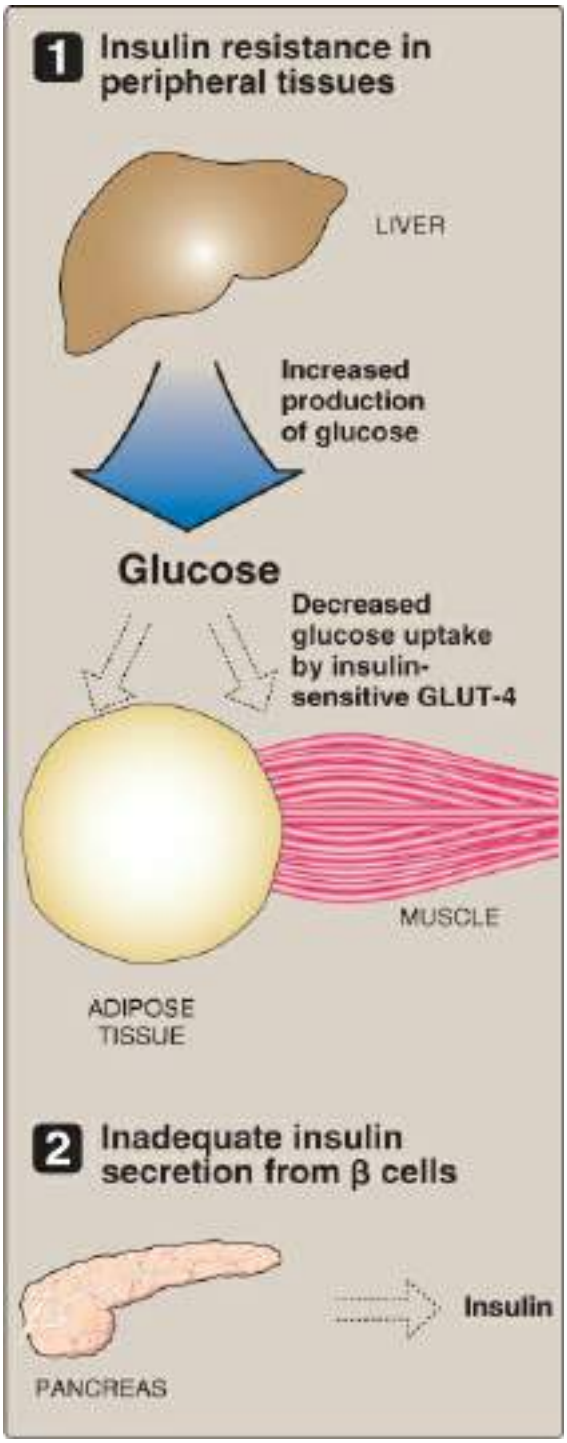


Figure 25.6
Major factors contributing to hyperglycemia observed in type 2 diabetes. GLUT = glucose transporter.

III. TYPE 2 DIABETES

T2D is the most common form of the disease, afflicting >90% of the U.S. population with

diabetes. In the United States, T2D is most common among Hispanic Americans, Native Americans, and African Americans, followed by Asian Americans. Non–Hispanic Caucasian/European American populations have the lowest incidence rates for T2D. Typically, T2D develops gradually without obvious symptoms. The disease is often detected by routine screening tests. However, many individuals with T2D have symptoms of polyuria and polydipsia of several weeks' duration. Polyphagia may be present but is less common. Patients with T2D have a combination of insulin resistance and dysfunctional β cells (Fig. 25.6), but do not require insulin to sustain life. However, in >90% of these patients, insulin will eventually be required to control hyperglycemia and keep HbA_{1c} <7%. The metabolic alterations observed in T2D are milder than those described for type 1, in part because insulin secretion in T2D, although inadequate, does restrain ketogenesis and blunts the development of DKA. (Note: Insulin suppresses the release of glucagon [see p. 348].) Diagnosis is based on the presence of hyperglycemia as described above. The pathogenesis does not involve viruses or autoimmune antibodies and is not completely understood. (Note: An acute complication of T2D in the elderly is a hyperosmolar hyperglycemic state characterized by severe hyperglycemia and dehydration and altered mental status.)

T2D is characterized by hyperglycemia, insulin resistance, impaired insulin secretion, and, ultimately, β-cell failure. The eventual need for insulin therapy has eliminated the designation of T2D as non–insulin-dependent diabetes.

A. Insulin resistance

Insulin resistance is the decreased ability of target tissues, such as the liver, white adipose, and skeletal muscle, to respond properly to normal (or elevated) circulating concentrations of insulin. For example, insulin resistance is characterized by increased hepatic glucose production, decreased glucose uptake by muscle and adipose tissue, and increased adipose lipolysis with production of free fatty acids (FFA).

1. Insulin resistance and obesity: Although obesity is the most common cause of insulin resistance and increases the risk of T2D, most people with obesity and insulin resistance do not develop diabetes. In the absence of a defect in β-cell function, obese individuals can compensate for insulin resistance with elevated levels of insulin. For example, Figure 25.7A shows that insulin secretion is two to three times higher in patients suffering from obesity than it is in individuals with leaner physiques. This higher insulin concentration compensates for the diminished effect of the hormone (as a result of insulin resistance) and produces blood glucose levels similar to those observed in lean individuals (Fig. 25.7B).

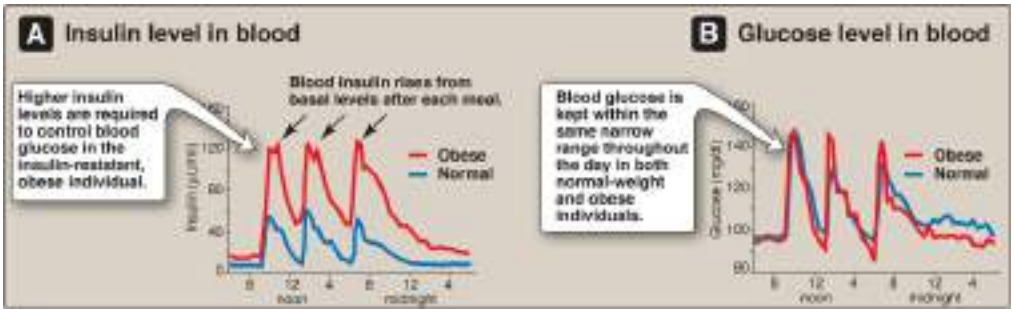


Figure 25.7
Daily blood insulin (A), and blood glucose (B), level changes in normal-weight and obese subjects.

2. Insulin resistance and type 2 diabetes: Insulin resistance alone will not lead to T2D. Rather, T2D develops in insulin-resistant individuals who also show impaired β-cell function. Insulin resistance and subsequent risk for the development of T2D is commonly observed in individuals who suffer from obesity, are physically inactive, are elderly, or in the 3% to 5% of pregnant women who develop gestational diabetes. These patients are unable to sufficiently compensate for insulin resistance with increased insulin release. Figure 25.8 shows the time course for the development of insulin resistance, hyperglycemia, and the loss of β-cell function. Prior to the age when a diagnosis of T2D is possible, an individual with insulin resistance is able to compensate by secreting higher than normal levels of insulin (>100% of normal). As a result, the individual is able to maintain glucose levels close to normal (although they may have glucose levels in the prediabetes range). At some point, the elevated secretion of insulin is no longer sufficient to compensate for the insulin resistance, and there is an increase in the blood glucose concentration above the diagnostic threshold (>125 mg/dl or ≥6.5% HbA_{1c}). After the initial diagnosis, the β-cell defect may result in declining insulin secretion and worsening hyperglycemia. The decreasing insulin secretion may eventually decrease to well below 100% normal.

3. Causes of insulin resistance: Insulin resistance increases with weight gain and decreases with weight loss. Excess adipose tissue (particularly in the abdomen) is key in the development of insulin resistance. Adipose is not simply an energy storage tissue, but also a secretory tissue. With obesity, there are changes in adipose secretions that result in insulin resistance (Fig. 25.9). These include secretion of proinflammatory cytokines such as interleukin 6 and tumor necrosis factor-α by activated macrophages (inflammation is associated with insulin resistance); increased synthesis of leptin, a protein with proinflammatory effects (see p. 394 for additional effects of leptin); and decreased secretion of adiponectin (see p. 391), a protein with anti-inflammatory effects. The net result is chronic, low-grade inflammation. One effect of insulin resistance is increased lipolysis and production of FFA (see Fig. 25.9). FFA availability decreases use of glucose, contributing to hyperglycemia, and increases ectopic deposition of

TAG in liver (hepatic steatosis). (Note: Steatosis results in nonalcoholic fatty liver disease (NAFLD). If accompanied by inflammation, a more serious condition, nonalcoholic steatohepatitis [NASH], can develop.) FFA also have a proinflammatory effect. In the long term, FFA impair insulin signaling. (Note: Adiponectin increases FA β oxidation [see p. 391]. Consequently, a decrease in this adipocyte protein contributes to FFA availability.)

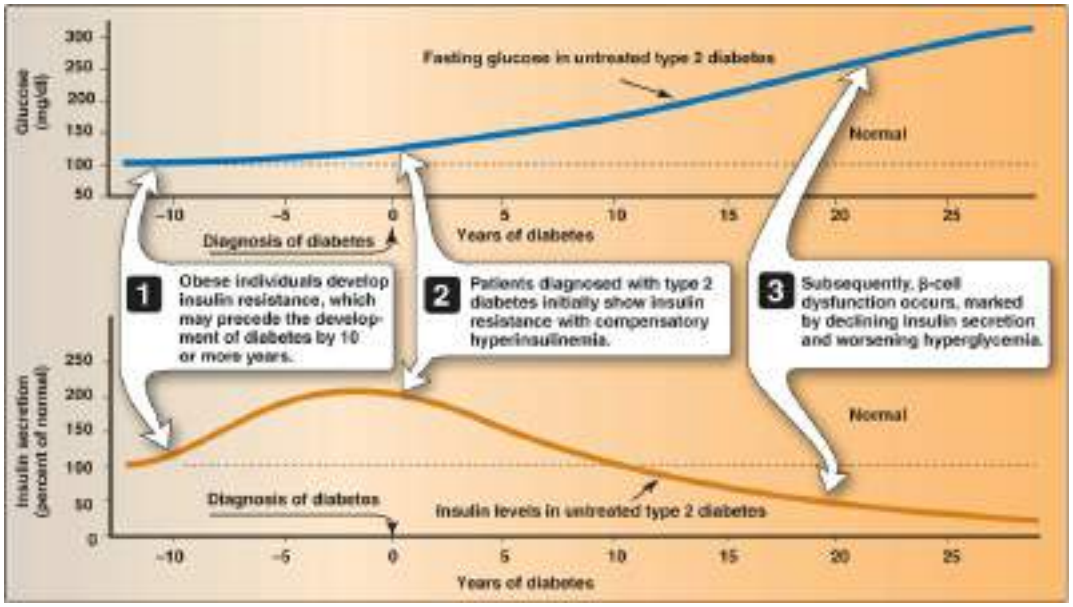


Figure 25.8
Progression of blood glucose and insulin levels in patients with type 2 diabetes.

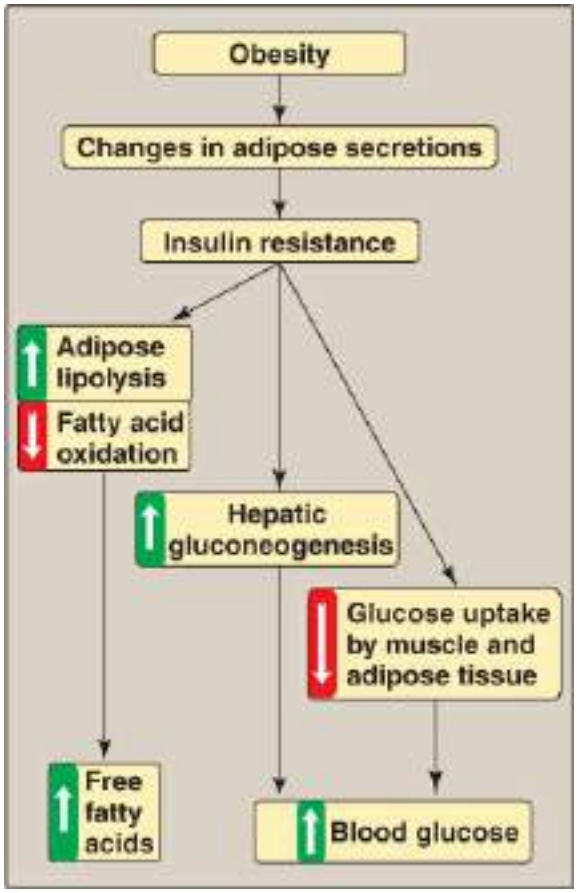


Figure 25.9
Obesity, insulin resistance, and hyperglycemia. (Note: Inflammation also is associated with insulin resistance.)

B. Dysfunctional β cells

In T2D, the pancreas initially retains β-cell capacity, resulting in insulin levels that vary from above normal to below normal. However, with time, the β cell becomes increasingly dysfunctional and fails to secrete enough insulin to correct the prevailing hyperglycemia. For example, insulin levels are high in individuals who suffer from T2D as well as obesity, compared to individuals who similarly suffer from obesity but without T2D. Thus, the natural progression of the disease results in a declining ability to control hyperglycemia with endogenous secretion of insulin (Fig. 25.10). Deterioration of β-cell function may be accelerated by the toxic effects of sustained hyperglycemia and elevated FFA and a proinflammatory environment.

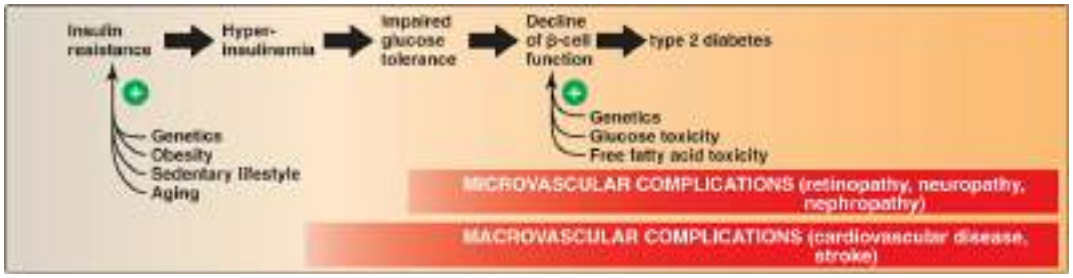


Figure 25.10
Typical progression of type 2 diabetes.

C. Metabolic changes

The abnormalities of glucose and TAG metabolism in T2D are the result of insulin resistance that occurs primarily in liver, skeletal muscle, and white adipose tissue (Fig. 25.11).

1. Hyperglycemia: Hyperglycemia is caused by increased hepatic production of glucose, combined with diminished use of glucose by muscle and adipose tissues (due to insulin resistance). Ketonemia is usually minimal or absent in patients with T2D because the presence of insulin, even in the presence of insulin resistance, restrains any increase in hepatic ketogenesis.

2. Dyslipidemia: In the liver, FFA are converted to TAGs, which are packaged in VLDL and secreted into the bloodstream. Dietary TAG–rich chylomicrons are synthesized and secreted by the intestinal mucosal cells following a meal. Because lipoprotein TAG degradation catalyzed by lipoprotein lipase in adipose tissue is low in diabetes, the plasma chylomicron and VLDL levels are elevated, resulting in hypertriacylglycerolemia (Fig. 25.10). Low levels of high-density lipoproteins are also associated with T2D, likely as a result of increased degradation.

D. Treatment

The goal in treating T2D is to maintain blood glucose concentrations within normal limits and to prevent the development of long-term complications. Weight reduction, exercise, and medical nutrition therapy (dietary modifications) can often correct the hyperglycemia of newly diagnosed T2D. Oral antihyperglycemic agents can be used by T2D patients to reduce blood glucose levels. (Note: Antihyperglycemics are also referred to as hypoglycemic agents, as hypoglycemia can result from their use.) Antihyperglycemic agents include biguanides such as metformin (decreases hepatic gluconeogenesis), sulfonylureas and meglitinides (increase insulin secretion; see p. 344), thiazolidinediones (decrease FFA levels and increase peripheral insulin sensitivity), α-glucosidase inhibitors (decrease absorption of dietary carbohydrate), incretins (decrease glucagon secretion, increase insulin secretion, feeling of satiety), and SGLT inhibitors (decrease renal reabsorption of glucose), or insulin therapy may also be required to achieve satisfactory plasma glucose levels. (Note:

Bariatric surgery in individuals suffering from morbid obesity and T2D has been shown to result in disease remission in most patients. Remission may not be permanent.) Antihyperglycemic agents and their tissue-specific effects on glucose and lipid metabolism is summarized in Figure 25.12.

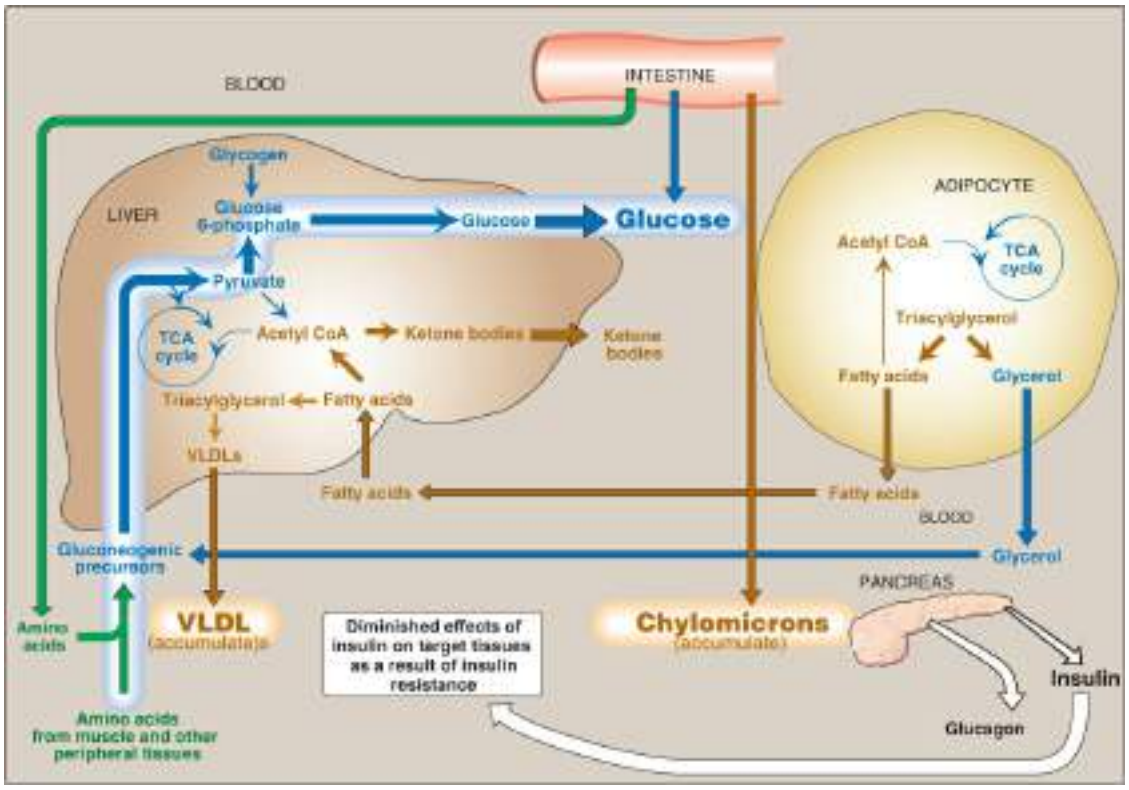


Figure 25.11
Intertissue relationships in type 2 diabetes. (Note: Ketogenesis is restrained as long as insulin action is adequate.) TCA = tricarboxylic acid; CoA = coenzyme A; VLDLs = very–low-density lipoproteins.

IV. CHRONIC EFFECTS AND PREVENTION

As noted previously, available therapies moderate the hyperglycemia of diabetes but fail to completely normalize metabolism. The long-standing elevation of blood glucose is associated with the chronic vascular complications of diabetes including cardiovascular disease (CVD) and stroke (macrovascular complications) as well as retinopathy, nephropathy, and neuropathy (microvascular). Intensive insulin treatment (see p. 379) delays the onset and slows the progression of some long-term complications. For example, the incidence of retinopathy decreases as control of blood glucose improves and HbA_{1c} levels decrease (Fig. 25.13). (Note: Data concerning the effect of tight control on CVD in T2D are less clear.) The benefits of tight control of blood glucose outweigh the increased risk of severe hypoglycemia in most patients. How hyperglycemia causes the chronic complications of diabetes is unclear. In cells in which glucose uptake is not dependent on insulin, elevated blood glucose leads to increased intracellular glucose and its metabolites. For example, increased intracellular sorbitol

contributes to cataract formation (see p. 154) in diabetes. Additionally, hyperglycemia promotes glycation of cellular proteins in a reaction analogous to the formation of HbA_{1c}. These glycated proteins undergo additional reactions and become advanced glycation end products (AGEs) that mediate some of the early microvascular changes of diabetes and can reduce wound healing. Some AGEs bind to a membrane receptor (RAGE), causing the release of proinflammatory molecules. There is currently no preventative treatment for T1D. The risk for T2D can be significantly decreased by a combined regimen of medical nutrition therapy, weight loss, exercise, and aggressive control of hypertension and dyslipidemias. For example, Figure 25.14 shows the incidence of disease in normal and overweight individuals with varying degrees of exercise.

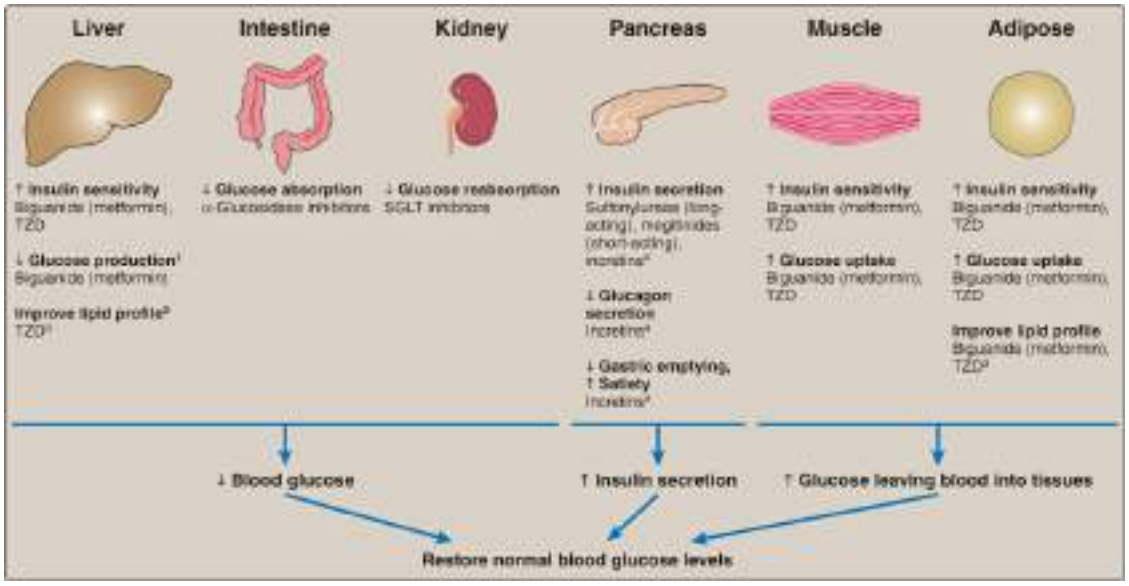


Figure 25.12
Antihyperglycemic agents and their tissue-specific effects on glucose and lipid metabolism in type 2 diabetes. **1.** Decrease hepatic gluconeogenesis and glycogenolysis. **2.** Combination of increasing HDL, decreasing triacylglycerides, and/or decreasing adipocyte lipolysis. **3.** Increase in adiponectin release from adipocytes, increased β-oxidation. **4.** Incretins and DPP4 inhibitors. TZD = Thiazolidinediones. DPP4 = dipeptidyl peptidase 4, SGLT = sodium-glucose cotransporter.

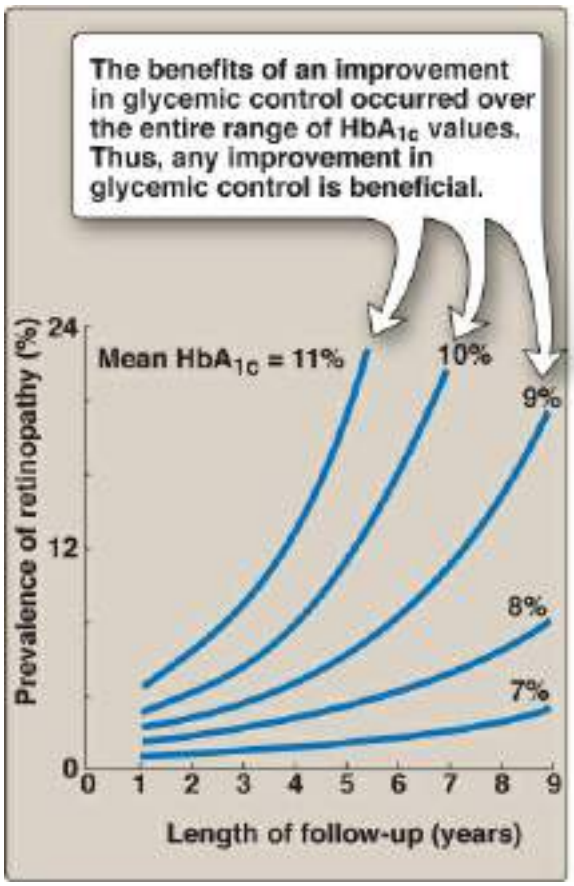


Figure 25.13
Relationship of glycemic control and diabetic retinopathy. HbA_{1c} = glycated hemoglobin.

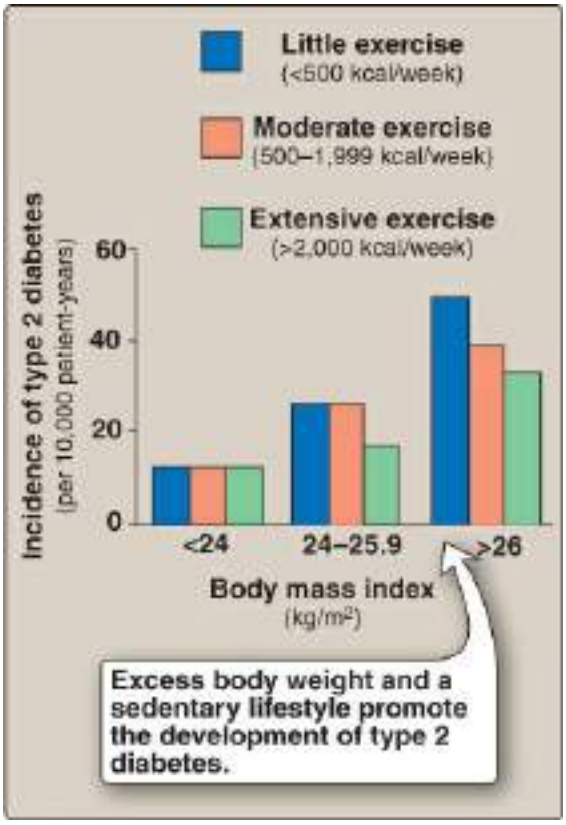


Figure 25.14
Effect of body mass index and exercise on the development of type 2 diabetes.

V. Chapter Summary

- **Diabetes mellitus** is a heterogeneous group of syndromes characterized by an **elevation** of **FBG** that is caused by a relative or absolute deficiency of insulin (Fig. 25.15).
- Diabetes is the leading cause of **adult blindness** and **amputation** and a major cause of **renal failure**, **nerve damage**, **heart attacks**, and **stroke**.
- Diabetes can be classified into two groups, **T1D** and **T2D**.
- T1D constitutes ~10% of >30 million cases of diabetes in the United States. The disease is characterized by an **absolute deficiency** of **insulin** caused by an **autoimmune attack** on the **pancreatic β cells**. This destruction requires an **environmental stimulus** (such as a viral infection) and a **genetic determinant** that causes the β cell to be mistakenly identified as "nonself." The **metabolic abnormalities** of T1D include **hyperglycemia**, **DKA**, and **hypertriacylglycerolemia** that result from a deficiency of insulin. Those with T1D must rely on **exogenous insulin** delivered subcutaneously to control hyperglycemia and ketoacidosis.
- **T2D** has a strong **genetic** component. It results from a combination of **insulin resistance** and **dysfunctional β cells**. Insulin resistance is the decreased ability of target tissues, such as liver, white adipose, and skeletal muscle, to respond properly to normal (or elevated) circulating concentrations of insulin. **Obesity** is the most common cause of insulin resistance. However, most people with obesity and insulin resistance do not develop diabetes. In the absence of a defect in β-cell function, obese individuals without diabetes can compensate for insulin resistance with elevated levels of insulin. Insulin resistance alone will not lead to T2D. Rather, T2D develops in insulin-resistant individuals who also show impaired β-cell function. The acute **metabolic alterations** observed in T2D are **milder** than those described for the completely insulin-dependent type 1 form of the disease, in part because insulin secretion in T2D, although inadequate, does restrain **ketogenesis** and blunts the development of DKA.
- Available treatments for diabetes moderate the hyperglycemia but fail to completely normalize metabolism. The long-standing elevation of blood glucose is associated with the **chronic complications** of diabetes including **CVD** and **stroke** (**macrovascular**) as well as **retinopathy**, **nephropathy**, and **neuropathy** (**microvascular**).

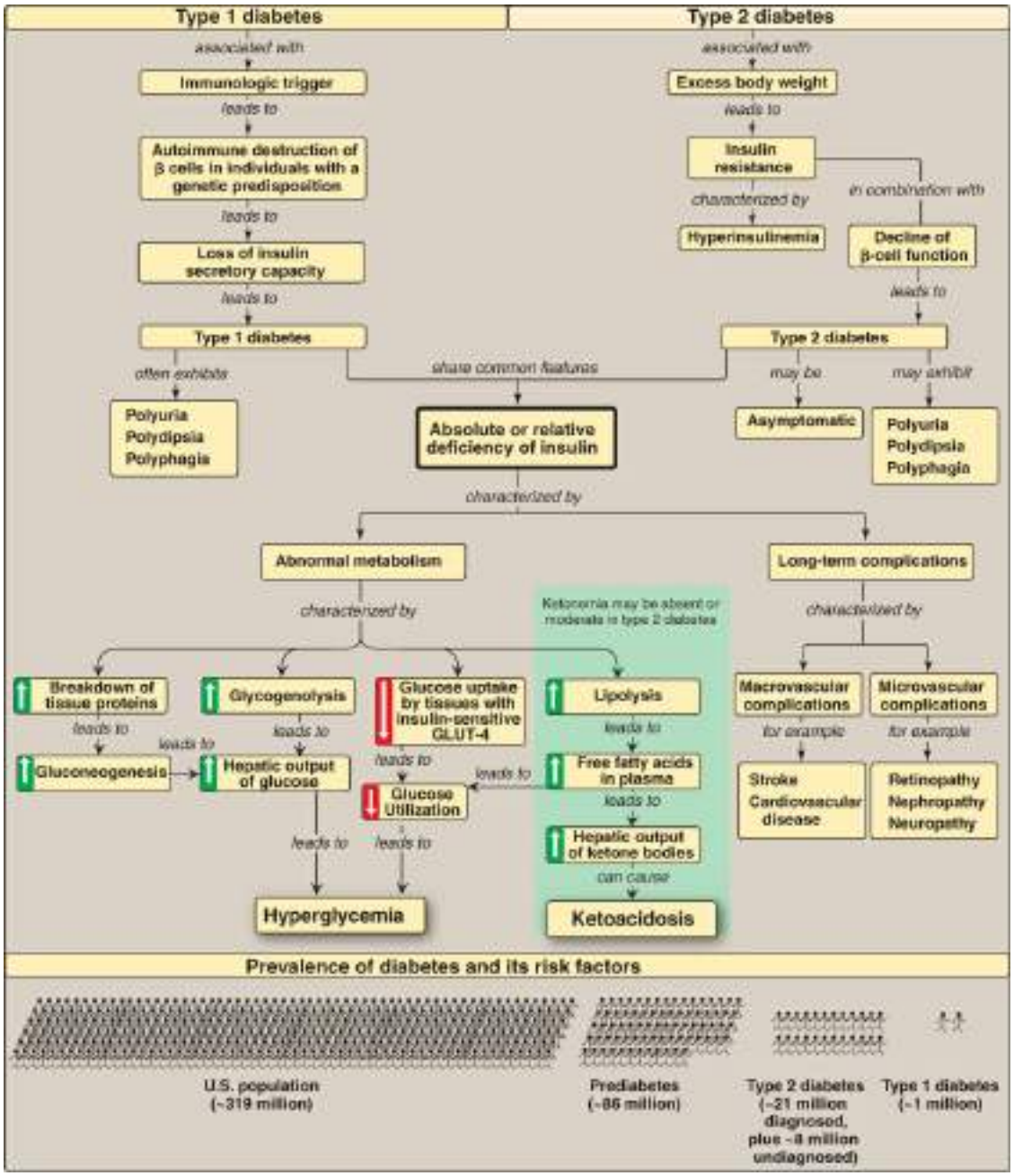
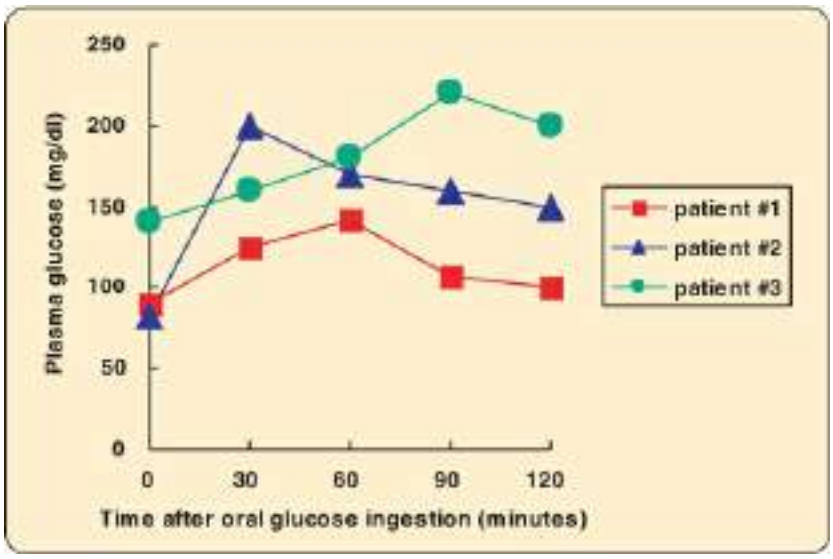


Figure 25.15
Key concept map for diabetes. (Note: Data are from 2014.) GLUT = glucose transporter.

Study Questions

Choose the ONE best answer.

25.1 Three patients being evaluated for gestational diabetes are given an oral glucose tolerance test. Based on the data shown below, which patient is prediabetic?



A. Patient #1
B. Patient #2
C. Patient #3
D. All
E. None

Correct answer = B. Patient #2 has a normal fasting blood glucose (FBG) but an impaired glucose tolerance (GT) as reflected in her blood glucose level at 2 hours and, so, is described as prediabetic. Patient #1 has a normal FBG and GT, whereas patient #3 has diabetes.

25.2 Relative or absolute lack of insulin in humans would result in which one of the following reactions in the liver?

A. Decreased activity of hormone-sensitive lipase
B. Decreased gluconeogenesis from lactate
C. Decreased glycogenolysis
D. Increased formation of 3-hydroxybutyrate
E. Increased glycogenesis

Correct answer = D. Low insulin levels favor the liver producing ketone bodies, using acetyl coenzyme A generated by β oxidation of the fatty acids provided by hormone-sensitive lipase (HSL) in adipose tissue (not liver). Low insulin also causes activation of HSL, decreased glycogen synthesis, and increased gluconeogenesis and glycogenolysis.

25.3 Which one of the following is characteristic of untreated diabetes regardless of the type?

A. Hyperglycemia
B. Ketoacidosis
C. Low levels of hemoglobin A_{1c}
D. Normal levels of C-peptide
E. Obesity
F. Simple inheritance pattern

Correct answer = A. Elevated blood glucose occurs in type 1 diabetes (T1D) as a result of a lack of insulin. In type 2 diabetes (T2D), hyperglycemia is due to a defect in β-cell function and insulin resistance. The hyperglycemia results in elevated hemoglobin A_{1c} levels. Ketoacidosis is rare in T2D, whereas obesity is rare in T1D. C (connecting)-peptide is a measure of insulin synthesis. It would be virtually absent in T1D and initially increased

then decreased in T2D. Both forms of the disease show complex genetics.

25.4 An individual suffering from obesity as well as T2D typically:

A. benefits from receiving insulin about 6 hours after a meal.
B. has a higher plasma level of glucagon than does a normal individual.
C. has a lower plasma level of insulin than does a normal individual early in the disease process.
D. shows improvement in glucose tolerance if body weight is reduced.
E. shows sudden onset of symptoms.

Correct answer = D. Many individuals with type 2 diabetes are obese, and almost all show some improvement in blood glucose with weight reduction. Symptoms usually develop gradually. These patients have elevated insulin levels and usually do not require insulin (certainly not 6 hours after a meal) until late in the disease. Glucagon levels are typically normal or low.

For questions 25.5 to 25.7, match the antihyperglycemic drug with it therapeutic mechanism of action.

A. Decreases FFA levels and increases peripheral insulin sensitivity
B. Increases insulin secretion
C. Decrease dietary carbohydrate absorption
D. Decreases gluconeogenesis
E. Decreases renal reabsorption of glucose

25.5. Sulfonylureas

25.6. SGLT inhibitors

25.7. Thiazolidinediones

Correct answers = B, E, A, respectively. Sulfonylureas are insulin secretagogues that stimulate secretion of insulin from the pancreas. SGLT inhibitors decrease reabsorption of glucose in the kidneys, so excess glucose is excreted in the urine. Thiazolidinediones signal through peroxisome proliferator-activated receptors that mediate storage of FFA in adipocytes, thereby increasing insulin sensitivity in peripheral tissues. α-Glucosidase inhibitors decrease intestinal dietary glucose absorption. Metformin decreases hepatic glucose production.

Obesity 26

I. OVERVIEW

Obesity is a disorder of body weight regulatory systems characterized by an accumulation of excess body fat. In primitive societies, in which daily life required a high level of physical activity and food was only available intermittently, a genetic tendency favoring storage of excess calories as fat may have had a survival value. Today, however, the sedentary lifestyle and abundance and wide variety of palatable, inexpensive foods in industrialized societies has undoubtedly contributed to an obesity epidemic. As adiposity has increased, so has the risk of developing associated diseases, such as type 2 diabetes (T2D), cardiovascular disease (CVD), hypertension, cancer, and arthritis. Particularly alarming is the explosion of obesity in children and adolescents, which has shown a threefold increase in prevalence over the last four decades. (Note: Approximately 1 in 5 children and adolescents age 6 to 19 years are obese.) In the United States, the lifetime risk of becoming overweight or obese is ~50% and 25%, respectively. Obesity has increased globally, and, by some estimates, there are more obese than undernourished individuals worldwide.

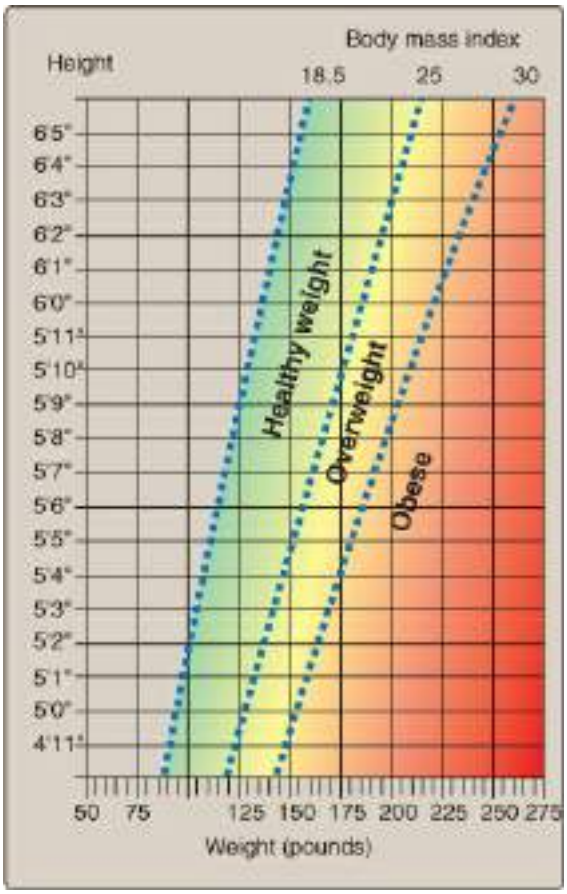


Figure 26.1
To use this body mass index (BMI) chart, find height in the left-hand column. Move across the row to weight. Height and weight intersect at the individual's BMI. (Note: To calculate BMI using pounds and inches, use BMI = weight in pounds/[height in inches]^2 × 703. Anyone >100-lb overweight is considered morbidly obese.]

II. ASSESSMENT

Because the amount of body fat is difficult to measure directly, it is usually determined from an indirect measure, the body mass index (BMI), which has been shown to correlate with the amount of body fat in most individuals. (Note: Exceptions are athletes who have large amounts of lean muscle mass.) Measuring the waist size with a tape measure is also used to screen for obesity, because this measurement reflects the amount of fat in the central abdominal area of the body. The presence of excess central fat is associated with an increased risk for morbidity and mortality, independent of the BMI. (Note: A waist size ≥40 in [men] and ≥35 in [women] is considered a risk factor.)

A. Body mass index

The BMI (defined as weight in kg/[height in m]^2) provides a measure of relative weight, adjusted for height. This allows comparisons within and between populations. The healthy range for the BMI is between 18.5 and 24.9. Individuals

with a BMI between 25 and 29.9 are considered overweight, those with a BMI ≥30 are defined as obese, and a BMI >40 is considered severely (morbidly) obese (Fig. 26.1). These cutoffs are based on studies examining the relationship of BMI to premature death and are similar in men and women. Nearly two-thirds of U.S. adults are overweight, and more than one third of those are obese. Children with a BMI-for-age above the 95th percentile are considered obese.

B. Anatomic differences in fat deposition

The anatomic distribution of body fat has a major influence on associated health risks. A waist/hip ratio (WHR) >0.8 for women and >1.0 for men is defined as android, apple-shaped, or upper-body obesity and is associated with more fat deposition in the trunk (Fig. 26.2A). In contrast, a lower WHR reflects a preponderance of fat distributed in the hips and thighs and is called gynoid, pear-shaped, or lower-body obesity. It is defined as a WHR of <0.8 for women and <1.0 for men. The pear shape, more commonly found in women, presents a much lower risk of metabolic disease, and some studies indicate that it may actually be protective. Thus, the clinician can use simple indices of body shape to identify those who may be at higher risk for metabolic diseases associated with obesity.

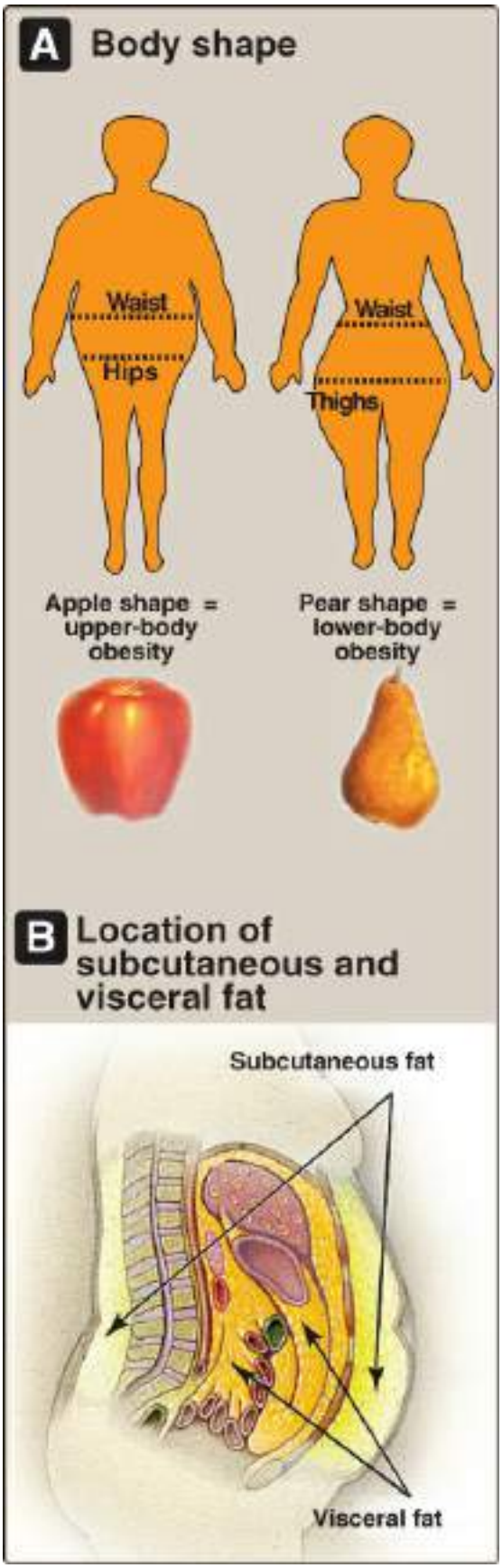


Figure 26.2
A: Individuals with upper-body obesity (**left**) have greater health risks than individuals with lower-body obesity (**right**). **B:** Visceral fat is located inside the abdominal cavity, packed in between the internal organs. Subcutaneous fat is found underneath the skin.

About 80% to 90% of human body fat is stored in subcutaneous (subq) depots in the abdominal (upper body) and the gluteal–femoral (lower body) regions. The remaining 10% to 20% is in visceral depots located deep within the abdominal cavity (Fig. 26.2B). Excess fat in visceral and abdominal subq stores increases health risks associated with obesity.

C. Biochemical differences in regional fat depots

The regional types of fat described above are biochemically different. Subq adipocytes from the lower body, particularly in women, are larger, very efficient at fat (triacylglycerol [TAG]) deposition, and tend to mobilize fatty acids (FA) more slowly than subq adipocytes from the upper body. Visceral adipocytes are the most metabolically active. In obese individuals, both abdominal subq and visceral depots have high rates of lipolysis and contribute to increased availability of free fatty acids (FFA). These metabolic differences may contribute to the higher health risk found in individuals with upper body (abdominal) obesity. (Note: FFA impair insulin signaling and are proinflammatory [see p. 382].)

1. Endocrine function: White adipose tissue, once thought to be a passive reservoir of TAG, is now known to play an active role in body weight regulatory systems. For example, the adipocyte is an endocrine cell that secretes a number of protein regulators (adipokines), such as the hormones leptin and adiponectin. Leptin regulates appetite as well as metabolism (see p. 394). Adiponectin reduces FFA levels in the blood (by increasing FA oxidation in muscles) and has been associated with improved lipid profiles, increased insulin sensitivity resulting in better glycemic control, and reduced inflammation in patients with diabetes. (Note: Adiponectin levels decrease as body weight increases, whereas leptin levels increase.)

2. Importance of portal circulation: With obesity, there is increased release of FFA and secretion of proinflammatory cytokines, such as interleukin 6 (IL-6) and tumor necrosis factor-α (TNF-α), from adipose tissue. (Note: Cytokines are small proteins that regulate the immune system.) One hypothesis for why abdominal adipose depots have such a large influence on metabolic dysfunction in obesity is that the FFA and cytokines released from these depots enter the portal vein and, therefore, have direct access to the liver. In the liver, they may lead to insulin resistance (see p. 382) and increased hepatic synthesis of TAG, which are released as components of very–low-density lipoprotein particles and contribute to the hypertriacylglycerolemia associated with obesity. By contrast, FFA from lower body subq adipose depots enter the general circulation, where they can be oxidized in muscle and, therefore, reach the liver in lower

concentration.

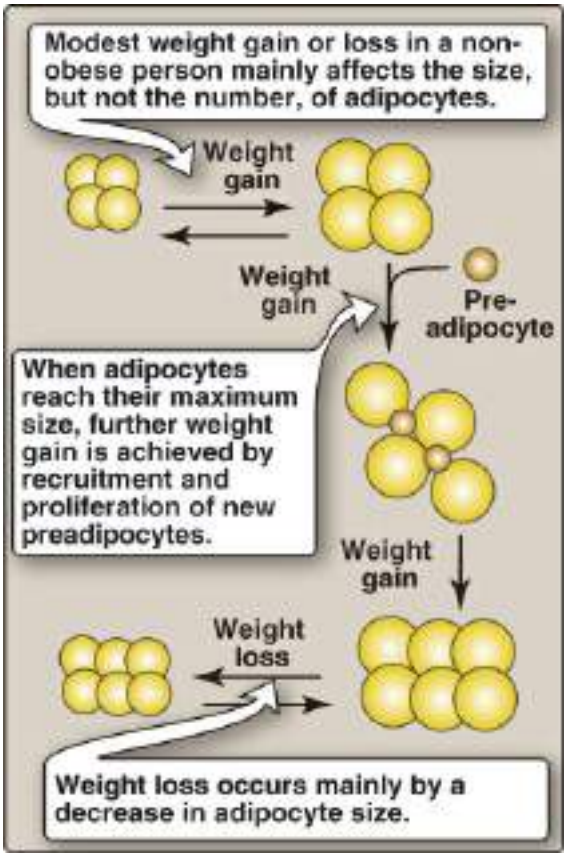


Figure 26.3
Hypertrophic (increased size) and hyperplastic (increased number) changes to adipocytes are thought to occur in severe obesity.

D. Adipocyte size and number

As TAGs are stored, adipocytes can expand to an average of two to three times their normal volume (Fig. 26.3). However, the ability of fat cells to expand is limited. With prolonged overnutrition, preadipocytes within adipose tissue are stimulated to proliferate and differentiate into mature fat cells, increasing the number of adipocytes. Thus, most obesity is due to a combination of increased fat cell size (hypertrophy) and number (hyperplasia). Obese individuals can have up to five times the normal number of adipocytes. (Note: Like other tissues, the adipose tissue undergoes continuous remodeling. Contrary to early dogma, we now know that adipocytes can die. The estimated average lifespan of an adipocyte is 10 years.) If excess calories cannot be accommodated within adipose tissue, the excess FA "spill over" into other tissues, such as muscle and the liver. The amount of this ectopic fat is strongly associated with insulin resistance. With weight loss in an obese individual, the size of the fat cells is reduced, but the number is not usually affected. Thus, a normal amount of body fat is achieved by decreasing the size of the fat cell below normal. However, small fat cells are very efficient at

reaccumulating fat, and this may drive appetite and weight regain.

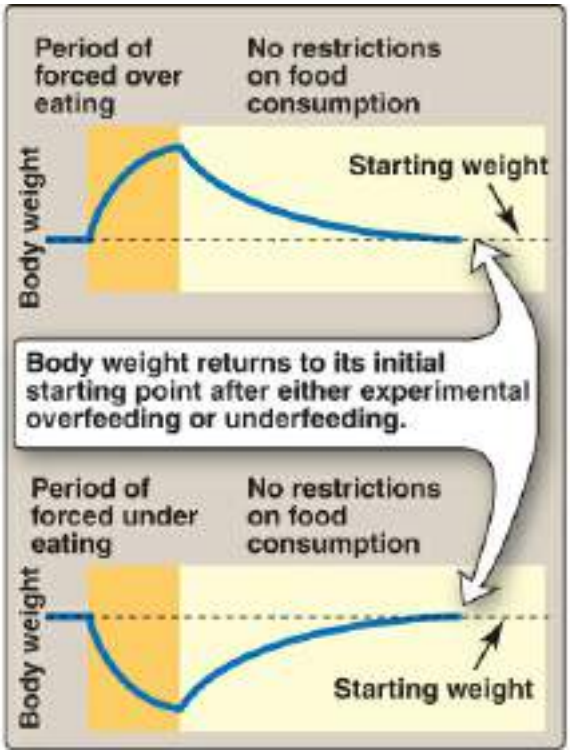


Figure 26.4
Weight changes following episodes of overfeeding or underfeeding followed by feeding with no restrictions.

III. BODY WEIGHT REGULATION

The body weight of most individuals tends to be relatively stable over time. This observation prompted the hypothesis that each individual has a biologically predetermined “set point” for body weight. The body attempts to add to adipose stores when the body weight falls below the set point and to lose adipose from stores when the body weight rises above the set point. Thus, the body defends the set point. For example, with weight loss, appetite increases and energy expenditure falls, whereas with overfeeding, appetite falls and energy expenditure may slightly increase (Fig. 26.4). However, a strict set point model explains neither why some individuals fail to revert to their starting weight after a period of overeating nor the current epidemic of obesity.

A. Genetic contributions

It is now evident that genetic mechanisms play a major role in determining body weight.

1. Biologic origin: The importance of genetics as a determinant of obesity is indicated by the observation that children who are adopted usually show a body weight that correlates with their biologic rather than adoptive parents.

Furthermore, identical twins have very similar BMI, whether reared together or apart, and their BMI are more similar than those of nonidentical, dizygotic twins.

2. Mutations: Rare, single gene mutations can cause human obesity. For example, mutations in the gene for leptin (causing decreased production) or its receptor (decreased function) result in hyperphagia (increased appetite for and consumption of food) and severe obesity (Fig. 26.5), underscoring the importance of the leptin system in regulating human body weight (see IV below). (Note: Most obese humans have elevated leptin levels but are resistant to the appetite-regulating effects of this hormone.)

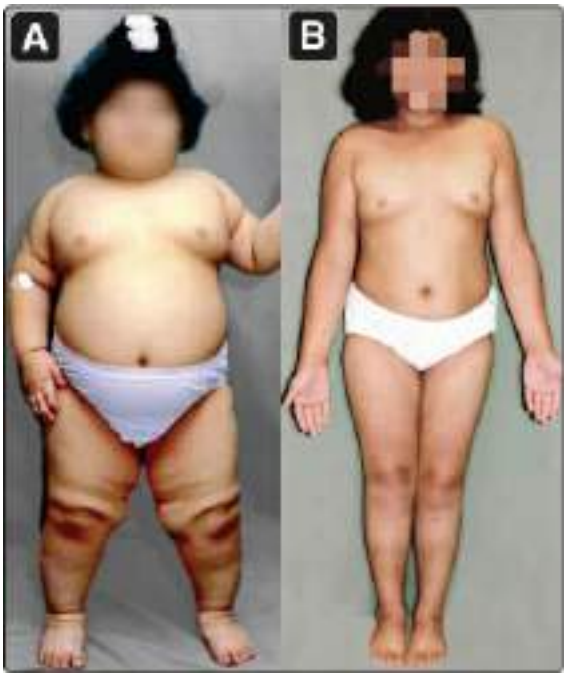


Figure 26.5
A: Patient with leptin deficiency before initiation of therapy at age 5 years. **B:** Patient at age 9 years after 48 months of therapy with subcutaneous injections of recombinant leptin.

B. Environmental and behavioral contributions

The epidemic of obesity occurring over the last several decades cannot be simply explained by changes in genetic factors, which are stable on this short time scale. Clearly, environmental factors, such as the ready availability of palatable, energy-dense foods, play a role. Furthermore, sedentary lifestyles decrease physical activity and enhance the tendency to gain weight. Eating behaviors, such as portion size, variety of foods consumed, an individual's food preferences, and the number of people present during eating, also influence food consumption. However, it is important to note that many individuals in this same environment do not become obese. The susceptibility to obesity appears to be explained, at least in part, by an interaction of an individual's genes and his or her environment and can be influenced by additional factors such as maternal under- or overnutrition that may

"set" the body regulatory systems to defend a higher or lower level of body fat. Thus, epigenetic changes (see p. 526) likely influence the risk for obesity.

IV. MOLECULAR INFLUENCES

The cause of obesity can be summarized in a deceptively simple application of the first law of thermodynamics: Obesity results when energy (caloric) intake exceeds energy expenditure. However, the mechanism underlying this imbalance involves a complex interaction of biochemical, neurologic, environmental, and psychological factors. The basic neural and humoral pathways that regulate appetite, energy expenditure, and body weight involve systems that regulate short-term food intake (meal to meal), and signals for the long-term (day to day, week to week, year to year) regulation of body weight (Fig. 26.6).

A. Long-term signals

Long-term signals reflect the status of fat (TAG) stores.

1. Leptin: Leptin is an adipocyte peptide hormone that is made and secreted in proportion to the size of fat stores. It acts on the brain to regulate food intake and energy expenditure. When we consume more calories than we need, body fat increases, and leptin production by adipocytes increases. The body adapts by increasing energy use (increasing activity) and decreasing appetite (an anorexigenic effect). When body fat decreases, the opposite effects occur. Unfortunately, most obese individuals are leptin resistant, and the leptin system may be better at preventing weight loss than preventing weight gain. (Note: Leptin's effects are mediated through binding to receptors in the arcuate nucleus of the hypothalamus.)

2. Insulin: Many obese individuals are also hyperinsulinemic, as a compensatory mechanism to insulin resistance (see p. 381). Like leptin, insulin acts on hypothalamic neurons to dampen appetite. (See Chapter 23 for the effects of insulin on metabolism.)

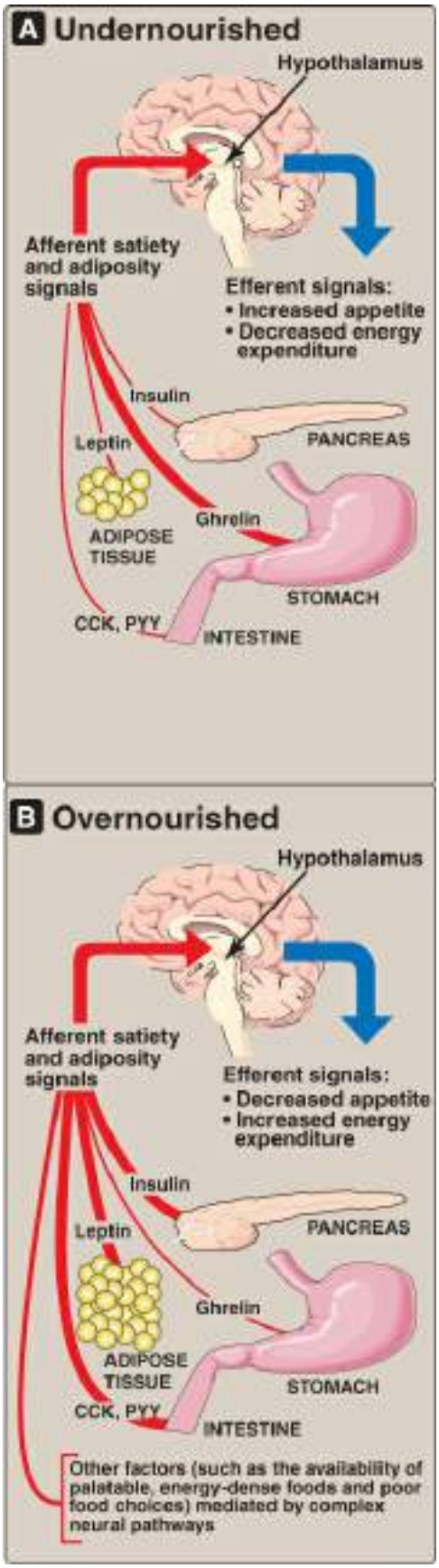


Figure 26.6
Some signals that influence appetite and satiety in undernourished (**A**) and overnourished (**B**) states. CCK = cholecystokinin; PYY = peptide YY.

B. Short-term signals

Short-term signals from the gastrointestinal (GI) tract control hunger and satiety, which affect the size and number of meals over a time course of minutes to hours. In the absence of food intake (between meals), the stomach produces ghrelin, an orexigenic (appetite-stimulating) hormone that drives hunger. As food is consumed, GI hormones, including cholecystokinin and peptide YY, among others, induce satiety (an anorexigenic effect), thereby terminating eating, through actions on gastric emptying and neural signals to the hypothalamus. Within the hypothalamus, neuropeptides (such as orexigenic neuropeptide Y [NPY] and anorexigenic α-melanocyte–stimulating hormone [α-MSH]) and neurotransmitters (such as anorexigenic serotonin and dopamine) are important in regulating hunger and satiety. Long-term and short-term signals interact, insofar as leptin increases secretion of α-MSH and decreases secretion of NPY. Thus, there are many complex regulatory loops that control the size and number of meals in relationship to the status of body fat stores. (Note: α-MSH, a cleavage product of proopiomelanocortin, binds to the melanocortin-4 receptor [MC4R]. Loss-of-function mutations to MC4R are associated with early-onset obesity.)

V. METABOLIC EFFECTS

The primary metabolic effects of obesity include dyslipidemias, glucose intolerance, and insulin resistance expressed primarily in the liver, skeletal muscle, and adipose tissue. These metabolic abnormalities reflect molecular signals originating from the increased mass of adipocytes (see Fig. 25.9, p. 382, and Fig. 26.6). (Note: About 30% of obese individuals do not show these metabolic abnormalities.)

A. Metabolic syndrome

Abdominal obesity is associated with a cluster of metabolic abnormalities (hyperglycemia, insulin resistance, hyperinsulinemia, atherogenic dyslipidemia [high levels of small low-density lipoprotein (LDL), low levels of high-density lipoprotein (HDL) and elevated TAG], and hypertension) that is referred to as metabolic syndrome (Fig. 26.7). It is a risk factor for developing CVD and T2D. The low-grade, chronic, systemic inflammation seen with obesity contributes to the pathogenesis of insulin resistance and T2D and likely plays a role in metabolic syndrome. In obesity, adipocytes release proinflammatory mediators such as IL-6 and TNF-α. Additionally, levels of adiponectin, which normally dampens inflammation and sensitizes tissues to insulin, are low.

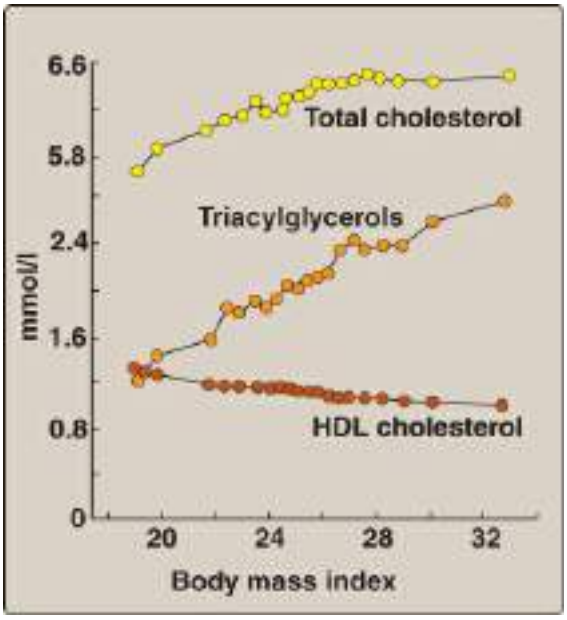


Figure 26.7
Body mass index and changes in blood lipids. HDL = high-density lipoprotein.

B. Nonalcoholic liver disease

Obesity and insulin resistance is associated with increased lipolysis of WAT TAG and free FA in circulation. This leads to ectopic deposition of TAG in the liver (hepatic steatosis) and results in an increased risk for nonalcoholic fatty liver disease ([NAFLD], see p. 382).

VI. OBESITY AND HEALTH

Obesity is correlated with an increased risk of death (Fig. 26.8) and is a risk factor for a number of chronic conditions, including T2D, dyslipidemias, hypertension, CVD, some cancers, gallstones, arthritis, gout, pelvic floor disorders (e.g., urinary incontinence), NAFLD, and sleep apnea. The relationship between obesity and associated morbidities is stronger among individuals age <55 years. After age 74 years, there is no longer an association between increased BMI and mortality. (Note: Obesity also has social consequences [e.g., stigmatization and discrimination].) Weight loss in obese individuals leads to decreased blood pressure, plasma TAG, and blood glucose levels. HDL levels also increase.

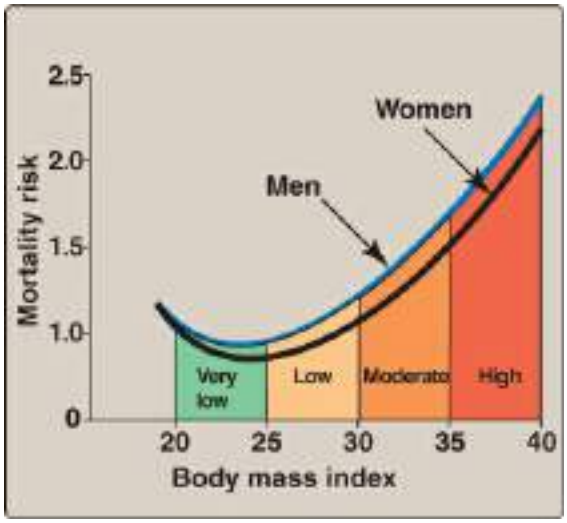


Figure 26.8
Body mass index and the relative risk of death.

VII. WEIGHT REDUCTION

Weight reduction can help reduce the complications of obesity. To achieve weight reduction, the obese patient must decrease energy intake or increase energy expenditure, although decreasing energy intake is thought to contribute more to inducing weight loss. Typically, a plan for weight reduction combines dietary change; increased physical activity; and behavioral modification, which can include nutrition education and meal planning, recording food intake through food diaries, modifying factors that lead to overeating, and relearning cues to satiety. Medications or surgery may be recommended. Once weight loss is achieved, weight maintenance is a separate process that requires vigilance because the majority of patients regain weight after they stop their weight-loss efforts.

A. Caloric restriction

Dieting is the most commonly practiced approach to weight control. Because 1 lb of adipose tissue corresponds to ~3,500 kcal, the effect that caloric restriction will have on the amount of adipose tissue can be estimated. Weight loss on calorie-restricted diets is determined primarily by caloric intake and not nutrient composition. (Note: However, compositional aspects can affect glycemic control and the blood lipid profile.) Caloric restriction is ineffective over the long term for many individuals. Over 90% of people who attempt to lose weight regain the lost weight when dietary intervention is suspended. Nonetheless, although few individuals will reach their ideal weight with treatment, weight losses of 10% of body weight over a 6-month period often reduce blood pressure and lipid levels and enhance control of T2D.

B. Physical activity

An increase in physical activity can create an energy deficit. Although adding exercise to a hypocaloric regimen may not produce a greater weight loss initially, exercise is a key component of programs directed at maintaining weight loss. In addition, physical activity increases cardiopulmonary fitness and reduces the risk of CVD, independent of weight loss. Persons who combine caloric restriction and exercise with behavioral treatment may expect to lose ~5% to 10% of initial body weight over a period of 4 to 6 months. Studies show that individuals who maintain their exercise program regain less weight after their initial weight loss.

C. Pharmacologic treatment

The U.S. Food and Drug Administration has approved several weight-loss medications for use in adults. They include orlistat (decreases absorption of dietary fat), lorcaserin, and phentermine in combination with topiramate (promote satiety through serotonin signaling), liraglutide (an incretin mimetic, decreases appetite by activating the glucagon-like peptide 1 receptor, see Fig. 25.12), and buproprion in combination with naltrexone (increase metabolism by reducing appetite). Their effects on weight reduction tend to be modest. (Note: Pharmacologic activation of brown adipocytes [see p. 86] is being explored.)

D. Surgical treatment

Gastric bypass and restriction surgeries are effective in causing weight loss in severely obese individuals. Through mechanisms that remain poorly understood, these operations greatly improve glycemic control in morbidly obese diabetic individuals. (Note: Implantation of a device that electrically stimulates the vagus nerve to decrease food intake has been approved.)

VIII. Chapter Summary

- **Obesity**, the accumulation of excess body fat, results when **energy** (**caloric**) **intake** exceeds **energy expenditure** (Fig. 26.9). Obesity is increasing in industrialized countries because of a reduction in daily energy expenditure and an increase in energy intake resulting from the increasing availability of palatable, inexpensive foods.
- The **BMI** is easy to determine and highly correlated to body fat. Nearly 69% of U.S. adults are **overweight** (BMI ≥25), and >33% of this group are **obese** (BMI ≥30).
- The anatomic distribution of body fat has a major influence on associated health risks. Excess fat located in the **abdomen** (upper body, apple shape), as reflected in **waist size**, is associated with greater risk for **hypertension**, **insulin resistance**, **diabetes**, **dyslipidemia**, and **coronary heart disease** as compared to fat located in the hips and thighs (lower body, pear shape).
- A person's weight is determined by genetic and environmental factors.
- **Appetite** is influenced by afferent, or incoming, signals (i.e., neural signals, circulating hormones such as **leptin**, and metabolites) that are integrated by the **hypothalamus**. These diverse signals prompt release of hypothalamic peptides (such as **NPY** and **α-MSH**) and activate outgoing, efferent neural signals.
- Obesity is correlated with an increased risk of **death** and is also a risk factor for a number of **chronic conditions**.
- **Weight reduction** is achieved best with **negative energy balance**, that is, by decreasing caloric intake and increasing physical activity. Virtually all diets that limit particular groups of foods or macronutrients lead to short-term weight loss. Long-term maintenance of weight loss is difficult to achieve.
- Modest reduction in food intake occurs with **pharmacologic treatment**. **Surgical procedures**, such as gastric bypass, designed to limit food intake are an option for the severely obese patient who has not responded to other treatments.

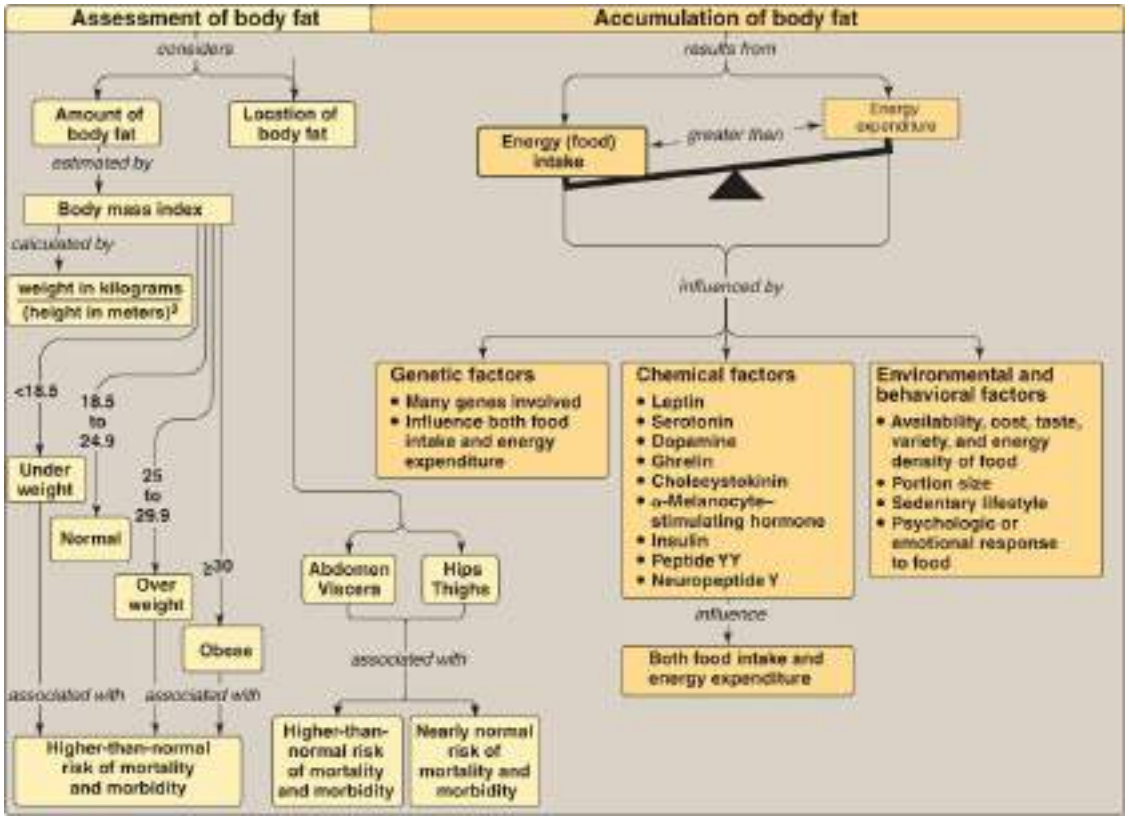


Figure 26.9
Key concept map for obesity. (Note: Body mass index may also be calculated by weight in pounds/[height in inches]^2 × 703.)

Study Questions

Choose the ONE best answer.

For Questions 26.1 and 26.2, use the following scenario.

A 40-year-old female, 5-ft, 1-in (155-cm) tall and weighing 188 lb (85.5 kg), seeks your advice on how to lose weight. Her waist measured 41 in and her hips 39 in. The remainder of the physical examination and the blood laboratory data were all within the normal range. Her only child (who is age 14 years), her sister, and both of her parents are overweight. The patient recalls being overweight throughout her childhood and adolescence. Over the past 15 years, she had been on seven different diets for periods of 2 weeks to 3 months, losing from 5 to 25 lb each time. On discontinuation of the diets, she regained weight, returning to 185 to 190 lb.

26.1 Calculate and interpret the body mass index for the patient.

Body mass index (BMI) = weight in kg/(height in m)^2 = 85.5/1.55^2 = 35.6. Because her BMI is >30, the patient is classified as obese.

26.2 Which one of the following statements best describes the patient?

A. She has approximately the same number of adipocytes as an individual of normal weight, but each adipocyte is larger.
B. She shows an apple pattern of fat distribution.
C. She would be expected to show higher-than-normal levels of adiponectin.
D. She would be expected to show lower-than-normal levels of circulating leptin.
E. She would be expected to show lower-than-normal levels of circulating triacylglycerols.

Correct answer = B. Her waist/hip ratio (WHR) is 1.05 (41/39). Apple shape is defined as a WHR of >0.8 for women and >1.0 for men. Therefore, she has an apple pattern of fat distribution, more commonly seen in males. Compared with other women of the same body weight who have a gynoid (pear-shaped) fat pattern, her android fat pattern places her at greater risk for diabetes, hypertension, dyslipidemia, and coronary heart disease. Individuals with marked obesity and a history dating to early childhood have a fat depot made up of too many adipocytes, each fully loaded with triacylglycerol (TAG). Plasma leptin levels are proportional to fat mass, suggesting that resistance to leptin, rather than its deficiency, occurs in human obesity. Adiponectin levels decrease with increasing fat mass. The elevated circulating free fatty acids characteristic of obesity are carried to the liver and converted to TAG. The TAGs are released as components of very–low-density lipoproteins, resulting in elevated plasma TAG levels, or are stored in the liver, resulting in hepatic steatosis.

26.3 Which one of the following metabolic abnormalities is associated with abdominal obesity and metabolic syndrome?

A. Higher-than-normal levels of glucose.
B. Higher-than-normal levels of HDL.
C. Lower-than-normal blood pressure.
D. Lower-than-normal levels of insulin.
E. Lower-than-normal levels of TAG.

Correct answer = A. Abdominal obesity is associated with metabolic syndrome, which is defined as a cluster of metabolic abnormalities including hyperglycemia, insulin resistance, hyperinsulinemia, atherogenic dyslipidemia (high levels of small low-density lipoprotein [LDL], low levels of high-density lipoprotein [HDL] and elevated TAG), and hypertension. Metabolic syndrome is a risk factor for developing CVD and T2D. Low-grade, chronic, systemic

inflammation seen with obesity contributes to insulin resistance and T2D and likely plays a role in metabolic syndrome.

26.4 Which one of the following statements about leptin is correct?

A. Leptin expression and secretion is inversely proportional to the size of fat stores.
B. Leptin signals to decrease appetite and increase energy expenditure.
C. Leptin signals to decrease secretion of α-melanocyte–stimulating hormone (α-MSH).
D. Leptin signals to increase secretion of adiponectin from adipocytes.
E. Leptin signals to increase secretion of neuropeptide Y (NPY).

Correct answer = B. As we consume more calories than we need, body fat increases. Leptin levels increase, whereas adiponectin levels decrease as body weight increases. The body adapts to increasing energy by increasing energy use (increasing activity) and decreasing appetite (an anorexigenic effect). Leptin signaling increases secretion of anorexigenic α-MSH and decreases secretion of orexigenic NPY.

26.5 Which one of the following statements best describes the relationship between obesity and nonalcoholic fatty liver disease?

A. Chylomicrons increase delivery of FA to the liver.
B. Increased free FA in circulation lead to deposition of TAG in the liver.
C. Increased NADH:NAD^+ ratio increases glycerol 3-phosphate dehydrogenase activity.
D. Insulin resistance leads to decreased lipolysis of WAT TAG stores.
E. Obesity leads to increased hepatic FA synthesis.

Correct answer = B. Obesity and insulin resistance is associated with increased lipolysis of WAT TAG and free FA in circulation, not chylomicrons. This leads to ectopic deposition of TAG in the liver (hepatic steatosis) and results in an increased risk for nonalcoholic fatty liver disease (NAFLD). Obesity and insulin resistance would lead to a decrease in hepatic FA synthesis. An increase in NADH:NAD^+ ratio results from chronic alcoholism.

UNIT VI:
Medical Nutrition

Nutrition: Overview and Macronutrients 27

I. OVERVIEW

Nutrients are the constituents of food necessary to sustain the normal functions of the body. All energy (calories) is provided by three classes of nutrients: fats, carbohydrates, and protein (Fig. 27.1). Because the intake of these energy-rich molecules is larger (g amounts) than that of the other dietary nutrients (mg to µg amounts), they are called macronutrients. Although alcohol is also an energy source, it is not a nutrient and it interferes with growth, maintenance, and repair. This chapter focuses on the kinds and amounts of macronutrients that are needed to maintain optimal health and prevent chronic disease. Those nutrients needed in lesser amounts (mg or µg), vitamins and minerals, are called micronutrients and are considered in Chapters 28 and 29. The names macronutrient and micronutrients do not signify their relative importance, but rather denote their relative dietary intake requirements. A nutrient is a micronutrient when less than a gram is required daily.

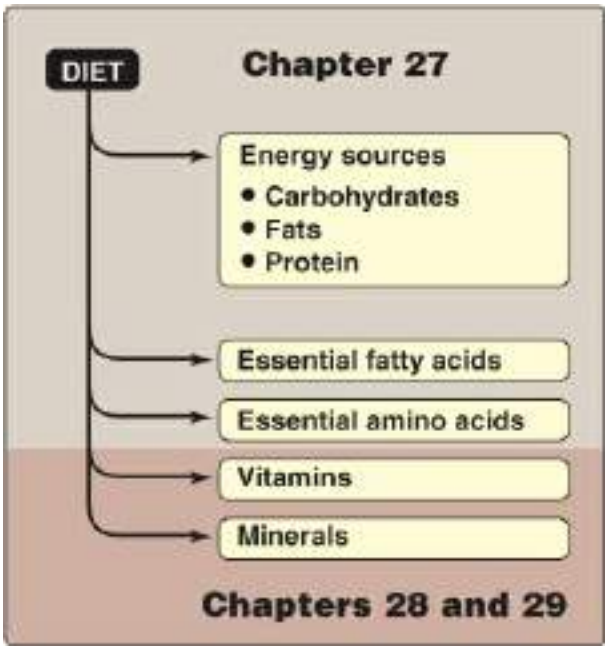


Figure 27.1
Essential nutrients obtained from the diet. (Note: Ethanol may provide a significant contribution to the daily caloric intake of some individuals.).

II. DIETARY REFERENCE INTAKES

Committees of US and Canadian experts organized by the Food and Nutrition Board of the Institute of Medicine of the National Academy of Sciences have compiled Dietary Reference Intakes (DRI), which are estimates of the amounts of nutrients required to prevent deficiencies and maintain optimal health and growth. The DRI expands on the Recommended Dietary Allowances (RDAs), which have been published with periodic revisions since 1941. Unlike the RDA, the DRI establishes upper limits on the consumption of some nutrients and incorporates the role of nutrients in lifelong health, going beyond mere prevention of deficiency diseases. Both the DRI and the RDA refer to long-term average daily nutrient intakes, because it is not necessary to consume the full RDA every day.

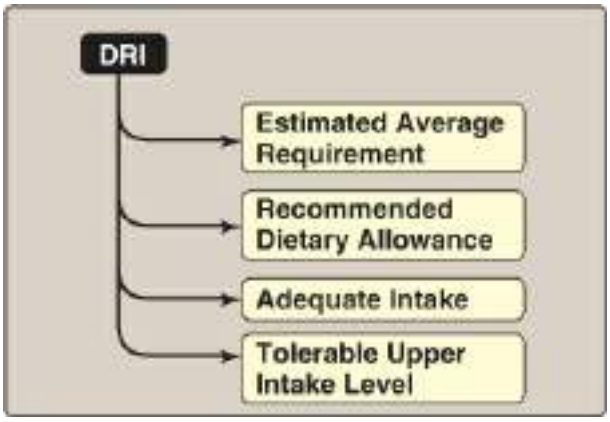


Figure 27.2
Components of the Dietary Reference Intakes (DRI).

A. Definition

The DRI consists of four dietary reference standards for the intake of nutrients designated for specific life stage (age) groups, physiologic states, and gender (Fig. 27.2).

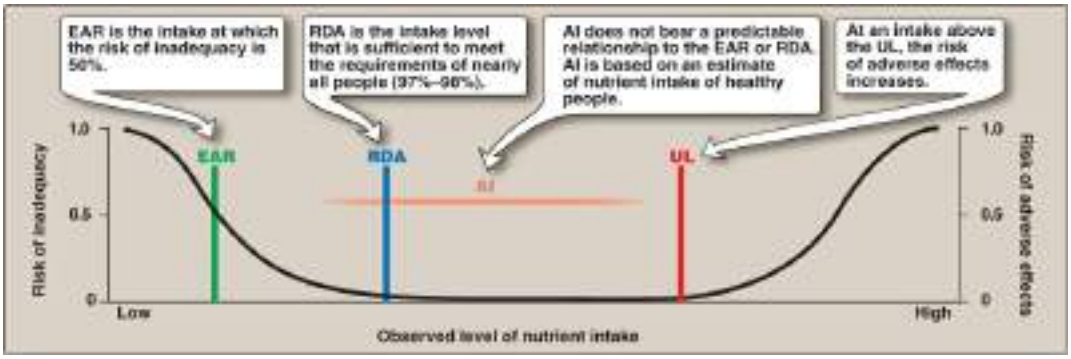


Figure 27.3
Comparison of the components of the Dietary Reference Intakes. EAR = estimated average requirement; RDA = recommended dietary allowance; AI = adequate intake; UL = tolerable upper intake level.

1. Estimated average requirement: The average daily nutrient intake level estimated to meet the requirement of 50% of the healthy individuals in a particular life stage and gender group is the Estimated Average Requirement (EAR) (Fig. 27.3). It is useful in estimating the actual requirements in groups and individuals.

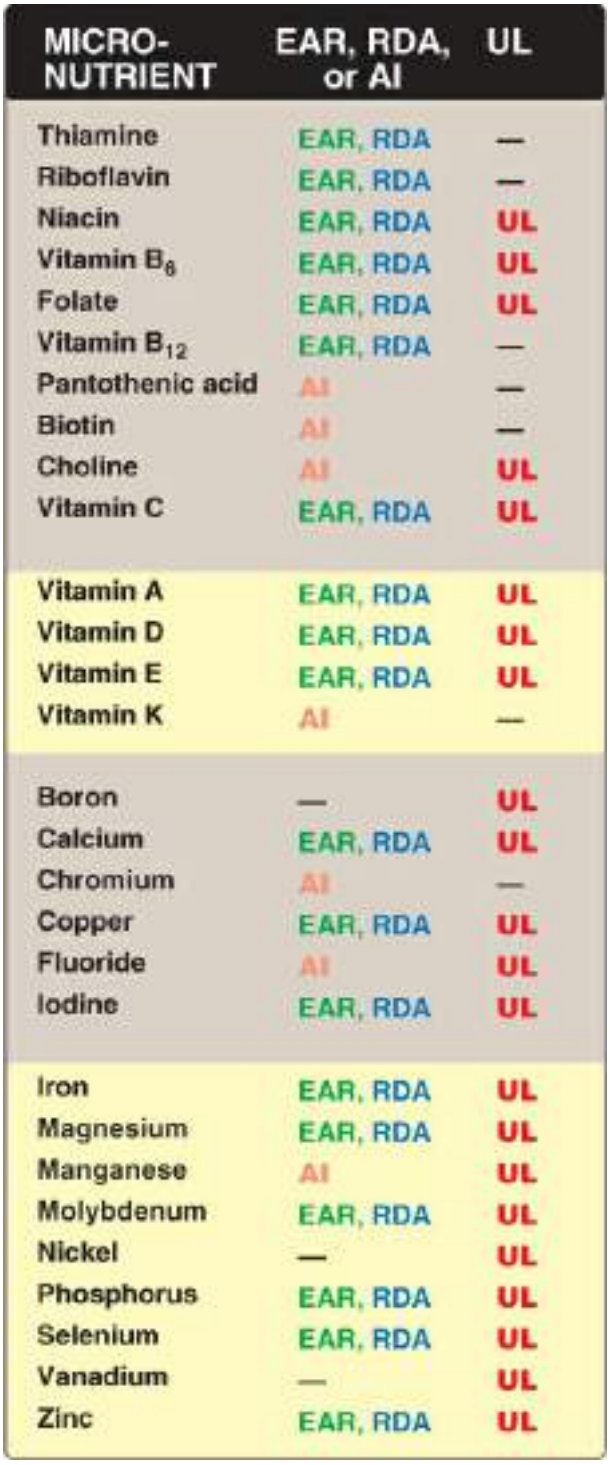
2. Recommended dietary allowance: The RDA is the average daily nutrient intake level that is sufficient to meet the requirements of nearly all (97% to 98%) individuals in a particular life stage and gender group (Fig. 27.3). The RDA is not the minimal requirement for healthy individuals, but it is intentionally set to provide a margin of safety for most individuals. The EAR serves as the foundation for setting the RDA. If the standard deviation (SD) of the EAR is available and the requirement for the nutrient is normally distributed, the RDA is set at 2 SD above the EAR (i.e., RDA = EAR + 2 SD_{EAR}).

3. Adequate intake: An Adequate Intake (AI) is set instead of an RDA if sufficient scientific evidence is not available to calculate an EAR or RDA. The AI is based on estimates of nutrient intake by a group (or groups) of apparently healthy people. For example, the AI for young infants, for whom human milk is the recommended sole source of food for the first 6 months, is based on the estimated daily mean nutrient intake supplied by human milk for healthy, full-term infants who are exclusively breast-fed.

4. Tolerable upper intake level: The highest average daily nutrient intake level that is likely to pose no risk of adverse health effects to almost all individuals in the general population is the Tolerable Upper Intake Level (UL). As intake increases above the UL, the potential risk of adverse effects may increase. The UL is useful because of the increased availability of fortified foods and the increased use of dietary supplements. For some nutrients, there may be insufficient data on which to develop a UL.

B. Using the dietary reference intakes

Most nutrients have a set of DRIs (Fig. 27.4). Usually a nutrient has an EAR and a corresponding RDA. Most are set by age and gender and may be influenced by special factors, such as pregnancy and lactation in women (see Section IX). When the data are not sufficient to estimate an EAR (or an RDA), an AI is designated. Intakes below the EAR need to be improved because the probability of adequacy is ≤50% (Fig. 27.3). Intakes between the EAR and RDA likely need to be improved because the probability of adequacy is <98%, and intakes at or above the RDA can be considered adequate. Intakes above the AI can be considered adequate. Intakes between the UL and the RDA can be considered to have no risk for adverse effects. (Note: Because the DRI is designed to meet the nutritional needs of the healthy, it does not include any special needs of the sick.)



MICRO-NUTRIENT	EAR, RDA, or AI	UL
Thiamine	EAR, RDA	—
Riboflavin	EAR, RDA	—
Niacin	EAR, RDA	UL
Vitamin B_6	EAR, RDA	UL
Folate	EAR, RDA	UL
Vitamin B_{12}	EAR, RDA	—
Pantothenic acid	AI	—
Biotin	AI	—
Choline	AI	UL
Vitamin C	EAR, RDA	UL
Vitamin A	EAR, RDA	UL
Vitamin D	EAR, RDA	UL
Vitamin E	EAR, RDA	UL
Vitamin K	AI	—
Boron	—	UL
Calcium	EAR, RDA	UL
Chromium	AI	—
Copper	EAR, RDA	UL
Fluoride	AI	UL
Iodine	EAR, RDA	UL
Iron	EAR, RDA	UL
Magnesium	EAR, RDA	UL
Manganese	AI	UL
Molybdenum	EAR, RDA	UL
Nickel	—	UL
Phosphorus	EAR, RDA	UL
Selenium	EAR, RDA	UL
Vanadium	—	UL
Zinc	EAR, RDA	UL

Figure 27.4
Dietary Reference Intakes for vitamins and minerals in individuals age 1 year and older. (Note: An RDA has been set for carbohydrate and protein [macronutrients] but not for fat.) EAR = Estimated Average Requirement; RDA = Recommended Dietary Allowance; AI = Adequate Intake; UL = Tolerable Upper Intake Level; — = no value established.

III. ENERGY REQUIREMENT IN HUMANS

The Estimated Energy Requirement (EER) is the average dietary energy intake predicted to maintain an energy balance (i.e., the calories consumed are equal to the energy expended) in a healthy adult of a defined age, gender, and height whose weight and level of physical activity are consistent with good health. Differences in the genetics, body composition, metabolism, and behavior of individuals make it difficult to accurately predict a person's caloric requirements. However, some simple approximations can provide useful estimates. For example, sedentary adults require approximately 30 kcal/kg/day to maintain body weight, moderately active adults require 35 kcal/kg/day, and very active adults require 40 kcal/kg/day.

A. Energy content of food

The energy content of food is calculated from the heat released by the total combustion of food in a calorimeter. It is expressed in kilocalories (kcal, or Cal). The standard conversion factors for determining the metabolic caloric value of fat, protein, and carbohydrate are shown in Figure 27.5. A calorie is the amount of energy needed to raise the temperature of 1 gram of water by one-degree Celsius. Kilocalorie is the amount of energy needed to raise 1,000 grams (1 kg) of water by one-degree Celsius. In Nutrition, 1,000-calorie units are known as kilocalories or Cal. That is to say "one gram of carbohydrate is equivalent to 4 calories" in nutrition is actually "one gram of carbohydrate is equivalent to 4000 calories."

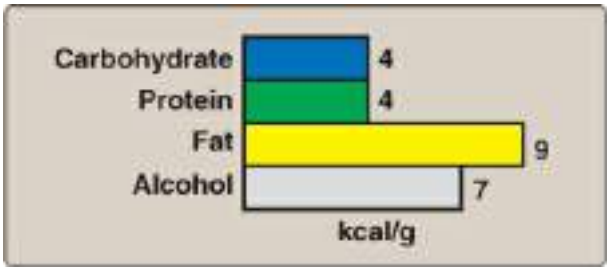


Figure 27.5
Average energy available from the macronutrients and alcohol.

Note that the energy content of fat is more than twice that of carbohydrate or protein, whereas the energy content of ethanol is intermediate between those of fat and carbohydrate. (Note: The joule [J] is the International System of Units [SI] used for energy and it is widely used in countries other than the United States. One cal = 4.2 J; 1 kcal [1 Cal, 1 food calorie] = 4.2 kJ. For uniformity, many scientists are promoting the use of joules rather than calories in the United States. However, kcal still predominates and is used throughout this text.)

B. Use of food energy in the body

The energy generated by metabolism of the macronutrients is used for three energy-requiring processes that occur in the body: resting metabolic rate (RMR), physical activity, and the thermic effect of food. Another minor process which

requires energy is thermogenesis (not shown in Fig. 27.7). The number of kcal expended by these processes in a 24-hour period is the total energy expenditure (TEE).

1. Resting metabolic rate: RMR is the energy expended by an individual in a resting, postabsorptive state. It represents the energy required to carry out the normal body functions, such as respiration, blood flow, and ion transport. RMR can be determined by various methods such as calorimetry, doubly labeled water or mathematical formulas. However, indirect calorimetry is the most commonly used method to quantify RMR by measuring oxygen (O_2) consumed or carbon dioxide (CO_2) produced. The ratio of CO_2 to O_2 is the respiratory quotient (RQ). It reflects the metabolic fuel or substrate being oxidized for energy in tissues (Fig. 27.6). RQ for carbohydrates, proteins and fats are 1.0, 0.84, and 0.71 respectively. For example, complete oxidation of glucose uses 6 O_2 and produces 6 CO_2, therefore the ratio is 1. On the other hand, the most common fatty acid, palmitate when oxidized use 23 O_2 and produces 16 CO_2, hence the ratio of RQ = CO_2/O_2 = 0.7. An RQ close to 0.8 reflects the oxidation of the mixture of fat and carbohydrate in the diet.

SUBSTRATE	RQ
Carbohydrate	1.00
Protein	0.84
Fat	0.71

Figure 27.6
The respiratory quotient (RQ). (Note: For protein, the nitrogen is removed and excreted, and the α-keto acids are oxidized.)

RMR also can be estimated using equations that include sex and age (RMR reflects lean muscle mass, which is highest in men and the young) as well as height and weight. A commonly used rough estimate is 1 kcal/kg/hr for men and 0.9 kcal/kg/hr for women. (Note: A basal metabolic rate [BMR] can be determined if more stringent environmental conditions are used, but it is not routinely done. RMR is approximately 10% higher than the BMR.) In an adult, the 24-hour RMR, known as the resting energy expenditure (REE), is approximately 1,800 kcal for men (70 kg) and 1,300 kcal for women (50 kg). From 60% to 75% of the TEE in sedentary individuals is attributable to the REE (Fig. 27.7). (Note: Hospitalized individuals are commonly hypercatabolic, and the RMR is multiplied by an injury factor that ranges from 1.0 [mild infection] to 2.0 [severe burns] in calculating their TEE.)

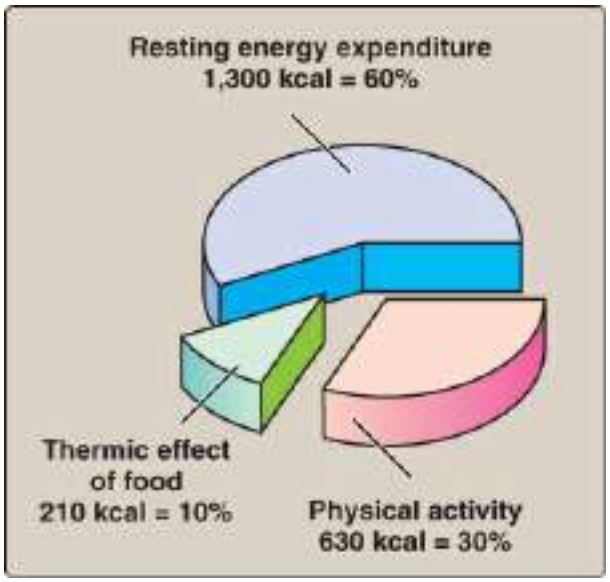


Figure 27.7
Estimated total energy expenditure in a healthy 20-year-old woman, 5 ft, 4 in (165 cm) tall, weighing 110 lb (50 kg), and engaged in light activity.

2. Physical activity: Muscular activity provides the greatest variation in the TEE. The amount of energy consumed depends on the duration and intensity of the exercise. This energy cost is expressed as a multiple of the RMR (range is 1.1 to >8.0) that is referred to as the physical activity ratio (PAR) or the metabolic equivalent of the task (MET). In general, a lightly active person requires approximately 30% to 50% more calories than the RMR (see Fig. 27.7), whereas a highly active individual may require ≥100% calories above the RMR.

3. Thermic effect of food: The production of heat by the body increases as much as 30% above the resting level during the digestion and absorption of food. This is called the thermic effect of food, or diet-induced thermogenesis. The thermic response to food intake may amount to 5% to 10% of the TEE.

4. Thermogenesis: There are two types of thermogenesis: adaptive and nonexercise activity thermogenesis (NEAT). Adaptive thermogenesis is the regulated production of heat in response to environmental changes in temperature and diet, for example, shivering in response to cold. NEAT includes the common daily activities, such as fidgeting, walking to work, pacing while talking on the phone and standing.

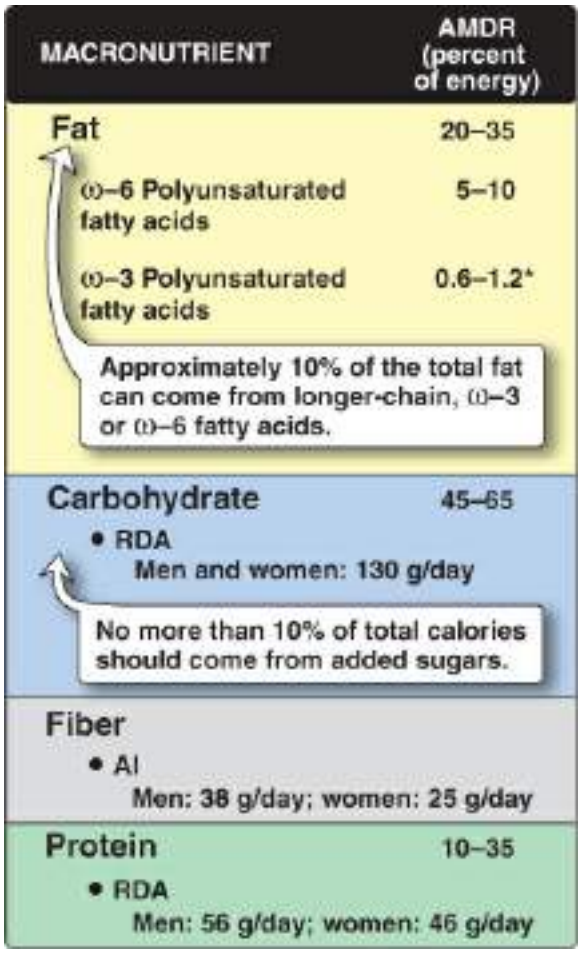


Figure 27.8
Acceptable Macronutrient Distribution Ranges (AMDR) in adults. (Note: *A growing body of evidence suggests that higher levels of ω-3 polyunsaturated fatty acids provide protection against coronary heart disease.) RDA = recommended dietary allowance; AI = adequate intake.

IV. ACCEPTABLE MACRONUTRIENT DISTRIBUTION RANGES

Acceptable Macronutrient Distribution Ranges (AMDRs) are defined as a range of intakes for a particular macronutrient that is associated with reduced risk of chronic disease while providing adequate amounts of essential nutrients. The AMDR for adults is 45% to 65% of their total calories from carbohydrates, 20% to 35% from fat, and 10% to 35% from protein (Fig. 27.8). The biologic properties of dietary fat, carbohydrate, and protein are described below.

V. DIETARY FATS

The incidence of a number of chronic diseases is significantly influenced by the kinds and amounts of nutrients consumed (Fig. 27.9). Dietary fats most strongly influence the incidence of coronary heart disease (CHD), but evidence linking dietary fat and the risk for cancer or obesity is much weaker.

Earlier recommendations emphasized decreasing the total amount of dietary fat. Unfortunately, this resulted in increased consumption of refined grains and added sugars. Data now show that the type of fat is a more important risk factor than the total amount of fat.

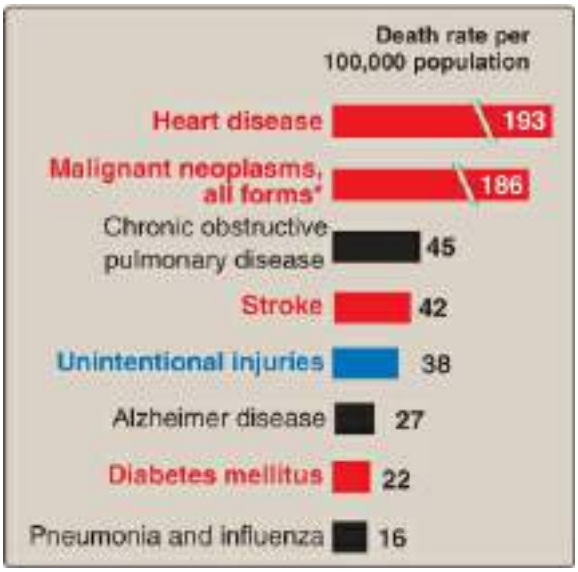


Figure 27.9
Influence of nutrition on some common causes of death in the United States in the year 2010. Red indicates causes of death in which the diet plays a significant role. Blue indicates causes of death in which excessive alcohol consumption plays a part. (Note: *Diet plays a role in only some forms of cancer.)

A. Plasma lipids and coronary heart disease

Plasma cholesterol is derived from the diet or from endogenous biosynthesis. In either case, cholesterol is transported between the tissues in combination with protein and phospholipids as lipoproteins.

1. Low-density and high-density lipoproteins: The level of plasma cholesterol is not precisely regulated but, rather, varies in response to diet. Elevated levels of total cholesterol (hypercholesterolemia) result in an increased risk for CHD (Fig. 27.10). A much stronger correlation exists between CHD and the level of cholesterol in low-density lipoproteins ([LDL-C] see Chapter 18). As LDL-C increases, CHD increases. In contrast, elevated levels of high-density lipoprotein cholesterol (HDL-C) have been associated with a decreased risk for heart disease (see Chapter 18). (Note: Elevated plasma triacylglycerol [TAG] is associated with CHD, but a causative relationship has yet to be demonstrated.) Abnormal levels of plasma lipids (dyslipidemias) act in combination with smoking, obesity, sedentary lifestyle, insulin resistance, and other risk factors to increase the risk of CHD.

2. Benefits of lowering plasma cholesterol: Dietary or pharmacologic management of hypercholesterolemia has been shown to be effective in decreasing LDL-C,

increasing HDL-C, and reducing the risk for cardiovascular events. The diet-induced changes in plasma cholesterol concentrations are modest, typically 10% to 20%, whereas treatment with statin drugs decreases plasma cholesterol by 30% to 60% (see p. 249). (Note: Dietary and drug treatment can also lower TAG.)

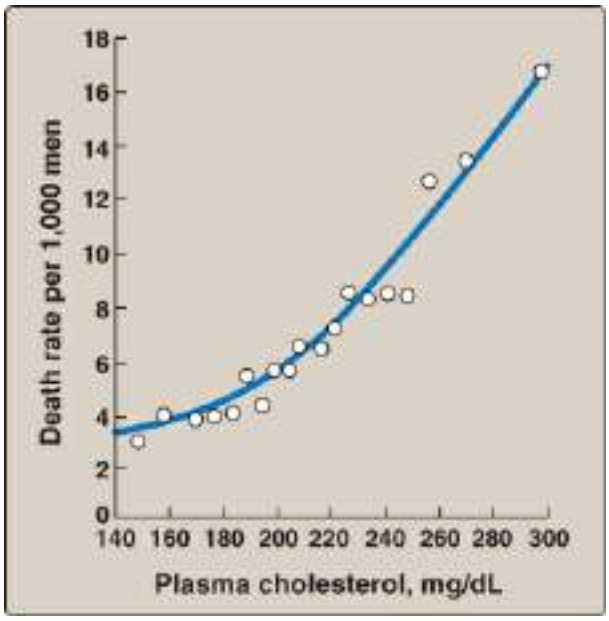


Figure 27.10
Correlation of the death rate from coronary heart disease with the concentration of plasma cholesterol. (Note: The data were obtained from a multiyear study of men with the death rate adjusted for age.)

B. Dietary fats and plasma lipids

TAGs are quantitatively the most important class of dietary fats. The influence of TAG on blood lipids is determined by the chemical nature of their constituent fatty acids. The absence or presence and number of double bonds (saturated vs. mono- and polyunsaturated), the location of the double bonds (ω-6 vs. ω-3), and the cis versus trans configuration of the unsaturated fatty acids are the most important structural features that influence blood lipids.

1. Saturated fats: TAG composed primarily of fatty acids whose hydrocarbon chains do not contain any double bonds are referred to as saturated fats. Consumption of saturated fats is positively associated with high levels of total plasma cholesterol and LDL-C and an increased risk of CHD. The main sources of saturated fatty acids are dairy and meat products and some vegetable oils, such as coconut and palm oils (a major source of fat in Latin America and Asia, although not in the United States). Many experts strongly advise limiting intake of saturated fats to <10% of total caloric intake and replacing them with unsaturated fats (and whole grains).

Saturated fatty acids with carbon chain lengths of 14 (myristic) and 16 (palmitic) are most potent in increasing the plasma cholesterol level. Stearic acid (18 carbons, found in many foods including chocolate) has little effect on blood cholesterol.

2. Monounsaturated fats: TAGs containing primarily fatty acids with one double bond are referred to as monounsaturated fats (MUFA). Fatty acids containing more than one double bond are termed polyunsaturated fatty acids (PUFA). MUFA are generally obtained from plant-based oils. When substituted for saturated fatty acids in the diet, MUFA lower both total plasma cholesterol and LDL-C and maintain or increase HDL-C. This ability of MUFA to favorably modify lipoprotein levels may explain, in part, the observation that Mediterranean cultures, with diets rich in olive oil (high in monounsaturated oleic acid), show a low incidence of CHD. (Note: Although there is no AMDR for MUFA, it is recommended that the fats in diet should be mostly unsaturated fatty acids [MUFA and PUFA].)

a. The Mediterranean diet: The Mediterranean diet is an example of a diet rich in MUFA (from olive oil, olives, nuts and fish) and PUFA (from fish oils, plant oils, and some nuts) but low in saturated fat. For example, Figure 27.11 shows the composition of the Mediterranean diet in comparison with both a Western diet similar to that consumed in the United States and a typical low-fat diet. The Mediterranean diet contains seasonally fresh food, with an abundance of plant material, low amounts of red meat, and olive oil as the principal source of fat. The Mediterranean diet is associated with decreased plasma total cholesterol, LDL-C, and TAG, and increased HDL-C when compared with a typical Western diet higher in saturated fats.

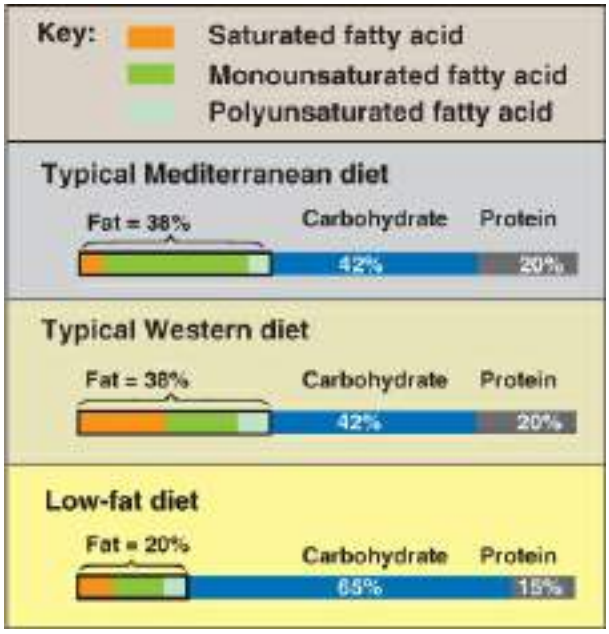
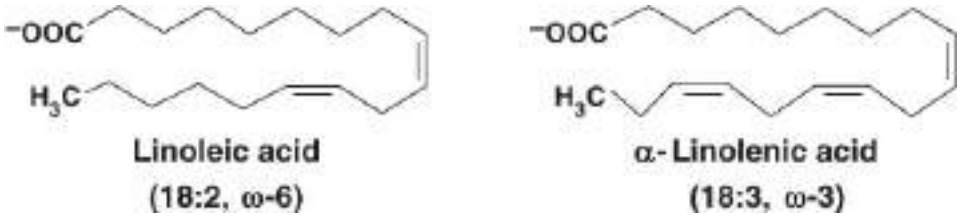


Figure 27.11

Composition of typical Mediterranean, Western, and low-fat diets.

3. Polyunsaturated fats: TAGs containing primarily fatty acids with more than one double bond are referred to as polyunsaturated fats. The effects of PUFA on cardiovascular disease are influenced by the location of the double bonds within the molecule.

a. ω-6 Fatty acids: These are long-chain PUFA, with the first double bond beginning at the sixth bond position when starting from the methyl (ω) end of the fatty acid molecule. (Note: They are also called n-6 fatty acids [see Chapter 16].) Consumption of fats containing ω-6 PUFA, principally linoleic acid (18:2 [9,12]), obtained from vegetable oils, lowers plasma cholesterol when substituted for saturated fats. Plasma LDL-C is lowered, but HDL-C, which protects against CHD, is also lowered, partially offsetting the benefits of lowering LDL-C. Nuts, avocados, olives, soybeans, and various oils, including sunflower and corn oil, are common sources of these fatty acids. The AMDR for linoleic acid is 5% to 10%. (Note: The lower recommendation for intake of PUFA relative to MUFA is because of concern that free radical–mediated oxidation [peroxidation] of PUFA may lead to deleterious products.)



b. ω-3 Fatty acids: These are long-chain PUFA, with the first double bond beginning at the third bond position from the methyl (ω) end. Dietary ω-3 PUFA suppress cardiac arrhythmias, reduce plasma TAG, decrease the tendency for thrombosis, lower blood pressure, and substantially reduce risk of cardiovascular mortality, but they have little effect on LDL-C or HDL-C levels. Evidence suggests that they have anti-inflammatory effects. The ω-3 PUFA, principally α-linolenic acid, 18:3(9,12,15), are found in plant oils, such as flaxseed and canola, and some nuts, such as walnuts. The AMDR for α-linolenic acid is 0.6% to 1.2%. Fish oil contains the long-chain ω-3 docosahexaenoic acid (DHA, 22:6) and eicosapentaenoic acid (EPA, 20:5). Two meals containing fatty fish (e.g., salmon, mackerel, anchovies, sardines, herring) per week are recommended. (Note: DHA is included in infant formulas to promote brain development.) Linoleic and α-linolenic acids are essential fatty acids (EFAs) required for membrane fluidity and synthesis of eicosanoids (see Chapter 17, VIII). EFA deficiency, caused primarily by fat malabsorption, is characterized by scaly dermatitis as a result of the depletion of skin ceramides with long-chain fatty acids (see Chapter 17, III. F).

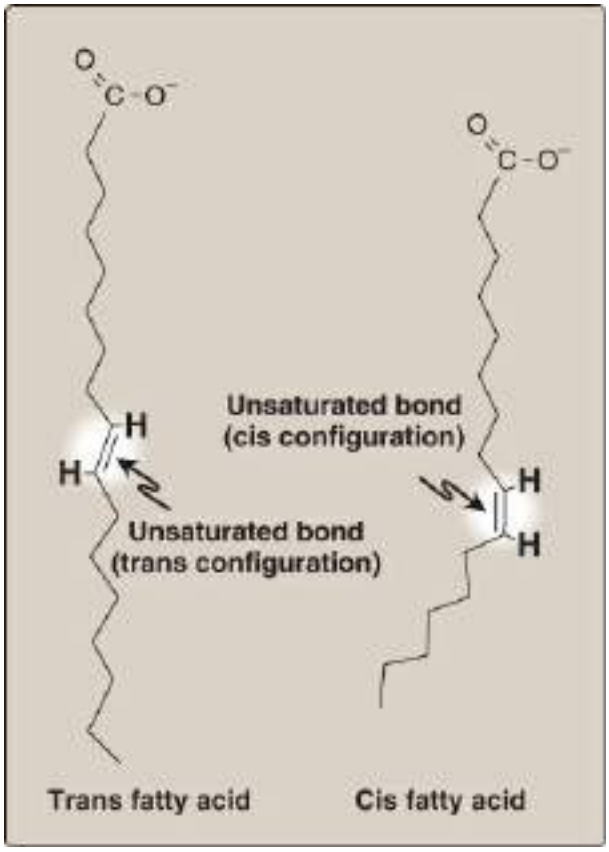


Figure 27.12
Structure of cis and trans fatty acids.

4. Trans fatty acids: Trans fatty acids (Fig. 27.12) are chemically classified as unsaturated fatty acids but behave more like saturated fatty acids in the body because they elevate LDL-C and lower HDL-C, thereby increasing the risk of CHD. Trans fatty acids do not occur naturally in plants but occur in small amounts in animals. However, trans fatty acids are formed during the hydrogenation of vegetable oils (e.g., in the manufacture of margarine and partially hydrogenated vegetable oil). Trans fatty acids are a major component of many commercial baked goods, such as cookies, and most deep-fried foods. Many manufacturers have reformulated their products to be free of trans fats. In 2006, the U.S. Food and Drug Administration required that Nutrition Facts labels (see Section VIII B2) portray the trans-fat content of packaged food and has taken steps to eliminate artificial trans fats in processed foods.

5. Dietary cholesterol: Cholesterol is found only in animal products. The America Heart Association declared in 2015 that “there is insufficient evidence to determine whether lowering dietary cholesterol reduces LDL-C” and the Dietary Guidelines Advisory Committee concluded that the “available evidence shows no appreciable relationship between consumption of dietary cholesterol and serum cholesterol.

C. Other dietary factors affecting coronary heart disease

Moderate consumption of alcohol (up to 1 drink/day for women and up to 2 drinks/day for men) decreases the risk of CHD, because there is a positive correlation between moderate alcohol (ethanol) consumption and the plasma concentration of HDL-C. However, because of the potential dangers of alcohol abuse, health professionals are reluctant to recommend increased alcohol consumption to their patients. Red wine may provide cardioprotective benefits in addition to those resulting from its alcohol content (e.g., red wine contains phenolic compounds that inhibit lipoprotein oxidation). (Note: These antioxidants are also present in raisins and grape juice.) Figure 27.13 summarizes the effects of dietary fats. (Note: Recent studies [including meta-analyses] have raised questions concerning the current guidelines for dietary fat in the prevention of CHD.)

VI. DIETARY CARBOHYDRATES

The primary role of dietary carbohydrates is to provide energy. Although self-reported caloric intake in the United States peaked in 2003 and is now declining, the incidence of obesity has dramatically increased (see Chapter 26). During this same period, carbohydrate consumption has significantly increased (as fat consumption decreased), leading some observers to link obesity with carbohydrate consumption. However, obesity has also been related to increasingly inactive lifestyles and to calorie-dense foods served in expanded portion size. Carbohydrates are not inherently fattening.

TYPE OF FAT	METABOLIC EFFECTS	EFFECTS ON DISEASE PREVENTION
Trans fatty acid	↓ LDL ↓ HDL	↑ Incidence of coronary heart disease
Saturated fatty acid	↑ LDL Little effect on HDL	↑ Incidence of coronary heart disease; may increase risk of prostate, colon cancer
Monounsaturated fatty acid	↓ LDL Maintain or increase HDL	↓ Incidence of coronary heart disease
Polyunsaturated ω-6 fatty acids such as linoleic acid	↓ LDL ↓ HDL Provide arachidonic acid, which is an important precursor of prostaglandins and leukotrienes	↓ Incidence of coronary heart disease
Polyunsaturated ω-3 fatty acids such as DHA	Little effect on LDL Little effect on HDL Suppress cardiac arrhythmias, reduce serum triacylglycerols, decrease the tendency for thrombosis, lower blood pressure, reduce inflammation	↓ Incidence of coronary heart disease ↓ Risk of sudden cardiac death

Figure 27.13
Effects of dietary fats. LDL = low-density lipoprotein; HDL = high-density lipoprotein; DHA = docosahexaenoic acid.

A. Classification

Dietary carbohydrates are classified as simple sugars (monosaccharides and disaccharides), complex sugars (polysaccharides), and fiber.

1. Monosaccharides: Glucose and fructose are the principal monosaccharides found in food. Glucose is abundant in fruits, sweet corn, corn syrup, and honey. Free fructose is found together with free glucose in honey and fruits (e.g., apples).

    a. High-fructose corn syrup: High-fructose corn syrups (HFCSs) are prepared through enzymatic processing to convert glucose into fructose. Pure corn syrup (100% glucose) is then added to fructose to produce a desired sweetness. In the United States, HFCS 55 (containing 55% fructose and 42% glucose) is commonly used as a substitute for sucrose in beverages, including soft drinks, with HFCS 42 used in processed foods. The composition and metabolism of HFCS and sucrose are similar, the major difference being that HFCS is ingested as a mixture of monosaccharides (Fig. 27.14). Most studies have shown no significant difference between sucrose and HFCS meals in either postprandial glucose or insulin responses. (Note: The rise in the use of HFCS parallels the rise in obesity, but a causal relationship has not been demonstrated.)

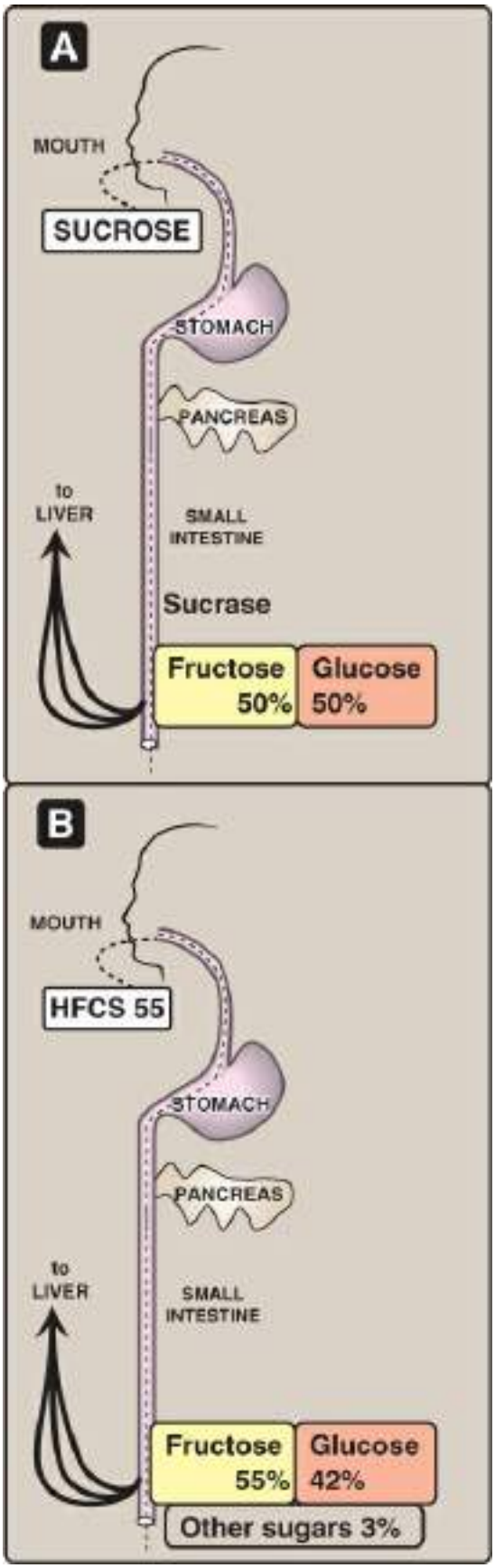


Figure 27.14
Digestion of sucrose, (A), or high-fructose corn syrup (HFCS) 55, (B), leads to absorption of glucose plus fructose.

2. Disaccharides: The most abundant disaccharides are sucrose (glucose + fructose), lactose (glucose + galactose), and maltose (glucose + glucose). Sucrose is ordinary table sugar and is abundant in molasses and maple syrup. Lactose is the principal sugar found in milk. Maltose is a product of enzymic digestion of glycogen and starches in the intestine. It is also found in significant quantities in beer and malt liquors as maltose is found in germinating grains. The term “sugar” refers to monosaccharides and disaccharides. “Added sugars” are those sugars and syrups (such as HFCS) added to foods during processing or preparation.

3. Polysaccharides: Complex carbohydrates are composed of oligosaccharides and polysaccharides which are predominantly polymers of glucose. Examples of polysaccharides are starch, glycogen, and dietary fiber. Starch is an example of a complex carbohydrate that is found in abundance in plants. Common sources include wheat and other grains, potatoes, dried peas and beans (legumes), and vegetables.

4. Fiber: Dietary fiber is the edible part of plants which is nondigestible, nonstarch carbohydrates and lignin (a noncarbohydrate polymer of aromatic alcohols). Although soluble fiber is resistant to digestion and absorption in the human small intestine, it is completely or partially fermented by the gut bacteria to short-chain fatty acids (SCFA) in the large intestine. SCFAs play an essential role in regulating host metabolism, immune system, and cell proliferation. Insoluble fiber passes through the digestive track largely unchanged. Dietary fiber provides little energy but has several beneficial effects. First, it adds bulk to the diet (Fig. 27.15). Fiber can absorb 10 to 15 times its own weight in water, drawing fluid into the lumen of the intestine and increasing bowel motility and promoting bowel movements (laxation). Soluble fiber delays gastric emptying and can result in a sensation of fullness (satiety). This delayed emptying also results in reduced spikes in blood glucose following a meal. Second, consumption of soluble fiber has been shown to lower LDL-C levels by increasing fecal bile acid excretion and interfering with bile acid reabsorption (see Chapter 18 Section V). For example, diets rich (25 to 50 g/day) in the soluble fiber oat bran are associated with a modest, but significant, reduction in risk for CHD by lowering total cholesterol and LDL-C levels. Also, fiber-rich diets decrease the risk for constipation, hemorrhoids, and diverticulosis. The AI for dietary fiber is 25 g/day for women and 38 g/day for men. However, most American diets are far lower in fiber at approximately 15 g/day. (Note: “Functional fiber” is the term used for isolated fiber that has proven health benefits such as commercially available fiber supplements.) Introduction of fiber into the diet should be gradual as it can lead to abdominal discomfort, gas, diarrhea, and even constipation.

B. Dietary carbohydrate and blood glucose

Some carbohydrate-containing foods produce a rapid rise followed by a steep fall in blood glucose concentration, whereas others result in a gradual rise followed by a slow decline (Fig. 27.16). Thus, they differ in their glycemic response (GR). (Note: Fiber blunts the GR.) The glycemic index (GI) ranks carbohydrate-rich foods on a scale of 0 to 100 based on the GR they cause relative to the GR caused by the same amount (50 g) of carbohydrate eaten in the form of white bread or glucose. A low GI is <55, whereas a high GI is ≥70. Evidence suggests that a low-GI diet improves glycemic control in diabetic individuals. Food with a low GI tends to create a sense of satiety over a longer period of time and may be helpful in limiting caloric intake. (Note: How much a typical serving size of a food raises blood glucose is referred to as the glycemic load (GL). A food (e.g., carrots) can have a high GI and a low GL.)

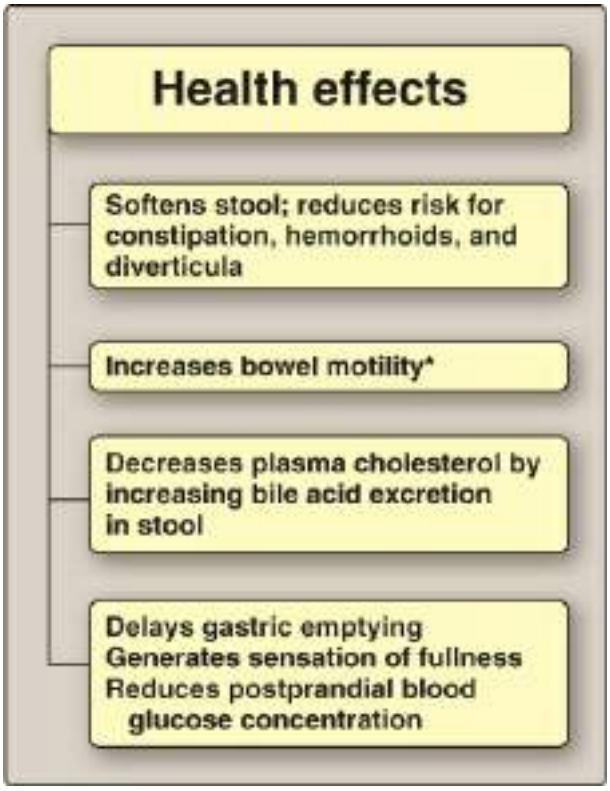


Figure 27.15
Actions of dietary fiber. (Note: *Increasing bowel motility decreases exposure time of the intestines to carcinogens.)

C. Carbohydrate requirements

Carbohydrates are not essential nutrients, because the carbon skeletons of most amino acids can be converted into glucose (see Chapter 20 Section II A). However, they supply essential nutrients such as vitamins and minerals. In addition, the absence of dietary carbohydrate leads to ketogenesis (see Chapter 16 Section V)

and degradation of body protein whose constituent amino acids provide carbon skeletons for gluconeogenesis (see Chapter 10 Section II C). The RDA for carbohydrate is set at 130 g/day for adults and children, based on the amount of glucose used by carbohydrate-dependent tissues, such as the brain and erythrocytes. However, this level of intake is usually exceeded. Adults should consume 45% to 65% of their total calories from carbohydrates. It is now recommended that added sugars represent no more than 10% of total energy intake because of concerns that they may displace nutrient-rich foods from the diet. (Note: Added sugars are associated with increased body weight and type 2 diabetes.)

D. Simple sugars and disease

There is no direct evidence that the consumption of simple sugars naturally present in food is harmful. Contrary to folklore, diets high in sucrose do not lead to diabetes or hypoglycemia. Also contrary to popular belief, carbohydrates are not inherently fattening. They yield 4 kcal/g (the same as protein and less than one half that of fat; see Fig. 27.5) and result in fat synthesis only when consumed in excess of the body's energy needs. However, there is an association between sucrose consumption and dental caries, particularly in the absence of fluoride treatment (see Chapter 29 Section III E).

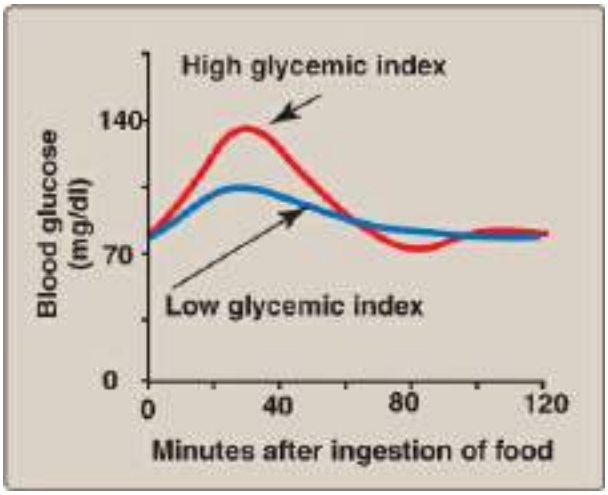


Figure 27.16
Blood glucose concentrations following ingestion of food with a low or high glycemic index (GI). (Note: The GI is defined as the area under the blood glucose curve.)

VII. DIETARY PROTEIN

The AMDR for protein is 10% to 35%. Dietary protein provides the essential amino acids (EAAs) (see Fig. 20.2). Nine of the 20 amino acids needed for the synthesis of body proteins are essential (i.e., they cannot be synthesized in humans).

A. Protein quality

The quality of a dietary protein is a measure of its ability to provide the EAAs required for tissue maintenance. Most government agencies have adopted the Protein Digestibility–Corrected Amino Acid Score (PDCAAS) as the standard by which to evaluate protein quality. PDCAAS is based on the profile of EAA after correcting for the digestibility of the protein. The highest possible score under these guidelines is 1.00. This amino acid score provides a method to balance intakes of poorer-quality proteins with high-quality dietary proteins.

Source	PDCAAS value
Animal proteins	
Egg	1.00
Milk protein	1.00
Beef/poultry/fish	0.82–0.92
Gelatin	0.08
Plant proteins	
Soybean protein	1.00
Kidney beans	0.68
Whole wheat bread	0.40

Figure 27.17
Relative quality of some common dietary proteins. PDCAAS = Protein Digestibility–Corrected Amino Acid Score.

1. Proteins from animal sources: Proteins from animal sources (meat, poultry, milk, and fish) have a high quality because they contain all the EAAs in proportions similar to those required for synthesis of human tissue proteins (Fig. 27.17), and they are more readily digested. (Note: Gelatin prepared from animal collagen is an exception. It has a low biologic value as a result of deficiencies in several EAA.)

2. Proteins from plant sources: Plant proteins have a lower quality than do animal proteins since they have low amounts of more than one of the EAAs. Therefore, they are called incomplete proteins. Animal proteins such as egg, milk and meat are known as complete proteins because they contain adequate levels of all EAAs. Proteins from different plant sources may be combined in such a way that the result is equivalent in nutritional value to animal protein. For example, wheat (lysine deficient but methionine rich) may be combined with kidney beans (methionine poor but lysine rich) to produce a higher biologic value than either of the component proteins (Fig. 27.18). (Note: Animal proteins can also complement the biologic value of plant proteins.)

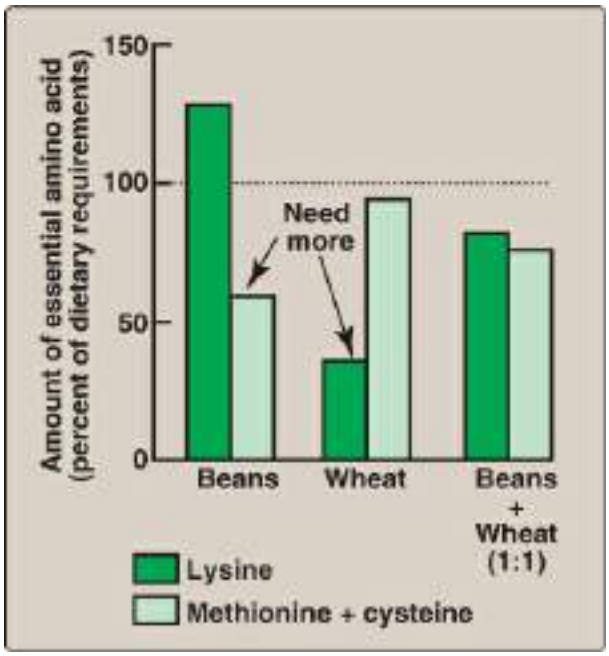


Figure 27.18
Combining two incomplete proteins that have complementary amino acid deficiencies results in a mixture with a higher biologic value.

B. Nitrogen balance

Nitrogen balance occurs when the amount of nitrogen consumed equals that of the nitrogen excreted in the urine (primarily as urinary urea nitrogen, or UUN), sweat, and feces. Most healthy adults are normally in nitrogen balance. (Note: There is, on average, 1 g nitrogen in 6.25 g protein.)

1. Positive nitrogen balance: This occurs when nitrogen intake exceeds nitrogen excretion. It is observed during situations in which tissue growth occurs, for example, in childhood, pregnancy, or during recovery from an emaciating illness.

2. Negative nitrogen balance: This occurs when nitrogen loss is greater than nitrogen intake. It is associated with inadequate dietary protein; lack of an EAA; or during physiologic stresses, such as trauma, burns, illness, or surgery.

Nitrogen (N) balance (g N_{in} − g N_{out}) in a 24-hour period can be determined by the formula, N balance = protein intake in g/6.25 − (UUN + 4 g), where 4 g accounts for urinary loss in forms other than UUN plus loss in skin and feces.

C. Protein requirements

The amount of dietary protein required in the diet varies with its biologic value. The greater the proportion of animal protein in the diet, the less protein is required. The

RDA for protein is computed for proteins of mixed biologic value at 0.8 g/kg of body weight for adults, or approximately 56 g of protein for a 70-kg individual. People who exercise strenuously on a regular basis may benefit from extra protein to maintain muscle mass, and a daily intake of approximately 1 g/kg has been recommended for athletes. Women who are pregnant or lactating require up to 30 g/day in addition to their basal requirements. To support growth, infants should consume 2 g/kg/day. (Note: Disease states influence protein needs. Protein restriction may be needed in kidney disease, whereas burns require increased protein intake.)

1. Consumption of excess protein: There is no physiologic advantage to the consumption of more protein than the RDA. Protein consumed in excess of the body's needs is deaminated, and the resulting carbon skeletons are metabolized to provide energy or acetyl coenzyme A for fatty acid synthesis. When excess protein is eliminated from the body as urinary nitrogen, it is often accompanied by increased urinary calcium, thereby increasing the risk of nephrolithiasis (kidney stones) and osteoporosis.

2. The protein-sparing effect of carbohydrates: The dietary protein requirement is influenced by the carbohydrate content of the diet. When the intake of carbohydrates is low, amino acids are deaminated to provide carbon skeletons for the synthesis of glucose that is needed as a fuel by the central nervous system. If carbohydrate intake is <130 g/day, substantial amounts of protein are metabolized to provide precursors for gluconeogenesis. Therefore, carbohydrate is considered to be "protein-sparing," because it allows amino acids to be used for repair and maintenance of tissue protein rather than for gluconeogenesis.

D. Protein-energy (calorie) malnutrition

In developed countries, protein-energy malnutrition (PEM), also known as protein-energy undernutrition (PEU), is most commonly seen in patients with medical conditions that decrease appetite or alter how nutrients are digested or absorbed or in hospitalized patients with major trauma or infections. (Note: Such highly catabolic patients frequently require intravenous [IV, or parenteral] or tube-based [enteral] administration of nutrients.) PEM may also be seen in children or the elderly who are malnourished. In developing countries, an inadequate intake of protein and/or calories is the primary cause of PEM. Affected individuals show a variety of symptoms, including a depressed immune system with a reduced ability to resist infection. Death from secondary infection is common. PEM is a spectrum of degrees of malnutrition, and two extreme forms are kwashiorkor and marasmus (Table 27.1). (Note: Marasmic kwashiorkor has features of both forms.)

Table 27.1 Physical Features of Extreme Protein-Energy Malnutrition (PEM) in Children

Type of PEM	Weight for Age (% Expected)	Weight for Height	Edema	Muscle and Fat Content
Kwashiorkor	60–80	Normal or ↓	Present	↓
Marasmus	<60	Markedly ↓	Absent	Markedly ↓

Note: The fatty liver and skin and hair changes of kwashiorkor are not seen in marasmus.

1. Kwashiorkor: Kwashiorkor occurs when protein deprivation is relatively greater than the reduction in total calories. Protein deprivation is associated with severely decreased synthesis of visceral protein. Kwashiorkor is commonly seen in developing countries in children after weaning at about age 1 year, when their diet consists predominantly of carbohydrates. Typical symptoms include stunted growth, skin lesions, depigmented hair, anorexia, fatty liver, bilateral pitting edema, and decreased serum albumin concentration. Edema results from the lack of adequate blood proteins, primarily albumin, to maintain the distribution of water between blood and tissues. It may mask muscle and fat loss. Therefore, chronic malnutrition is reflected in the level of serum albumin. (Note: Because caloric intake from carbohydrates may be adequate, insulin levels suppress lipolysis and proteolysis. Kwashiorkor is, therefore, nonadapted malnutrition.)

2. Marasmus: Marasmus occurs when calorie deprivation is relatively greater than the reduction in protein. It usually occurs in developing countries in children younger than age 1 year when breast milk is supplemented or replaced with watery gruels of native cereals that are usually deficient in both protein and calories. Typical symptoms include arrested growth, extreme muscle wasting and depletion of subcutaneous fat (emaciation), weakness, and anemia (Fig. 27.19). Individuals with marasmus do not show the edema observed in kwashiorkor. (Note: Refeeding severely malnourished individuals can result in hypophosphatemia (see Chapter 29 Section II A2), because any available phosphate is used to phosphorylate carbohydrate intermediates. Milk is frequently given because it is rich in phosphate.)

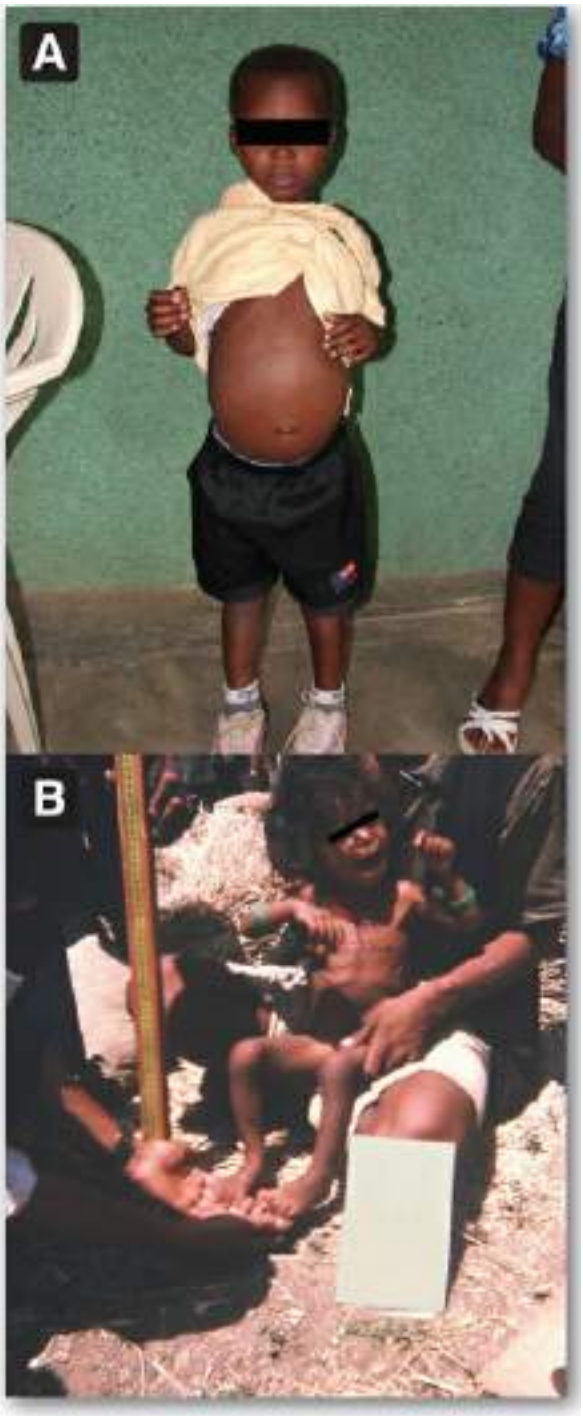


Figure 27.19
A: A child with kwashiorkor. Note the swollen belly and lower legs. **B:** Child suffering with marasmus.

Cachexia, a wasting disorder characterized by loss of appetite and muscle atrophy (with or without increased lipolysis) that cannot be reversed by conventional nutritional support, is seen with a number of chronic diseases, such as cancer and chronic pulmonary and renal disease. It is associated with decreased treatment tolerance and response and decreased survival time.

VIII. NUTRITION TOOLS

A set of tools has been developed that gives consumers information about what (and how much) they should eat as well as the nutritional content of the foods they do eat. Additional tools allow medical professionals to assess whether or not the nutritional needs of an individual are being met.

A. MyPlate

MyPlate was designed by the U.S. Department of Agriculture (USDA) to graphically illustrate its recommendations as to what food groups and how much of each should be consumed daily. In MyPlate, the relative amounts of each of five food groups (vegetables, grains, protein, fruit, and dairy) are represented by the relative size of their section on the plate (Fig. 27.20). The number of servings depends on variables that include age and sex. The Healthy Eating Plate created by experts at Harvard School of Public Health and Harvard Medical School is also used. It differs from MyPlate which recommends limiting milk and substituting with water. In addition, it recommends healthy oils and physical activity.

Figure 27.20
MyPlate.

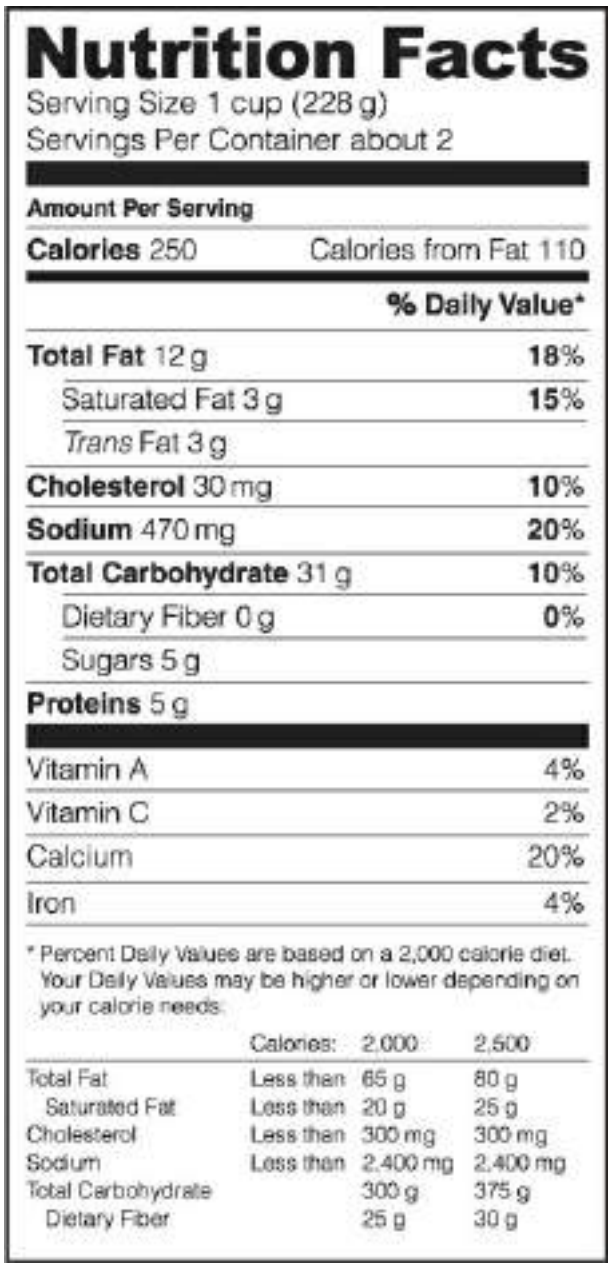
B. Nutrition facts label

Most types of packaged goods are required to have a Nutrition Facts label, or “food label” (Fig. 27.21), that includes the size of a single serving, the Cal it provides, and the number of servings per container. In addition, a percent daily value (%DV) is shown for most nutrients listed. (Note: The %DV is based on a 2,000-Cal diet for healthy adults.)

1. Percent daily value: The %DV compares the amount of a given nutrient in a

single serving of a product to the recommended daily intake for that nutrient. For example, the %DV for the micronutrients listed, as well as for total carbohydrates and fiber, are based on their recommended minimum daily intake. Thus, if the label lists 20% for calcium, one serving provides 20% of the minimum recommended amount of calcium needed each day. In contrast, the %DV for saturated fat, cholesterol, and sodium are based on their recommended maximum daily intake, and the %DV reflects what percentage of this maximum a serving provides. There is no %DV for protein because the recommended intake depends on body weight. (Note: “Sugars” represents mono- and disaccharides. The remainder of the carbohydrate [total carbohydrate – (fiber + sugars)] is the oligo- and polysaccharides.)

2. Nutrition Facts labels: In 2014, the USDA proposed the following changes to the Nutrition Facts label: Added sugars, vitamin D, and potassium are to be included; vitamins A and C, total fat, and Cal from fat are to be removed since the type of fat is more important than the amount; and serving size is to be adjusted to reflect the amounts people are now consuming. Additionally, design was changed to highlight key parts of the label (Fig. 27.22).



Nutrition Facts

Serving Size 1 cup (228 g)
Servings Per Container about 2

Amount Per Serving

Calories 250	Calories from Fat 110
	% Daily Value*
Total Fat 12 g	**18%**
Saturated Fat 3 g	**15%**
Trans Fat 3 g	
Cholesterol 30 mg	**10%**
Sodium 470 mg	**20%**
Total Carbohydrate 31 g	**10%**
Dietary Fiber 0 g	**0%**
Sugars 5 g	
Proteins 5 g	
Vitamin A	4%
Vitamin C	2%
Calcium	20%
Iron	4%

* Percent Daily Values are based on a 2,000 calorie diet. Your Daily Values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65 g	80 g
Saturated Fat	Less than	20 g	25 g
Cholesterol	Less than	300 mg	300 mg
Sodium	Less than	2,400 mg	2,400 mg
Total Carbohydrate		300 g	375 g
Dietary Fiber		25 g	30 g

Figure 27.21
Nutrition Facts label (food label).

C. Nutrition assessment

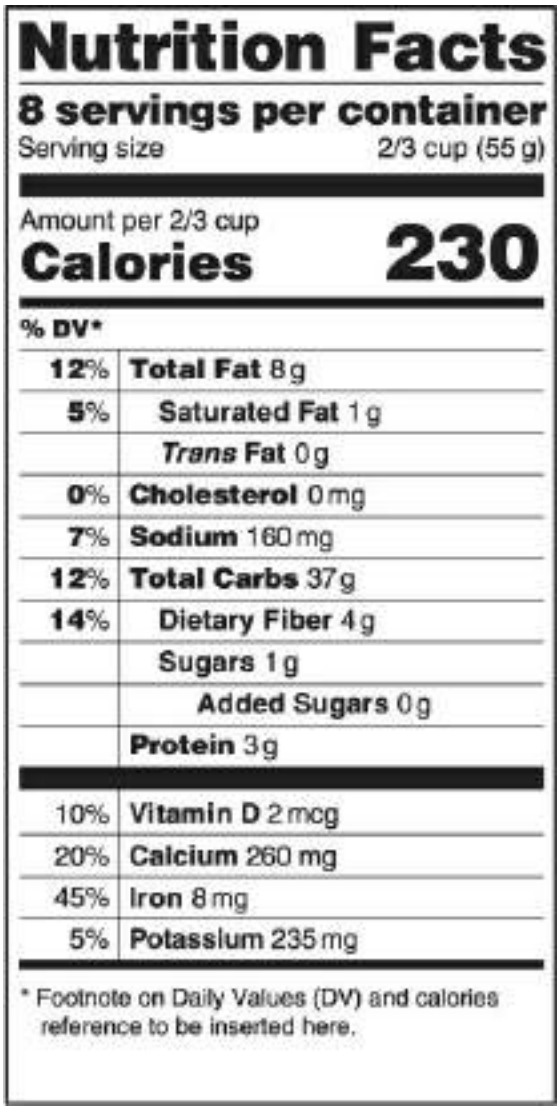
Nutrition assessment evaluates nutritional status based on clinical information. It includes (but is not limited to) dietary history, anthropometric measures, and laboratory data. Assessment findings may result in medical nutrition therapy (MNT), which is an evidence-based medical approach for the treatment of certain medical conditions using a personalized nutrition plan. For example, MNT for hyperlipidemia involves reducing the quantity and types of fats and often calories in the diet.

1. Dietary history: This is a record of food intake over a period of time. For a food

diary, the specific types and exact amounts of food eaten are recorded in “real time” (as soon as possible after eating) for a period of 3 to 7 days. Retrospective approaches include a food frequency questionnaire (e.g., what fruits were eaten and how often they were eaten in a typical day, week, or month) and a 24-hour recall of the specific foods and the amounts eaten in the last 24 hours.

2. Anthropometric measures: These are physical measures of the body. They include (but are not limited to) weight, height, body mass index (an indicator of obesity, see Chapter 26 II A), skin-fold thickness (an indicator of subcutaneous fat), and waist circumference (an indicator of abdominal fat, see Chapter 26 II). (Note: Ideal body weight can be calculated using the Hamwi method: 106 lb [for males] or 100 lb [for females] for the first 5 ft of height + 5 lb for every inch over 5 ft, with an adjustment of −10% for a small frame and +10% for a large one.)

3. Laboratory data: These are obtained by tests performed on body fluids, tissues, and waste. They can include plasma LDL-C (for cardiovascular risk), fecal fat (for malabsorption), red cell indices (for vitamin deficiencies), and N balance and serum proteins (such as albumin and transthyretin [prealbumin]) for protein–energy status. (Note: These proteins are made in the liver and transport molecules such as fatty acids and thyroxine [see Chapter 29 Section IV A] through blood. Low albumin levels correlate with increased morbidity and mortality in hospitalized patients. The short half-life (2 to 3 days) of transthyretin as compared to that of albumin [20 days] has led to its use in monitoring the progress of hospitalized patients.)



Nutrition Facts

8 servings per container

Serving size 2/3 cup (55 g)

Amount per 2/3 cup

Calories 230

% DV*	
12%	**Total Fat** 8 g
5%	**Saturated Fat** 1 g
	***Trans* Fat** 0 g
0%	**Cholesterol** 0 mg
7%	**Sodium** 160 mg
12%	**Total Carbs** 37 g
14%	**Dietary Fiber** 4 g
	Sugars 1 g
	Added Sugars 0 g
	Protein 3 g
10%	**Vitamin D** 2 mcg
20%	**Calcium** 260 mg
45%	**Iron** 8 mg
5%	**Potassium** 235 mg

* Footnote on Daily Values (DV) and calories reference to be inserted here.

Figure 27.22
Nutrition Facts label showing changes proposed in 2014 for implementation by 2018.

Nutritional insufficiency can be the result of inadequate nutrient intake (caused, e.g., by an inability to eat, loss of appetite, or decreased availability), inadequate absorption, decreased utilization, increased excretion, or increased requirements.

IX. NUTRITION AND THE LIFE STAGES

Macronutrient energy sources, micronutrients, EFA, and EAA are required at every life stage. Additionally, each stage has specific nutrition needs.

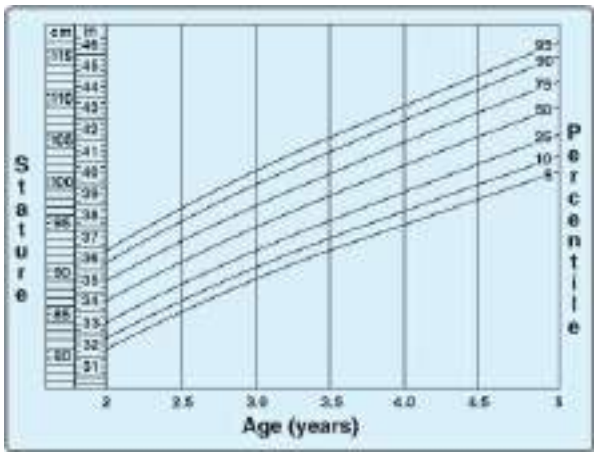


Figure 27.23
Clinical growth chart of stature-for-age for boys age 2 to 5 years from the Centers for Disease Control and Prevention (CDC) (see https://www.cdc.gov/growthcharts/). Charts for girls are pink.

A. Infancy, childhood, and adolescence

The rapid growth and development in infancy (birth to age 1 year) and childhood (age 1 year to adolescence) necessitate higher energy and protein needs relative to body size than are required in subsequent life stages. In adolescence, the marked increases in height and weight that occur increase nutritional needs. Growth charts (Fig. 27.23) are used to compare an individual's stature (height) and/or weight to the expected values for others of the same age (≤20 years) and sex. They are based on data from large numbers of normal individuals over time. (Note: Deviations from the expected growth curve, as reflected in the crossing of two or more percentile lines, raise concern.)

1. Infants: Ideal infant nutrition is based on human breast milk because it provides calories and most micronutrients in amounts appropriate for the human infant. Carbohydrates, protein, and fat are present in a 7:3:1 ratio. (Note: In addition to the disaccharide lactose, human milk contains nearly 200 unique oligosaccharides. About 90% of the microbiota [the population of microbes] in the breast-fed infant's intestine is represented by one type, *Bifidobacterium infantis*, which expresses all the enzymes needed to degrade these complex sugars. The sugars, in turn, act as prebiotics that support the growth of *B. infantis*, a probiotic [helpful bacteria].) Breast milk is low in vitamin D; however, exclusively breast-fed babies require vitamin D supplementation. (Note: Human milk provides antibodies and other proteins that reduce the risk of infection.)

> The microbiota in and on the human body plus their genomes are referred to as the microbiome. It is acquired at birth from the environment and changes with the life stages. The gut microbiome influences host nutrition by facilitating processing of food consumed and is itself influenced by that food. Its relationship with undernutrition, obesity, and diabetes is under investigation.

2. Children: As with infants, children have increased need for calories and nutrients. The primary concerns in this stage, however, are deficiencies of iron and calcium.

3. Adolescents: In the teen years, the increases in height and weight increase the need for calories, protein, calcium, iron, and phosphorus. Eating patterns in this stage can result in overconsumption of fat, sodium, and sugar and underconsumption of vitamin A, thiamine, and folic acid. (Note: Eating disorders and obesity are concerns in this age group.)

B. Adulthood

Overnutrition is a concern in young adults, whereas malnutrition is a concern in older adults.

1. Young adults: Nutrition in young adults focuses on the maintenance of good health and the prevention of disease. The goal is a diet rich in plant-based foods (with a focus on fiber and whole grains), limited intake of saturated fat and trans fatty acids, and balanced intake of ω-3 and ω-6 PUFA.

2. Pregnant or lactating women: The requirements for calories, protein, and virtually all micronutrients increase in pregnancy and lactation. Supplementation with folic acid (to prevent neural tube defects [see Chapter 28 Section II 2]), vitamin D, calcium, iron, iodine, and DHA is typically recommended.

3. Older adults: Aging increases the risk of malnutrition. Decreased appetite resulting from a reduced sense of taste (dysgeusia) and smell (hyposmia) decreases nutrient intake. (Note: Physical limitations, including problems with dentition, and psychosocial factors, such as isolation, may also play a role in reduced intake.) Inadequate intake of protein, calcium, and vitamins D and B_{12} is common. B_{12} deficiency can result from decreased absorption caused by achlorhydria (reduced stomach acid, see Chapter 28 IV). In aging, lean muscle mass decreases and fat increases, resulting in decreased RMR. (Note: Drug–nutrient interactions can occur at any life stage but are more common as the number of medications increases as in aging.)

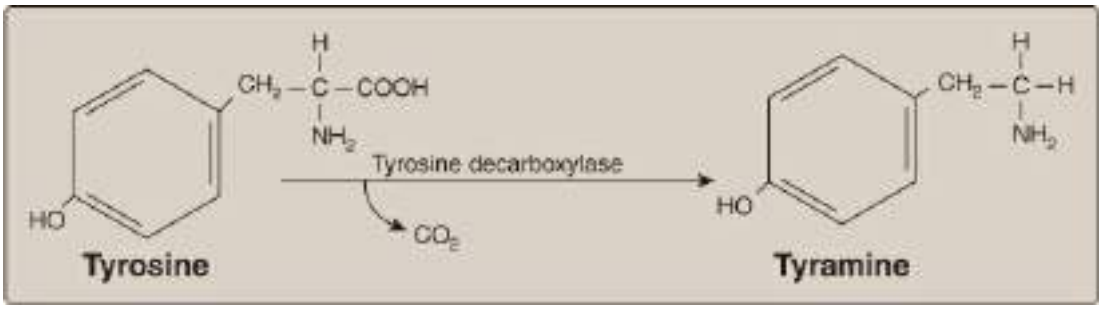


Figure 27.24
Decarboxylation of tyrosine to tyramine. CO_2 = carbon dioxide.

Clinical Application 27.1: Monoamine Oxidase Inhibitors

Monoamine oxidase inhibitors (MAOIs), used to treat depression (see Chapter 21 Section III A4) and early Parkinson disease, can interact with tyramine-containing foods. Tyramine is a monoamine derived from the decarboxylation of tyrosine during the curing, aging, or fermentation of food (Fig. 27.24). It causes the release of norepinephrine, increasing blood pressure, and heart rate. Patients who take MAOIs and consume such foods are at risk for a hypertensive crisis.

X. Chapter Summary

- The **Dietary Reference Intakes** (**DRI**) provide estimates of the amounts of nutrients required to prevent deficiencies and maintain optimal health and growth.
- DRIs consist of the **Estimated Average Requirement** (**EAR**), the **Recommended Dietary Allowance** (**RDA**), the **Adequate Intake** (**AI**), and the **Tolerable Upper Intake Level** (**UL**).
- **Estimated Average Requirement** (**EAR**) is the average daily nutrient intake level estimated to meet the requirement of 50% of the healthy individuals in a particular life stage (age) and gender group.
- **Recommended Dietary Allowance** (**RDA**) is the average daily dietary intake level that is sufficient to meet the nutrient requirements of nearly all (97% to 98%) individuals in a life stage and gender group.
- **Adequate Intake** (**AI**) is set instead of an RDA if sufficient scientific evidence is not available to calculate the RDA.
- **Tolerable Upper Intake Level** (**UL**) is the highest average daily nutrient intake level that is likely to pose no risk of adverse health effects to almost all individuals in the general population.
- The energy generated by the metabolism of the **macronutrients** (9 kcal/g of fat and 4 kcal/g of protein or carbohydrate) is used for three energy-requiring processes that occur in the body: **resting metabolic rate**, **physical activity**, and the **thermic effect of food**.
- **Acceptable Macronutrient Distribution Ranges** (**AMDR**) are defined as the ranges of intake for a particular macronutrient that are associated with reduced risk of chronic disease while providing adequate amounts of essential nutrients.
- Adults should consume **45% to 65%** of their **total calories** from **carbohydrates**, **20% to 35%** from **fat**, and **10% to 35%** from **protein** (Fig. 27.25).
- Elevated levels of cholesterol in low-density lipoproteins (LDL-C) result in increased risk for **coronary heart disease** (**CHD**).
- Elevated levels of cholesterol in high-density lipoproteins (HDL-C) have been associated with a decreased risk for CHD.
- Dietary or drug treatment of **hypercholesterolemia** is effective in decreasing LDL-C, increasing HDL-C, and reducing the risk for CHD.
- Consumption of **saturated fats** is strongly associated with high levels of total plasma and LDL-C. When substituted for saturated fatty acids in the diet, **monounsaturated fats** lower both total plasma cholesterol and LDL-C but maintain or increase HDL-C.
- Consumption of fats containing ω**-6 polyunsaturated fatty acids** lowers plasma LDL-C, but HDL-C, which protects against CHD, is also lowered.
- Dietary ω**-3 polyunsaturated fats** suppress cardiac arrhythmias and reduce plasma triacylglycerols, decrease the tendency for thrombosis, and substantially reduce the risk of cardiovascular mortality.
- **Carbohydrates** provide **energy** and **fiber** to the diet. When they are consumed as part of a diet in which caloric intake is equal to energy expenditure, they do not promote obesity.
- Dietary **protein** provides **essential amino acids**.
- **Protein quality** is a measure of its ability to provide the essential amino acids required for tissue maintenance. Proteins from animal sources, in general, have a higher-quality protein than that derived from plants. However, proteins from different plant sources may be combined in such a way that the result is equivalent in nutritional value to animal protein.
- **Positive nitrogen** (**N**) **balance** occurs when N intake exceeds N excretion. It is observed in situations in which tissue growth occurs, for example, in childhood, pregnancy, or during recovery from an emaciating illness.
- **Negative N balance** occurs when N losses are greater than N intake. It is associated with inadequate dietary protein; lack of an essential amino acid; or during physiologic stresses such as trauma, burns, illness, or surgery.
- **Kwashiorkor** occurs when protein deprivation is relatively greater than the reduction in total calories. It is characterized by edema.
- **Marasmus** occurs when calorie deprivation is relatively greater than the reduction in protein. No edema is seen. Both are extreme forms of **protein-energy malnutrition** (**PEM**). **Nutrition Facts labels** give

consumers information about the nutritional content of packaged foods.

- Medical assessment of nutritional status includes **dietary history**, **anthropometric measures**, and **laboratory data**. Each life stage has specific nutrition needs.
- **Growth charts** are used to monitor the growth pattern of an individual from birth through adolescence.
- **Drug–nutrient interactions** are of concern, especially in older adults.

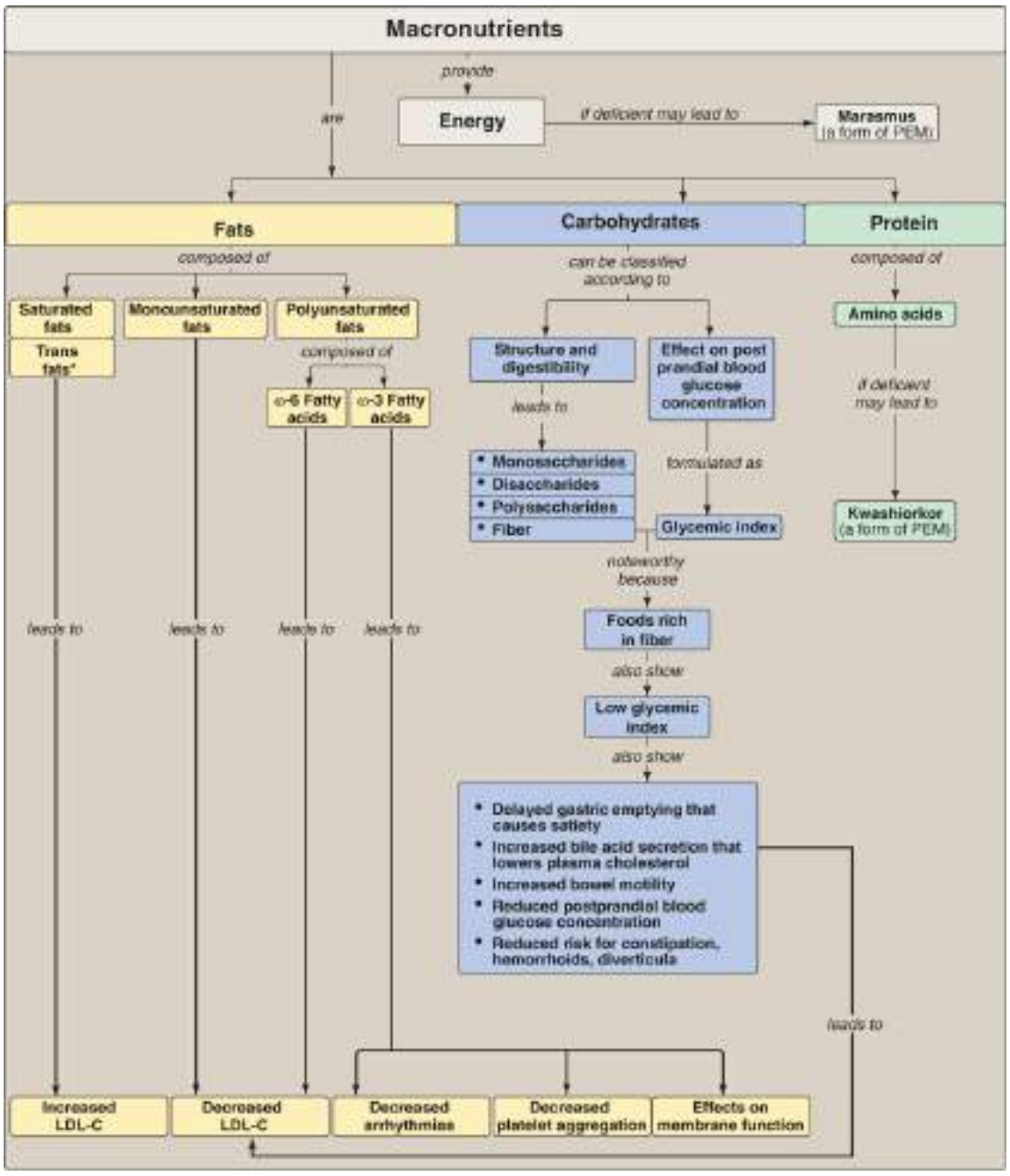


Figure 27.25
Key concept map for the macronutrients. (Note: *Trans fatty acids are chemically classified as unsaturated.) PEM = protein-energy malnutrition; LDL = low-density lipoprotein; C = cholesterol.

Study Questions

Choose the ONE best answer.

27.1 For the child shown at right, which of the statements would support a diagnosis of kwashiorkor? The child:

A. appears plump due to increased deposition of fat in adipose tissue.
B. displays abdominal and peripheral edema.
C. has a serum albumin level above normal.
D. has markedly decreased weight for height.

The correct answer = B. Kwashiorkor is caused by inadequate protein intake in the presence of fair to good energy (calorie) intake. Typical findings in a patient with kwashiorkor include abdominal and peripheral edema (note the swollen belly and legs) caused largely by a decreased serum albumin concentration. Body fat stores are depleted, but weight for height can be normal because of edema. Treatment includes a diet adequate in calories and protein.

27.2 Which one of the following statements concerning dietary fat is correct?

A. Coconut oil is rich in monounsaturated fats, and olive oil is rich in saturated fats.
B. Fatty acids containing trans double bonds, unlike the naturally occurring cis isomers, raise high-density lipoprotein cholesterol levels.
C. The polyunsaturated fatty acids linoleic and linolenic acids are required components.
D. Triacylglycerols obtained from plants generally contain less unsaturated fatty acids than do those from animals.

Correct answer = C. Humans are unable to make linoleic and linolenic fatty acids. Consequently, these fatty acids are essential in the diet. Coconut oil is rich in saturated fats, and olive oil is rich in monounsaturated fats. Trans fatty acids raise plasma levels of low-density lipoprotein cholesterol, not high-density lipoprotein cholesterol. Triacylglycerols obtained from plants generally contain more unsaturated fatty acids than do those from animals.

27.3 Given the information that a 70-kg man is consuming a daily average of 275 g of carbohydrate, 75 g of protein, and 65 g of fat, which one of the following conclusions can reasonably be drawn?

A. About 20% of calories are derived from fats.
B. The diet contains a sufficient amount of fiber.
C. The individual is in nitrogen balance.
D. The proportions of carbohydrate, protein, and fat in the diet conform to current recommendations.
E. The total energy intake per day is about 3,000 kcal.

Correct answer = D. The total energy intake is (275 g carbohydrate × 4 kcal/g) + (75 g protein × 4 kcal/g) + (65 g fat × 9 kcal/g) = 1,100 + 300 + 585 = 1,985 total kcal/day. The percentage of calories derived from carbohydrate is 1,100/1,985 = 55, from protein is 300/1,985 = 15, and from fat is 585/1,985 = 30. These are very close to current recommendations. The amount of fiber or nitrogen balance cannot be deduced from the data presented. If the protein is of low biologic value, a negative nitrogen balance is possible.

27.4 In chronic bronchitis, excessive mucus production causes airway obstruction that results in hypoxemia (low blood oxygen level), impaired expiration, and hypercapnia (carbon dioxide retention). Why might a high-fat, low-carbohydrate diet be recommended for a patient with chronic obstructive pulmonary disease caused by chronic bronchitis?

A. Fat contains more oxygen atoms relative to carbon or hydrogen atoms than do carbohydrates.
B. Fat is calorically less dense than carbohydrates.
C. Fat metabolism generates less carbon dioxide.
D. The respiratory quotient (RQ) for fat is higher than the RQ for carbohydrates.

Correct answer = C. A treatment goal for the chronic obstructive pulmonary disease (COPD) caused by acute bronchitis is to insure appropriate nutrition without increasing the respiratory quotient (RQ), which is the ratio of carbon dioxide (CO_2) produced to oxygen consumed, thereby minimizing the production of CO_2. Less CO_2 is produced from the metabolism of fat (RQ = 0.7) than from the catabolism of carbohydrate (RQ = 1.0). Fat contains fewer oxygen atoms. Fat is calorically denser than is carbohydrate. (Note: RQ is determined by indirect calorimetry.)

27.5 A 32-year-old male who was rescued from a house fire was admitted to the hospital with burns over 45% of his body (severe burns). The patient weighs 154 lb (70 kg) and is 72 in (183 cm) tall. Which one of the following is the best rapid estimate of the immediate daily caloric needs of this patient?

A. 1,345 kcal
B. 1,680 kcal
C. 2,690 kcal
D. 3,360 kcal

Correct answer = D. A commonly used rough estimate of the total energy expenditure (TEE) for men is 1 kcal/1 kg body weight/24 hrs. (Note: It is 0.8 kcal for females.) For this patient, that value is 1,680 kcal (1 kcal/kg/hr × 24 hours × 70 kg). In addition, an injury factor of 2 for severe burns must be included in the calculation: 1,680 kcal × 2 = 3,360 kcal.

27.6 Which one of the following is the best advice to give a patient who asks about the notation “%DV” (percent daily value) on the Nutrition Facts label?

A. Achieve 100% daily value for each nutrient each day.
B. Select foods that have the highest percent daily value for all nutrients.
C. Select foods with a low percent daily value for the micronutrients.
D. Select foods with a low percent daily value for saturated fat.

Correct answer = D. The percent daily value (%DV) compares the amount of a given nutrient in a single serving of a product to the recommended daily intake for that nutrient. The %DV for the micronutrients listed on the label, as well as for total carbohydrates and fiber, are based on their recommended minimum daily intake, whereas the %DV for saturated fat, cholesterol, and sodium are based on their recommended maximum daily intake.

For Questions 27.7 and 27.8, use the following case.

A sedentary 50-year-old male weighing 176 lb (80 kg) requests a physical. He denies any health problems. Routine blood analysis is unremarkable except for plasma total cholesterol of 295 mg/dL. (Reference value is <200 mg.) The man refuses drug therapy for his hypercholesterolemia. Analysis of a 1-day dietary recall showed the following:

Kilocalories	3,475 kcal	Cholesterol	822 mg
Protein	102 g	Saturated fat	69 g
Carbohydrate	383 g	Total fat	165 g
Fiber	6 g		

27.7 Decreasing which one of the following dietary components would have the greatest effect in lowering the patient's plasma cholesterol?

A. Carbohydrates
B. Cholesterol
C. Fiber
D. Monounsaturated fat
E. Polyunsaturated fat
F. Saturated fat

Correct answer = F. The intake of saturated fat most strongly influences plasma cholesterol in this diet. The patient is consuming a high-calorie, high-fat diet with 42% of the fat as saturated fat. The most important dietary recommendations are to lower total caloric intake, substitute monounsaturated and polyunsaturated fats for saturated fats, and increase dietary fiber. A decrease in dietary cholesterol would be helpful but is not a primary objective.

27.8 What information would be necessary to estimate the patient's total energy expenditure?

The daily basal energy expenditure (estimated resting metabolic rate/hour × 24 hours) and a physical activity ratio (PAR) based on the type and duration of physical activities are needed variables. An additional 10% would be added to account for the thermic effect of food. Note that if the patient were hospitalized, an injury factor (IF) would be included in the calculation, and the PAR would be modified. Tables of PAR and IF are available.

Micronutrients: Vitamins 28

I. OVERVIEW

Vitamins are organic molecules that cannot be synthesized in adequate quantities by humans and, therefore, must be supplied by the diet. Nine vitamins (folic acid, cobalamin, ascorbic acid, pyridoxine, thiamine, niacin, riboflavin, biotin, and pantothenic acid) are classified as water soluble. Because they are readily excreted in the urine, toxicity is rare. However, deficiencies can develop quickly. Four vitamins (A, D, K, and E) are termed fat soluble (Fig. 28.1). They are released, absorbed, and transported (in chylomicrons, see Chapter 18 Section VI B) with dietary fat. They are stored in the liver and adipose tissue and are eliminated slower than the water-soluble vitamins. In fact, consumption of vitamins A and D in excess of the Dietary Reference Intakes (see Chapter 27) can lead to accumulation of toxic quantities of these compounds. Vitamins are required to perform specific cellular functions. For example, many of the water-soluble vitamins are precursors of coenzymes for the enzymes of intermediary metabolism. In contrast to the water-soluble vitamins, only one fat-soluble vitamin (vitamin K) has a coenzyme function.

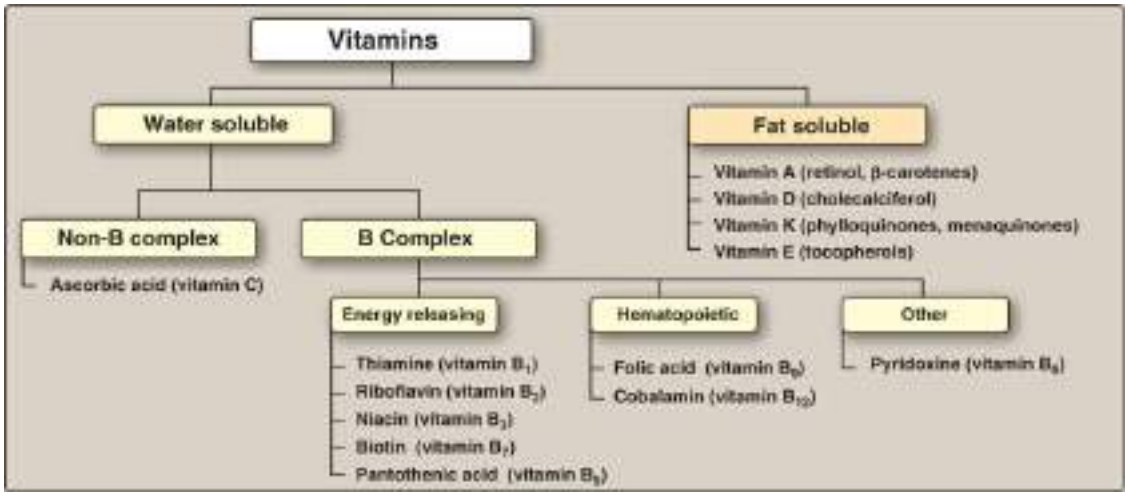


Figure 28.1
Classification of the vitamins. Because they are required in lesser amounts than the macronutrients (carbohydrate, protein, and lipid), vitamins are termed micronutrients.

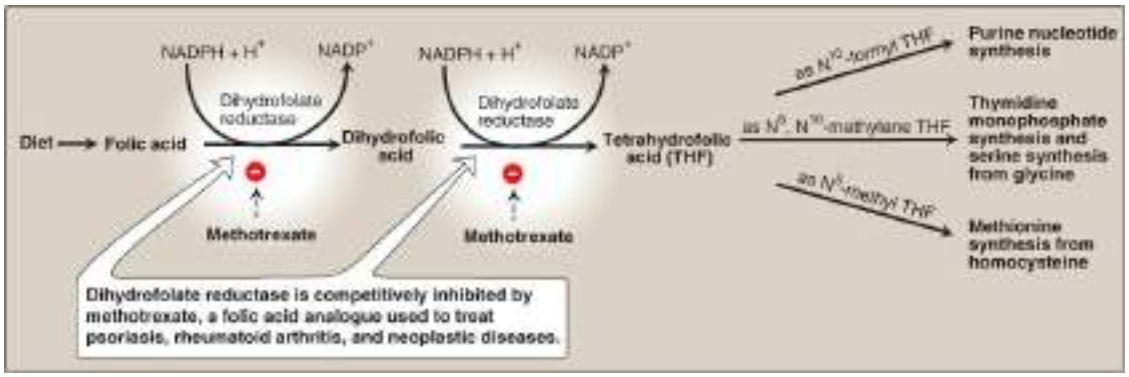


Figure 28.2
Production and use of tetrahydrofolate. NADP(H) = nicotinamide adenine dinucleotide phosphate.

II. FOLIC ACID (VITAMIN B_9)

Vitamin B_9 describes many forms of naturally occurring folate. Folic acid is the synthetic form of folate that is used in supplements and in fortification of foods. However, these two terms, folic acid and folate, are often used interchangeably. Folic acid plays a key role in one-carbon metabolism, and it is essential for the biosynthesis of several compounds. Folic acid deficiency is probably the most common vitamin deficiency in the United States, particularly among pregnant women and individuals with alcoholism. (Note: Leafy, dark-green vegetables are a good source of folic acid.)

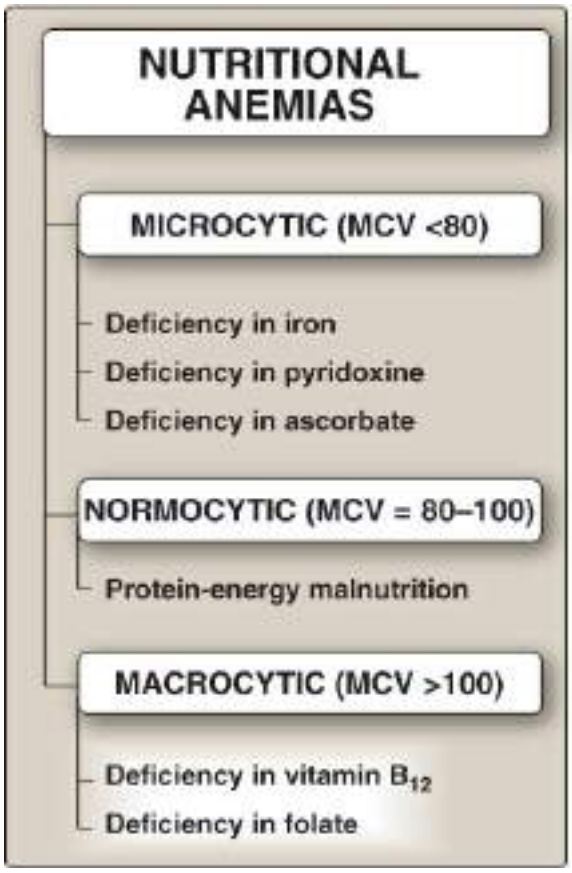


Figure 28.3
Classification of nutritional anemias by red cell size. The normal mean corpuscular volume (MCV) for people older than age 18 years is 80 to 100 μm^3. (Note: Microcytic anemia is also seen with heavy metal [e.g., lead] poisoning.)

A. Function

Tetrahydrofolate (THF), the reduced, coenzyme form of folate, receives one-carbon fragments from donors such as serine, glycine, and histidine and transfers them to intermediates in the synthesis of amino acids, purine nucleotides, and thymidine monophosphate (TMP), a pyrimidine nucleotide incorporated into DNA (Fig. 28.2).

B. Nutritional anemias

Anemia is a condition in which the blood has a lower than normal concentration of hemoglobin, which results in a reduced ability to transport oxygen (O_2). Nutritional anemias (i.e., those caused by inadequate intake of one or more essential nutrients) can be classified according to the size of the red blood cells (RBCs), or mean corpuscular volume (MCV), observed in the blood (Fig. 28.3). Microcytic anemia (MCV below normal), caused by lack of iron, is the most common form of nutritional anemia. The second major category of nutritional anemia, macrocytic (MCV above normal), results from a deficiency in folic acid or vitamin B_{12}. (Note: These macrocytic anemias are commonly called megaloblastic because a deficiency of

either vitamin [or both] causes accumulation of large, immature RBC precursors, known as megaloblasts, in the bone marrow and the blood [Fig. 28.4]. Hypersegmented neutrophils are also seen.)

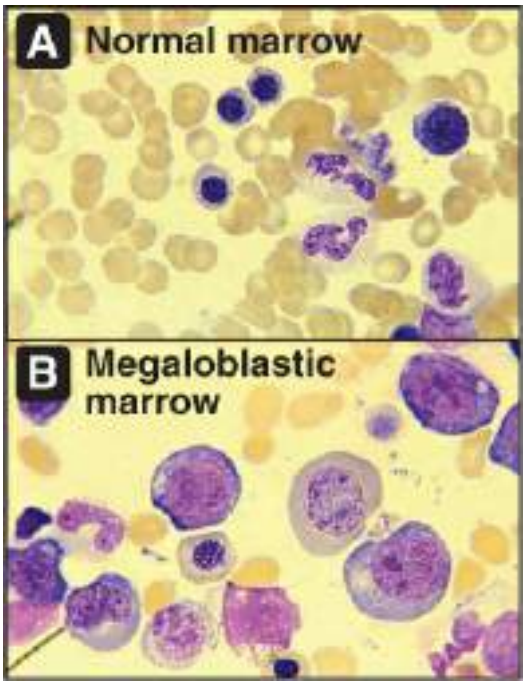


Figure 28.4
Bone marrow histology in normal (**A**) and folate-deficient (**B**) individuals.

1. Folate and anemia: Inadequate serum levels of folate can be caused by increased demand (e.g., pregnancy and lactation; see Chapter 27 Section IX), poor absorption caused by pathology of the small intestine, alcoholism, or treatment with drugs (e.g., methotrexate) that are dihydrofolate reductase inhibitors (see Fig. 28.2). A folate-free diet can cause a deficiency within a few weeks. A primary result of folic acid deficiency is megaloblastic anemia (see Fig. 28.4), caused by diminished synthesis of purine nucleotides and TMP, which leads to an inability of cells (including RBC precursors) to make DNA and, therefore, an inability to divide.

2. Folate and neural tube defects: Spina bifida and anencephaly, the most common neural tube defects (NTDs), affect ~3,000 pregnancies in the United States annually. Folic acid supplementation before conception and during the first trimester has been shown to significantly reduce NTD. Therefore, all women of childbearing age are advised to consume 0.4 mg/day (400 µg/day) of folic acid to reduce the risk of having a pregnancy affected by NTD and ten times that amount if a previous pregnancy was affected. Adequate folate nutrition must occur at the time of conception because critical folate-dependent development occurs in the first weeks of fetal life, at a time when many women are not yet aware of their pregnancy. In 1998, the U.S. Food and Drug

Administration authorized the fortification of cereal grain products with folic acid and also recommended folate supplementation in the form of pills resulting in a dietary supplementation of ~0.1 mg/day. This supplementation allows ~50% of all reproductive-aged women to receive 0.4 mg of folate from all sources.

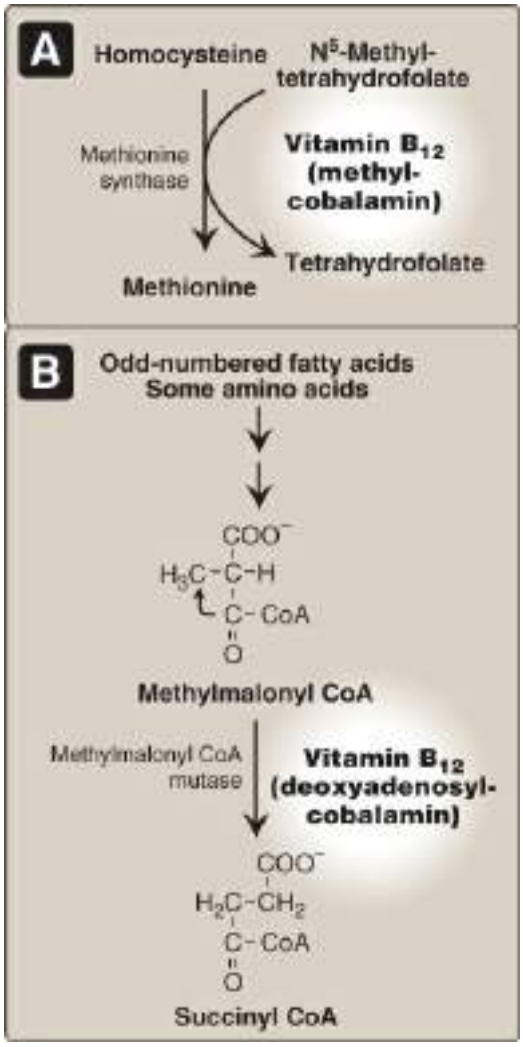


Figure 28.5
A, B: Reactions requiring coenzyme forms of vitamin B_{12}. CoA = coenzyme A.

III. COBALAMIN (VITAMIN B_{12})

Vitamin B_{12} is required in humans for two essential enzymatic reactions: the remethylation of homocysteine (Hcy) to methionine and the isomerization of methylmalonyl coenzyme A (CoA), which is produced during the degradation of some amino acids (isoleucine, valine, threonine, and methionine) and fatty acids (FA) with odd numbers of carbon atoms (Fig. 28.5). When cobalamin is deficient, unusual (branched) FA accumulate and become incorporated into cell membranes, including those of the central nervous system (CNS). This may account for some of the neurologic

manifestations of vitamin B_{12} deficiency. (Note: Folic acid [as N^5-methyl THF] is also required in the remethylation of Hcy. Therefore, deficiency of B_{12} or folate results in elevated Hcy levels.)

A. Structure and coenzyme forms

Cobalamin contains a corrin ring system that resembles the porphyrin ring of heme (see Chapter 21), but differs in that two of the pyrrole rings are linked directly rather than through a methene bridge. Cobalt (see Chapter 29 Section IV) is held in the center of the corrin ring by four coordination bonds with the nitrogens of the pyrrole groups. The remaining coordination bonds of the cobalt are with the nitrogen of 5,6-dimethylbenzimidazole and with cyanide in commercial preparations of the vitamin in the form of cyanocobalamin (Fig. 28.6). The physiologic coenzyme forms of cobalamin are 5′-deoxyadenosylcobalamin and methylcobalamin, in which cyanide is replaced with 5′-deoxyadenosine or a methyl group, respectively (see Fig. 28.6).

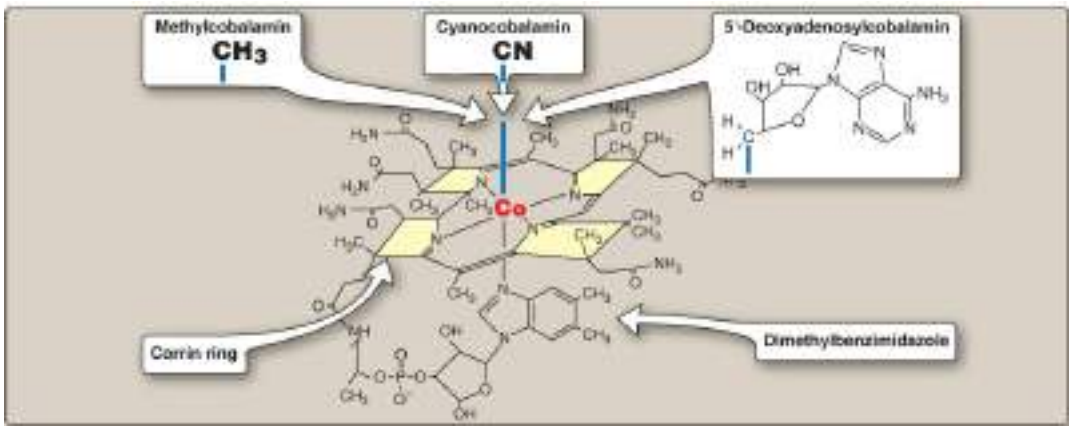


Figure 28.6
Structure of vitamin B_{12} (cyanocobalamin) and its coenzyme forms (methylcobalamin and 5′-deoxyadenosylcobalamin).

B. Distribution

Vitamin B_{12} is synthesized only by microorganisms, and it is not present in plants. Animals obtain the vitamin preformed from their intestinal microbiota (see Chapter 27 Section IX A) or by eating foods derived from other animals. Cobalamin is present in appreciable amounts in liver, red meat, fish, eggs, dairy products, and fortified cereals.

C. Folate trap hypothesis

The effects of cobalamin deficiency are most pronounced in rapidly dividing cells, such as the erythropoietic tissue of bone marrow and the mucosal cells of the intestine. Such tissues need both the N^5,N^{10}-methylene and N^{10}-formyl forms of THF for the synthesis of nucleotides required for DNA replication (see pp. 325 and

336). However, in vitamin B_{12} deficiency, the utilization of the N^5-methyl form of THF in the B_{12}-dependent methylation of Hcy to methionine is impaired. Because the methylated form cannot be converted directly to other forms of THF, folate is trapped in the N^5-methyl form, which accumulates. The levels of the other forms decrease. Thus, cobalamin deficiency leads to a deficiency of the THF forms needed in purine and TMP synthesis, resulting in the symptoms of megaloblastic anemia.

D. Clinical indications for cobalamin

In contrast to other water-soluble vitamins, significant amounts (2 to 5 mg) of vitamin B_{12} are stored in the body. As a result, it may take several years for the clinical symptoms of B_{12} deficiency to develop as a result of decreased intake of the vitamin. (Note: Deficiency happens much more quickly [in months] if absorption is impaired [see below]. The Schilling test evaluates B_{12} absorption.) B_{12} deficiency can be determined by the level of methylmalonic acid in blood, which is elevated in individuals with low intake or decreased absorption of the vitamin.

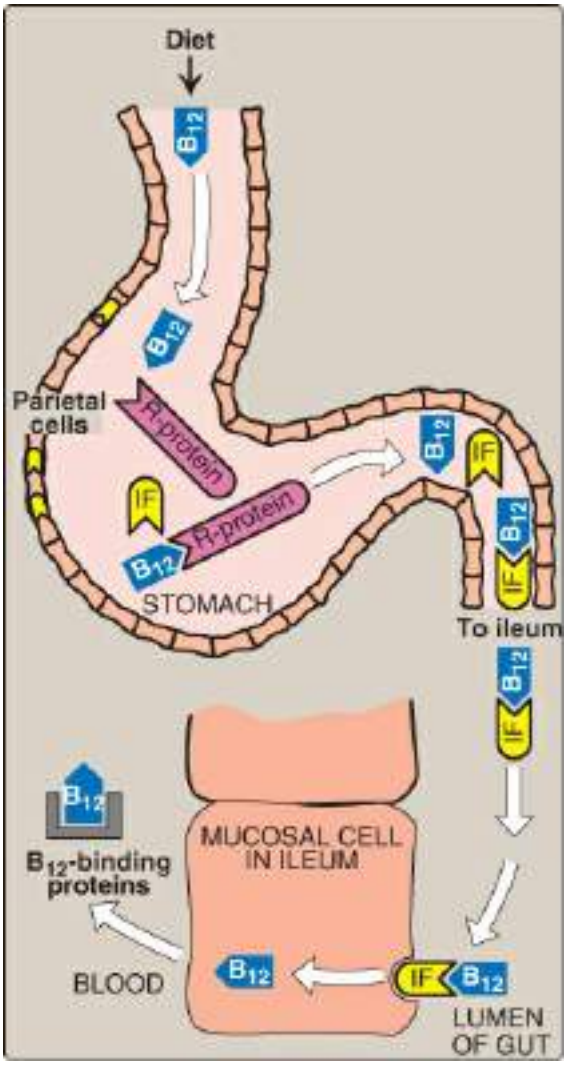


Figure 28.7
Absorption of vitamin B_{12}. (Note: Acid-dependent release of B_{12} from food is not shown.) IF = intrinsic factor.

1. Pernicious anemia: Vitamin B_{12} deficiency is most commonly seen in patients who fail to absorb the vitamin from the intestine (Fig. 28.7). B_{12} is released from food in the acidic environment of the stomach. (Note: Malabsorption of cobalamin in the elderly is most often due to reduced secretion of gastric acid [achlorhydria].) Free B_{12} then binds a glycoprotein (R-protein or haptocorrin), and the complex moves into the intestine. B_{12} is released from the R-protein by pancreatic enzymes and binds another glycoprotein, intrinsic factor (IF). The cobalamin–IF complex travels through the intestine and binds to a receptor (cubilin) on the surface of mucosal cells in the ileum. The cobalamin is transported into the mucosal cell and, subsequently, into the general circulation, where it is carried by its binding protein (transcobalamin). B_{12} is taken up and stored in the liver, primarily. It is released into bile and efficiently reabsorbed in

the ileum. Severe malabsorption of vitamin B_{12} leads to pernicious anemia. This disease is most commonly a result of an autoimmune destruction of the gastric parietal cells that are responsible for the synthesis of IF (lack of IF prevents B_{12} absorption). (Note: Patients who have had a partial or total gastrectomy become IF deficient and, therefore, B_{12} deficient.) Individuals with cobalamin deficiency are usually anemic (folate recycling is impaired), and they show neuropsychiatric symptoms as the disease develops. The CNS effects are irreversible. Pernicious anemia requires lifelong treatment with either high-dose oral B_{12} or intramuscular injection of cyanocobalamin. (Note: Supplementation works even in the absence of IF because ~1% of B_{12} uptake is by IF-independent diffusion.)

Folic acid supplementation can partially reverse the hematologic abnormalities of B_{12} deficiency and, therefore, can mask a cobalamin deficiency. Thus, to prevent the later CNS effects of B_{12} deficiency, therapy for megaloblastic anemia is initiated with both vitamin B_{12} and folic acid until the cause of the anemia can be determined.

IV. ASCORBIC ACID (VITAMIN C)

The active form of vitamin C is ascorbic acid (Fig. 28.8). Its main function is as a reducing agent. Vitamin C is a coenzyme in hydroxylation reactions (e.g., hydroxylation of prolyl and lysyl residues in collagen, and hydroxylation of dopamine to norepinephrine in epinephrine synthesis), where its role is to keep the iron (Fe) of **hydroxylases** in the reduced, ferrous (Fe^{+2}) form. Thus, vitamin C is required for the maintenance of normal connective tissue as well as for wound healing. Vitamin C also facilitates the absorption of dietary nonheme iron from the intestine by reduction of the ferric form (Fe^{+3}) to the ferrous form (Fe^{+2}) (see Chapter 29 Section III B).

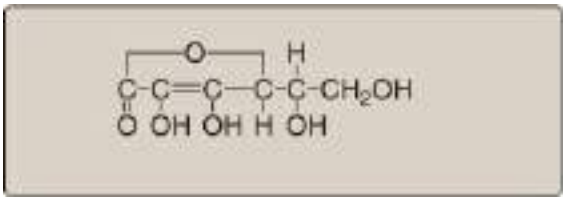


Figure 28.8
Structure of ascorbic acid.

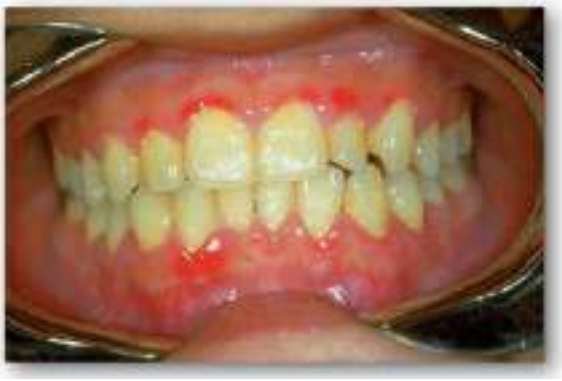


Figure 28.9
Oral manifestations in a patient with scurvy.

A. Deficiency

Ascorbic acid deficiency results in scurvy, a disease characterized by sore and spongy gums, loose teeth, fragile blood vessels, hemorrhage, swollen joints, bone changes, and fatigue (Fig. 28.9). Many of the deficiency symptoms can be explained by the decreased hydroxylation of collagen, resulting in defective connective tissue. A microcytic anemia caused by decreased absorption of iron may also be seen.

B. Chronic disease prevention

Vitamin C is one of a group of nutrients that includes vitamin E (see p. 442) and β-carotene (see Section XI A), which are known as antioxidants. (Note: Vitamin C regenerates the functional, reduced form of vitamin E.) Even though there is a belief that Vitamin C or Vitamin E supplementation may reduce the incidence of some chronic diseases, there is no evidence to support these claims.

V. PYRIDOXINE (VITAMIN B_6)

Vitamin B_6 is a collective term for pyridoxine, pyridoxal, and pyridoxamine, all derivatives of pyridine. They differ only in the nature of the functional group attached to the ring (Fig. 28.10). Pyridoxine occurs primarily in plants, whereas pyridoxal and pyridoxamine are found in foods obtained from animals. All three compounds can serve as precursors of the biologically active coenzyme, pyridoxal phosphate (PLP). PLP functions as a coenzyme for a large number of enzymes, particularly those that catalyze reactions involving amino acids, for example, in the transsulfuration of Hcy to cysteine, and in the synthesis of dopamine and serotonin. (Note: PLP is also required by **glycogen phosphorylase** [see Chapter 11].)

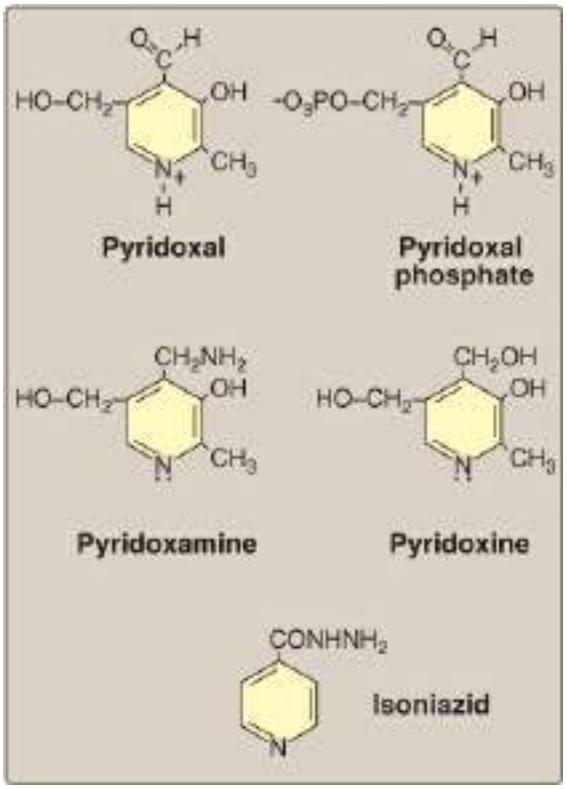


Figure 28.10
Structures of vitamin B_6 and the antituberculosis drug isoniazid.

Reaction type	Example
Transamination	Oxaloacetate + glutamate ⇄ aspartate + α-ketoglutarate
Deamination	Serine → pyruvate + NH_3
Decarboxylation	Histidine → histamine + CO_2
Condensation	Glycine + succinyl CoA → δ-aminolevulinic acid

A. Clinical indications for pyridoxine

Isoniazid, a drug commonly used to treat tuberculosis, can induce a vitamin B_6 deficiency by forming an inactive derivative with PLP. Thus, B6 supplementation is essential for some patients to prevent the development of peripheral neuropathy. Otherwise, dietary deficiencies in pyridoxine are rare but have been observed in newborn infants fed formulas low in B_6, in women taking oral contraceptives, and in those with alcoholism.

B. Toxicity

Vitamin B_6 is the only water-soluble vitamin with significant toxicity. Neurologic symptoms (sensory neuropathy) occur at intakes above 500 mg/day, an amount nearly 400 times the recommended dietary allowance (RDA) and over five times the

tolerable upper limit (UL). (See Chapter 27 for a discussion of RDA and UL.) Substantial improvement, but not complete recovery, occurs when the vitamin is discontinued.

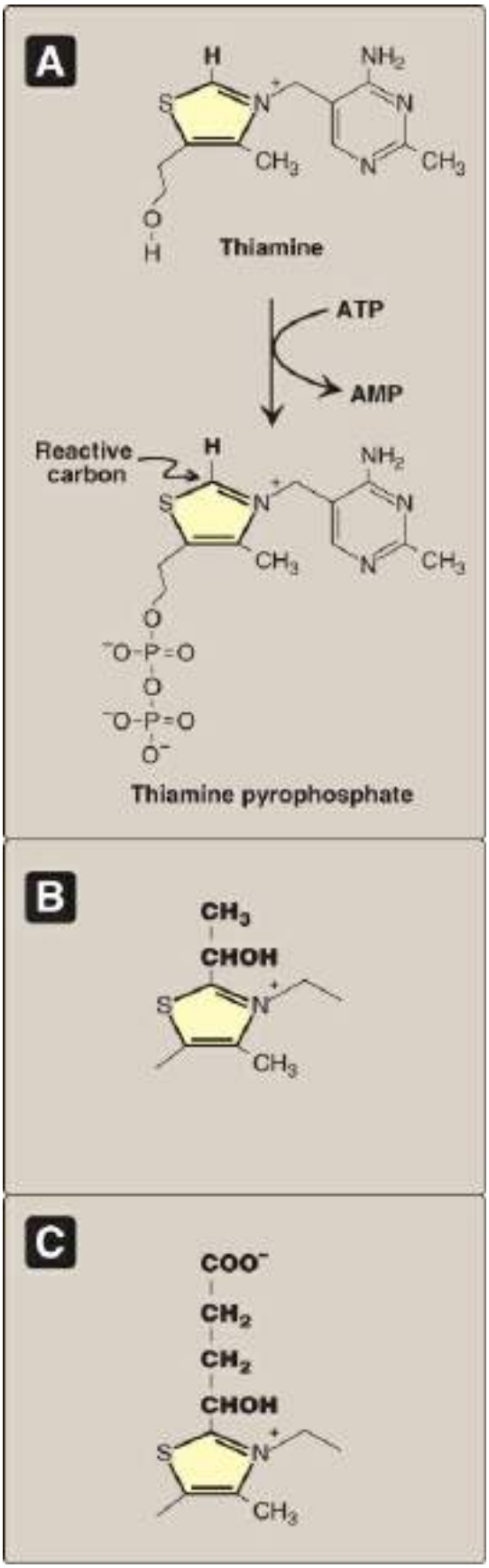


Figure 28.11
A: Structure of thiamine and its coenzyme form, thiamine pyrophosphate. **B:** Structure of intermediate formed in the reaction catalyzed by pyruvate dehydrogenase. **C:** Structure of intermediate formed in the reaction catalyzed by α-ketoglutarate dehydrogenase. AMP = adenosine monophosphate.

VI. THIAMINE (VITAMIN B_1)

Thiamine pyrophosphate (TPP) is the biologically active form of the vitamin, formed by the transfer of a pyrophosphate group from ATP to thiamine (Fig. 28.11). TPP serves as a coenzyme in the formation or degradation of α-ketols by **transketolase** (Fig. 28.12A) and in the oxidative decarboxylation of α-keto acids (Fig. 28.12B).

A. Clinical indications for thiamine

The oxidative decarboxylation of pyruvate and α-ketoglutarate, which plays a key role in energy metabolism of most cells, is particularly important in tissues of the CNS. In thiamine deficiency, the activity of these two dehydrogenase-catalyzed reactions is decreased, resulting in decreased production of ATP and, therefore, impaired cellular function. TPP is also required by branched-chain α-keto acid dehydrogenase of muscle (see p. 295). (Note: It is the decarboxylase of each of these α-keto acid dehydrogenase multienzyme complexes that requires TPP.) Thiamine deficiency is diagnosed by an increase in erythrocyte transketolase activity observed with addition of TPP.

1. Beriberi: This severe thiamine-deficiency syndrome is found in areas where there is severe malnutrition or in areas where starchy, low-thiamine food, such as polished rice is the major component of the diet. Adult beriberi is classified as dry (characterized by peripheral neuropathy, especially in the legs) or wet (characterized by edema because of dilated cardiomyopathy).

2. Wernicke–Korsakoff syndrome: In the United States, thiamine deficiency, which is seen primarily in association with chronic alcoholism, is due to dietary insufficiency or impaired intestinal absorption of the vitamin. Some individuals with alcoholism develop Wernicke–Korsakoff syndrome, a thiamine-deficiency state characterized by mental confusion, gait ataxia, nystagmus (a to-and-fro motion of the eyeballs), and ophthalmoplegia (weakness of eye muscles) with Wernicke encephalopathy as well as memory problems and hallucinations with Korsakoff dementia. The syndrome is treatable with thiamine supplementation, but recovery of memory is typically incomplete.

VII. NIACIN (VITAMIN B_3)

Niacin, or nicotinic acid, is a substituted pyridine derivative. The biologically active coenzyme forms are nicotinamide adenine dinucleotide (NAD^+) and its phosphorylated derivative, nicotinamide adenine dinucleotide phosphate ($NADP^+$), as shown in Figure

28.13. Nicotinamide, a derivative of nicotinic acid that contains an amide instead of a carboxyl group, also occurs in the diet. Nicotinamide is readily deaminated in the body and, therefore, is nutritionally equivalent to nicotinic acid. NAD^+ and $NADP^+$ serve as coenzymes in oxidation–reduction reactions in which the coenzyme undergoes reduction of the pyridine ring by accepting two electrons from a hydride ion, as shown in Figure 28.14. The reduced forms of NAD^+ and $NADP^+$ are NADH and NADPH, respectively. (Note: A metabolite of tryptophan, quinolinate, can be converted to NAD[P]. In comparison, 60 mg of tryptophan = 1 mg of niacin.)

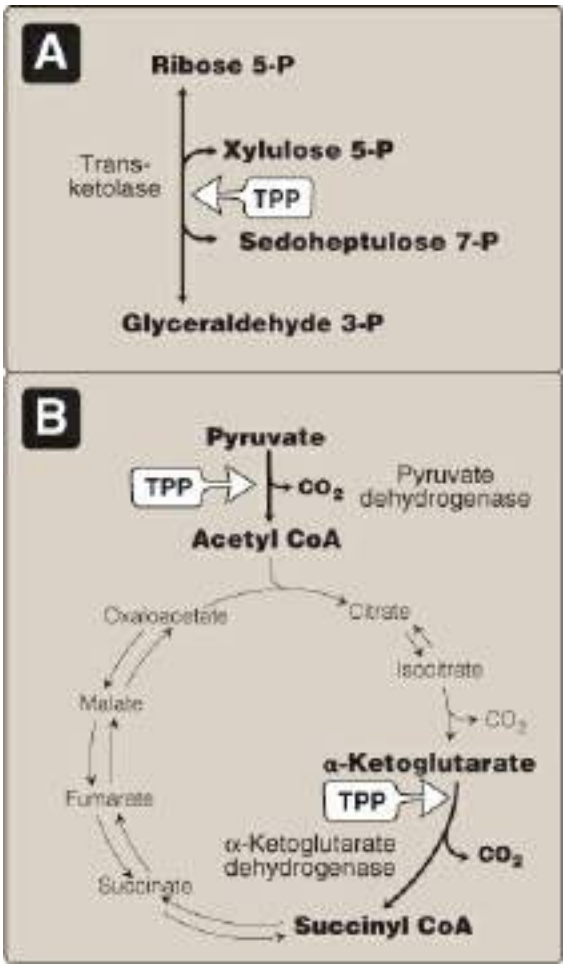


Figure 28.12
Reactions that use thiamine pyrophosphate (TPP) as coenzyme. **A:** Transketolase. **B:** Pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. (Note: TPP is also used by branched-chain α-keto acid dehydrogenase.) P = phosphate; CoA = coenzyme A; CO_2 = carbon dioxide.

A. Distribution

Niacin is found in unrefined and enriched grains and cereal, milk, and lean meats (especially liver).

B. Clinical indications for niacin

1. Deficiency: A deficiency of niacin causes pellagra, a disease involving the skin, gastrointestinal tract, and CNS. The symptoms of pellagra progress through the three Ds: dermatitis (photosensitive), diarrhea, and dementia. If untreated, death (a fourth D) occurs. Hartnup disorder, characterized by defective absorption of tryptophan, can result in pellagra-like symptoms. (Note: Corn is low in both niacin and tryptophan. Corn-based diets can cause pellagra.)

2. Hyperlipidemia treatment: Niacin at doses of 1.5 g/day, or 100 times the RDA, strongly inhibits lipolysis in adipose tissue, the primary producer of circulating free fatty acids (FFAs). The liver normally uses these circulating FFA as a major precursor for triacylglycerol (TAG) synthesis. Thus, niacin causes a decrease in liver TAG synthesis, which is required for very–low-density lipoprotein ([VLDL] see p. 256) production. Low-density lipoprotein (LDL, the cholesterol-rich lipoprotein) is derived from VLDL in the plasma. Thus, both plasma TAG (in VLDL) and cholesterol (in LDL) are lowered. Therefore, niacin is particularly useful in the treatment of type IIb hyperlipoproteinemia, in which both VLDL and LDL are elevated. The high doses of niacin required can cause acute, prostaglandin-mediated flushing. Aspirin can reduce this side effect by inhibiting prostaglandin synthesis (see p. 237). Itching may also occur. (Note: Niacin raises high-density lipoprotein and lowers Lp[a] levels [see p. 262].)

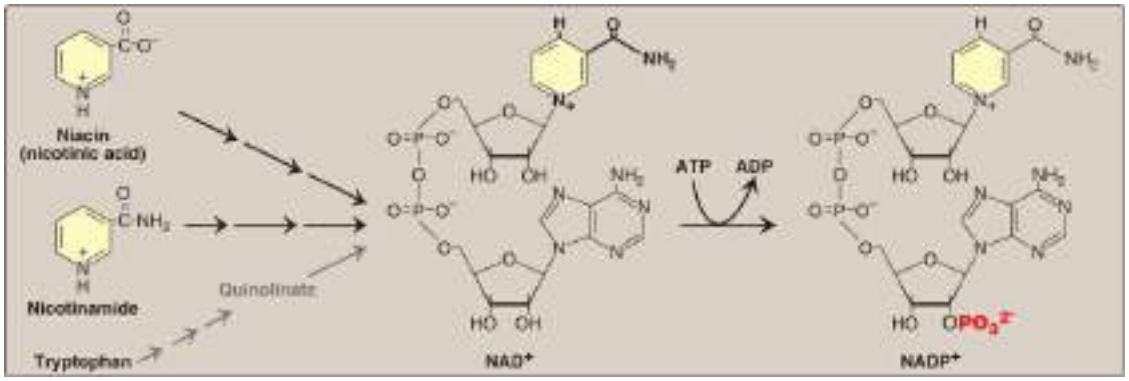


Figure 28.13
Structure and biosynthesis of oxidized nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$). ADP = adenosine diphosphate.

VIII. RIBOFLAVIN (VITAMIN B_2)

The two biologically active forms of B_2 are flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), formed by the transfer of an adenosine monophosphate moiety from ATP to FMN (Fig. 28.15). FMN and FAD are each capable of reversibly accepting two hydrogen atoms, forming $FMNH_2$ or $FADH_2$, respectively. FMN and FAD are bound tightly, sometimes covalently, to flavoenzymes (e.g., NADH dehydrogenase [FMN] and succinate dehydrogenase [FAD]) that catalyze the oxidation or reduction of a substrate. Riboflavin deficiency is not associated with a major human disease, although

it frequently accompanies other vitamin deficiencies. Deficiency symptoms include dermatitis, cheilosis (fissuring at the corners of the mouth), and glossitis (the tongue appearing smooth and dark). (Note: Because riboflavin is light sensitive, phototherapy for hyperbilirubinemia [see p. 317] may require supplementation with the vitamin.)

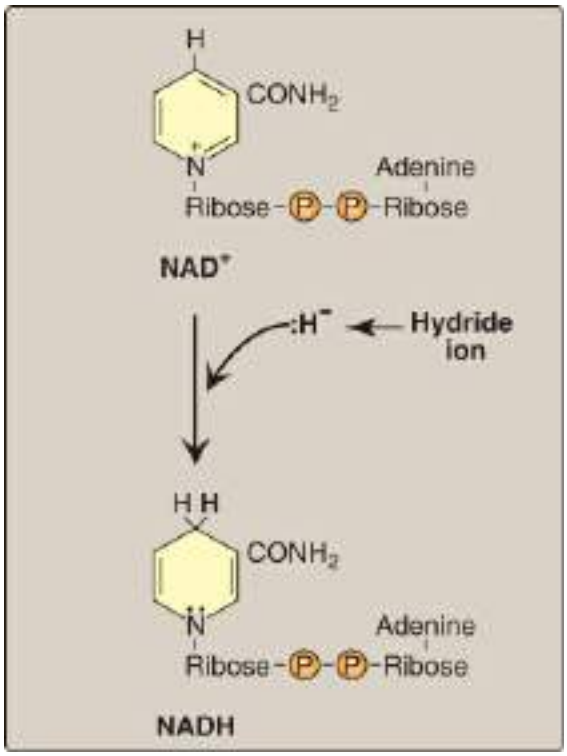


Figure 28.14
Reduction of oxidized nicotinamide adenine dinucleotide (NAD^+) to NADH. (Note: The hydride ion consists of a hydrogen [H] atom plus an electron.) (P) = phosphate.

IX. BIOTIN (VITAMIN B_7)

Biotin is a coenzyme in carboxylation reactions, in which it serves as a carrier of activated carbon dioxide (CO_2) for the mechanism of biotin-dependent carboxylations. Biotin is covalently bound to the ε-amino group of lysine residues in biotin-dependent enzymes (Fig. 28.16). Biotin deficiency does not occur naturally because the vitamin is widely distributed in food. Also, a large percentage of the biotin requirement in humans is supplied by intestinal bacteria. However, the addition of raw egg white to the diet as a source of protein can induce symptoms of biotin deficiency, namely, dermatitis, hair loss, loss of appetite, and nausea. Raw egg white contains the glycoprotein avidin, which tightly binds biotin and prevents its absorption from the intestine. With a normal diet, however, it has been estimated that 20 eggs/day would be required to induce a deficiency syndrome. (Note: Inclusion of raw eggs in the diet is not recommended because of the possibility of salmonellosis caused by infection with *Salmonella enterica*.)

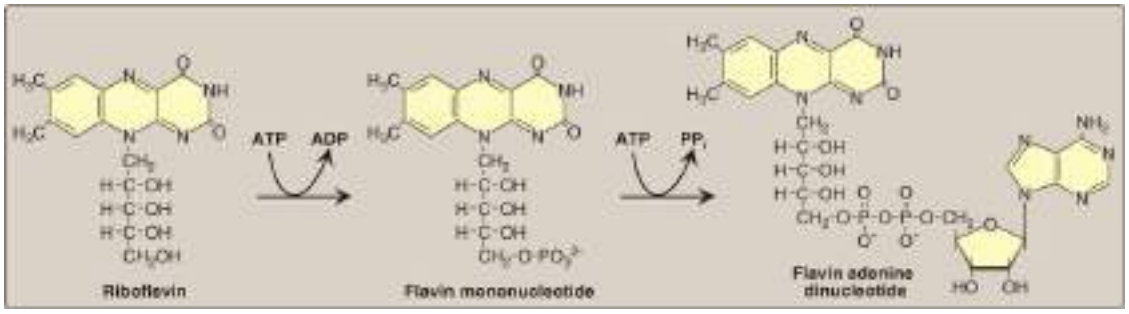


Figure 28.15
Structure and biosynthesis of the oxidized forms of flavin mononucleotide and flavin adenine dinucleotide. ADP = adenosine diphosphate; PP_i = pyrophosphate.

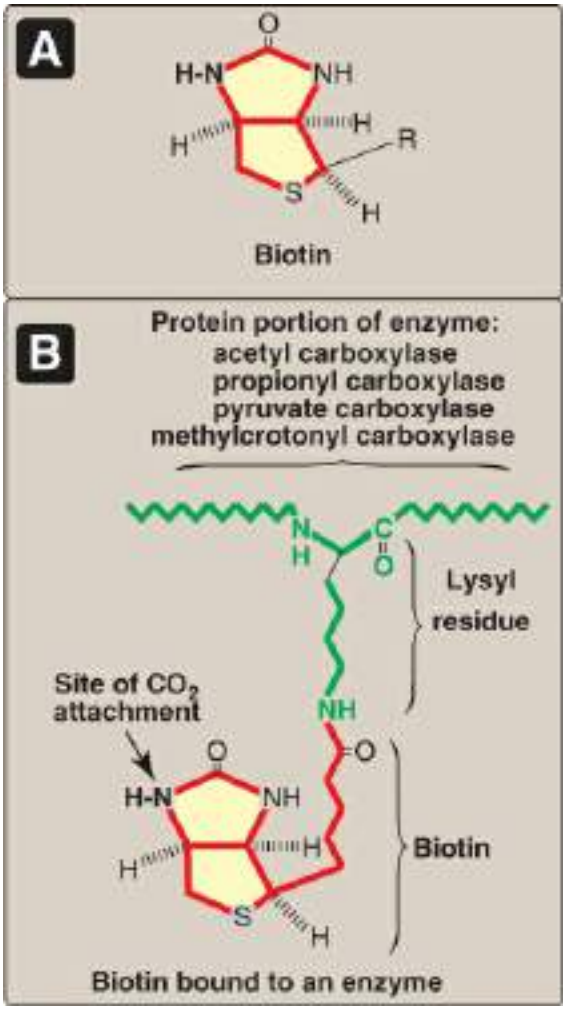


Figure 28.16
A: Structure of biotin. **B:** Biotin covalently bound to a lysyl residue of a biotin-dependent enzyme. CO_2 = carbon dioxide.

Multiple carboxylase deficiency results from decreased ability to add biotin to carboxylases during their synthesis or to remove it during their degradation. Treatment is biotin supplementation.

X. PANTOTHENIC ACID (VITAMIN B_5)

Pantothenic acid is a component of CoA, which functions in the transfer of acyl groups (Fig. 28.17). CoA contains a thiol group that carries acyl compounds as activated thiol esters. Examples of such structures are succinyl CoA, fatty acyl CoA, and acetyl CoA. Pantothenic acid is also a component of the acyl carrier protein domain of **fatty acid synthase**. Eggs, liver, and yeast are the most important sources of pantothenic acid, although the vitamin is widely distributed. Pantothenic acid deficiency is not well characterized in humans, and no RDA has been established.

XI. VITAMIN A

Vitamin A is a fat-soluble vitamin that comes primarily from animal sources as retinol (preformed vitamin A), a retinoid. The retinoids, a family of structurally related molecules, are essential for vision, reproduction, growth, and maintenance of epithelial tissues. They also play a role in immune function. Retinoic acid, derived from oxidation of retinol, mediates most of the actions of the retinoids, except for vision, which depends on retinal, the aldehyde derivative of retinol.

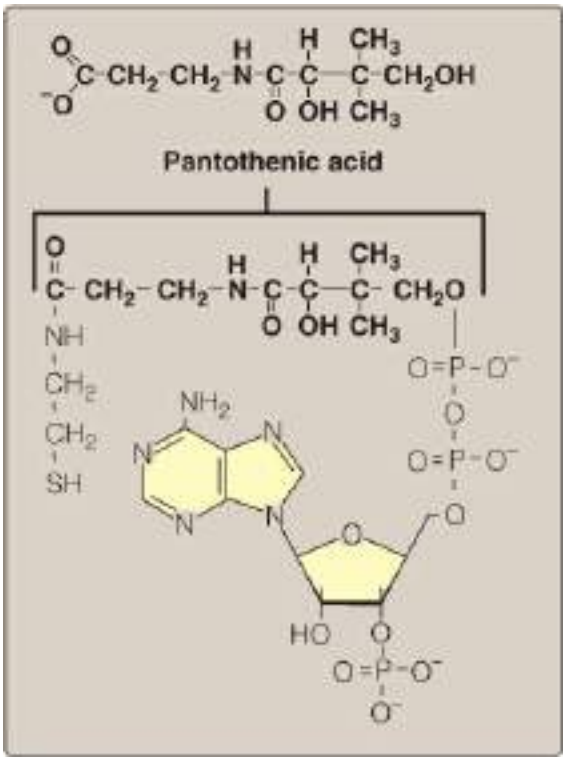


Figure 28.17
Structure of coenzyme A.

A. Structure

The retinoids include the natural forms of vitamin A, retinol and its metabolites (Fig. 28.18), and synthetic forms (drugs).

1. Retinol: A primary alcohol containing a β-ionone ring with an unsaturated side chain, retinol is found in animal tissues as a retinyl ester with long-chain FA. It is the storage form of vitamin A.
2. Retinal: This is the aldehyde derived from the oxidation of retinol. Retinal and retinol can readily be interconverted.
3. Retinoic acid: This is the acid derived from the oxidation of retinal. Retinoic acid cannot be reduced in the body and, therefore, cannot give rise to either retinal or retinol.
4. β-Carotene: Plant foods contain β-carotene (provitamin A), which can be oxidatively and symmetrically cleaved in the intestine to yield two molecules of retinal. In humans, the conversion is inefficient, and the vitamin A activity of β-carotene is only about 1/12 that of retinol.

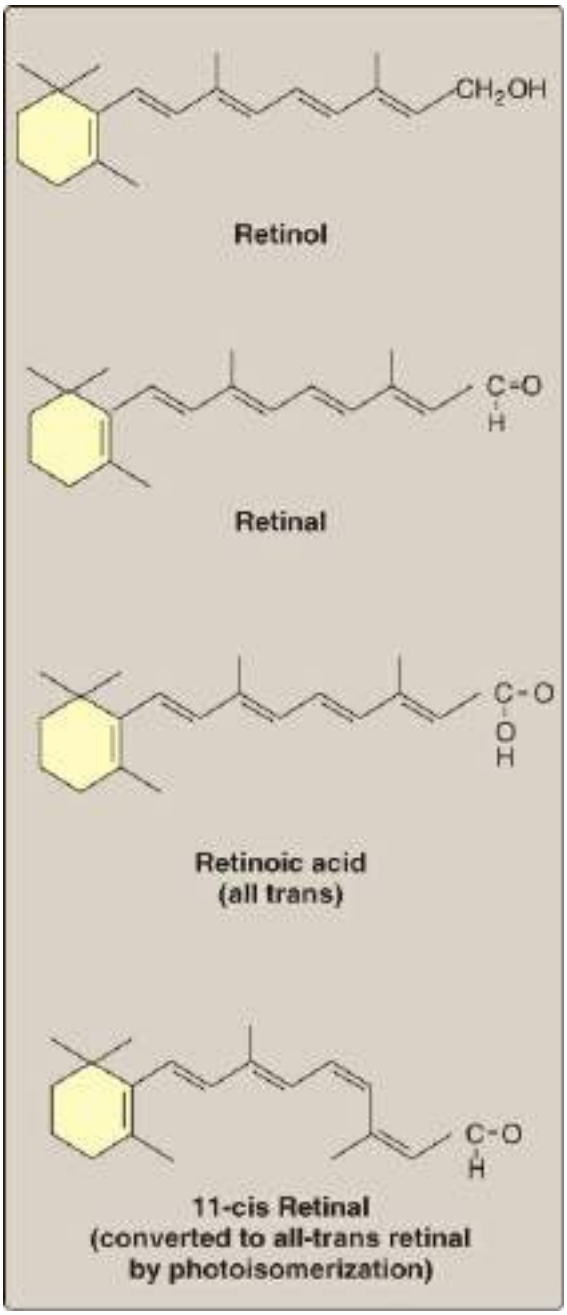


Figure 28.18
Structure of the retinoids.

B. Absorption and transport to the liver

Retinyl esters from the diet are hydrolyzed in the intestinal mucosa, releasing retinol and FFA (Fig. 28.19). Retinol derived from esters and from the reduction of retinal from β-carotene cleavage is reesterified to long-chain FA within the enterocytes and secreted as a component of chylomicrons into the lymphatic system. Retinyl esters contained in chylomicron remnants are taken up by, and stored in, the liver. (Note: All fat-soluble vitamins are carried in chylomicrons.)

C. Release from the liver

When needed, retinol is released from the liver and transported through the blood to extrahepatic tissues by retinol-binding protein complexed with transthyretin (see Fig. 28.19). The ternary complex binds to a transport protein on the surface of the cells of peripheral tissues, permitting retinol to enter. An intracellular retinol-binding protein carries retinol to sites in the nucleus where the vitamin regulates transcription in a manner analogous to that of steroid hormones.

D. Retinoic acid mechanism of action

Retinol is oxidized to retinoic acid. Retinoic acid binds with high affinity to specific receptor proteins (retinoic acid receptors [RARs]) present in the nucleus of target tissues such as epithelial cells (Fig. 28.20). The activated retinoic acid–RAR complex binds to response elements on DNA and recruits activators or repressors to regulate retinoid-specific RNA synthesis, resulting in control of the production of specific proteins that mediate several physiologic functions. For example, retinoids control the expression of the gene for keratin in most epithelial tissues of the body. (Note: The RAR proteins are part of the superfamily of transcriptional regulators that includes the nuclear receptors for steroid and thyroid hormones and vitamin D, all of which function in a similar way [see p. 265].)

E. Functions

1. Visual cycle: Vitamin A is a component of the visual pigments of rod and cone cells. Rhodopsin, the visual pigment of the rod cells in the retina, consists of 11-cis retinal bound to the protein opsin (see Fig. 28.19). When rhodopsin, a G protein–coupled receptor, is exposed to light, a series of photochemical isomerizations occurs, which results in the bleaching of rhodopsin and release of all-trans retinal and opsin. This process activates the G protein transducin, triggering a nerve impulse that is transmitted by the optic nerve to the brain. Regeneration of rhodopsin requires isomerization of all-trans retinal back to 11-cis retinal. All-trans retinal is reduced to all-trans retinol, esterified, and isomerized to 11-cis retinol that is oxidized to 11-cis retinal. The latter combines with opsin to form rhodopsin, thus completing the cycle. Similar reactions are responsible for color vision in the cone cells.

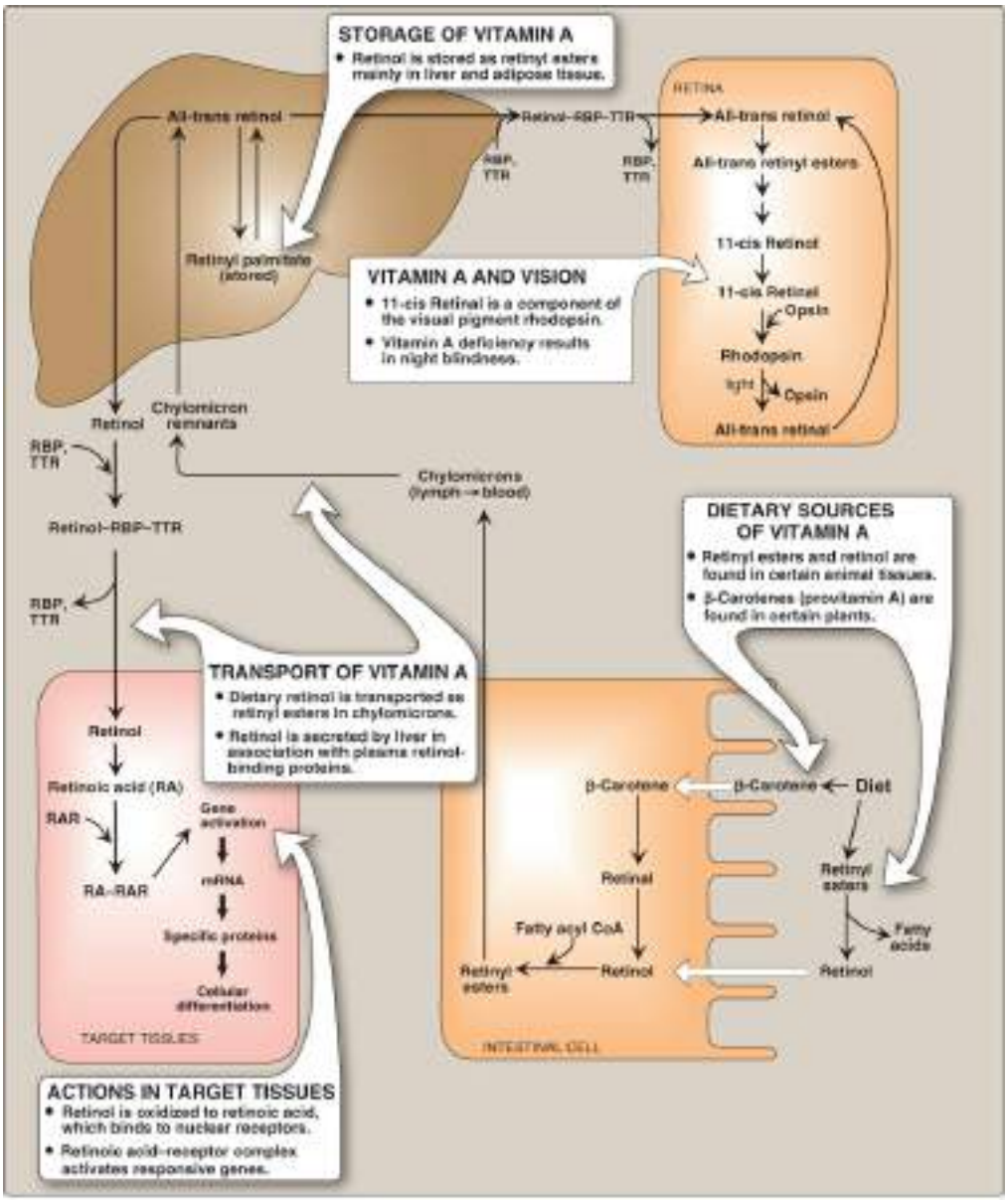


Figure 28.19
Absorption, transport, and storage of vitamin A and its derivatives. (Note: β-Carotene is a carotenoid, a plant pigment with antioxidant activity.) RBP = retinol-binding protein; TTR = transthyretin; RAR = retinoic acid receptor; CoA = coenzyme A; mRNA = messenger RNA.

2. Epithelial cell maintenance: Vitamin A is essential for normal differentiation of epithelial tissues and mucus secretion and, thus, supports the body's barrier-based defense against pathogens.

3. Reproduction: Retinol and retinal are essential for normal reproduction, supporting spermatogenesis in the male and preventing fetal resorption in the female. Retinoic acid is inactive in maintaining reproduction and in the visual cycle but promotes growth and differentiation of epithelial cells.

F. Distribution

Liver, kidney, cream, butter, and egg yolk are good sources of preformed vitamin A. Yellow, orange, and dark-green vegetables and fruits are good sources of the carotenes (provitamin A).

G. Requirement

The RDA for adults is 900 retinol activity equivalents (RAE) for males and 700 RAE for females. In comparison, 1 RAE = 1 μg of retinol, 12 μg of β-carotene, or 24 μg of other carotenoids.

H. Clinical indications for vitamin A

Although chemically related, retinoic acid and retinol have distinctly different therapeutic applications. Retinol and its carotenoid precursor are used as dietary supplements, whereas various forms of retinoic acid are useful in dermatology (Fig. 28.21).

1. Deficiency: Vitamin A, administered as retinol or retinyl esters, is used to treat patients who are deficient in the vitamin. Night blindness (nyctalopia) is one of the earliest signs of vitamin A deficiency. The visual threshold is increased, making it difficult to see in dim light. Prolonged deficiency leads to an irreversible loss in the number of visual cells. Severe deficiency leads to xerophthalmia, a pathologic dryness of the conjunctiva and cornea, caused, in part, by increased keratin synthesis. If untreated, xerophthalmia results in corneal ulceration and, ultimately, in blindness because of the formation of opaque scar tissue. The condition is most commonly seen in children in developing tropical countries. Over 500,000 children worldwide are blinded each year by xerophthalmia caused by insufficient vitamin A in the diet.

2. Skin conditions: Dermatologic problems such as acne are effectively treated with retinoic acid or its derivatives (see Fig. 28.21). Mild cases of acne and skin aging are treated with tretinoin (all-trans retinoic acid). Tretinoin is too toxic for systemic (oral) administration in treating skin conditions and is confined to topical application. (Note: Oral tretinoin is used in treating acute promyelocytic leukemia.) In patients with severe cystic acne unresponsive to conventional therapies, isotretinoin (13-cis retinoic acid) is administered orally. An oral synthetic retinoid is used to treat psoriasis.

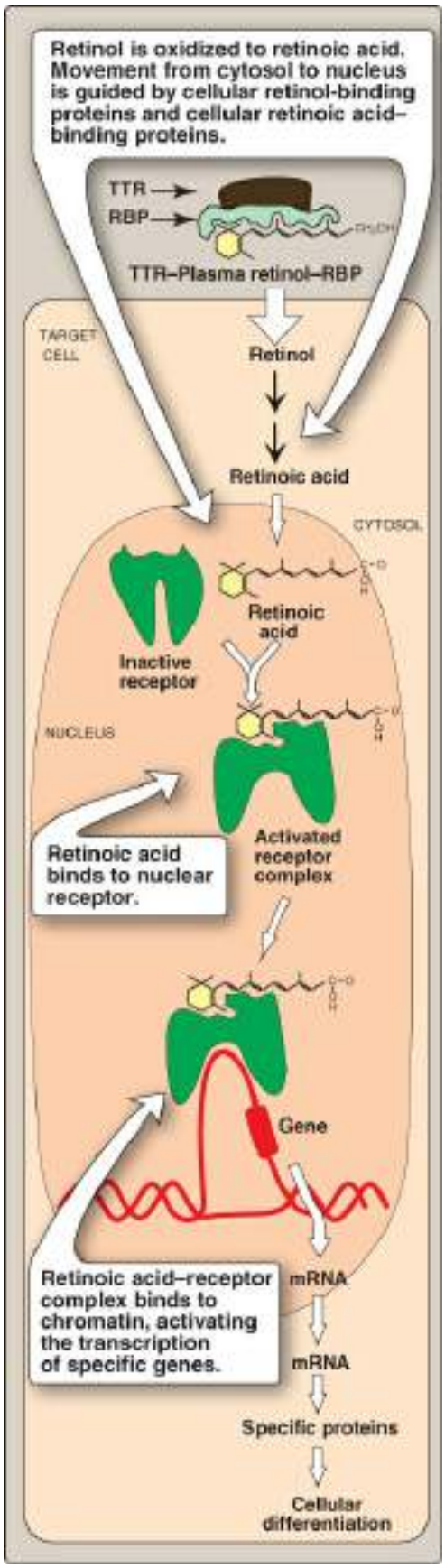


Figure 28.20
Action of the retinoids. (Note: Retinoic acid–receptor complex forms a dimer, but is shown as monomer for simplicity.) TTR = transthyretin; RBP = retinol-binding protein; mRNA = messenger RNA.

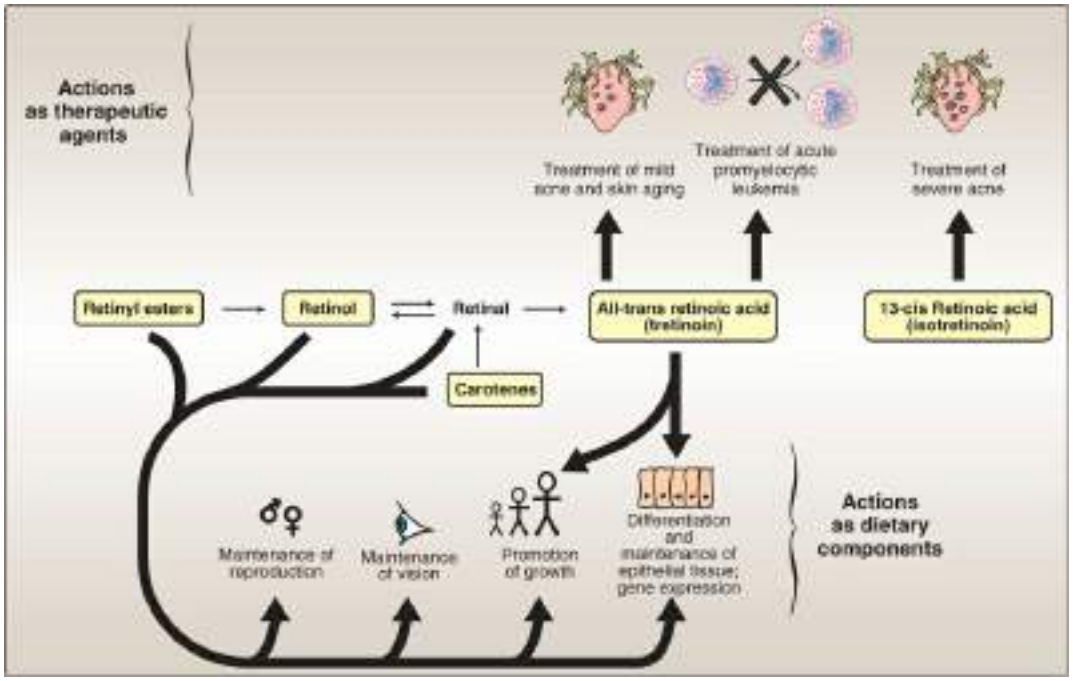


Figure 28.21
Summary of actions of retinoids. Compounds in boxes are available as dietary components or as pharmacologic agents.

I. Retinoid toxicity

1. Vitamin A: Excessive intake of vitamin A (but not carotene) produces a toxic syndrome called hypervitaminosis A. Amounts exceeding 7.5 mg/day of retinol should be avoided. Early signs of chronic hypervitaminosis A are reflected in the skin, which becomes dry and pruritic (because of decreased keratin synthesis); in the liver, which becomes enlarged and can become cirrhotic; and in the CNS, where a rise in intracranial pressure may mimic the symptoms of a brain tumor. Pregnant women, in particular, should not ingest excessive quantities of vitamin A because of its potential for teratogenesis (causing congenital malformations in the developing fetus). UL is 3,000 µg preformed vitamin A/day. (Note: Vitamin A promotes bone growth. In excess, however, it is associated with decreased bone mineral density and increased risk of fractures.)

2. Isotretinoin: The drug, an isomer of retinoic acid, is teratogenic and absolutely contraindicated in women with childbearing potential unless they have severe, disfiguring cystic acne that is unresponsive to standard therapies. Pregnancy must be excluded before treatment begins, and birth control must be used. Prolonged treatment with isotretinoin can result in an increase in TAG and

cholesterol, providing some concern for an increased risk of CVD.

XII. VITAMIN D

The D vitamins are a group of sterols that have a hormone-like function. The active molecule, 1,25-dihydroxycholecalciferol ([1,25-diOH-D_3], or calcitriol), binds to intracellular receptor proteins. The 1,25-diOH-D_3–receptor complex interacts with response elements in the nuclear DNA of target cells in a manner similar to that of vitamin A (see Fig. 28.20) and either selectively stimulates or represses gene transcription. The most prominent actions of calcitriol are to regulate the serum levels of calcium and phosphorus.

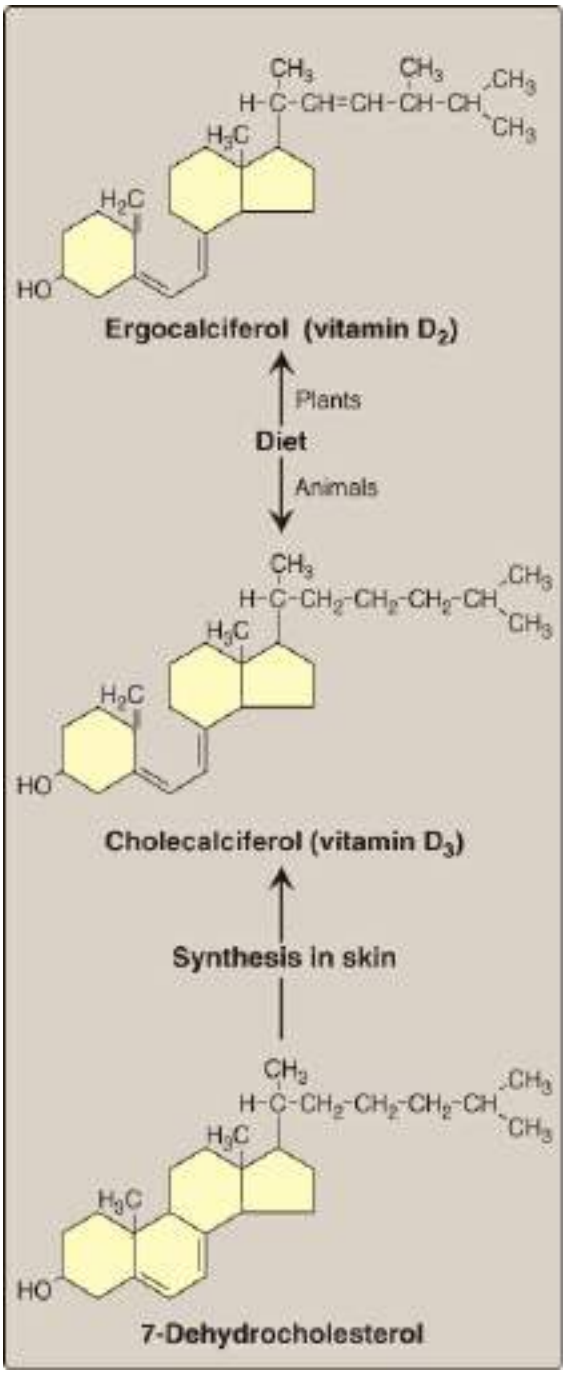


Figure 28.22
Sources of vitamin D. Vitamins D_2 and D_3 are first converted to calcidiol and then to calcitriol (active vitamin D). (Note: 7-Dehydrocholesterol [provitamin D_3] is decreased in the skin of older adults.)

A. Distribution

1. Endogenous vitamin precursor: 7-Dehydrocholesterol, an intermediate in cholesterol synthesis, is converted to cholecalciferol in the dermis and epidermis of humans exposed to sunlight and transported to liver bound to vitamin D–

binding protein.

2. Diet: Ergocalciferol (vitamin D_2), found in plants, and cholecalciferol (vitamin D_3), found in animal tissues, are sources of preformed vitamin D activity (Fig. 28.22). Vitamin D_2 and vitamin D_3 differ chemically only in the presence of an additional double-bond and methyl group in the plant sterol. Dietary vitamin D is packaged into chylomicrons. (Note: Preformed vitamin D is a dietary requirement only in individuals with limited exposure to sunlight.)

B. Metabolism

1. 1,25-Dihydroxycholecalciferol formation: Vitamins D_2 and D_3 are not biologically active but are converted *in vivo* to calcitriol, the active form of the D vitamin, by two sequential hydroxylation reactions (Fig. 28.23). The first hydroxylation occurs at the 25 position and is catalyzed by a specific 25-hydroxylase in the liver. The product of the reaction, 25-hydroxycholecalciferol ([25-OH-D_3], calcidiol), is the predominant form of vitamin D in the serum and the major storage form. 25-OH-D_3 is further hydroxylated at the 1 position by 25-hydroxycholecalciferol 1-hydroxylase found primarily in the kidney, resulting in the formation of 1,25-diOH-D_3 (calcitriol). (Note: Both hydroxylases are cytochrome P450 proteins [see Chapter 13].)

2. Hydroxylation regulation: Calcitriol is the most potent vitamin D metabolite. Its formation is tightly regulated by the level of serum phosphate (PO_4^{3-}) and calcium ions (Ca^{2+}) as shown in Figure 28.24. 25-Hydroxycholecalciferol 1-hydroxylase activity is increased directly by low serum PO_4^{3-} or indirectly by low serum Ca^{2+}, which triggers the secretion of parathyroid hormone (PTH) from the chief cells of the parathyroid gland. PTH upregulates the 1-hydroxylase. Thus, hypocalcemia caused by insufficient dietary Ca^{2+} results in elevated levels of serum 1,25-diOH-D_3. (Note: 1,25-diOH-D_3 inhibits expression of PTH, forming a negative-feedback loop. It also inhibits activity of the 1-hydroxylase.)

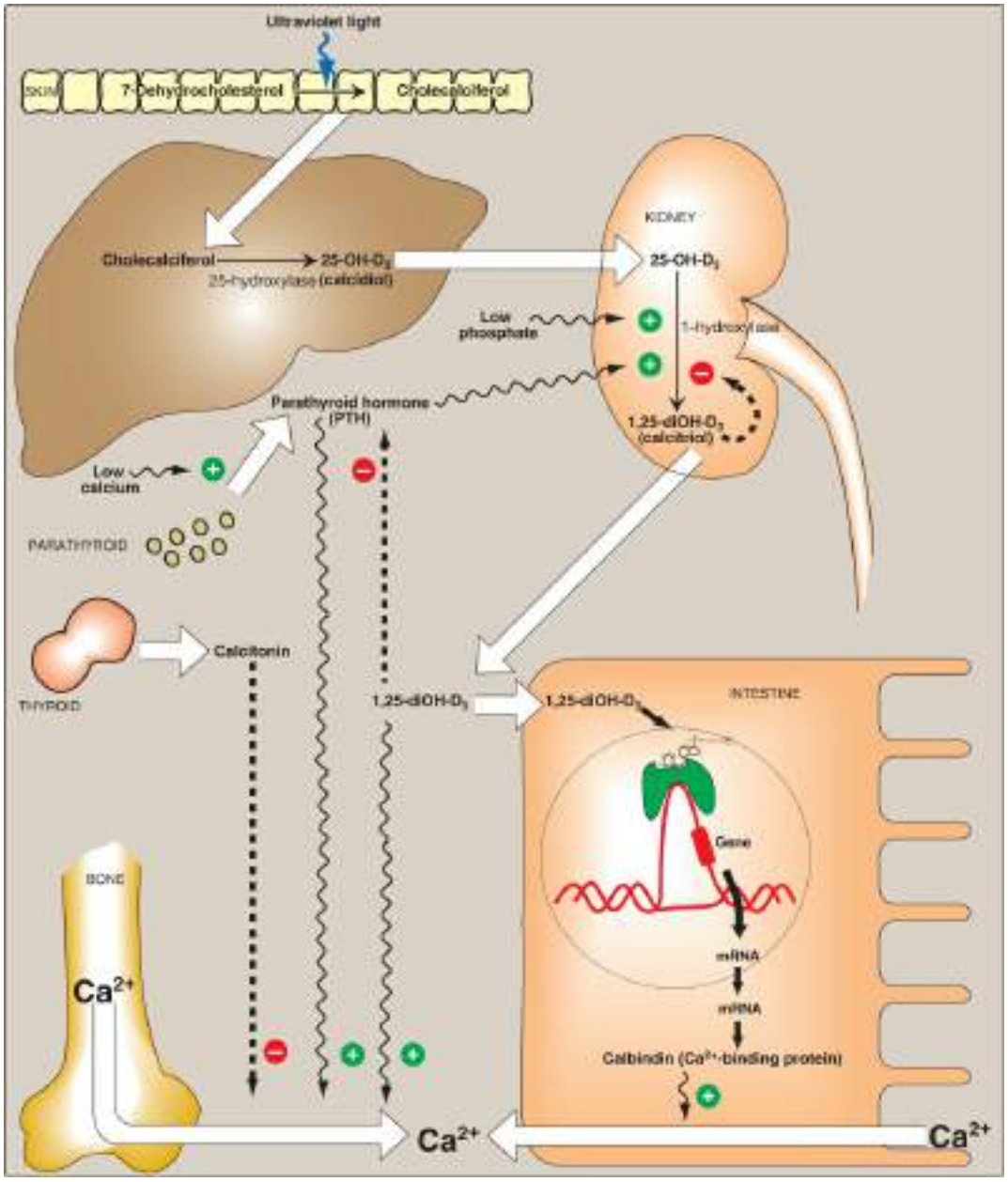


Figure 28.23
Metabolism and actions of vitamin D. (Note: Calcitonin, a thyroid hormone, decreases blood calcium [Ca^{2+}] by inhibiting mobilization from bone, absorption from the intestine, and reabsorption by the kidney. It opposes the actions of PTH.) mRNA = messenger RNA; 25-OH-D_3 = 25-hydroxycholecalciferol; 1,25-diOH-D_3 = 1,25-dihydroxycholecalciferol.

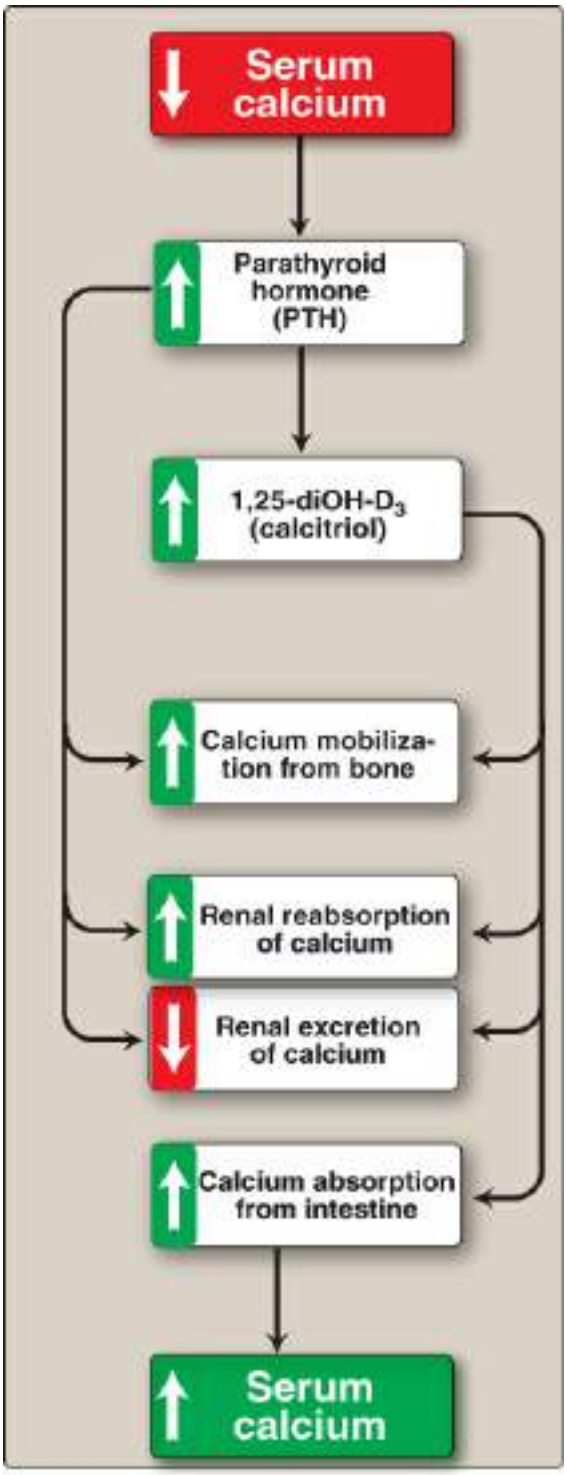


Figure 28.24
Response to low serum calcium. (Note: Calcitriol also increases intestinal absorption and renal reabsorption of phosphate. In contrast, PTH decreases renal reabsorption of phosphate.). 1,25-diOH-D_3 = 1,25-dihydroxycholecalciferol.

C. Function

The overall function of calcitriol is to maintain adequate serum levels of Ca^{2+}. It performs this function by (1) increasing uptake of Ca^{2+} by the intestine, (2) minimizing loss of Ca^{2+} by the kidney by increasing reabsorption, and (3) stimulating resorption (demineralization) of bone when blood Ca^{2+} is low (see Fig. 28.23).

1. Effect on the intestine: Calcitriol stimulates intestinal absorption of Ca^{2+} by first entering the intestinal cell and binding to a cytosolic receptor. The 1,25-diOH-D_3–receptor complex then moves to the nucleus where it selectively interacts with response elements on the DNA. As a result, Ca^{2+} uptake is enhanced by increased expression of the calcium-binding protein calbindin. Thus, the mechanism of action of 1,25-diOH-D_3 is typical of steroid hormones (see p. 265).

2. Effect on bone: Bone is composed of collagen and crystals of $Ca_5(PO_4)_3OH$ (hydroxylapatite). When blood Ca^{2+} is low, 1,25-diOH-D_3 stimulates bone resorption by a process that is enhanced by PTH. The result is an increase in serum Ca^{2+}. Therefore, bone is an important reservoir of Ca^{2+} that can be mobilized to maintain serum levels. (Note: PTH and calcitriol also work together to prevent renal loss of Ca^{2+}.)

D. Distribution and requirement

Vitamin D occurs naturally in fatty fish, liver, and egg yolk. Milk, unless it is artificially fortified, is not a good source. The RDA for individuals of ages 1 to 70 years is 15 µg/day and 20 µg/day if over age 70 years. Experts disagree, however, on the optimal level of vitamin D needed to maintain health. (Note: 1 µg vitamin D = 40 international units [IU].) Because breast milk is a poor source of vitamin D, supplementation is recommended for breastfed babies.

E. Clinical indications for vitamin D

1. Nutritional rickets: Vitamin D deficiency causes a net demineralization of bone, resulting in rickets in children and osteomalacia in adults (Fig. 28.25). Rickets is characterized by the continued formation of the collagen matrix of bone, but incomplete mineralization results in soft, pliable bones. In osteomalacia, demineralization of pre-existing bones increases their susceptibility to fracture. Insufficient exposure to daylight and/or deficiencies in vitamin D consumption occurs predominantly in infants and the elderly. Vitamin D deficiency is more common in the northern latitudes, because less vitamin D synthesis occurs in the skin as a result of reduced exposure to ultraviolet light. (Note: Loss-of-function mutations in the vitamin D receptor result in hereditary vitamin D–deficient rickets.)

2. Renal osteodystrophy: Chronic kidney disease causes decreased ability to form active vitamin D as well as increased retention of PO_4^{3-}, resulting in

hyperphosphatemia and hypocalcemia. The low blood Ca^{2+} causes a rise in PTH and associated bone demineralization with release of Ca^{2+} and PO_4^{3-}. Supplementation with vitamin D is an effective therapy. However, supplementation must be accompanied by PO_4^{3-} reduction therapy to prevent further bone loss and precipitation of calcium phosphate crystals.

3. Hypoparathyroidism: Lack of PTH causes hypocalcemia and hyperphosphatemia. (Note: PTH increases phosphate excretion.) Patients may be treated with vitamin D and calcium supplementation.

F. Toxicity

Like all fat-soluble vitamins, vitamin D can be stored in the body and is only slowly metabolized. High doses (100,000 IU for weeks or months) can cause loss of appetite, nausea, thirst, and weakness. Enhanced Ca^{2+} absorption and bone resorption result in hypercalcemia, which can lead to deposition of calcium salts in soft tissue (metastatic calcification). The UL is 100 µg/day (4,000 IU/day) for individuals ages 9 years or older, with a lower level for those under age 9 years. (Note: Toxicity is only seen with use of supplements. Excess vitamin D produced in the skin is converted to inactive forms.)

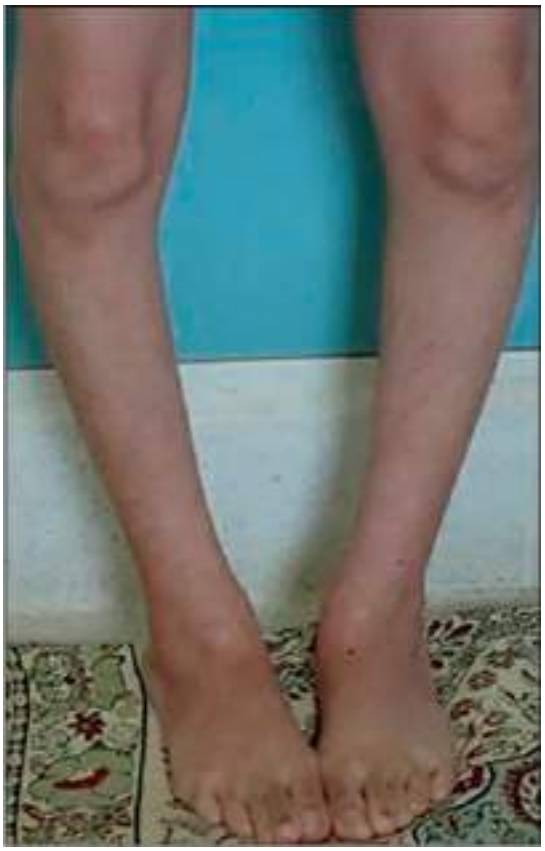


Figure 28.25
Bowed legs of middle-aged man with osteomalacia, a nutritional vitamin D deficiency that results in

demineralization of the skeleton.

XIII. VITAMIN K

The principal role of vitamin K is in the posttranslational modification of a number of proteins (most of which are involved with blood clotting), in which it serves as a coenzyme in the carboxylation of certain glutamic acid residues in these proteins. Vitamin K exists in several active forms, for example, in plants as phylloquinone (or vitamin K_1), and in intestinal bacteria as menaquinone (or vitamin K_2). A synthetic form of vitamin K, menadione, is able to be converted to K_2.

A. Function

1. γ-Carboxyglutamate formation: Vitamin K is required for the posttranslational modification of coagulation factors prothrombin, FVII, FIX and FX (See Chapter 35) which are synthesized in the liver. Formation of the functional versions of these enzyme factors requires the vitamin K–dependent carboxylation of several glutamic acid residues to γ-carboxyglutamate (Gla) residues (Fig. 28.26). The carboxylation reaction requires γ-glutamyl carboxylase, O_2, CO_2, and the hydroquinone form of vitamin K (which gets oxidized to the epoxide form). The formation of Gla residues is sensitive to inhibition by warfarin, a synthetic analog of vitamin K that inhibits vitamin K epoxide reductase (VKOR), the enzyme required to regenerate the functional hydroquinone form of vitamin K.

2. Prothrombin interaction with membranes: The Gla residues are good chelators of positively charged calcium ions, because of their two adjacent, negatively charged carboxylate groups. With prothrombin, for example, the prothrombin–calcium complex is able to bind to negatively charged membrane phospholipids on the surface of damaged endothelium and platelets. Attachment to membrane increases the rate at which the proteolytic conversion of prothrombin to thrombin can occur (Fig. 28.27).

3. γ-Carboxyglutamate residues in other proteins: Gla residues are also present in proteins other than those involved in forming a blood clot. For example, osteocalcin and matrix Gla protein of bone and proteins C and S (involved in limiting the formation of blood clots) also undergo γ-carboxylation.

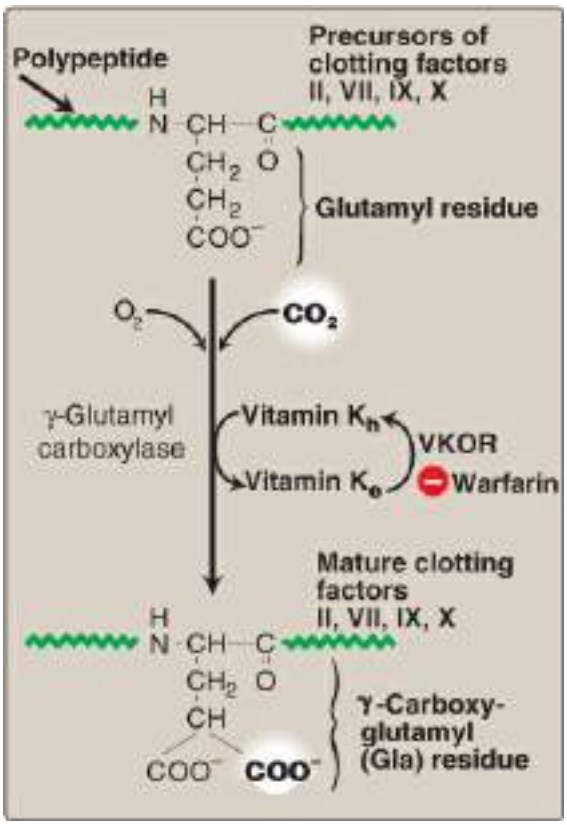


Figure 28.26
Carboxylation of glutamate to form γ-carboxyglutamate. h = hydroquinone; e = epoxide; VKOR = vitamin K epoxide reductase.

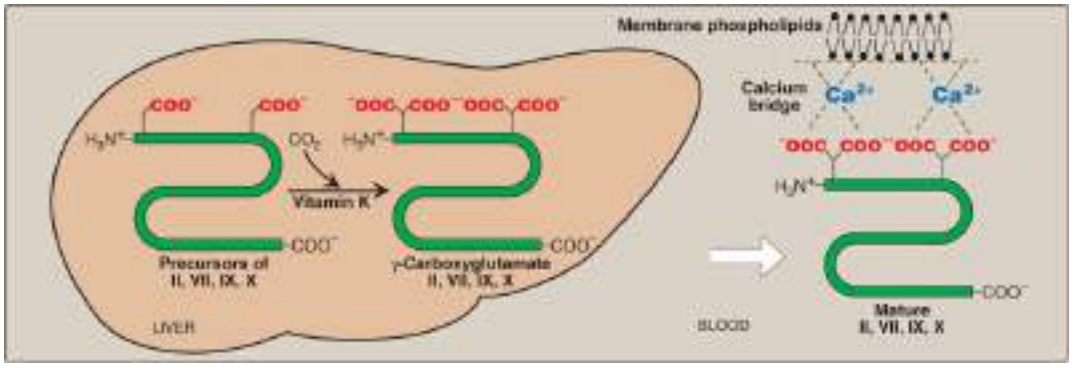


Figure 28.27
Role of vitamin K in blood coagulation. CO_2 = carbon dioxide.

B. Distribution and requirement

Vitamin K is found in cabbage, kale, spinach, egg yolk, and liver. The adequate intake for vitamin K is 120 µg/day for adult males and 90 µg for adult females. There is also synthesis of the vitamin by the gut microbiota.

C. Clinical indications for vitamin K

1. Deficiency: A true vitamin K deficiency is unusual because adequate amounts are generally obtained from the diet and produced by intestinal bacteria. If the bacterial population in the gut is decreased (e.g., by antibiotics), the amount of endogenously formed vitamin is decreased, and this can lead to hypoprothrombinemia in the marginally malnourished individual (e.g., a debilitated geriatric patient). This condition may require supplementation with vitamin K to correct the bleeding tendency. In addition, certain cephalosporin antibiotics (e.g., cefamandole) cause hypoprothrombinemia, apparently by a warfarin-like mechanism that inhibits VKOR. Consequently, their use in treatment is usually supplemented with vitamin K. Deficiency can also affect bone health.

2. Deficiency in the newborn: Because newborns have sterile intestines, they initially lack the bacteria that synthesize vitamin K. Because human milk provides only about one fifth of the daily requirement for vitamin K, it is recommended that all newborns receive a single intramuscular dose of vitamin K as prophylaxis against hemorrhagic disease of the newborn.

D. Toxicity

Prolonged administration of large doses of menadione can produce hemolytic anemia and jaundice in the infant, because of toxic effects on the RBC membrane. Therefore, it is no longer used to treat vitamin K deficiency. No UL for the natural form has been set.

XIV. VITAMIN E

The E vitamins consist of eight naturally occurring tocopherols, of which α-tocopherol is the most active (Fig. 28.28). Vitamin E functions as an antioxidant in prevention of nonenzymic oxidations (e.g., oxidation of LDL and peroxidation of polyunsaturated FA by O_2 and free radicals). (Note: Vitamin C regenerates active vitamin E.)

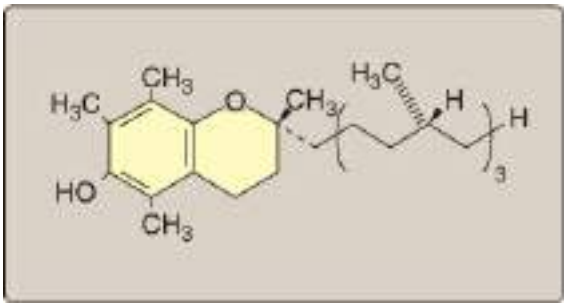


Figure 28.28
Structure of vitamin E (α-tocopherol).

VITAMIN	OTHER NAMES	ACTIVE FORM	FUNCTION
Vitamin B_9	Folic acid	Tetrahydrofolic acid	Transfer one-carbon units; synthesis of methionine, serine, purine nucleotides, and thymidine monophosphate
Vitamin B_{12}	Cobalamin	Methylcobalamin Deoxyadenosyl cobalamin	Coenzyme for reactions: Homocysteine → methionine Methylmalonyl CoA → succinyl CoA
Vitamin C	Ascorbic acid	Ascorbic acid	Antioxidant Coenzyme for hydroxylation reactions, for example: in procollagen: Proline → hydroxyproline Lysine → hydroxylysine
Vitamin B_6	Pyridoxine Pyridoxamine Pyridoxal	Pyridoxal phosphate	Coenzyme for enzymes, particularly in amino acid metabolism
Vitamin B_1	Thiamine	Thiamine pyrophosphate	Coenzyme of enzymes catalyzing: Pyruvate → acetyl CoA α-Ketoglutarate → Succinyl CoA Ribose 5-P + xylulose 5-P → Sedoheptulose 7-P + Glyceraldehyde 3-P Branched-chain α-keto acid oxidation
Vitamin B_3	Niacin Nicotinic acid	NAD^+, $NADP^+$	Electron transfer
Vitamin B_2	Riboflavin	FMN, FAD	Electron transfer
Vitamin B_7	Biotin	Enzyme-bound biotin	Carboxylation reactions
Vitamin B_5	Pantothenic acid	Coenzyme A	Acyl carrier **WATER SOLUBLE**
Vitamin A	Retinol Retinal Retinoic acid β-Carotene	Retinol Retinal Retinoic acid	**FAT SOLUBLE** Maintenance of reproduction Vision Promotion of growth Differentiation and maintenance of epithelial tissues Gene expression
Vitamin D	Cholecalciferol Ergocalciferol	1,25-Dihydroxycholecalciferol	Calcium uptake Gene expression
Vitamin K	Menadione Menaquinone Phylloquinone	Menadione Menaquinone Phylloquinone	γ-Carboxylation of glutamate residues in clotting and other proteins
Vitamin E	α-Tocopherol	Any of several tocopherol derivatives	Antioxidant

DEFICIENCY	SIGNS AND SYMPTOMS	TOXICITY	NOTES
Megaloblastic anemia Neural tube defects	Anemia Birth defects	None	Administration of high levels of folate can mask vitamin B_{12} deficiency
Pernicious anemia Dementia Spinal degeneration	Megaloblastic anemia Neuropsychiatric symptoms	None	Pernicious anemia is treated with intramuscular or high-dose oral vitamin B_{12}
Scurvy	Sore, spongy gums Loose teeth Poor wound healing Bleeding	None	Benefits of supplementation not established in controlled trials
Rare	Glossitis Neuropathy	Yes	Deficiency can be induced by isoniazid Sensory neuropathy occurs at high doses
Beriberi Wernicke-Korsakoff syndrome (most common in alcoholism)	Peripheral neuropathy (dry form), edema and cardiomyopathy (wet form) Confusion, ataxia, memory loss, hallucinations, dysregulated eye movements	None	—
Pellagra	Dermatitis Diarrhea Dementia	None	High doses of niacin used to treat hyperlipidemia
Rare	Dermatitis Angular stomatitis	None	—
Rare	Dermatitis	None	Consumption of large amounts of raw egg whites (which contains a protein, avidin, that binds biotin) can induce a biotin deficiency
Rare	—	None	—
			WATER SOLUBLE
			FAT SOLUBLE
Night blindness Xerophthalmia Infertility Growth retardation	Increased visual threshold Dryness of cornea	Yes	β-Carotene not acutely toxic, but supplementation is not recommended Excess vitamin A can increase incidence of fractures
Rickets (in children) Osteomalacia (in adults)	Soft, pliable bones	Yes	Vitamin D is not a true vitamin because it can be synthesized in skin; application of sunscreen lotions or presence of dark skin color decreases this synthesis.
Newborn Rare in adults	Bleeding	Rare	Vitamin K produced by intestinal bacteria. Vitamin K deficiency common in newborns Intramuscular treatment with vitamin K is recommended at birth
Rare	Red blood cell fragility leads to hemolytic anemia	None	Benefits of supplementation for disease prevention not established in controlled trials

Figure 28.29
Summary of vitamins. (Note: Choline, like vitamin D, is considered an essential micronutrient in humans even though we are able to synthesize it.) P = phosphate; NAD(P) = nicotinamide adenine dinucleotide (phosphate); FMN = flavin mononucleotide; FAD = flavin adenine dinucleotide; CoA = coenzyme A.

A. Distribution and requirements

Vegetable oils are rich sources of vitamin E, whereas liver and eggs contain moderate amounts. The RDA for α-tocopherol is 15 mg/day for adults. The vitamin E requirement increases as the intake of polyunsaturated FA increases to limit FA peroxidation.

B. Deficiency

Newborns have low reserves of vitamin E, but breast milk (and formulas) contains the vitamin. Very–low-birth-weight infants may be given supplements to prevent the hemolysis and retinopathy associated with vitamin E deficiency. When observed in adults, deficiency is usually associated with defective lipid absorption or transport. (Note: Abetalipoproteinemia, caused by a defect in the formation of chylomicrons [and VLDL], results in vitamin E deficiency [see p. 256].)

C. Clinical indications for vitamin E

Vitamin E is not recommended for the prevention of chronic disease, such as CVD or cancer. Clinical trials using vitamin E supplementation have been uniformly disappointing. For example, subjects in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study trial who received high doses of vitamin E not only lacked cardiovascular benefit but also had an increased incidence of stroke. (Note: Vitamins E and C are used to slow the progression of age-related macular degeneration.)

D. Toxicity

Vitamin E is the least toxic of the fat-soluble vitamins, and no toxicity has been observed at doses of 300 mg/day (UL = 1,000 mg/day).

> Populations consuming diets high in fruits and vegetables show decreased incidence of some chronic diseases. However, clinical trials have failed to show a definitive benefit from supplements of folic acid; vitamins A, C, or E; or antioxidant combinations for the prevention of cancer or CVD.

XV. Chapter Summary

The vitamins are summarized in Figure 28.29.

Study Questions

Choose the ONE best answer.

For Questions 28.1–28.5, match the vitamin deficiency to the clinical consequence.

A. Folic acid
B. Niacin
C. Vitamin A
D. Vitamin B_{12}
E. Vitamin C
F. Vitamin D
G. Vitamin E
H. Vitamin K

Correct answers = H, B, A, C, E. Vitamin K is required for formation of the γ-carboxyglutamate residues in several proteins required for blood clotting. Consequently, a deficiency of vitamin K results in a tendency to bleed. Niacin deficiency is characterized by the three Ds: diarrhea, dermatitis, and dementia (and death, a fourth D, if untreated). Folic acid deficiency can result in neural tube defects in the developing fetus. Night blindness is one of the first signs of vitamin A deficiency. Rod cells in the retina detect white and black images and work best in low light, for example, at night. Rhodopsin, the visual pigment of the rod cells, consists of 11-cis retinal bound to the protein opsin. Vitamin C is required for the hydroxylation of proline and lysine during collagen synthesis. Severe vitamin C deficiency (scurvy) results in defective connective tissue, characterized by sore and spongy gums, loose teeth, capillary fragility, anemia, and fatigue.

28.1 Bleeding

28.2 Diarrhea and dermatitis

28.3 Neural tube defects

28.4 Night blindness (nyctalopia)

28.5 Sore, spongy gums and loose teeth

28.6 A 52-year-old female presents with fatigue of several months' duration. Blood studies reveal a macrocytic anemia, reduced levels of hemoglobin, elevated levels of homocysteine, and normal levels of methylmalonic acid. Which of the following is most likely deficient in this patient?

A. Folic acid
B. Folic acid and vitamin B_{12}
C. Iron
D. Vitamin C

Correct answer = A. Macrocytic anemia is seen with deficiencies of folic acid, vitamin B_{12}, or both. Vitamin B_{12} is utilized in only two reactions in the body: the remethylation of homocysteine (Hcy) to methionine, which also requires folic acid (as tetrahydrofolate [THF]), and the isomerization of methylmalonyl coenzyme A to succinyl coenzyme A, which does not require THF. The elevated Hcy and normal methylmalonic acid levels in the patient's blood reflect a deficiency of folic acid as the cause of the macrocytic anemia. Iron deficiency causes microcytic anemia, as can vitamin C deficiency.

28.7 A 10-month-old African American female, whose family recently located from Maine to Virginia, is being evaluated for the bowed appearance of her legs. The parents report that the baby is still being breastfed and takes no supplements. Radiologic studies confirm the suspicion of rickets caused by vitamin D deficiency. Which one of the following statements concerning vitamin D is correct?

A. A deficiency results in an increased secretion of calbindin.
B. Chronic kidney disease results in overproduction of 1,25-dihydroxycholecalciferol (calcitriol).
C. 25-Hydroxycholecalciferol (calcidiol) is the active form of the vitamin.
D. It is required in the diet of individuals with limited exposure to sunlight.
E. Its actions are mediated through binding to G protein–coupled receptors.
F. It opposes the effect of parathyroid hormone.

Correct answer = D. Vitamin D is required in the diet of individuals with limited exposure to sunlight, such as those living at northern latitudes like Maine and those with dark skin. Note that breast milk is low in vitamin D, and the lack of supplementation increases the risk of a deficiency. Vitamin D deficiency results in decreased synthesis of calbindin. Chronic kidney disease decreases production of calcitriol (1,25-dihydroxycholecalciferol), the active form of the vitamin. Vitamin D binds to nuclear receptors and alters gene transcription. Its effects are synergistic with parathyroid hormone.

28.8 Why might a deficiency of vitamin B_6 result in a fasting hypoglycemia? Deficiency of what other vitamin could also result in hypoglycemia?

Vitamin B_6 is required for glycogen degradation by glycogen phosphorylase. A deficiency would result in fasting hypoglycemia. Additionally, a deficiency of biotin (required by pyruvate carboxylase of gluconeogenesis) would also result in fasting hypoglycemia.

Micronutrients: Minerals 29

I. OVERVIEW

Minerals are inorganic substances (elements) required in small amounts by the body. They function in a number of processes including formation of bones and teeth, fluid balance, nerve conduction, muscle contraction, signaling, and catalysis. (Note: Several minerals are essential enzyme cofactors.) Like the organic vitamins (see Chapter 28), minerals are micronutrients required in mg or µg amounts. Those required by adults in the largest amounts (>100 mg/day) are referred to as the macrominerals. Minerals required in amounts between 1 and 100 mg/day are the microminerals (trace minerals). Ultratrace minerals are required in amounts <1 mg/day (Fig. 29.1). (Note: The classification of specific minerals into these categories can vary among sources.) Mineral concentrations in the body are influenced by their rates of absorption and excretion.

MINERAL CLASSIFICATIONS	RDA (OR AI*) FOR ADULTS
MACROMINERALS	
Calcium (Ca)	1,000–2000 mg
Chloride (Cl)	1,800–2,300 mg*
Magnesium (Mg)	310–420 mg
Phosphorus (P)	700 mg
Potassium (K)	4,700 mg*
Sodium (Na)	1,500 mg*
MICROMINERALS (TRACE)	
Chromium (Cr)	30–35 mg
Copper (Cu)	900 µg
Fluorine (as fluoride [F^-])	3–4 mg
Iron (Fe)	8–18 mg
Manganese (Mn)	1.8–2.3 mg*
Zinc (Zn)	8–11 mg
MICROMINERALS (ULTRATRACE)	
Iodine (I)	150 µg
Molybdenum (Mo)	45 µg
Selenium (Se)	55 µg

Figure 29.1
Classification of minerals and recommended amounts to be consumed/day by adults. (Note: *An adequate intake [AI] is set if insufficient scientific evidence is available to calculate a Recommended Dietary Allowance [RDA].)

II. MACROMINERALS

The macrominerals include calcium (Ca^{2+}), phosphorus ([P] as inorganic phosphate [P_i, or PO_4^{3-}]), magnesium (Mg^{2+}), sodium (Na^+), chloride (Cl^-), and potassium (K^+). (Note: The free ionic forms are electrolytes.)

A. Calcium and phosphorus

These macrominerals are considered together because they are components of hydroxylapatite ($Ca_5[PO_4]_3OH$), which makes up bones and teeth.

1. Calcium: Ca^{2+} is the most abundant mineral in the body, with approximately 98% being found in bones. The remainder is involved in a number of processes such as signaling, muscle contraction, and blood clotting. Ca^{2+} binds to a variety of proteins including calmodulin (see Chapter 11), phospholipase A_2 (see p. 236), and protein kinase C (see p. 227) and alters their activity. (Note: Calbindin is a vitamin D–induced intracellular Ca^{2+}-binding protein involved in Ca^{2+} absorption in the intestine [see p. 439].) Dairy products, many green vegetables (e.g., broccoli, but not spinach), and fortified orange juice are good dietary sources. Although dietary deficiency syndromes are unknown, average Ca^{2+} intake in the United States is insufficient for optimal bone health. Toxicity is seen only with supplements (tolerable upper limit [UL] = 2,500 mg/day for adults). Hypercalcemia (elevated serum Ca^{2+}) can result from overproduction of parathyroid hormone (PTH). This may cause constipation and kidney stones. Hypocalcemia (low serum Ca^{2+}) can result from a deficiency of PTH or vitamin D. It can lead to bone demineralization (resorption). (Note: The hormonal regulation of serum Ca^{2+} levels was presented in the vitamin D section of Chapter 28 and is reviewed in 3. below.)

> Bone mass increases from infancy through the early reproductive years and then shows an age-related loss in both men and women that increases the risk for fracture. This loss is greatest in postmenopausal Caucasian women. Some studies have shown that supplementation with Ca^{2+} and vitamin D decreases this risk.

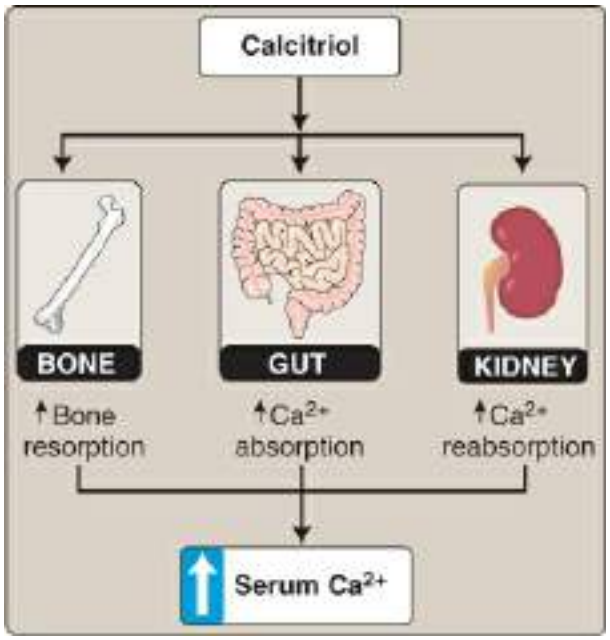


Figure 29.2
Effect of calcitriol on serum calcium (Ca^{2+}).

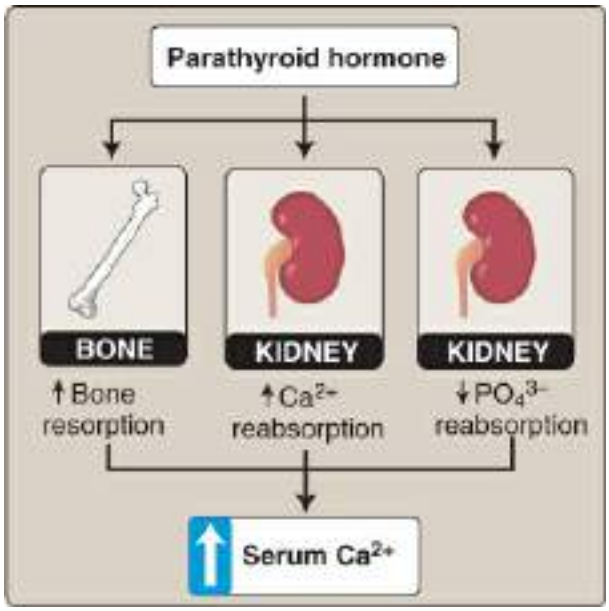


Figure 29.3
Effect of parathyroid hormone on serum calcium (Ca^{2+}). PO_4^{3-} = phosphate.

2. Phosphorus: Free phosphate (P_i) is the most abundant intracellular anion. However, 85% of the body's phosphorus is in the form of inorganic hydroxylapatite, with most of the remainder in intracellular organic compounds such as phospholipids, nucleic acids, ATP, and creatine phosphate. Phosphate is supplied as ATP for kinases and as P_i for phosphorylases (e.g., glycogen phosphorylase, see Chapter 11). (Note: Its addition by kinases or removal by phosphatases is an important means of covalent regulation of enzymes [see Chapter 24].) Phosphorus is widely distributed in food (milk is a good source), and dietary deficiency is rare. Hypophosphatemia can be caused by refeeding carbohydrates to malnourished patients (refeeding syndrome, see p. 414), overuse of aluminum-containing antacids (aluminum chelates P_i), and increased urinary loss in response to increased production of PTH (see below). Muscle weakness is a common symptom. Hyperphosphatemia is caused primarily by decreased PTH levels. The excess P_i can combine with Ca^{2+} and form crystals that deposit in soft tissue (metastatic calcification). (Note: The Ca^{2+}/P_i ratio is important for bone formation [the ratio is approximately 2/1 in bone], and some experts are concerned that replacement of Ca^{2+}-rich milk by Ca^{2+}-poor, P_i-rich soft drinks can affect bone health.)

3. Hormonal regulation: Serum levels of Ca^{2+} and P_i are primarily controlled by calcitriol (1,25-dihydroxycholecalciferol, the active form of vitamin D) and PTH,

both of which respond to a decrease in serum Ca^{2+}. Calcitriol, produced by the kidneys, increases serum Ca^{2+} and P_i by increasing bone resorption and intestinal absorption and renal reabsorption of Ca^{2+} and P_i (Fig. 29.2). PTH (from the parathyroid glands) increases serum Ca^{2+} by increasing bone resorption, increasing renal reabsorption of Ca^{2+}, and activating the renal 1-hydroxylase that produces calcitriol from calcidiol (25-OH-D3) (Fig. 29.3). In contrast to calcitriol, PTH decreases P_i reabsorption in the kidneys, lowering serum P_i. (Note: High serum P_i increases PTH and decreases calcitriol.) A third hormone, calcitonin (from the C cells of the thyroid gland), responds to elevated serum Ca^{2+} levels by promoting bone mineralization and increasing renal excretion of Ca^{2+} (and P_i).

B. Magnesium

About 60% of the body's Mg^{2+} is in bone, but it accounts for just 1% of the bone mass. The mineral is required by a variety of enzymatic reactions, including phosphorylation by **kinases** (Mg^{2+} binds the ATP cosubstrate) and phosphodiester bond formation by **DNA** and **RNA polymerases**. Mg^{2+} is widely distributed in foods, but the average intake in the United States is below the recommended level. Hypomagnesemia can result from decreased absorption or increased excretion of Mg^{2+}. Symptoms include hyperexcitability of skeletal muscles and nerves and cardiac arrhythmias. With hypermagnesemia, hypotension is seen. (Note: Magnesium sulfate is used in the treatment of preeclampsia, a hypertensive disorder of pregnancy.)

C. Sodium, chloride, and potassium

These macrominerals are considered together because they play important roles in several physiologic processes. For example, they maintain water balance, osmotic equilibrium, acid–base balance (pH), and the electrical gradients across cell membranes (membrane potential) that are essential for the functioning of neurons and myocytes. (Note: These processes are discussed in *Lippincott ® Illustrated Reviews: Physiology*.)

1. Sodium and chloride: Na^+ and Cl^- are primarily extracellular electrolytes. They are readily absorbed from foods containing salt (NaCl), much of which comes from processed foods. (Note: Na^+ is required for the intestinal absorption [and renal reabsorption] of glucose and galactose [see Chapter 7] and free amino acids [see p. 275] by Na^+-linked transporters. Cl^- is used to form hydrochloric acid required for digestion [see p. 274].) In the United States, the average daily consumption of NaCl is 1.5 to 3 times the adequate intake (AI) of 3.8 mg/day (UL = 5.8 g/day). Dietary deficiency is rare.

a. Hypertension: Na^+ intake is related to blood pressure (BP). Ingestion of Na^+

stimulates thirst centers in the brain and secretion of antidiuretic hormone from the pituitary, leading to water retention. This results in an increase in plasma volume and, consequently, an increase in BP. Chronic hypertension can damage the heart, kidneys, and blood vessels. Modest reductions in Na^+ intake have been shown to result in modest reductions in BP.

b. Hyper- and hyponatremia: Hypernatremia, typically caused by excess water loss, and hyponatremia, typically caused by decreased ability to excrete water, can result in severe brain damage. (Note: Chronic hyponatremia increases Ca^{2+} excretion and can result in osteoporosis [low bone mass].)

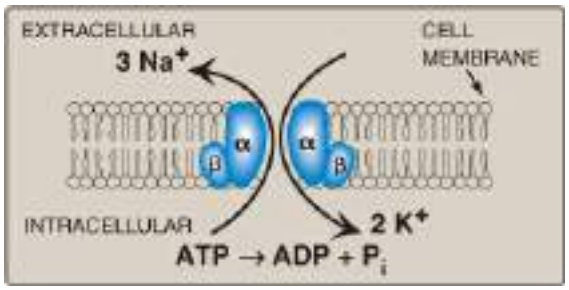


Figure 29.4
Na^+/K^+ ATPase. Na^+ = sodium; K^+ = potassium; ADP = adenosine diphosphate; P_i = phosphate.

2. Potassium: In contrast to Na^+, K^+ is primarily an intracellular electrolyte. (Note: The concentration differential of Na^+ and K^+ across the cell membrane is maintained by the Na^+/K^+ ATPase [Fig. 29.4].) In contrast to Na^+ and Cl^-, K^+ (like Mg^{2+}) is underingested in Western diets because its primary sources, fruits and vegetables, are underingested. (Note: Increasing dietary K^+ decreases BP by increasing Na^+ excretion.) There is a narrow range for normal serum K^+ levels, and even modest changes (up or down, resulting in hyper- or hypokalemia) can result in cardiac arrhythmias and skeletal muscle weakness. (Note: Hypokalemia can result from the inappropriate use of laxatives to lose weight.) No UL for K^+ has been established.

Cu-REQUIRING ENZYME	FUNCTION
Cytochrome c oxidase	Transfers electrons from cytochrome c to oxygen in the ETC
Dopamine β-hydroxylase	Hydroxylates dopamine to norepinephrine
Ferroxidases	Oxidize iron
Lysyl oxidase	Forms cross-links in collagen and elastin
Tyrosinase	Synthesizes melanin
Superoxide dismutase (nonmitochondrial form; also requires zinc)	Converts superoxide to hydrogen peroxide

Figure 29.5
Examples of enzymes that require copper (Cu). ETC = electron transport chain.

III. TRACE MINERALS (MICROMINERALS)

The trace minerals include copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn). They are required by adults in amounts between 1 and 100 mg/day.

A. Copper

Cu is a key component of several enzymes that play critical functions in the body (Fig. 29.5). These include **ferroxidases** such as the **ceruloplasmin** and **hephaestin** involved in the oxidation of ferrous iron (Fe^{2+}) to the ferric form (Fe^{3+}) that is required for its intracellular storage or transport through blood (see B.1. below). Meat, shellfish, nuts, and whole grains are good dietary sources of Cu. Dietary deficiency is uncommon. If a deficiency does develop, anemia may be seen because of the effect on Fe metabolism. Toxicity from dietary sources is rare (UL = 10 mg/day). Menkes syndrome and Wilson disease are genetic causes of Cu deficiency and Cu overload, respectively.

1. Menkes syndrome: In Menkes syndrome (“kinky hair” disease), a rare X-linked (1:140,000 males) disorder, efflux of dietary Cu out of intestinal enterocytes into the circulation by a Cu-transporting *ATPase* (*ATP7A*) is impaired. This results in

systemic Cu deficiency. Consequently, urinary and serum-free (unbound) Cu are low, as is the concentration of ceruloplasmin, which carries over 90% of the Cu in the circulation (Fig. 29.6). Progressive neurologic degeneration and connective tissue disorders are seen, as are changes to hair. Parenteral administration of Cu has been used as a treatment with varying success. (Note: The mildest form of Menkes syndrome is called occipital horn syndrome.)

2. Wilson disease: In Wilson disease, an autosomal-recessive (AR) disorder affecting 1:35,000 live births, efflux of Cu from the liver by ATP7B is impaired. Cu accumulates in the liver; leaks into the blood; and is deposited in the brain, eyes, kidneys, and skin. In contrast to Menkes syndrome, urinary and serum-free Cu are high (see Fig. 29.6). Hepatic dysfunction and neurologic and psychiatric symptoms are seen. Kayser–Fleischer rings (corneal deposits of Cu) may be present (Fig. 29.7). Life-long use of Cu-chelating agents, such as penicillamine, is the treatment.

VARIABLE	MENKES	WILSON
Whole-body Cu	Low	High
Free serum Cu	Low	High
Urinary Cu	Low	High
Inheritance	X-linked	AR
Cu-transporting ATPase affected	ATP7A	ATP7B

Figure 29.6
Comparison of Menkes syndrome and Wilson disease. Cu = copper; AR = autosomal recessive.

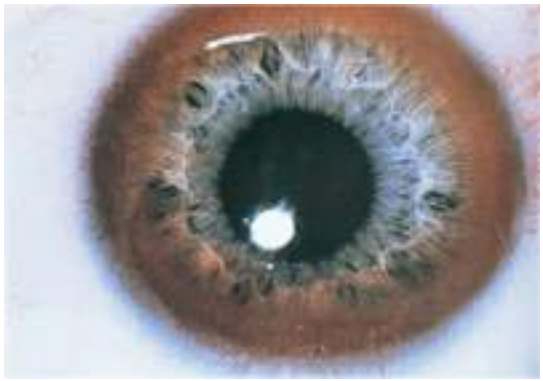


Figure 29.7
Kaiser–Fleischer rings.

The bioavailability (percent of the amount ingested that is able to be absorbed) of a mineral can be influenced by other minerals. For example, excess Zn decreases the absorption of Cu, and Cu is

needed for the absorption of Fe.

B. Iron

The adult body typically contains 3 to 4 g of Fe. It is a component of many proteins, both catalytic (e.g., **hydroxylases** such as **prolyl hydroxylase** and noncatalytic. Iron can be linked to sulfur (S) as seen in the Fe–S proteins of the electron transport chain, or it can be part of the heme prosthetic group in proteins such as hemoglobin (approximately 70% of all Fe), myoglobin, and the cytochromes. (Note: Free ionic Fe is toxic because it can cause production of the hydroxyl radical, a reactive oxygen species [ROS].) Dietary Fe is available as Fe^{2+} in heme (animal sources) and Fe^{3+} in nonheme sources (plants). Heme iron is less abundant, but it is better absorbed. Meat, poultry, some shellfish, iron-fortified foods such as breakfast cereals and grains, lentils, and green leafy vegetables are good dietary sources of Fe. About 10% of ingested Fe is absorbed. This amount, approximately 1 to 2 mg/day, is sufficient to replace Fe lost from the body primarily by the sloughing of cells.

1. Absorption, storage, and transport: Intestinal uptake of heme is by a heme carrier protein (Fig. 29.8). Within the enterocytes, heme oxygenase releases Fe^{2+} from heme (see p. 314). Nonheme Fe is taken up via the apical membrane protein divalent metal ion transporter-1 (DMT-1). (Note: Vitamin C enhances absorption of nonheme Fe because it is the coenzyme for duodenal cytochrome b [Dcytb], a ferrireductase that reduces Fe^{3+} to Fe^{2+}.) Absorbed Fe^{2+} from heme and nonheme sources has two possible fates: It can be (1) oxidized to Fe^{3+} and stored by the intracellular protein ferritin (up to 4,500 Fe^{3+}/ferritin) or (2) transported out of the enterocyte by the basolateral membrane protein ferroportin, oxidized by the Cu-containing membrane protein hephaestin, and taken up by the plasma transport protein transferrin (2 Fe^{3+}/transferrin), as shown in Figure 29.8. (Note: Cells other than enterocytes use the Cu-containing plasma protein ceruloplasmin in place of hephaestin.) In normal individuals, transferrin (Tf) is about one-third saturated with Fe^{3+}. Ferroportin, the only known exporter of Fe from cells to the blood in humans, is regulated by the hepatic peptide hepcidin that induces internalization and lysosomal degradation of ferroportin. Therefore, hepcidin is the central molecule in Fe homeostasis. (Note: Transcription of hepcidin is suppressed when Fe is deficient.)

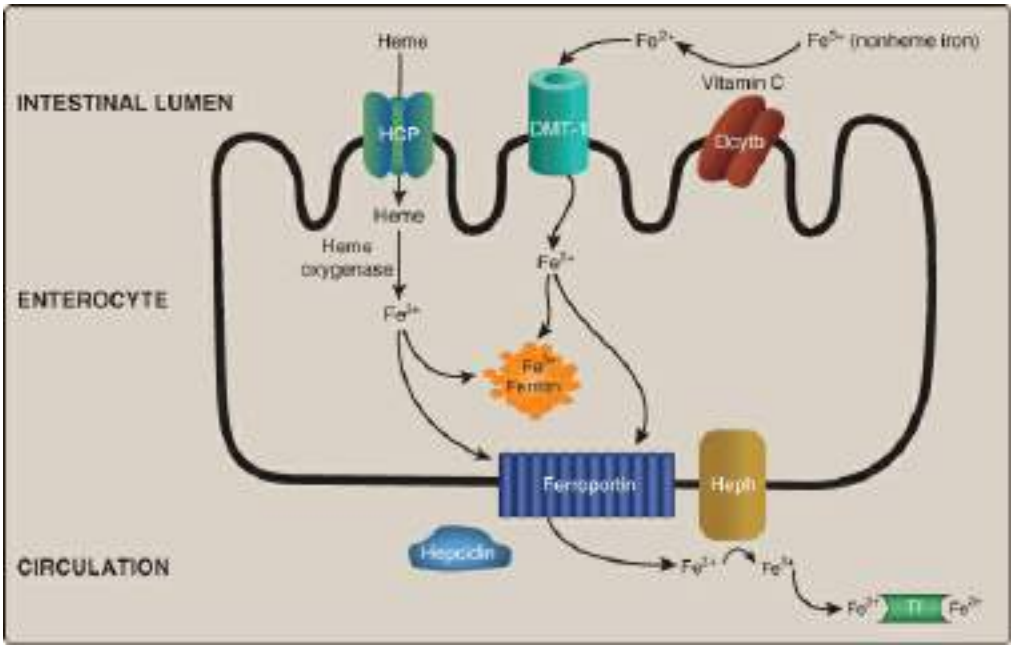


Figure 29.8
Absorption, storage, and transport of dietary iron (Fe). HCP = heme carrier protein; DMT = divalent metal ion transporter; Dcytb = duodenal cytochrome b (a ferrireductase); Heph = hephaestin; Tf = transferrin.

2. Recycling: Macrophages phagocytose old and/or damaged red blood cells (RBCs), freeing heme Fe that is sent out of the cells via ferroportin, oxidized by ceruloplasmin, and transported by Tf as described above. This recycled Fe meets approximately 90% of our daily need, which is predominantly for erythropoiesis.

3. Uptake: Tf-bound Fe^{3+} from enterocytes and macrophages binds to receptors (TfR) on erythroblasts and other Fe-requiring cells and is taken up by receptor-mediated endocytosis. The Fe^{3+} is released from Tf for use (or stored on ferritin), and the TfR (and Tf) is recycled in a process similar to the receptor-mediated endocytosis seen with low-density lipoprotein particles (see p. 257). (Note: Regulation of the translation of the messenger RNA for ferritin and the TfR by iron regulatory proteins and iron-responsive elements is discussed in Chapter 33.)

4. Deficiency: Fe deficiency can result in a microcytic, hypochromic anemia (Fig. 29.9), the most common anemia in the United States, as a result of decreased hemoglobin synthesis and, consequently, decreased RBC size. Treatment is the administration of Fe in various ways depending on the severity of anemia.

5. Excess: Fe overload can occur with accidental ingestion. (Note: Acute Fe poisoning is the most common cause of poisoning deaths of children age <6 years [UL = 40 mg/day for children, 45 mg/day for adults].) Treatment is use of an Fe chelator. Overload can also occur with genetic defects. An example is

hereditary hemochromatosis (HH), an AR disorder of Fe overload found primarily in those of Northern European ancestry. It is most commonly caused by mutations to the HFE (high Fe) gene. Hyperpigmentation with hyperglycemia (“bronze diabetes”) and damage to the liver (a major storage site for Fe), pancreas, and heart may be seen. In HH, serum Fe and Tf saturation are elevated. Treatment is phlebotomy or use of Fe chelators. (Note: Fe overload is seen with mutations to proteins of Fe metabolism that result in inappropriately low levels of hepcidin. It can result in hemosiderosis [the deposition of hemosiderin, an intracellular, insoluble storage form of Fe].)

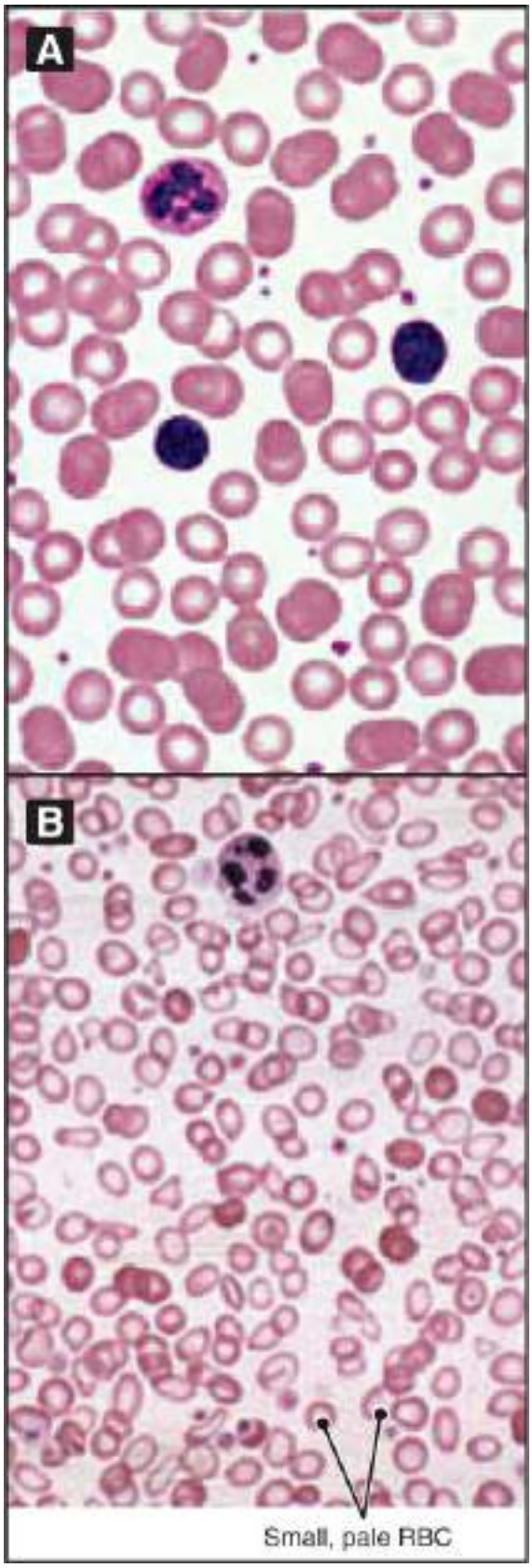


Figure 29.9

A: Normal red blood cells (RBCs). **B:** Small (microcytic), pale (hypochromic) RBC in microcytic anemia.

C. Manganese

Mn is important for the function of several enzymes (Fig. 29.10). Whole grains, legumes (e.g., beans and peas), nuts, and tea (especially green tea) are good sources of the mineral. Consequently, Mn deficiency in humans is rare. Toxicity from foods and/or supplements is also rare (UL = 11 mg/day for adults).

Mn-REQUIRING ENZYME	FUNCTION
Arginase-I	Hydrolyzes arginine to urea plus ornithine in the urea cycle
Glycosyltrans-ferases	Transfer sugars in proteoglycan synthesis
Pyruvate carboxylase	Carboxylates pyruvate to OAA in gluconeogenesis
Superoxide dismutase (mitochondrial form)	Converts superoxide to hydrogen peroxide

Figure 29.10
Examples of enzymes that require manganese (Mn). OAA = oxaloacetate.

D. Zinc

Zn plays important structural and catalytic functions in the body. Zinc fingers are supersecondary structures in proteins (e.g., transcription factors) that bind to DNA and regulate gene expression (Fig. 29.11). Hundreds of enzymes require Zn for activity. Examples include **alcohol dehydrogenase**, which oxidizes ethanol to acetaldehyde (see p. 352); **carbonic anhydrase**, which is important in the bicarbonate buffer system (see Chapter 3); **ALA dehydratase** (porphobilinogen synthase) of heme synthesis, which is inhibited by lead (lead replaces the zinc; see p. 310); and the nonmitochondrial isoform of **superoxide dismutase** (SOD), which also requires Cu (see Fig. 29.5). Dietary sources of Zn include meat, fish, eggs, and dairy products. Phytates (phosphate storage molecules in plants such as grains, seeds, legumes, some nuts) irreversibly bind Zn in the intestine, decreasing its absorption, and can result in a deficiency. (Note: Phytates may also bind Ca^{2+} and nonheme Fe.) Several drugs (e.g., penicillamine) chelate metals, and their use may cause Zn deficiency. (Note: Severe Zn deficiency is seen in acrodermatitis

enteropathica, an autosomal recessive disorder which arise due to a defect in the intestinal transporter for Zn. Symptoms include rashes around the orifices and in the limbs, slowed growth and development, diarrhea, and immune deficiencies. Vision problems may also occur because Zn is needed in the metabolism of vitamin A.)

> Eukaryotic cells infected with bacteria can restrict availability of the essential micronutrients Fe, Mn, and Zn to the pathogens. This decreases the intracellular survival of the pathogen and is known as “nutritional immunity.”

E. Other microminerals

Chromium (Cr) and fluorine (F) also play roles in the body. Cr potentiates the action of insulin by an unknown mechanism. It is found in fruits, vegetables, dairy products, and meat. F (as fluoride [F^-]) is added to water in many parts of the world to reduce the incidence of dental caries (Fig. 29.12). F^- replaces the hydroxyl group of hydroxylapatite, forming fluoroapatite that is more resistant to the enamel-dissolving acid produced by mouth bacteria.

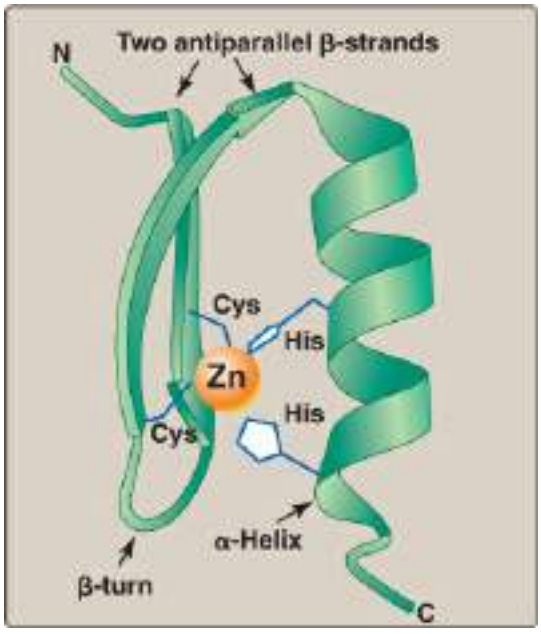


Figure 29.11
Zinc (Zn) finger is a common motif in proteins that bind DNA. Cys = cysteine; His = histidine.

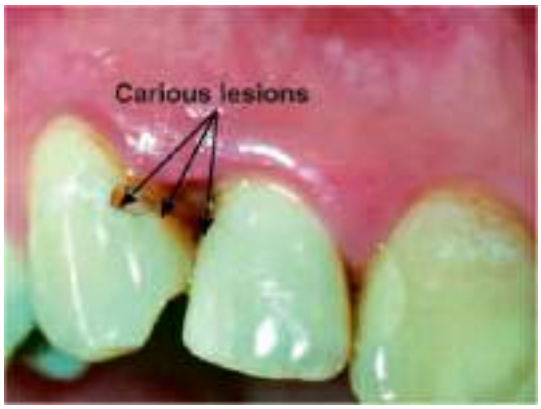


Figure 29.12
Dental caries (cavities).

IV. ULTRATRACE MINERALS

The ultratrace minerals include iodine (I), selenium (Se), and molybdenum (Mo). They are required by adults in amounts <1 mg/day.

A. Iodine

Iodine is utilized in the synthesis of the thyroid hormones triiodothyronine (T_3) and thyroxine (T_4) that are required for development, growth, and metabolism. Circulating iodide (I^-) is taken up ("trapped") and concentrated in the epithelial follicular cells of the thyroid gland. It then is sent into the colloid of the follicular lumen where it is oxidized to iodine (I_2) by **thyroperoxidase (TPO)**, as shown in Figure 29.13. *TPO* then uses I_2 to iodinate selected tyrosine residues in thyroglobulin (Tg), forming monoiodinated tyrosine (MIT) and diiodinated tyrosine (DIT), as shown in Figure 29.14. (Note: Tg is synthesized and secreted into colloid by follicular cells.) The coupling of two DIT on Tg gives T_4, whereas coupling one MIT and one DIT gives T_3. The iodinated Tg is endocytosed and stored in follicular cells until needed, at which time it is proteolytically digested to release T_3 and T_4, which are secreted into the circulation (see Fig. 29.13). Under normal conditions, approximately 90% of secreted thyroid hormone is T_4 that is carried by transthyretin. In target tissues (e.g., the liver and developing brain), T_4 is converted to T_3 (the more active form) by Se-containing **deiodinases**. T_3 binds to a nuclear receptor that binds DNA at thyroid response elements and functions as a transcription factor. (Note: Thyroid hormone production is controlled by thyrotropin [thyroid-stimulating hormone (TSH)] from the anterior pituitary. TSH secretion is itself controlled by thyrotropin-releasing hormone [TRH] from the hypothalamus.)

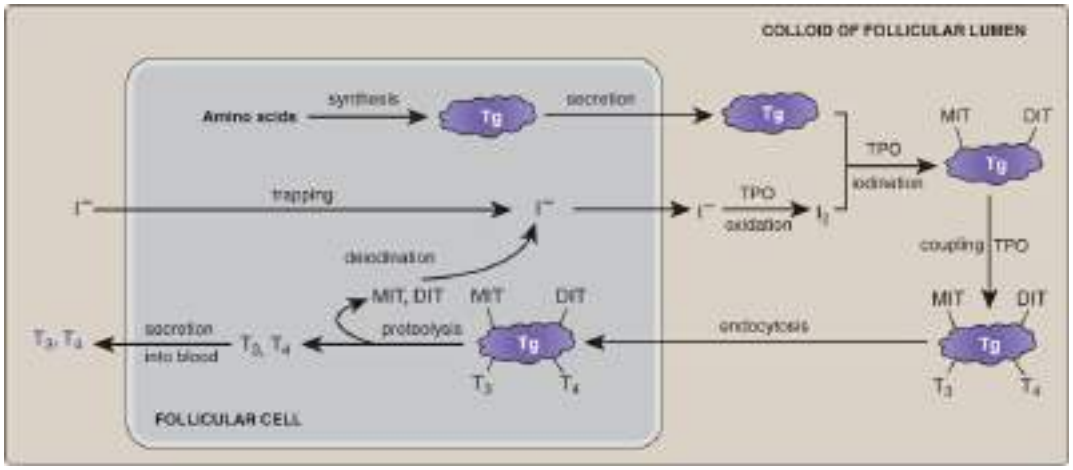


Figure 29.13
Thyroid hormone synthesis. Tg = thyroglobulin; I^- = iodide; I_2 = iodine; TPO = thyroperoxidase; MIT = monoiodinated tyrosine; DIT = diiodinated tyrosine; T_3 = triiodothyronine; T_4 = thyroxine.

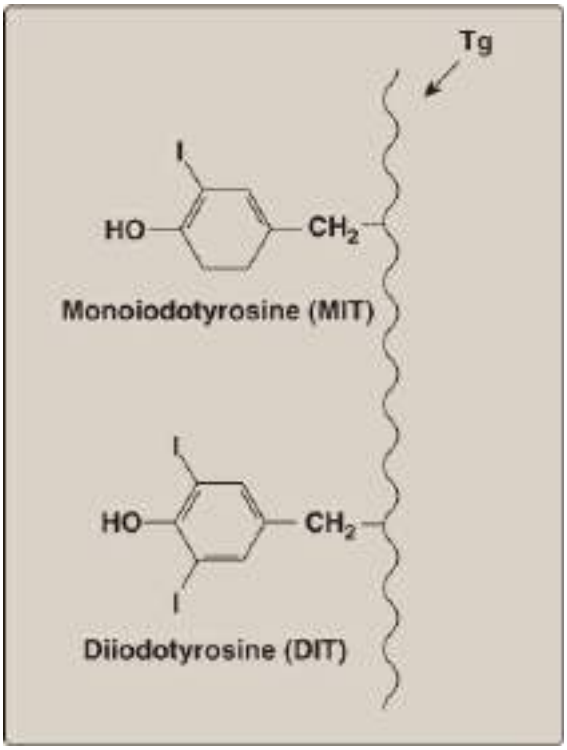


Figure 29.14
Iodination of thyroglobulin (Tg) with production of MIT and DIT.

1. Hypothyroidism: Underingestion of iodine (I) can result in goiter, an enlargement of the thyroid in response to excessive stimulation by TSH, as shown in Figure 29.15. More severe deficiency results in hypothyroidism that is characterized by fatigue, weight gain, decreased thermogenesis, and decreased metabolic rate (see p. 404). If hormone deficiency occurs during fetal and infant development (congenital hypothyroidism), irreversible intellectual disability (formerly called

"cretinism"), hearing loss, spasticity, and short stature can result. In the United States, dairy products, seafood, and meat are the primary sources of I. The use of iodized salt has greatly reduced dietary I deficiency. (Note: Autoimmune destruction of TPO is a cause of Hashimoto thyroiditis [a primary hypothyroidism].)

2. Hyperthyroidism: This condition is the result of overproduction of thyroid hormone. Although it can be caused by overingestion of I-containing supplements (UL = 1.1 g/day for adults), the most common cause of hyperthyroidism is Graves disease, in which an antibody that mimics the effect of TSH is produced, resulting in dysregulated production of thyroid hormone. This can cause nervousness, weight loss, increased perspiration and heart rate, protruding eyes (exophthalmos, Fig. 29.16), and goiter.

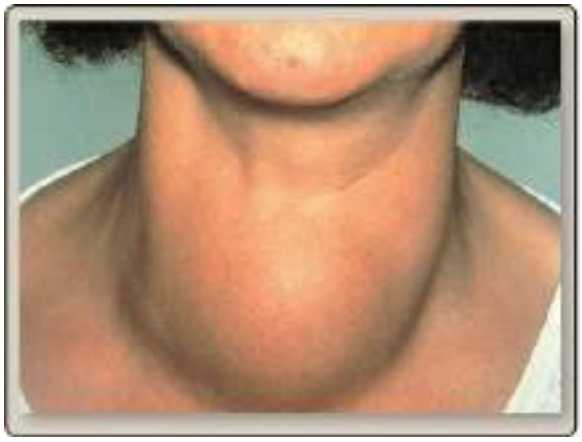


Figure 29.15
Goiter.

B. Selenium

Selenium (Se) is present in approximately 25 human proteins (selenoproteins) as a constituent of the amino acid selenocysteine, which is derived from serine (see p. 297). Selenoproteins include **glutathione peroxidase** that oxidizes glutathione in the reduction of hydrogen peroxide, a ROS, to water (see Chapter 13); **thioredoxin reductase** that reduces thioredoxin, a coenzyme of **ribonucleotide reductase** (see p. 330); and **deiodinases** that remove iodine from thyroid hormones. Meat, dairy products, and grains are important dietary sources. Keshan disease, first identified in China, is a cardiomyopathy caused by eating foods produced from Se-deficient soil. Toxicity (selenosis) caused by overingestion of supplements causes brittle nails and hair. Cutaneous and neurologic effects may also be seen (UL = 400 µg in adults).

C. Molybdenum

Molybdenum (Mo) functions as a cofactor for a small number of mammalian

oxidases (Fig. 29.17). Legumes are important dietary sources. No dietary deficiency syndromes are known. Mo has low toxicity in humans (UL = 2 mg/day in adults).

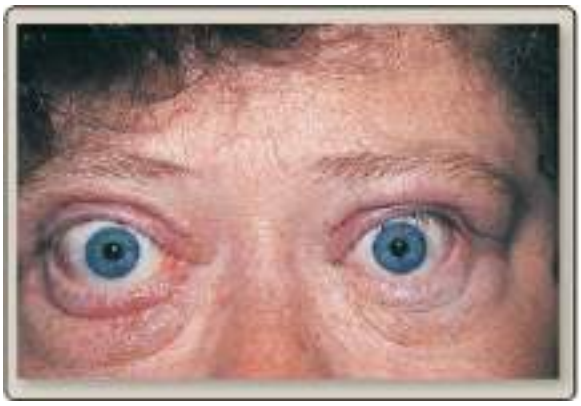


Figure 29.16
Exophthalmos.

Mo-REQUIRING ENZYME	FUNCTION
Aldehyde oxidase	Metabolizes drugs
Sulfite oxidase	Converts sulfite to sulfate in metabolism of the sulfur-containing amino acids methionine and cysteine
Xanthine oxidase	Oxidizes hypoxanthine to xanthine and xanthine to uric acid in purine degradation

Figure 29.17
Enzymes (oxidases) that require molybdenum (Mo).

Cobalt (Co), an ultratrace mineral, is a component of vitamin B_{12} (cobalamin, see p. 425), which is required as methylcobalamin in the remethylation of homocysteine to methionine (see p. 293) or adenosylcobalamin in the isomerization of methylmalonyl coenzyme A (CoA) to succinyl CoA (see p. 215). No Recommended Dietary Allowance or Daily Reference Intake (see p. 403) has been established for Co.

V. CHAPTER SUMMARY

The minerals are summarized in Figure 29.18.

CLASSIFICATION	FUNCTION(S)	NOTES
Macrominerals: >100 mg/day for adults		
Calcium (Ca)	Component of hydroxylapatite ($Ca_5[PO_4]_3OH$) of bone and teeth, muscle contraction, signaling, blood clotting	Dietary deficiencies unknown; toxicity from supplements; hypocalcemia with PTH or vitamin D deficiency causes kidney stones; hypercalcemia with increased PTH causes bone resorption
Chloride (Cl^-)	Fluid balance (along with Na, K), digestion	Dietary deficiency rare; overingested as NaCl
Magnesium (Mg)	Component (minor) of bone; regulates enzyme activity (binds substrate or enzyme)	Average U.S. intake is below recommended level; hyperexcitability and arrhythmias seen with hypomagnesemia; hypotension with hypermagnesemia
Phosphorus (P)	Component of hydroxylapatite of bone and teeth, energy storage, membrane structure, regulation	Dietary deficiency rare; hypophosphatemia with muscle weakness in refeeding syndrome, increased PTH, and use of aluminum-containing antacids; hyperphosphatemia with metastatic calcification in PTH deficiency
Potassium (K)	Membrane potential, blood pressure	Average U.S. intake is below recommended level; modest changes up or down in serum level result in arrhythmias and muscle weakness
Sodium (Na)	Membrane potential, blood volume and pressure; uptake of glucose, galactose, and amino acids	Dietary deficiency rare; overingested as NaCl; hyponatremia seen with excess water loss; hypernatremia with water retention
Microminerals (Trace): 1–100 mg/day		
Chromium (Cr)	Potentiates insulin action	Mechanism unknown
Copper (Cu)	Enzyme cofactor	Dietary deficiency rare; Menkes (genetic systemic Cu deficiency) and Wilson (genetic systemic Cu overload)
Fluorine (as fluoride [F^-])	Increases resistance to enamel-dissolving acid of mouth bacteria	Deficiency results in dental caries
Iron (Fe)	Enzyme cofactor, oxygen binding, Fe-S proteins	Dietary deficiency results in microcytic anemia; hereditary hemochromatosis, a genetic disease of Fe overload, with "bronze diabetes" (hyperglycemia, hyperpigmentation)
Manganese (Mn)	Enzyme cofactor	Dietary deficiency rare
Zinc (Zn)	Enzyme cofactor, protein structure (Zn finger)	Phytates and some drugs decrease absorption; severe deficiency (acrodermatitis enteropathica) with transporter defect
Microminerals (Ultratrace): <1 mg/day		
Iodine (I)	Thyroid hormone (T_3, T_4) synthesis	Undering estion causes goiter, hypothyroidism with fatigue, weight gain, and decreased metabolic rate; neurologic damage in congenital deficiency; hyperthyroidism (overproduction of T_3, T_4) in Graves disease
Molybdenum (Mo)	Enzyme cofactor	Dietary deficiency unknown
Selenium (Se)	Found (as selenocysteine) in selenoproteins	Dietary deficiency rare (Keshan disease with Se-deficient soil); toxicity from supplements

Figure 29.18
Summary of minerals. PTH = parathyroid hormone; Cl^- = chloride; S = sulfur; T_3 = triiodothyronine; T_4 = thyroxine.

Study Questions

For Questions 29.1–29.7, match the mineral to the most appropriate description.

A. Calcium
B. Chloride
C. Copper
D. Iodine
E. Iron
F. Magnesium
G. Manganese
H. Molybdenum
I. Phosphorus
J. Potassium
K. Selenium
L. Sodium
M. Zinc

Correct answers = L, B, I, K, M, A, D. Hypernatremia (elevation of serum sodium) can lead to water retention that can cause hypertension in salt-sensitive populations (e.g., African Americans). Chloride is the major extracellular anion. (Note: Sodium is the major extracellular cation, potassium is the major intracellular cation, and phosphate is the major intracellular anion. The concentration differential across the membrane is maintained by active transport.) Carbohydrate metabolism involves the generation of phosphorylated intermediates. Refeeding severely malnourished individuals traps phosphate and results in hypophosphatemia. Muscle weakness is a common symptom. Selenocysteine, an amino acid formed from serine and selenium, is found in proteins (selenoproteins) such as glutathione peroxidase, deiodinases, and thioredoxin reductase. Zinc fingers are a type of structural motif found in proteins (e.g., transcription factors) that bind to DNA. Severe deficiency of zinc as a result of mutations to its intestinal transporter can result in acrodermatitis enteropathica, which is characterized by dermatitis, diarrhea, and alopecia. Calcium is required for bone mineralization, muscle contraction, nerve conduction, and blood clotting. Its deficiency will affect all of these processes. Thyroid hormones are iodinated tyrosines released by proteolytic digestion of thyroglobulin. Underingestion of iodine causes enlargement of the thyroid in an attempt to increase hormone synthesis. (Note: Goiter can also result if too much hormone is made, as in Graves disease, or if too little is made, as in Hashimoto disease. Both are autoimmune diseases.) Thyroid hormone increases the resting metabolic rate.

29.1 Elevated levels of which mineral may result in hypertension in certain populations?

29.2 Which mineral is the major extracellular anion?

29.3 A decrease of which mineral is seen in refeeding syndrome and with overuse of aluminum-containing antacids?

29.4 Which mineral is a constituent of some amino acids found in proteins involved in antioxidant defense, thyroid hormone metabolism, and redox reactions?

29.5 Which mineral is required for the formation of a supersecondary protein structure that allows binding to DNA? (Its deficiency can result in a dermatitis.)

29.6 Deficiency of which mineral can cause bone pain, tetany (intermittent muscle spasms), paresthesia (a “pins and needles” sensation), and an increased tendency to bleed?

29.7 Deficiency of which mineral can result in goiter and a decreased metabolic rate?

29.8 DiGeorge syndrome is a congenital condition that results in structural anomalies and failure of the thymus and parathyroid glands to develop. Clinical manifestations include recurrent infections as a consequence of a deficiency in T cells. Which one of the following is an expected clinical consequence of the deficiency in parathyroid hormone?

A. Increased bone resorption
B. Increased calcium reabsorption in the kidney
C. Increased serum calcitriol
D. Increased serum phosphate

Correct answer = D. Parathyroid hormone (PTH) increases bone resorption (demineralization) resulting in the release of calcium and phosphate. It also increases the renal reabsorption of calcium, because PTH activates the renal hydroxylase that converts calcidiol to calcitriol. PTH also increases the renal excretion of phosphate. With the hypoparathyroidism of DiGeorge syndrome, all of these activities of PTH are impaired. Consequently, hypocalcemia and hyperphosphatemia are seen.

For questions 29.9 and 29.10, match the signs and symptoms to the pathology.

A. Graves disease
B. Hereditary hemochromatosis
C. Hypercalcemia
D. Hyperphosphatemia
E. Keshan disease
F. Menkes syndrome
G. Selenosis
H. Wilson disease

29.9 A 28-year-old male is seen for complaints of recent, severe, upper-right-quadrant pain. He also reports some difficulty with fine motor tasks. No jaundice is observed on physical examination. Laboratory tests were remarkable for elevated liver function tests (serum aspartate and alanine aminotransferases) and elevated urinary calcium and phosphate. Ophthalmology consult revealed Kayser–Fleischer rings in the cornea. The patient was started on penicillamine and zinc.

Correct answer = H. The patient has Wilson disease, an autosomal-recessive disorder that decreases copper efflux from the liver because of mutations to the hepatic copper transport protein ATP7B. Some copper leaks into the blood and is deposited in the brain, eyes, kidney, and skin. This results in liver and kidney damage, neurologic effects, and corneal changes caused by the excess copper. Administration of the metal chelator penicillamine is the treatment. (Note: Because zinc is also chelated, supplementation with zinc is common.) Graves disease results in hyperthyroidism. Hereditary hemochromatosis is a disorder of iron overload. Keshan disease is the result of selenium deficiency, whereas selenosis is caused by selenium excess. Menkes syndrome is the result of a systemic deficiency in copper as a result of mutations to ATP7A, an intestinal copper transport protein.

29.10 A 52-year-old female is seen because of unplanned changes in the pigmentation of her skin that give her a tanned appearance. Physical examination shows hyperpigmentation, hepatomegaly, and mild scleral icterus. Laboratory tests are remarkable for elevated serum transaminases (liver function tests) and fasting blood glucose. Results of other tests are pending.

Correct answer = B. The patient has hereditary hemochromatosis, a disease of iron overload that results from inappropriately low levels of hepcidin caused primarily by mutations to the HFE (high iron) gene. Hepcidin regulates ferroportin, the only known iron export protein in humans, by increasing its degradation. The increase in iron with hepcidin deficiency causes hyperpigmentation and hyperglycemia (“bronze diabetes”). Phlebotomy or use of iron chelators is the treatment. (Note: Pending lab tests would show an increase in serum iron and transferrin saturation.)

UNIT VII:
Storage and Expression of Genetic Information

DNA Structure, Replication, and Repair 30

I. OVERVIEW

Nucleic acids are required for the storage and expression of genetic information. There are two chemically distinct types of nucleic acids: deoxyribonucleic acid (DNA) and ribonucleic acid ([RNA], see Chapter 31). DNA, the repository of genetic information (the genome), is present not only in chromosomes in the nucleus of eukaryotic organisms, but also in mitochondria and the chloroplasts of plants. Prokaryotic cells, which lack nuclei, have a single chromosome but may also contain nonchromosomal DNA in the form of plasmids. The genetic information found in DNA is copied and transmitted to daughter cells through DNA replication. Each cell type is specialized, expressing only those genes that are required for it to perform its role in maintaining the organism. The DNA contained in a fertilized egg encodes the information that directs the development of an organism. This development may involve the production of billions of cells. Therefore, DNA must be able not only to replicate precisely each time a cell divides, but also to have the information that it contains be selectively expressed and processed for production of the set of functional RNA and protein products needed for cellular function. Transcription (RNA synthesis) is the first stage in the expression of genetic information (see Chapter 31). Next, the code contained in the nucleotide sequence of messenger RNA molecules is translated (protein synthesis; see Chapter 32), thus completing gene expression. The regulation of gene expression is discussed in Chapter 33.

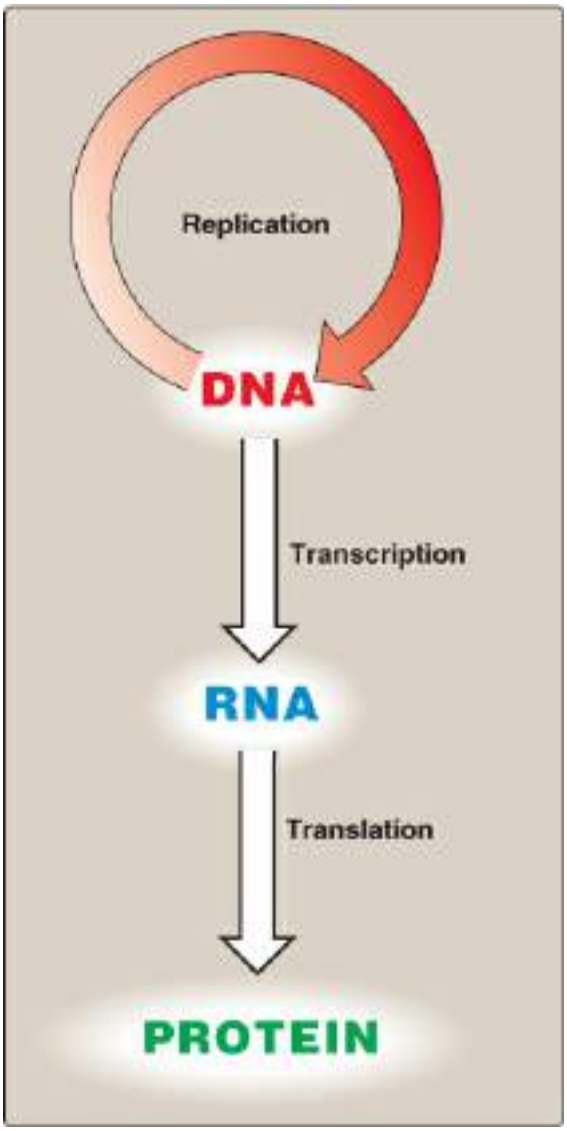


Figure 30.1
The “central dogma” of molecular biology.

The flow of information from DNA to RNA to protein is termed the “central dogma” of molecular biology (Fig. 30.1) and is descriptive of all organisms, with the exception of some viruses that have RNA as the repository of their genetic information.

II. DNA STRUCTURE

DNA is a polymer of deoxyribonucleoside monophosphates (dNMP), also called nucleotides, covalently linked by 3′-to-5′ phosphodiester bonds. With the exception of a few viruses that contain single-stranded DNA (ssDNA), DNA exists as a double-stranded molecule (dsDNA), in which the two strands wind around each other, forming a double helix. (Note: The sequence of the linked dNMP is primary structure, whereas the

double helix is secondary structure.) In eukaryotic cells, DNA is found associated with various types of proteins (known collectively as nucleoprotein) present in the nucleus, whereas in prokaryotes, the protein–DNA complex is present in a non–membrane-bound region known as the nucleoid.

A. 3′-to-5′ Phosphodiester bonds

Phosphodiester bonds join the 3′-hydroxyl group of the deoxyribose of one nucleotide to the 5′-hydroxyl group of the deoxyribose of an adjacent nucleotide through a phosphoryl group (Fig. 30.2). The resulting long, unbranched chain has polarity, with both a 5′ end (the end with the free phosphate) and a 3′ end (the end with the free hydroxyl) that are not attached to other nucleotides. By convention, the bases located along the resulting deoxyribose-phosphate backbone are always written in sequence from the 5′ end of the chain to the 3′ end. For example, the sequence of the DNA shown in Figure 30.2A is written TACG and is read “thymine, adenine, cytosine, guanine.” Phosphodiester linkages between nucleotides can be hydrolyzed enzymatically by a family of nucleases, deoxyribonucleases for DNA and ribonucleases for RNA, or cleaved hydrolytically by chemicals. (Note: Only RNA is cleaved by alkali.)

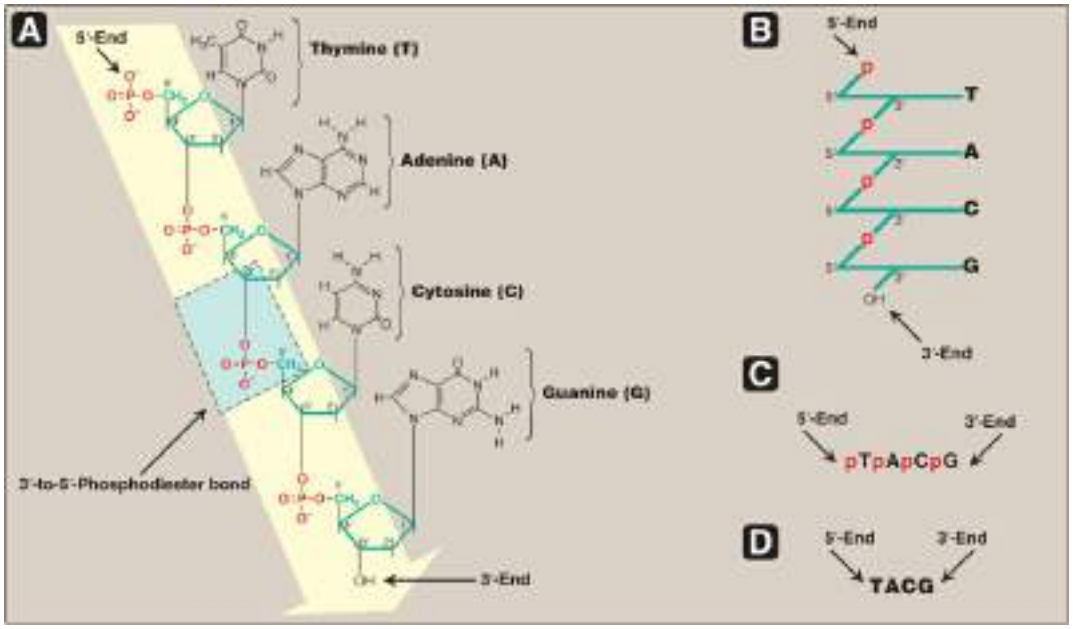


Figure 30.2
A: DNA with the nucleotide sequence shown written in the 5′→3′ direction. A 3′-to-5′ phosphodiester bond is shown highlighted in the *blue box*, and the deoxyribose-phosphate backbone is shaded in *yellow* with the *arrow* indicating the direction of DNA strand synthesis. **B:** DNA written in a more stylized form, emphasizing the deoxyribose-phosphate (p) backbone. **C:** A simpler representation of the nucleotide sequence. **D:** The simplest (and most common) representation. (Note: The nucleotide base sequence is assumed to be written in the 5′→3′ direction unless otherwise indicated.)

B. Double helix

In the double helix, the two chains are coiled around a common axis called the helical axis. The chains are paired in an antiparallel manner (i.e., the 5′ end of one strand is paired with the 3′ end of the other strand), as shown in Figure 30.3. In the DNA helix, the hydrophilic deoxyribose-phosphate backbone of each chain is on the outside of the molecule, whereas the hydrophobic bases are stacked inside. The overall structure resembles a twisted ladder. The spatial relationship between the two strands in the helix creates a major (wide) groove and a minor (narrow) groove. These grooves provide access for the binding of regulatory proteins to their specific recognition sequences along the DNA chain. (Note: Certain anticancer drugs, such as dactinomycin [actinomycin D], exert their cytotoxic effect by intercalating into the minor groove of the DNA double helix, thereby interfering with DNA [and RNA] synthesis.)

1. Base pairing: The bases of one strand of DNA are paired with the bases of the second strand, so that an adenine (A) is always paired with a thymine (T), and a cytosine (C) is always paired with a guanine (G). (Note: The base pairs [bps] are perpendicular to the helical axis [see Fig. 30.3].) Therefore, one polynucleotide chain of the DNA double helix is always the complement of the other. Given the sequence of bases on one chain, the sequence of bases on the complementary chain can be determined (Fig. 30.4). (Note: The specific base-pairing in DNA leads to the Chargaff rule, which states that in any sample of dsDNA, the amount of A equals the amount of T, the amount of G equals the amount of C, and the total amount of purines [A + G] equals the total amount of pyrimidines [T + C].) The base pairs are held together by hydrogen bonds: two between A and T and three between G and C (Fig. 30.5). The base pairs are also stacked along the axis so that the planes of their rings are parallel. The hydrogen bonds of the base pairs, plus the hydrophobic interactions between the stacked bases, stabilize the structure of the double helix.

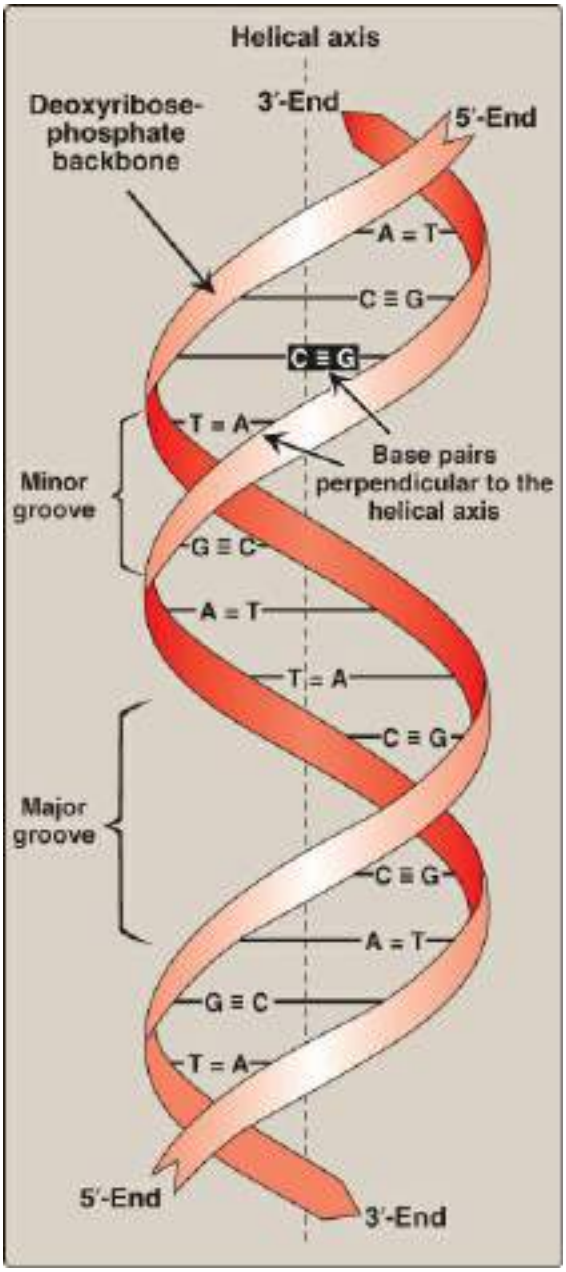


Figure 30.3
DNA double helix, illustrating some of its major structural features. T = thymine; A = adenine; C = cytosine; G = guanine.

2. DNA strand separation: The two strands of the double helix separate when hydrogen bonds between the paired bases are disrupted. Disruption can occur in the laboratory if the pH of the DNA solution is altered so that the nucleotide bases ionize, or if the solution is heated. (Note: Covalent phosphodiester bonds are not broken by such treatment.) When DNA is heated, the temperature at which one half of the helical structure is lost and single-stranded regions are formed is defined as the melting temperature (T_m). The loss of helical structure in DNA, called denaturation, can be monitored by measuring its absorbance at

260 nm. (Note: ssDNA has a higher relative absorbance at this wavelength than does dsDNA.) Because there are three hydrogen bonds between G and C but only two between A and T, DNA that contains high concentrations of A and T denatures at a lower temperature than does G- and C-rich DNA (Fig. 30.6). If the DNA solution is cooled or titrated to neutral pH, complementary DNA strands can reform the double helix by the process called renaturation (or, reannealing). (Note: Separation of the two strands over short regions occurs during both DNA and RNA synthesis.)

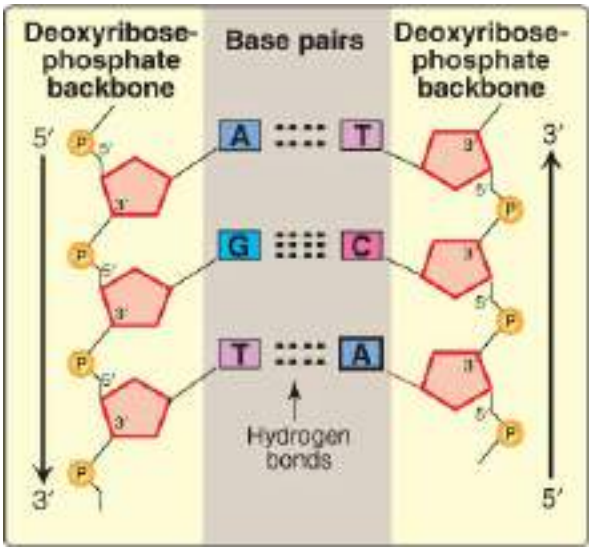


Figure 30.4
Two complementary DNA sequences. T = thymine; A = adenine; C = cytosine; G = guanine.

3. Structural forms: There are three major structural forms of DNA: the B form (described by Watson and Crick in 1953), the A form, and the Z form. The B form is a right-handed helix with 10 bps per 360-degree turn (or twist) of the helix, and with the planes of the bases perpendicular to the helical axis. Chromosomal DNA is thought to consist primarily of B-DNA (Fig. 30.7 shows a space-filling model of B-DNA). The A form is produced by moderately dehydrating the B form. It is also a right-handed helix, but there are 11 bps per turn, and the planes of the base pairs are tilted 20 degrees away from the perpendicular to the helical axis. The conformation found in DNA–RNA hybrids (see p. 466) or RNA–RNA double-stranded regions is probably very close to the A form. Z-DNA is a left-handed helix that contains 12 bps per turn (see Fig. 30.7). (Note: The deoxyribose-phosphate backbone zigzags, hence, the name Z-DNA.) Stretches of Z-DNA can occur naturally in regions of DNA that have a sequence of alternating purines and pyrimidines (e.g., poly GC). Transitions between the B and Z helical forms of DNA may play a role in regulating gene expression.

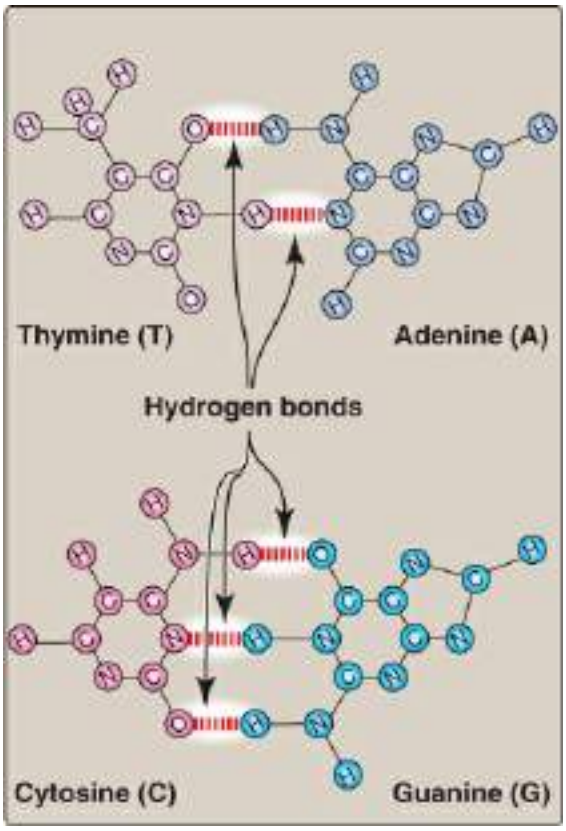


Figure 30.5
Hydrogen bonds between complementary bases.

C. Linear and circular DNA molecules

Each chromosome in the nucleus of a eukaryote consists of one long, linear molecule of dsDNA, which is bound by a complex mixture of proteins (histone and nonhistone, see p. 473) to form chromatin. Eukaryotes have closed, circular, dsDNA molecules in their mitochondria, as do plant chloroplasts. A prokaryotic organism typically contains a single, circular, dsDNA molecule. Each prokaryotic chromosome is associated with nonhistone proteins that help compact the DNA to form a nucleoid. In addition, most species of bacteria also contain small, circular, extrachromosomal DNA molecules called plasmids. Plasmid DNA carries genetic information and undergoes replication that may or may not be synchronized to chromosomal division. (Note: The use of plasmids as vectors in recombinant DNA technology is described in Chapter 34.)

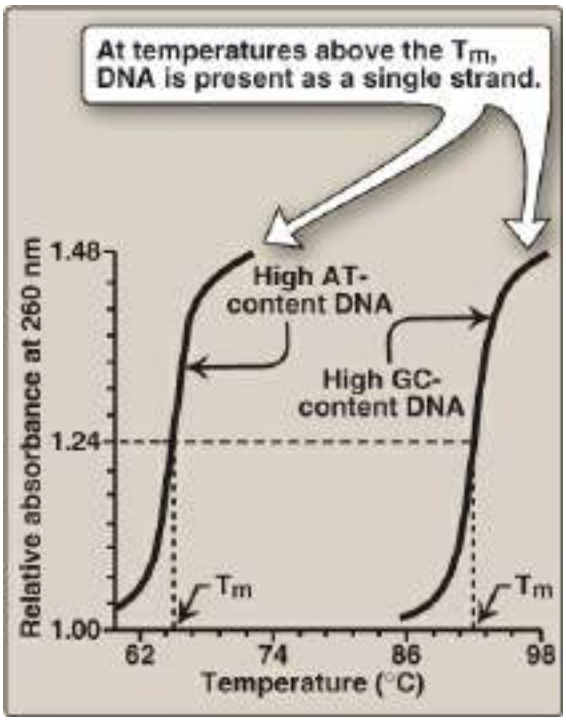


Figure 30.6
Melting temperatures (T_m) of DNA molecules with different nucleotide compositions.

Plasmids may carry genes that convey antibiotic resistance to the host bacterium and may facilitate the transfer of genetic information from one bacterium to another.

III. STEPS IN PROKARYOTIC DNA REPLICATION

When the two strands of dsDNA are separated, each can serve as a template for the replication (synthesis) of a new complementary strand. This produces two daughter molecules, each of which contains two DNA strands (one old, one new) in an antiparallel orientation (see Fig. 30.3). This process is called semiconservative replication because, although the parental duplex is separated into two halves (and, therefore, is not conserved as an entity), each of the parental strands remains intact in one of the two new duplexes (Fig. 30.8). The enzymes involved in DNA replication are template-directed, magnesium (Mg^{2+})-requiring polymerases that can synthesize the complementary sequence of each strand with extraordinary fidelity. The reactions described in this section were first known from studies of the bacterium *Escherichia coli (E. coli)*, and the description given below refers to the process in prokaryotes. DNA synthesis in higher organisms is more complex but involves the same types of mechanisms. In either case, initiation of DNA replication commits the cell to continue the process until the entire genome has been replicated.

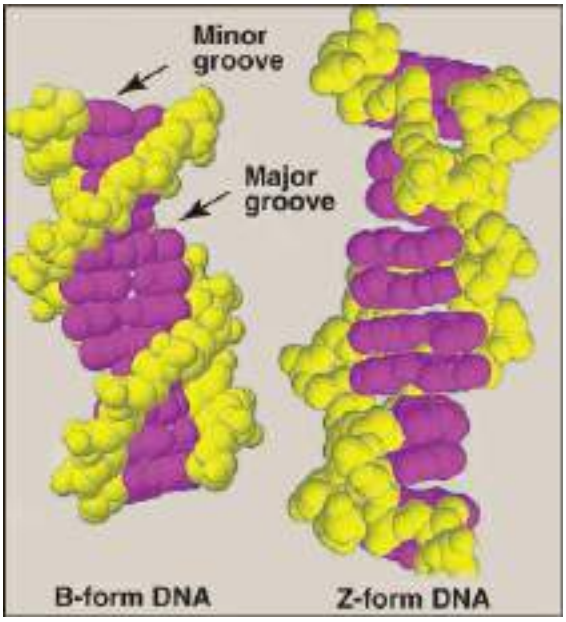


Figure 30.7
Structures of B-DNA and Z-DNA.

A. Complementary strand separation

In order for the two complementary strands of the parental dsDNA to be replicated, they must first separate (or “melt”) over a small region, because the polymerases use only ssDNA as a template. In prokaryotic organisms, DNA replication begins at a single, unique nucleotide sequence, a site called the origin of replication, or ori (oriC in *E. coli*), as shown in Figure 30.9A. (Note: This sequence is referred to as a consensus sequence, because the order of nucleotides at this site is essentially the same in different bacteria.) The ori includes short, AT-rich segments that facilitate melting. In eukaryotes, replication begins at multiple sites in each chromosome (Fig. 30.9B). Having multiple origins of replication provides a mechanism for rapidly replicating the great length of eukaryotic DNA molecules.

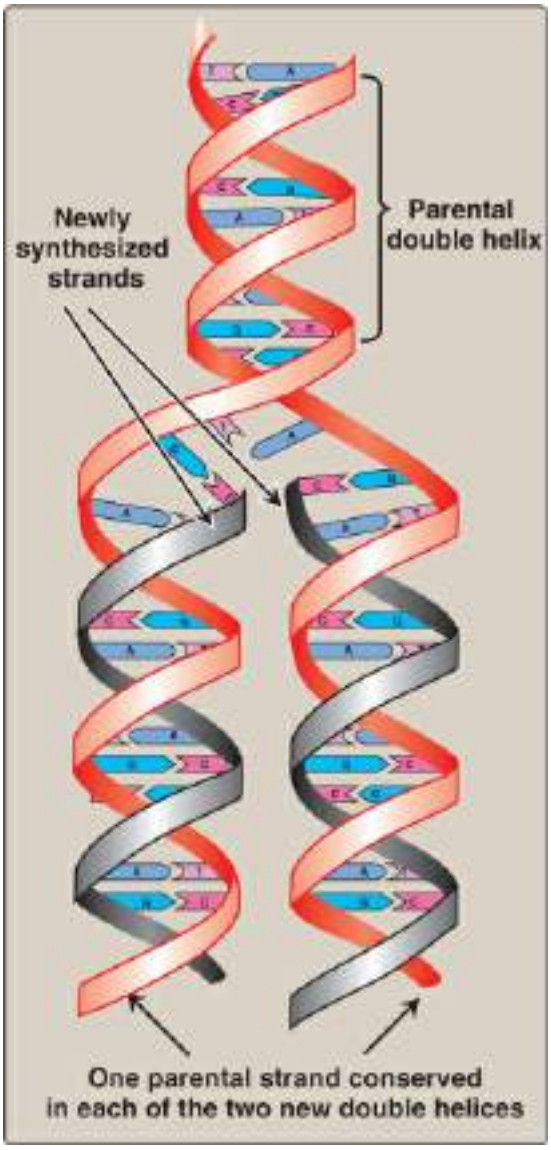


Figure 30.8
Semiconservative replication of DNA.

B. Replication fork formation

As the two strands unwind and separate, synthesis occurs at two replication forks that move away from the origin in opposite directions (bidirectionally), generating a replication bubble (see Fig. 30.9). (Note: The term “replication fork” derives from the Y-shaped structure in which the tines of the fork represent the separated strands [Fig. 30.10].)

1. Required proteins: Initiation of DNA replication requires the recognition of the origin (start site) by a group of proteins that form the prepriming complex. These proteins are responsible for melting at the ori, maintaining the separation of the parental strands, and unwinding the double helix ahead of the advancing replication fork. In *E. coli*, these proteins include the following.

a. DnaA protein: DnaA protein initiates replication by binding to specific nucleotide sequences (DnaA boxes) within oriC. Binding causes an AT-rich region (the DNA unwinding element) in the origin to melt. Melting (strand separation) results in a short, localized region of ssDNA.

b. DNA helicases: These enzymes bind to ssDNA near the replication fork and then move into the neighboring double-stranded region, forcing the strands apart (in effect, unwinding the double helix). Helicases require energy provided by ATP hydrolysis (see Fig. 30.10). Unwinding at the replication fork causes supercoiling in other regions of the DNA molecule. (Note: DnaB is the principal helicase of replication in *E. coli*. Binding of this hexameric protein to DNA requires DnaC.) Supercoiling is a type of tertiary structure in which the double helix of a chromosome crosses over on itself one or more times to relieve torsional strain in the DNA molecule.

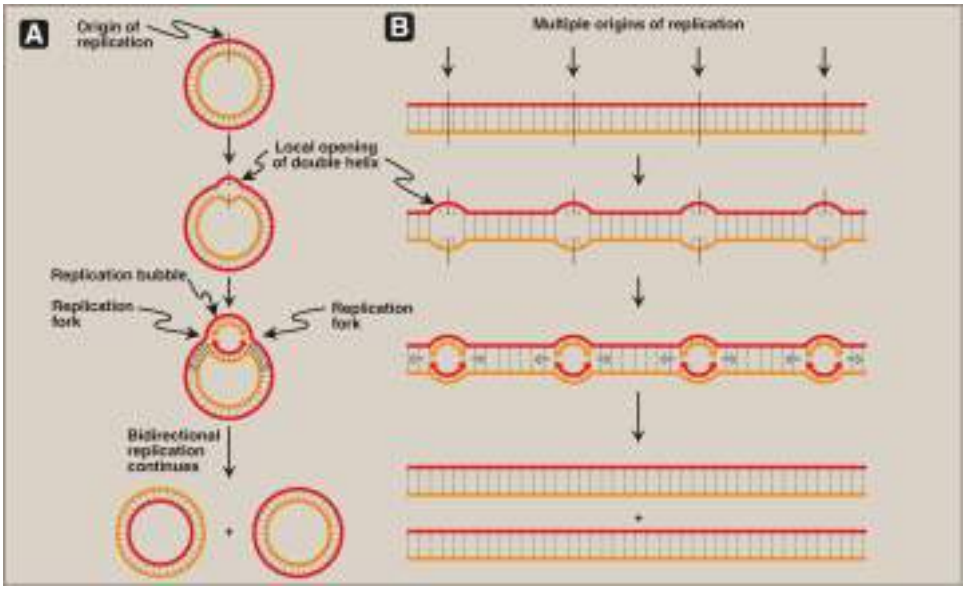


Figure 30.9
Replication of DNA: origins and replication forks. A: Small, circular prokaryotic DNA. B: Long, linear eukaryotic DNA.

c. Single-stranded DNA–binding protein: This protein binds to the ssDNA generated by helicases (see Fig. 30.10). Binding is cooperative (i.e., the binding of one molecule of single-stranded binding [SSB] protein makes it easier for additional molecules of SSB protein to bind tightly to the DNA strand). The SSB proteins are not enzymes, but rather serve to shift the equilibrium between dsDNA and ssDNA in the direction of the single-stranded forms. These proteins not only keep the two strands of DNA separated in the area of the replication origin, thus providing the single-stranded template required by polymerases, but also protect the DNA from nucleases that degrade ssDNA.

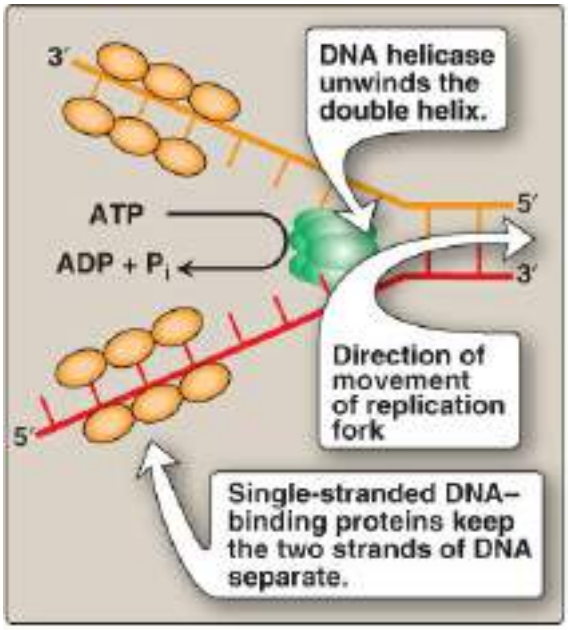


Figure 30.10
Proteins responsible for maintaining the separation of the parental strands and unwinding the double helix ahead of the advancing replication fork. ADP = adenosine diphosphate; P_i = inorganic phosphate.

2. Solving the problem of supercoils: Supercoiling can result from overwinding (positive supercoiling) or underwinding (negative supercoiling) of DNA. As the two strands of the double helix are separated, a problem is encountered, namely, the appearance of positive supercoils in the region of DNA ahead of the replication fork (Fig. 30.11) and negative supercoils in the region behind the fork. The accumulating positive supercoils interfere with further separation of the DNA strands. (Note: Supercoiling can be demonstrated by tightly grasping one end of a helical telephone cord while twisting the other end. If the cord is twisted in the direction of tightening the coils, the cord will wrap around itself in space to form positive supercoils. If the cord is twisted in the direction of loosening the coils, the cord will wrap around itself in the opposite direction to form negative supercoils.) To solve this problem, there is a group of enzymes called DNA topoisomerases, which are responsible for removing supercoils in the helix by transiently cleaving one or both of the DNA strands.

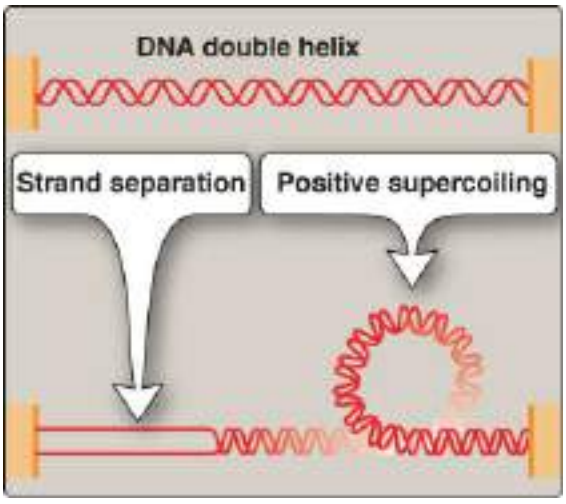


Figure 30.11
Positive supercoiling resulting from DNA strand separation.

a. Type I DNA topoisomerases: These enzymes reversibly cleave one strand of the double helix and form a covalent bond to the end of the nicked strand. They have both strand-cutting and strand-resealing activities. They do not require ATP, but rather appear to store the energy from the phosphodiester bond they cleave, reusing the energy to reseal the strand (Fig. 30.12). Each time an enzyme creates a transient nick in one DNA strand, it rotates around the intact DNA strand before resealing the nick, thus relieving (relaxing) accumulated supercoils. Type I topoisomerases relax negative supercoils (i.e., those that contain fewer turns of the helix than does relaxed DNA) in *E. coli* and both negative and positive supercoils (i.e., those that contain fewer or more turns of the helix than does relaxed DNA) in many prokaryotic cells (but not *E. coli*) and in eukaryotic cells.

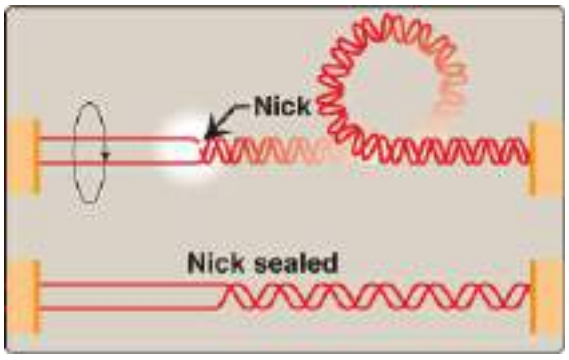


Figure 30.12
Action of type I DNA topoisomerases.

b. Type II DNA topoisomerases: These enzymes bind tightly to the DNA double helix and make transient breaks in both strands. The enzyme then passes a second part of the DNA double helix through the break and, finally, reseals

the break (Fig. 30.13). As a result, both negative and positive supercoils can be relieved by this ATP-requiring process. DNA gyrase, a type II topoisomerase found in bacteria and plants, has the unusual property of being able to introduce negative supercoils into circular DNA using energy from the hydrolysis of ATP. This facilitates the replication of DNA because the negative supercoils neutralize the positive supercoils introduced during opening of the double helix. It also aids in the transient strand separation required during transcription (see p. 485).

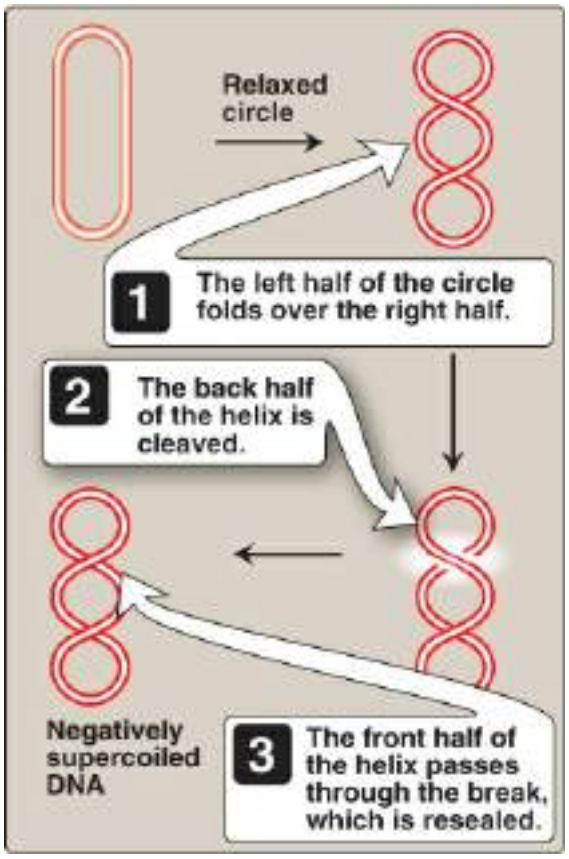


Figure 30.13
Action of type II DNA topoisomerase.

Anticancer agents, such as the camptothecins, target human type I topoisomerases, whereas etoposide targets human type II topoisomerases. Bacterial DNA gyrase is a unique target of a group of antimicrobial agents called fluoroquinolones (e.g., ciprofloxacin).

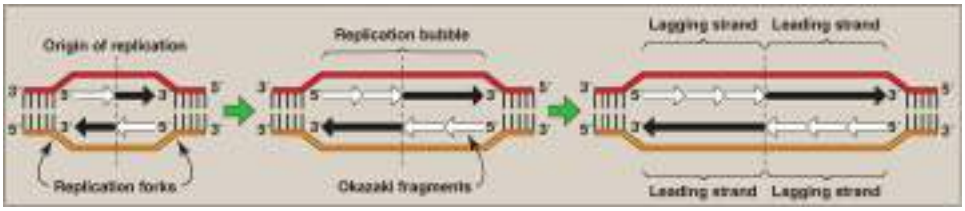


Figure 30.14
Semidiscontinuous synthesis of DNA. *Black arrows* = continuous synthesis; *white arrows* = discontinuous.

C. Direction of DNA replication

The DNA polymerases (DNA pols) responsible for copying the DNA templates are only able to read the parental nucleotide sequences in the 3′→5′ direction, and they synthesize the new DNA strands only in the 5′→3′ (antiparallel) direction. Therefore, beginning with one parental double helix, the two newly synthesized stretches of nucleotide chains must grow in opposite directions, one in the 5′→3′ direction toward the replication fork and one in the 5′→3′ direction away from the replication fork (Fig. 30.14). This feat is accomplished by a slightly different mechanism on each strand.

1. Leading strand: The strand that is being copied in the direction of the advancing replication fork is synthesized continuously and is called the leading strand.
2. Lagging strand: The strand that is being copied in the direction away from the replication fork is synthesized discontinuously, with small fragments of DNA being copied near the replication fork. These short stretches of discontinuous DNA, termed Okazaki fragments, are eventually joined (ligated) by ligase to become a single, continuous strand. The new strand of DNA produced by this mechanism is termed the lagging strand.

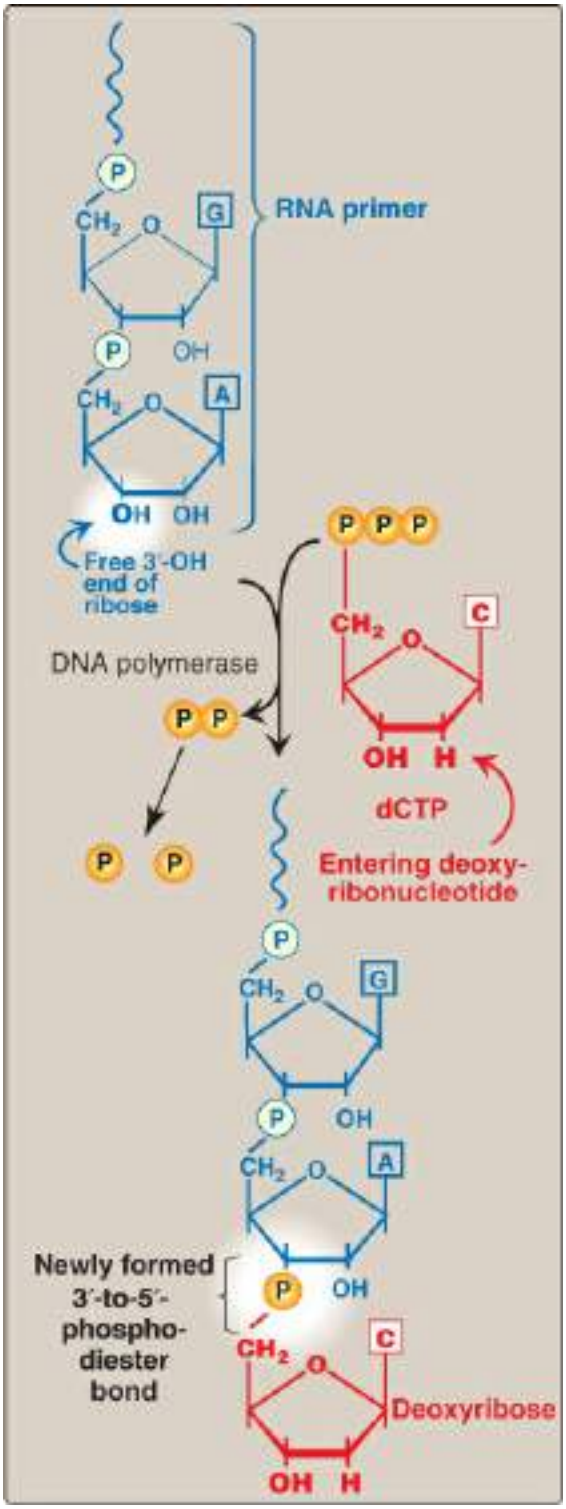


Figure 30.15
Use of an RNA primer to initiate DNA synthesis. (P) and (P) = phosphate; dCTP = deoxycytidine triphosphate.

D. RNA primer

DNA pols cannot initiate synthesis of a complementary strand of DNA on a totally single-stranded template. Rather, they require an RNA primer, which is a short piece of RNA base paired to the DNA template, thereby forming a double-stranded DNA–RNA hybrid. The free hydroxyl group on the 3′ end of the RNA primer serves as the first acceptor of a deoxynucleotide by action of a DNA pol (Fig. 30.15). (Note: Recall that glycogen synthase also requires a primer in the form of a short glycogen molecule [see p. 138].)

1. Primase: A specific RNA polymerase, called primase (DnaG), synthesizes the short stretches of RNA (~10 nucleotides long) that are complementary and antiparallel to the DNA template. In the resulting hybrid duplex, the uracil (U) in RNA pairs with A in DNA. As shown in Figure 30.16, these short RNA sequences are constantly being synthesized at the replication fork on the lagging strand, but only one RNA sequence at the origin of replication is required on the leading strand. The substrates for this process are 5′-ribonucleoside triphosphates, and pyrophosphate (PP_i) is released as each ribonucleoside monophosphate is added through formation of a 3′-to-5′ phosphodiester bond. (Note: The RNA primer is later removed, as described in F. below.)

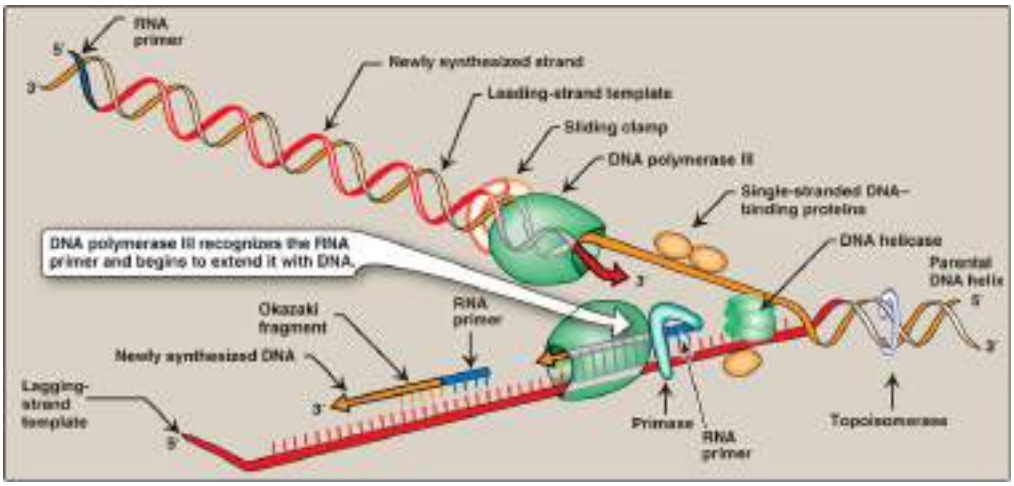


Figure 30.16
Elongation of the leading and lagging strands. (Note: The DNA sliding clamp is not shown for the lagging strand.)

2. Primosome: The addition of primase converts the prepriming complex of proteins required for DNA strand separation (see p. 463) to a primosome. The primosome makes the RNA primer required for leading-strand synthesis and initiates Okazaki fragment formation in discontinuous lagging-strand synthesis. As with DNA synthesis, the direction of synthesis of the primer is 5′→3′.

E. Chain elongation

Prokaryotic (and eukaryotic) DNA pols elongate a new DNA strand by adding

deoxyribonucleotides, one at a time, to the 3′ end of the growing chain (see Fig. 30.16). The sequence of nucleotides that are added is dictated by the base sequence of the parental strand, which serves as a template. Incoming nucleotides, used in the synthesis of the new strand, pair with the bases of the template.

1. DNA polymerase III: DNA chain elongation is catalyzed by the multisubunit enzyme, DNA pol III. Using the 3′-hydroxyl group of the RNA primer as the acceptor of the first deoxyribonucleotide, DNA pol III begins to add nucleotides along the single-stranded template that specifies the sequence of bases in the newly synthesized chain. DNA pol III is a highly processive enzyme (i.e., it remains bound to the template strand as it moves along and does not diffuse away and then rebind before adding each new nucleotide). The processivity of DNA pol III is the result of the β-subunits of the holoenzyme forming a ring that encircles and moves along the template strand of the DNA, thus serving as a sliding DNA clamp. (Note: Clamp formation is facilitated by a protein complex, the clamp loader, and ATP hydrolysis.) The new (daughter) strand grows in the 5′→3′ direction, antiparallel to the parental strand (see Fig. 30.16). The nucleotide substrates are 5′-deoxyribonucleoside triphosphates. PP_i is released when each new deoxynucleoside monophosphate is added to the free 3′-hydroxyl group of the growing chain through a 3′-to-5′ phosphodiester bond (see Fig. 30.15). Hydrolysis of PP_i to 2 P_i by pyrophosphatase means that a total of two high-energy bonds are used to drive the addition of each deoxynucleotide.

> The production of PP_i with subsequent hydrolysis to 2 P_i is a common theme in biochemistry. Removal of the PP_i drives the reaction that generates PP_i in the forward direction, making it essentially irreversible.

   All four substrates (deoxyadenosine triphosphate [dATP], deoxythymidine triphosphate [dTTP], deoxycytidine triphosphate [dCTP], and deoxyguanosine triphosphate [dGTP]) must be present for DNA elongation to occur. DNA synthesis stalls when the concentration of the nucleotide falls below the K_m for the polymerase binding to the nucleotide.)

2. Proofreading newly synthesized DNA: It is highly important for the survival of an organism that the nucleotide sequence of DNA be replicated with as few errors as possible. Misreading of the template sequence could result in deleterious, perhaps lethal, mutations. To ensure replication fidelity, DNA pol III has a proofreading activity (3′→5′ exonuclease, Fig. 30.17) in addition to its 5′→3′ polymerase activity. As each nucleotide is added to the chain, DNA pol III checks to make certain the base of the newly added nucleotide is, in fact, the complement of the base on the template strand. If it is not, the 3′→5′ exonuclease activity removes the erroneously added nucleotide in the direction opposite to polymerization. (Note: Because the exonuclease function of DNA pol III requires an improperly base-paired 3′-hydroxy terminus, it does not degrade

correctly paired nucleotide sequences.) For example, if the template base is C and the enzyme inserts an A instead of a G into the new chain, the 3′→5′ exonuclease activity hydrolytically removes the misplaced nucleotide. The 5′→3′ polymerase activity then repeats the nucleotide addition step and inserts the correct nucleotide containing G (see Fig. 30.17). (Note: The 5′→3′ polymerase and 3′→5′ exonuclease domains are located on different subunits of DNA pol III.)

> Sickle cell anemia is caused by a single nucleotide change, an error of inserting a T in the place of an A, in the β-globin gene. This mutation results in an incorrect amino acid (a valine in the place of a glutamate) in the β-globin protein that alters the function of the protein in the red blood cell.

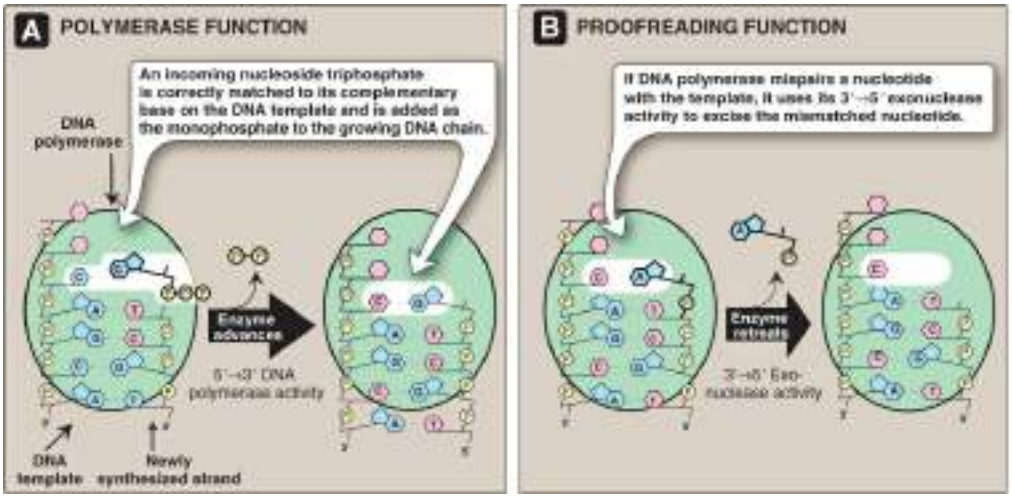


Figure 30.17
3′→5′ Exonuclease activity enables DNA polymerase III to proofread the newly synthesized DNA strand.

F. RNA primer excision and replacement by DNA

DNA pol III continues to synthesize DNA on the lagging strand until it nears the 5′ end of an RNA primer. When this occurs, the RNA is excised and the gap between Okazaki fragments is filled by DNA pol I.

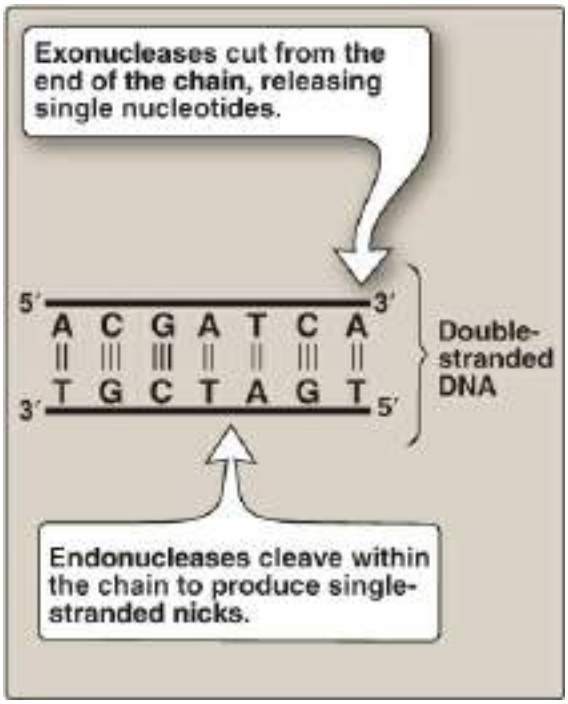


Figure 30.18
Endonuclease versus exonuclease activity. (Note: Restriction endonucleases (see p. 532) cleave both strands.)

1. 5′→3′ Exonuclease activity: In addition to having the 5′→3′ polymerase activity that synthesizes DNA and the 3′→5′ exonuclease activity that proofreads the newly synthesized DNA like DNA pol III, monomeric DNA pol I also has a 5′→3′ exonuclease activity that is able to hydrolytically remove the RNA primer. (Note: Exonucleases remove nucleotides from the end of the DNA chain, rather than cleaving the chain internally as do endonucleases [Fig. 30.18].) First, DNA pol I locates the space (nick) between the 3′ end of the DNA newly synthesized by DNA pol III and the 5′ end of the adjacent RNA primer. Next, DNA pol I hydrolytically removes the RNA nucleotides ahead of itself, moving in the 5′→3′ direction (5′→3′ exonuclease activity). As it removes ribonucleotides, DNA pol I replaces them with deoxyribonucleotides, synthesizing DNA in the 5′→3′ direction (5′→3′ polymerase activity). As it synthesizes the DNA, it also proofreads using its 3′→5′ exonuclease activity to remove errors. This removal/synthesis/proofreading continues until the RNA primer is totally degraded, and the gap is filled with DNA (Fig. 30.19). (Note: DNA pol I uses its 5′→3′ polymerase activity to fill in gaps generated during most types of DNA repair [see p. 476].)

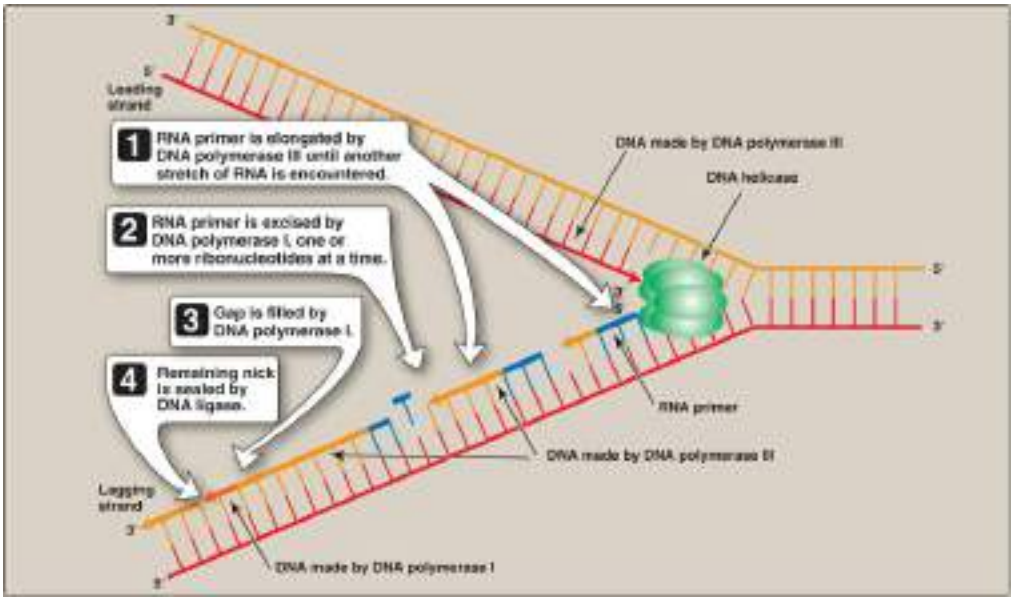


Figure 30.19
Removal of RNA primer and filling of the resulting gaps by DNA polymerase I.

2. Comparison of 5′→3′ and 3′→5′ exonuclease activities: The 5′→3′ exonuclease activity of DNA pol I allows the polymerase, moving 5′→3′, to hydrolytically remove one or more nucleotides at a time from the 5′ end of the ~10-nucleotide–long RNA primer. In contrast, the 3′→5′ exonuclease activity of DNA pol I and pol III allows these polymerases, moving 3′→5′, to hydrolytically remove one misplaced nucleotide at a time from the 3′ end of a growing DNA strand, increasing the fidelity of replication such that newly replicated DNA has no more than one error per 10^7 nucleotides.

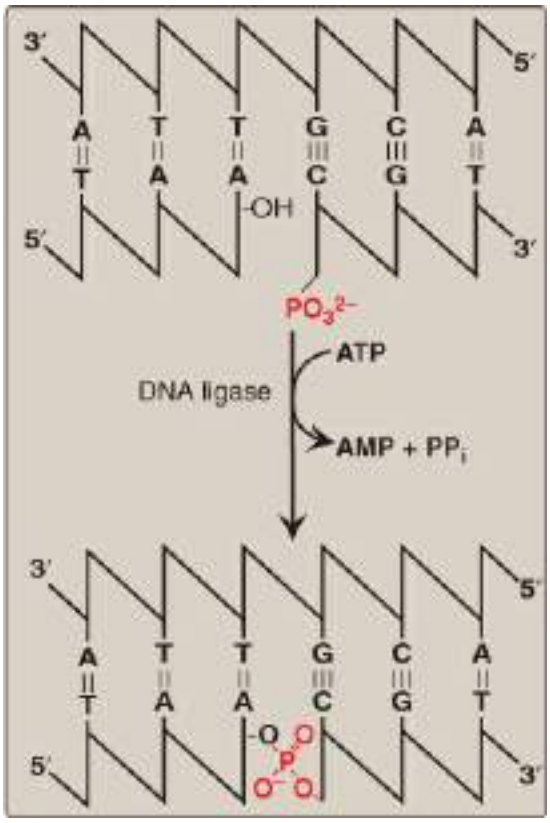


Figure 30.20
Formation of a phosphodiester bond by DNA ligase.

G. DNA ligase

DNA pol can only catalyze phosphodiester bond formation between a DNA strand and a mononucleotide and cannot join two sections of a DNA strand. The final phosphodiester linkage between the 5′-phosphate group on the DNA synthesized by DNA pol III and the 3′-hydroxyl group on the DNA made by DNA pol I is catalyzed by DNA ligase (Fig. 30.20). The joining (ligation) of these two stretches of DNA requires energy, which in most organisms is provided by the cleavage of ATP to adenosine monophosphate + PP_i.

H. Termination

Replication termination in *E. coli* is mediated by sequence-specific binding of the protein, terminus utilization substance (Tus) to replication termination (Ter) sites on the DNA, stopping the movement of the replication fork.

FUNCTION	PROTEIN(S)
Origin recognition	ORC
Helicase activity	*MCM*
ssDNA protection	RPA
Primer synthesis	Pol a/primase
Sliding clamp	PCNA
Primer removal	RNase H, FEN1

Figure 30.21
Proteins and their function in eukaryotic replication. ORC = origin recognition complex; MCM = minichromosome maintenance (complex); RPA = replication protein A; PCNA = proliferating cell nuclear antigen; FEN = flap endonuclease.

IV. EUKARYOTIC DNA REPLICATION

The process of eukaryotic DNA replication closely follows that of prokaryotic DNA synthesis. Some differences, such as the multiple origins of replication in eukaryotic cells versus single origins of replication in prokaryotes, have already been noted. Eukaryotic origin recognition proteins, ssDNA-binding proteins, and ATP-dependent DNA helicases have been identified, and their functions are analogous to those of the prokaryotic proteins previously discussed. In contrast, RNA primers are removed by RNase H and flap endonuclease 1 (FEN1) rather than by a DNA pol (Fig. 30.21).

A. Eukaryotic cell cycle

The events surrounding eukaryotic DNA replication and cell division (mitosis) are coordinated to produce the cell cycle (Fig. 30.22). The period preceding replication is called the Gap 1 phase (G_1). DNA replication occurs during the synthesis (S) phase. Following DNA synthesis, there is another phase (G_2, or Gap 2) before mitosis (M). Cells that have stopped dividing, such as mature T lymphocytes, are said to have gone out of the cell cycle into the G_0 phase. Such quiescent cells can be stimulated to reenter the G_1 phase to resume division. (Note: The cell cycle is controlled at a series of checkpoints that prevent entry into the next phase of the cycle until the preceding phase has been completed. Two key classes of proteins that control the progress of a cell through the cell cycle are the cyclins and cyclin-dependent kinases [Cdks].)

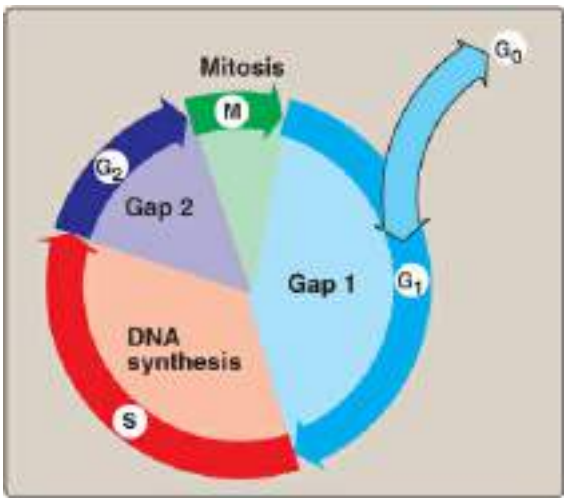


Figure 30.22
The eukaryotic cell cycle. (Note: Cells can leave the cell cycle and enter a reversible quiescent state called G_0.)

B. Eukaryotic DNA polymerases

At least five high-fidelity eukaryotic DNA pols have been identified and categorized on the basis of molecular weight, cellular location, sensitivity to inhibitors, and the templates or substrates on which they act. They are designated by Greek letters rather than by Roman numerals (Fig. 30.23).

1. Pol α: Pol α is a multisubunit enzyme. One subunit has primase activity, which initiates strand synthesis on the leading strand and at the beginning of each Okazaki fragment on the lagging strand. The primase subunit synthesizes a short RNA primer that is extended by the 5′→3′ polymerase activity of pol α, generating a short piece of DNA that is later extended by a more processive DNA pol such as pol ε or pol δ. (Note: Pol α is also referred to as pol α/primase.)

POLY-MERASE	FUNCTION	PROOF-READING*
Pol α (alpha)	• Contains primase • Initiates DNA synthesis	–
Pol β (beta)	• Repair	–
Pol δ (delta)	• Elongates Okazaki fragments of the lagging strand	+
Pol ε (epsilon)	• Elongates the leading strand	+
Pol γ (gamma)	• Replicates mitochondrial DNA	+

Figure 30.23
Activities of eukaryotic DNA polymerases (pol). (Note: The asterisk [*] denotes 3′→5′ exonuclease activity.)

2. Pol ε and pol δ: Pol ε is recruited to complete DNA synthesis on the leading strand, whereas pol δ elongates the Okazaki fragments of the lagging strand, each using 3′→5′ exonuclease activity to proofread the newly synthesized DNA. (Note: DNA pol ε associates with proliferating cell nuclear antigen [PCNA], a protein that serves as a sliding DNA clamp in much the same way the β subunits of DNA pol III do in *E. coli*, thus ensuring high processivity.)
3. Pol β and pol γ: Pol β is involved in gap filling in DNA repair. Pol γ replicates mitochondrial DNA.

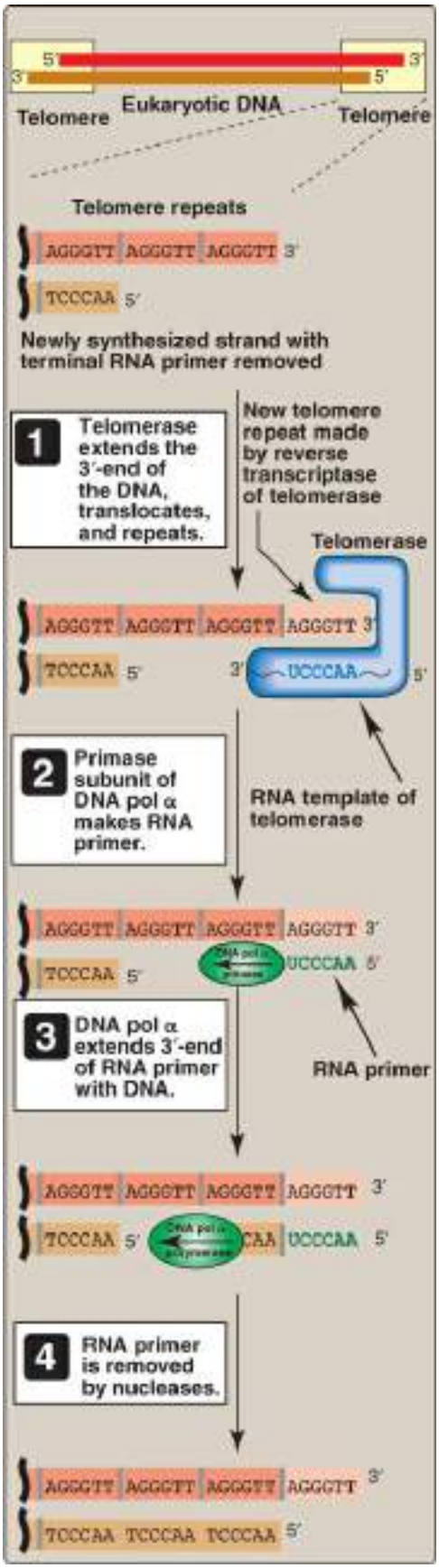


Figure 30.24
Mechanism of action of telomerase, a ribonucleoprotein. pol = polymerase.

C. Telomeres

Telomeres are complexes of DNA, associated with proteins (collectively known as shelterin) located at the ends of linear chromosomes. They maintain the structural integrity of the chromosome, preventing attack by nucleases, and allow repair systems to distinguish a true end from a break in dsDNA. In humans, telomeric DNA consists of several thousand tandem repeats of a noncoding hexameric sequence, AGGGTT, base paired to the repeating AACCCT sequence. The strand with AGGGTT repeats (the "G-rich strand") is longer than its complementary strand with AACCCT repeats (the "C-rich strand"), leaving ssDNA a few hundred nucleotides in length at the 3′ end. The single-stranded region is thought to fold back on itself, forming a loop structure that is stabilized by protein.

1. Telomere shortening: Eukaryotic cells face a special problem in replicating the ends of their linear DNA molecules. Following removal of the RNA primer from the extreme 5′ end of the lagging strand, there is no way to fill in the remaining gap with DNA. Consequently, in most normal human somatic cells, telomeres shorten with each successive cell division. Once telomeres are shortened beyond some critical length, the cell is no longer able to divide and is said to be senescent. In germ cells and stem cells, as well as in cancer cells, telomeres do not shorten and the cells do not senesce. This is a result of the activity of the ribonucleoprotein telomerase, which maintains telomeric length in these cells.

2. Telomerase: This complex contains a protein, TERT that acts as a reverse transcriptase and a short piece of RNA, TERC that acts as a template. The C-rich RNA template base pairs with the G-rich, single-stranded 3′ end of telomeric DNA (Fig. 30.24). The reverse transcriptase uses the RNA template to synthesize DNA in the usual 5′→3′ direction, extending the already longer 3′ end. Telomerase then translocates to the newly synthesized end, and the process is repeated. Once the G-rich strand has been lengthened, primase activity of DNA pol α can use it as a template to synthesize an RNA primer. The primer is extended by DNA pol α and then removed by nucleases.

> Telomeres may be viewed as mitotic clocks in that their length in most cells is inversely related to the number of times the cells have divided. The study of telomeres provides insight into the biology of normal aging, diseases of premature aging (the progerias), and cancer.

D. Reverse transcriptases

As seen with telomerase, reverse transcriptases are RNA-directed DNA pols. A reverse transcriptase is involved in the replication of retroviruses, such as human immunodeficiency virus (HIV). These viruses carry their genome in the form of

ssRNA molecules. Following infection of a host cell, the viral enzyme reverse transcriptase uses the viral RNA as a template for the 5′→3′ synthesis of viral DNA, which then becomes integrated into host chromosomes. Reverse transcriptase activity is also seen with transposons, DNA elements that can move about the genome (see p. 527). In eukaryotes, most transposons are transcribed to RNA, the RNA is used as a template for DNA synthesis by a reverse transcriptase encoded by the transposon, and the DNA is randomly inserted into the genome. (Note: Transposons that involve an RNA intermediate are called retrotransposons or retroposons.)

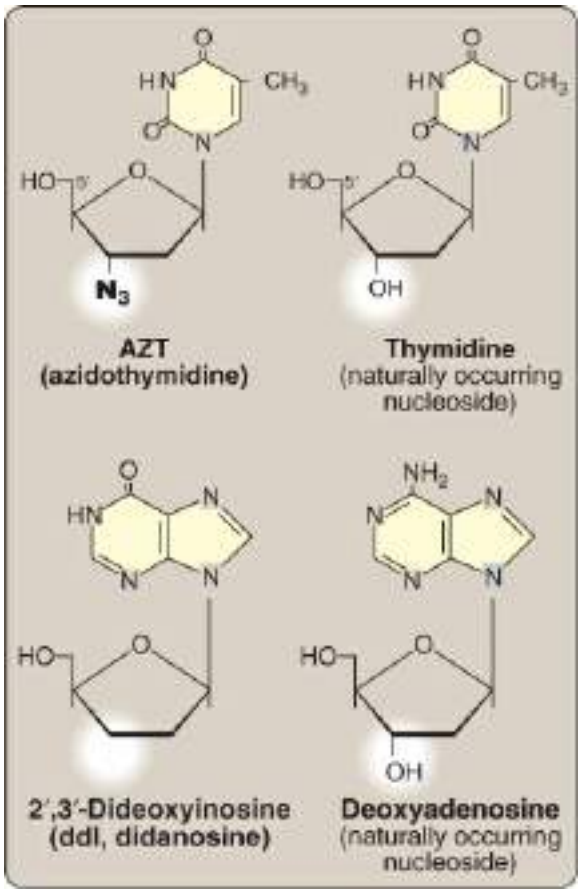


Figure 30.25
Examples of nucleoside analogs that lack a 3′-hydroxyl group. (Note: The ddI is converted to its active form [dideoxy ATP].)

E. DNA replication inhibition by nucleoside analogs

DNA chain growth can be blocked by the incorporation of certain nucleoside analogs that have been modified on the sugar portion (Fig. 30.25). For example, removal of the hydroxyl group from the 3′ carbon of the deoxyribose ring as in 2′,3′ dideoxyinosine ([ddI] also known as didanosine), or conversion of the deoxyribose to another sugar, such as arabinose, prevents further chain elongation. By blocking DNA synthesis, these compounds slow the division of rapidly proliferating cancer

cells and the replication of viruses. Cytosine arabinoside (cytarabine, or araC) has been used in anticancer chemotherapy, whereas adenine arabinoside (vidarabine, or araA) is an antiviral agent. Substitution on the sugar moiety, as seen in azidothymidine (AZT), also called zidovudine (ZDV), also terminates DNA chain elongation. (Note: These drugs are generally supplied as nucleosides, which are then converted to nucleotides by cellular kinases.)

V. EUKARYOTIC DNA ORGANIZATION

A typical (diploid) human somatic cell contains 46 chromosomes, whose total DNA is ~2 m long! It is difficult to imagine how such a large amount of genetic material can be effectively packaged into a volume the size of a cell nucleus so that it can be efficiently replicated and its genetic information expressed. To do so requires the interaction of DNA with a large number of proteins, each of which performs a specific function in the ordered packaging of these long molecules of DNA. Eukaryotic DNA is associated with tightly bound basic proteins, called histones. These serve to order the DNA into fundamental structural units, called nucleosomes, which resemble beads on a string. Nucleosomes are further arranged into increasingly more complex structures that organize and condense the long DNA molecules into chromosomes that can be segregated during cell division. (Note: The complex of DNA and protein found inside the nuclei of eukaryotic cells is called chromatin.)

A. Histones and nucleosome formation

There are five classes of histones, designated H1, H2A, H2B, H3, and H4. These small, evolutionally conserved proteins are positively charged at physiologic pH as a result of their high content of lysine and arginine. Because of their positive charge, they form ionic bonds with negatively charged DNA. Histones, along with ions such as Mg^{2+}, help neutralize the negatively charged DNA phosphate groups.

1. Nucleosomes: Two molecules each of H2A, H2B, H3, and H4 form the octameric core of the individual nucleosome “beads.” Around this structural core, a segment of dsDNA is wound nearly twice (Fig. 30.26). Winding eliminates a helical turn, causing negative supercoiling. (Note: The N-terminal ends of these histones can be acetylated, methylated, or phosphorylated. These reversible covalent modifications influence how tightly the histones bind to the DNA, thereby affecting the expression of specific genes. Histone modification is an example of epigenetics, or heritable changes in gene expression caused without alteration of the nucleotide sequence.) Neighboring nucleosomes are joined by linker DNA ~50 bp long. H1 is not found in the nucleosome core, but instead binds to the linker DNA chain between the nucleosome beads. H1 is the most tissue specific and species specific of the histones. It facilitates the packing of nucleosomes into more compact structures.

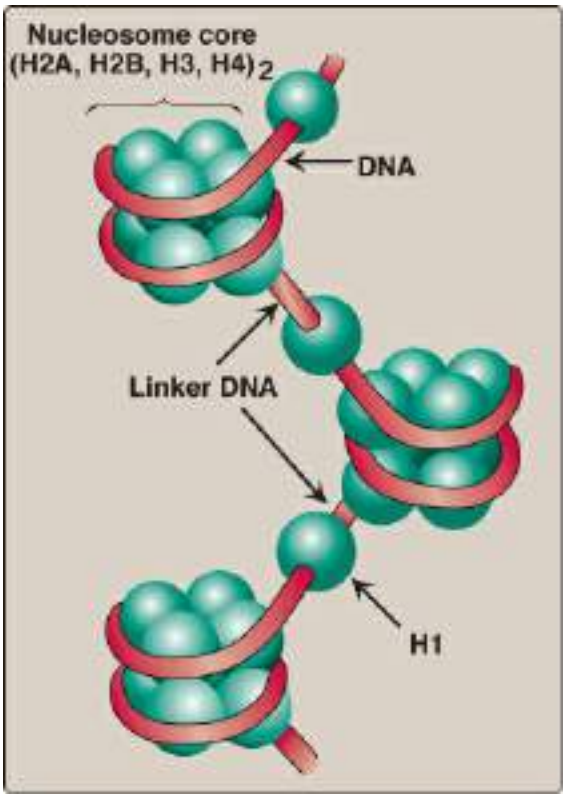


Figure 30.26
Organization of human DNA, illustrating the structure of nucleosomes. H = histone.

2. Higher levels of organization: Nucleosomes can be packed more tightly (stacked) to form a nucleofilament. This structure assumes the shape of a coil, often referred to as a 30-nm fiber. The fiber is organized into loops that are anchored by a nuclear scaffold containing several proteins. Additional levels of organization lead to the final chromosomal structure (Fig. 30.27).

B. Nucleosome fate during DNA replication

Parental nucleosomes are disassembled to allow access to DNA during replication. Once DNA is synthesized, nucleosomes form rapidly. Their histone proteins come both from *de novo* synthesis and from the transfer of parental histones.

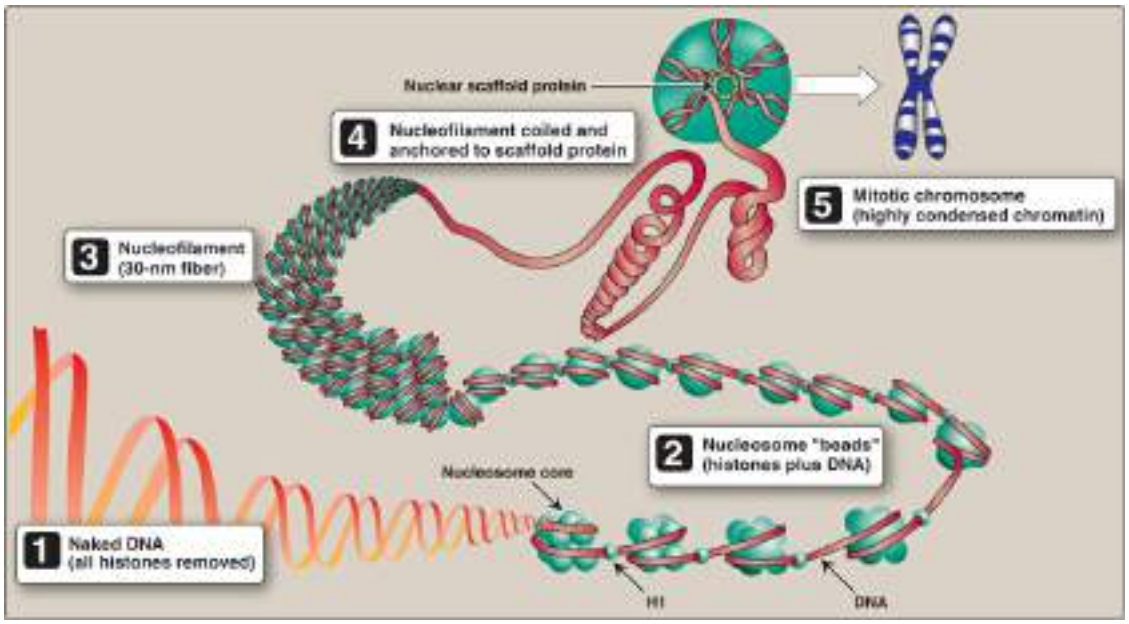


Figure 30.27
Structural organization of eukaryotic DNA. (Note: A 10^4 linear compaction is seen from 1–5.) H = histone.

VI. DNA REPAIR

Despite the elaborate proofreading system employed during DNA synthesis, errors (including incorrect base-pairing or insertion of one to a few extra nucleotides) can occur. In addition, DNA is constantly being subjected to environmental insults that cause the alteration or removal of nucleotide bases. The damaging agents can be either chemicals (e.g., nitrous acid, which can deaminate bases) or radiation (e.g., nonionizing ultraviolet [UV] radiation from sunlight, which can fuse two pyrimidines adjacent to each other in the DNA, and high-energy ionizing radiation, which can cause double-strand breaks). Bases are also altered or lost spontaneously from mammalian DNA at a rate of many thousands per cell per day. If the damage is not repaired, a permanent change (mutation) is introduced that can result in any of a number of deleterious effects, including loss of control over the proliferation of the mutated cell, leading to cancer. Luckily, cells are remarkably efficient at repairing damage done to their DNA, especially when the damage affects only one or two bases at a location on the same strand of the DNA duplex. Most of the repair systems (which are called excision repair systems) involve recognition of the damage (lesion) on the DNA, removal, or excision of the damage, filling the gap left by excision using the undamaged, complementary strand as a template for DNA synthesis, and ligation to restore the continuity of the repaired strand. These excision repair systems remove one to tens of nucleotides. (Note: Repair synthesis of DNA can occur outside of the S phase.) Damage may also affect both strands of the DNA at location (e.g., double-strand breaks). These forms of damage are repaired by different repair systems than those removing damage to one strand.

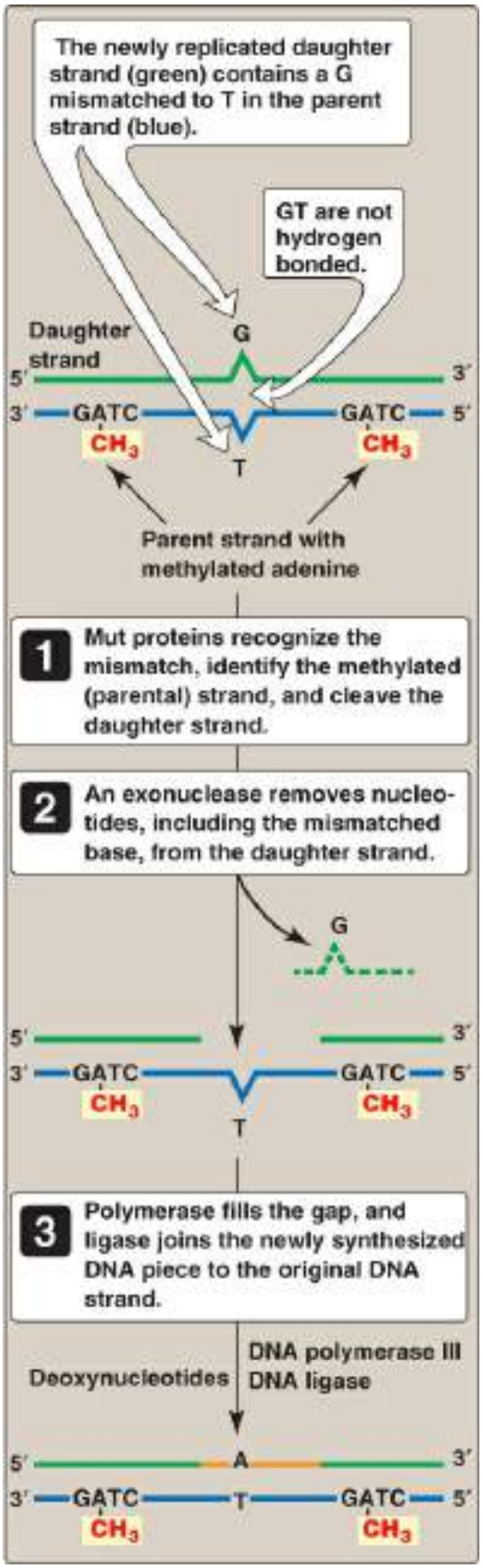


Figure 30.28
Methyl-directed mismatch repair in *Escherichia coli.* (Note: Mut S protein recognizes the mismatch and recruits Mut L. The complex activates Mut H, which cleaves the unmethylated [daughter] strand.)

A. Mismatch repair

Sometimes replication errors escape the proofreading activity during DNA synthesis, causing a mismatch of one to several bases. In *E. coli*, mismatch repair (MMR) is mediated by a group of proteins known as the Mut proteins (Fig. 30.28). Homologous proteins are present in humans. (Note: MMR occurs within minutes of replication and reduces the error rate of replication from 1 in 10^7 to 1 in 10^9 nucleotides.)

1. Mismatched strand identification: When a mismatch occurs, the Mut proteins that identify the mispaired nucleotide(s) must be able to discriminate between the correct strand and the strand with the mismatch. In prokaryotes, discrimination is based on the degree of methylation. GATC sequences, which are found once every thousand nucleotides, are methylated on the A residue by DNA adenine methylase (DAM). This methylation is not done immediately after synthesis, so the DNA is hemimethylated (i.e., the parental strand is methylated, but the daughter strand is not). The methylated parental strand is assumed to be correct, and it is the daughter strand that gets repaired. (Note: The exact mechanism by which the daughter strand is identified in eukaryotes is not yet known, but likely involves recognition of nicks in the newly synthesized strand.)

2. Repair procedure: When the strand containing the mismatch is identified, an endonuclease nicks the strand, and the mismatched nucleotide(s) is/are removed by an exonuclease. Additional nucleotides at the 5′ and 3′ ends of the mismatch are also removed. The gap left by removal of the nucleotides is filled, using the sister strand as a template, by a DNA pol, typically DNA pol III. The 3′ hydroxyl of the newly synthesized DNA is joined to the 5′ phosphate of the remaining stretch of the original DNA strand by DNA ligase.

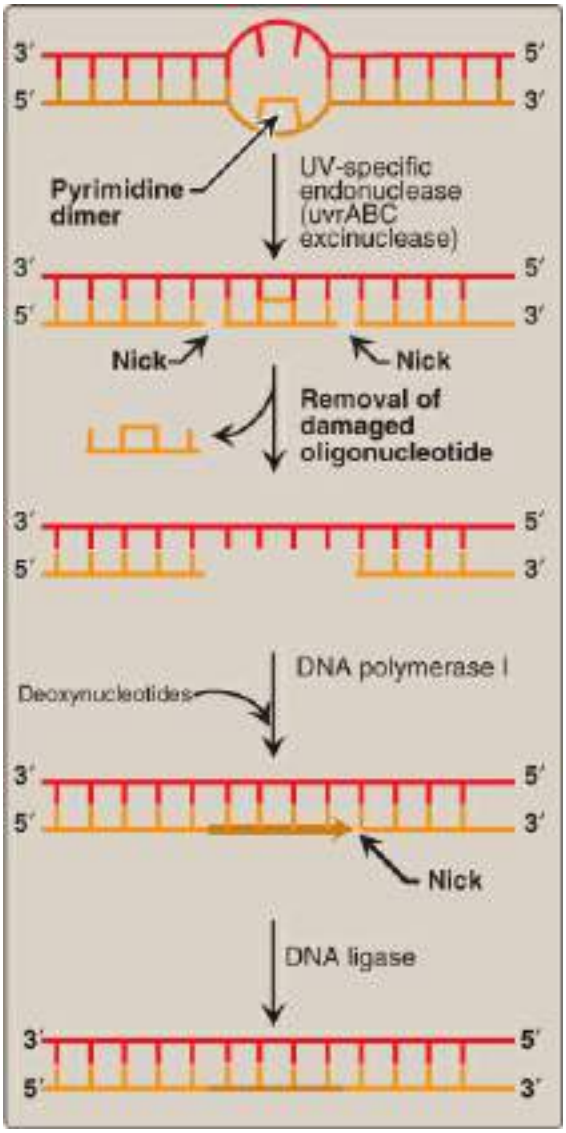


Figure 30.29
Nucleotide excision repair of pyrimidine dimers in *Escherichia coli* DNA. UV = ultraviolet.

Defects in the proteins involved in MMR in humans are associated with Lynch syndrome, also known as hereditary nonpolyposis colorectal cancer (HNPCC). Mutations in MSH2 and MLH1 (two human homologs of bacterial Mut proteins) account for 90% of patients with Lynch syndrome. Although HNPCC confers an increased risk for developing colon cancer (as well as other cancers), only about 5% of all colon cancer is the result of mutations in MMR.

B. Nucleotide excision repair

Exposure of a cell to UV radiation can result in the covalent joining of two adjacent pyrimidines (usually Ts), producing a dimer. These intrastrand cross-links prevent DNA pol from replicating the DNA strand beyond the site of dimer formation. T dimers are excised in bacteria by UvrABC proteins in a process known as

nucleotide excision repair (NER), as illustrated in Figure 30.29. The NER pathway is also present in humans (see 2. below). NER has two DNA damage recognition mechanisms, global genomic repair that finds damage throughout chromosomes and transcription-coupled repair that identifies DNA lesions encountered by RNA polymerases.

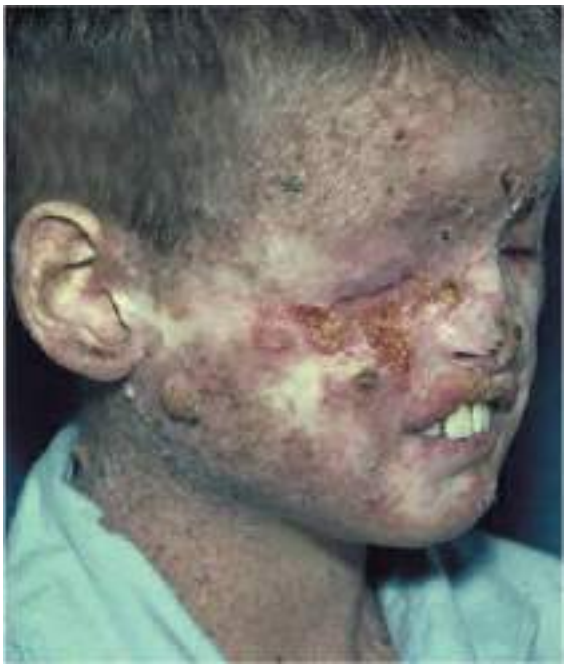


Figure 30.30
Patient with xeroderma pigmentosum.

1. Recognition and excision of UV-induced dimers: A UV-specific endonuclease (called uvrABC excinuclease) recognizes the bulky dimer and cleaves the damaged strand on both the 5′ side and 3′ side of the lesion. A short oligonucleotide containing the dimer is excised, leaving a gap in the DNA strand. This gap is filled in using DNA pol I and DNA ligase. The human NER pathway uses additional proteins to remove pyrimidine dimers that form in skin cells and to repair DNA damage that is created by chemical exposure, such as G adducts caused by benzo[a]pyrene from cigarette smoke. NER occurs throughout the cell cycle.
2. UV radiation and cancer: In the rare, human genetic disease xeroderma pigmentosum (XP), an individual's skin cells cannot repair pyrimidine dimers caused by sunlight, resulting in extensive accumulation of mutations and, consequently, early and numerous skin cancers (Fig. 30.30). XP can be caused by defects in seven genes that code for the XP proteins required for NER of UV damage.

C. Base excision repair

DNA bases can be altered, either spontaneously, as is the case with C, which slowly undergoes deamination (the loss of its amino group) to form U, or by the action of deaminating or alkylating compounds. For example, nitrous acid, which is formed by the cell from precursors such as the nitrates, deaminates C, A (to hypoxanthine), and G (to xanthine). Dimethyl sulfate can alkylate (methylate) A. Bases can also be lost spontaneously by hydrolysis from the deoxyribose sugar backbone. For example, ~10,000 purine bases are lost this way per cell per day. Lesions involving base alterations or loss can be corrected by base excision repair ([BER], Fig. 30.31).

1. Abnormal base removal: In BER, abnormal bases, such as U, which can occur in DNA by either deamination of C or improper use of dUTP instead of dTTP during DNA synthesis, are recognized by specific DNA glycosylases that hydrolytically cleave them from the deoxyribose-phosphate backbone of the strand. This leaves an apyrimidinic site, or apurinic if a purine was removed, both referred to as AP sites.

2. AP site recognition and repair: Specific AP endonucleases recognize that a base is missing and initiate the process of excision and gap filling by making an endonucleolytic cut just to the 5′ side of the AP site. A deoxyribose phosphate lyase removes the single, base-free, sugar phosphate residue. DNA pol I and DNA ligase complete the repair process.

D. Double-strand break repair

Ionizing radiation, chemotherapeutic agents such as doxorubicin, and oxidative free radicals (see p. 163) can cause double-strand breaks in DNA that can be lethal to the cell. (Note: Such breaks also occur naturally during genetic recombination.) dsDNA breaks cannot be corrected by the previously described strategy of excising the damage on one strand and using the undamaged strand as a template for replacing the missing nucleotide(s). Instead, they are repaired by one of two systems. The first is nonhomologous end joining (NHEJ), in which a group of proteins mediates the recognition, processing, and ligation of the ends of two DNA fragments. However, some DNA is lost in the process. Consequently, NHEJ is error prone and mutagenic. Defects in NHEJ are associated with a predisposition to cancer and immunodeficiency syndromes. The second repair system, homologous recombination (HR), uses the enzymes that normally perform genetic recombination between homologous chromosomes during meiosis. This system is much less error prone (“error free”) than NHEJ because any DNA that was lost is replaced using homologous DNA as a template. HR occurs in late S and G_2 of the cell cycle, whereas NHEJ can occur anytime. (Note: Mutations to the proteins BRCA1 or BRCA2 [breast cancer 1 or 2], which are involved in HR, increase the risk for developing breast and ovarian cancer.)

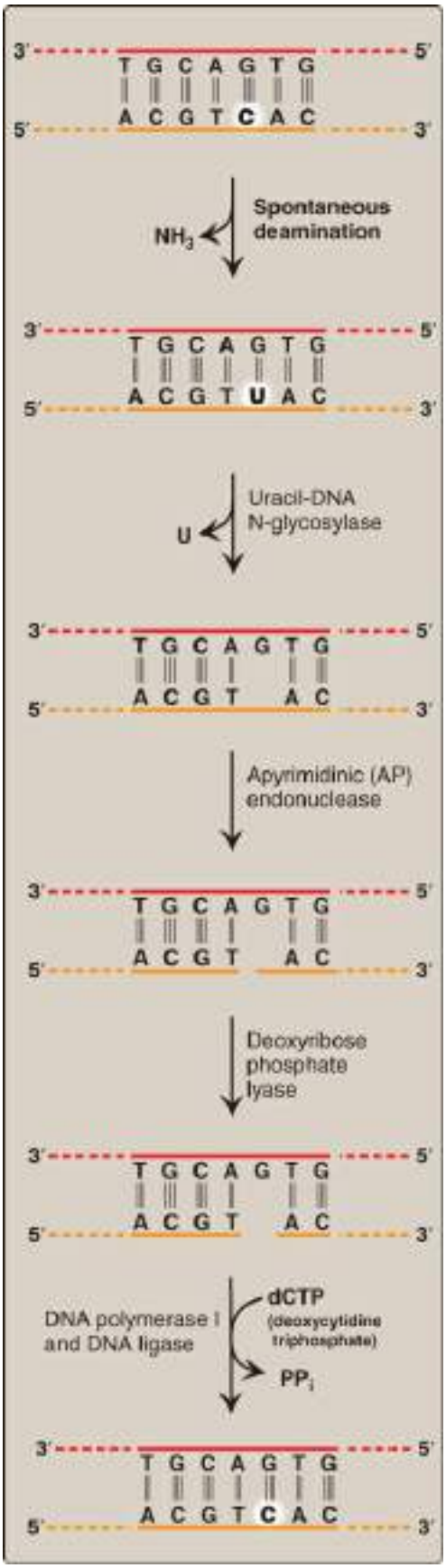


Figure 30.31
Correction of base alterations by base excision repair. C = cytosine; U = uracil; NH_3 = ammonia; PP_i = pyrophosphate.

VII. Chapter Summary

- DNA is composed of two polymers (chains) of dNMP (nucleotides). Each chain has **polarity**, with both a 5′ end (free phosphate) and a 3′ end (free hydroxyl). The nucleotide sequences of the chains are read from the 5′ end to the 3′ end (Fig. 30.32).
- DNA exists as a dsDNA, in which the two chains are paired in an **antiparallel** manner and form a **double helix**. Through hydrogen bonding, **A** pairs with **T**, and **C** pairs with **G**.
- Each DNA strand serves as a **template** for constructing a **complementary** daughter strand (**semiconservative replication**). DNA replication begins at an **origin of replication** where the two strands unwind and separate and synthesis occurs bidirectionally at two **replication forks** that move away from the origin. As **helicase separates the two strands**, positive **supercoils** are produced in the region of DNA ahead of the replication fork and negative supercoils behind the fork. **DNA topoisomerases types I** and **II** remove supercoils.
- **DNA pols** synthesize new DNA strands only in the **5′→3′** direction and require a short RNA **primer** created by **primase**. One of the new strands must grow in the 5′→3′ direction toward the replication fork (**leading strand**) while the other grows in the 5′→3′ direction away from the replication fork (**lagging strand**). Leading-strand synthesis is continuous from one RNA primer, whereas the lagging strand needs many primers (**discontinuous** synthesis involving **Okazaki fragments**).
- In *E. coli,* DNA chain elongation is catalyzed by **DNA pol III**, using **5′-deoxyribonucleoside triphosphates** as substrates. The enzyme **proofreads** the newly synthesized DNA, removing terminal mismatched nucleotides with its **3′→5′ exonuclease** activity. RNA primers are removed by **DNA pol I**, using its **5′→3′ exonuclease** activity. This enzyme fills the gaps with DNA, proofreading as it synthesizes. The final phosphodiester linkage is catalyzed by **DNA ligase**.
- There are at least five high-fidelity **eukaryotic DNA pols**. **Pol** α is a multisubunit enzyme, one subunit of which is a **primase**. Pol α 5′→3′ polymerase activity adds a short piece of DNA to the RNA primer. **Pol ε** completes DNA synthesis on the leading strand, whereas **pol** δ elongates each lagging strand fragment. **Pol β** is involved with DNA repair, and **pol** γ replicates mitochondrial DNA. Pols ε, δ, and γ use 3′→5′ exonuclease activity to proofread.
- **Nucleoside analogs** containing modified sugars can be used to block DNA chain growth. They are useful in anticancer and antiviral chemotherapy.
- **Telomeres** are stretches of **highly repetitive DNA** that are bound by protein and protect the **ends** of linear chromosomes. As most cells divide and age, these sequences are shortened, contributing to senescence. In cells that do not senesce (e.g., germline and cancer cells), the ribonucleoprotein **telomerase** employs its protein component **reverse transcriptase** to extend the telomeres, using its **RNA** component as a **template**.
- Pairs of the positively charged histone proteins (H2A, H2B, H3, and H4) form an octameric structural core around which DNA is wrapped, creating a **nucleosome**. The DNA connecting the nucleosomes, called **linker DNA**, is bound to H1. Nucleosomes are packed more tightly to form a nucleofilament. Additional levels of organization create a chromosome.
- Three types of DNA repair correct most DNA damage in chromosomes: NER, BER, and MMR. Each removes specific types of DNA damage (Fig. 30.33). NER removes **pyrimidine dimers** caused by UV radiation, BER replaces abnormal bases and AP sites, and MMR corrects mispaired bases caused by DNA pol errors. Defects in the **XP proteins** needed for NER in humans result in **XP**. Defective MMR in humans, mainly caused by mutations in the MSH2 and MLH1 genes, is associated with **HNPCC**.
- Double-strand breaks in DNA are repaired by **NHEJ** (error prone) and template-requiring **HR** (“error free”).

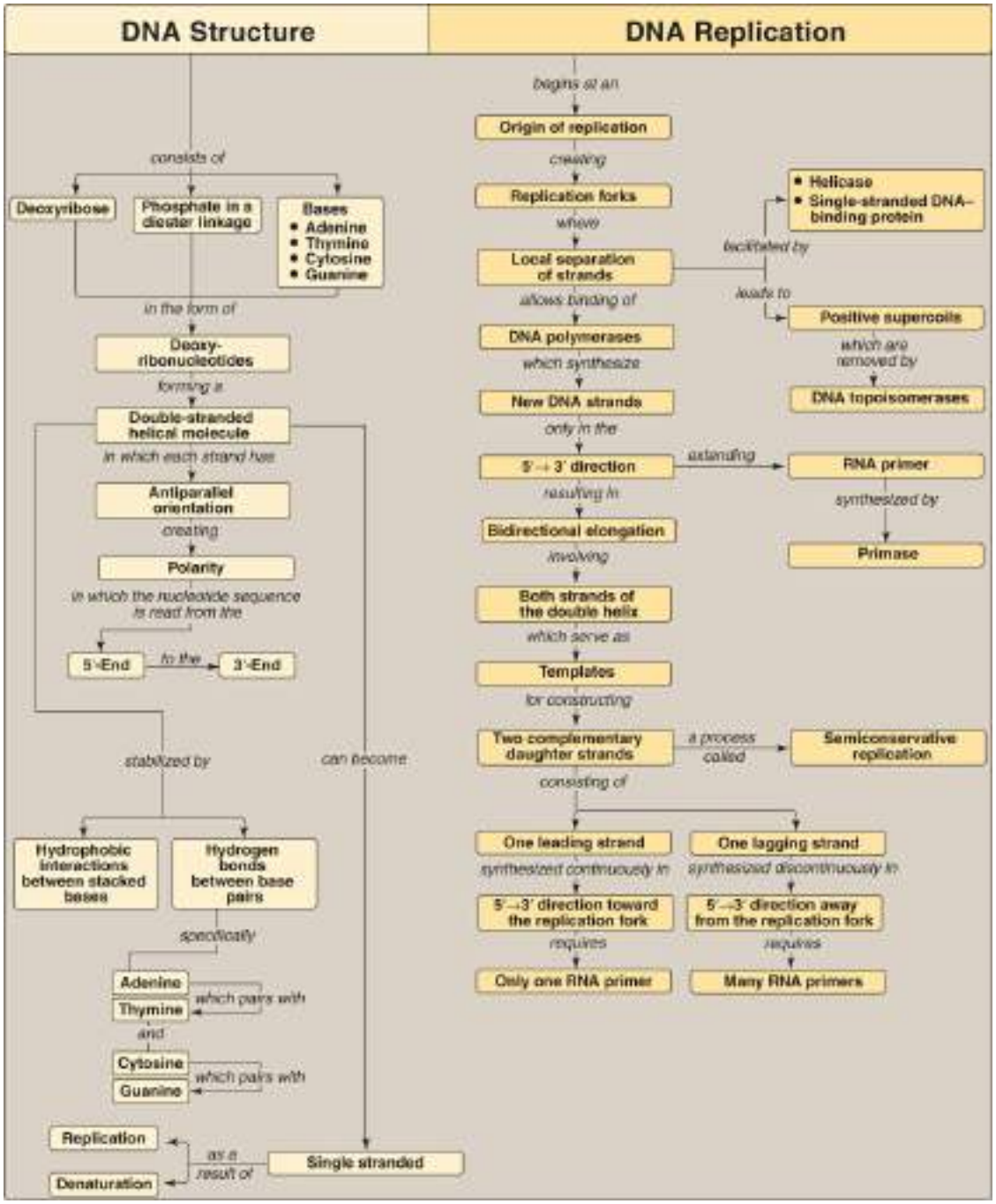


Figure 30.32
Key concept map for DNA structure and replication.

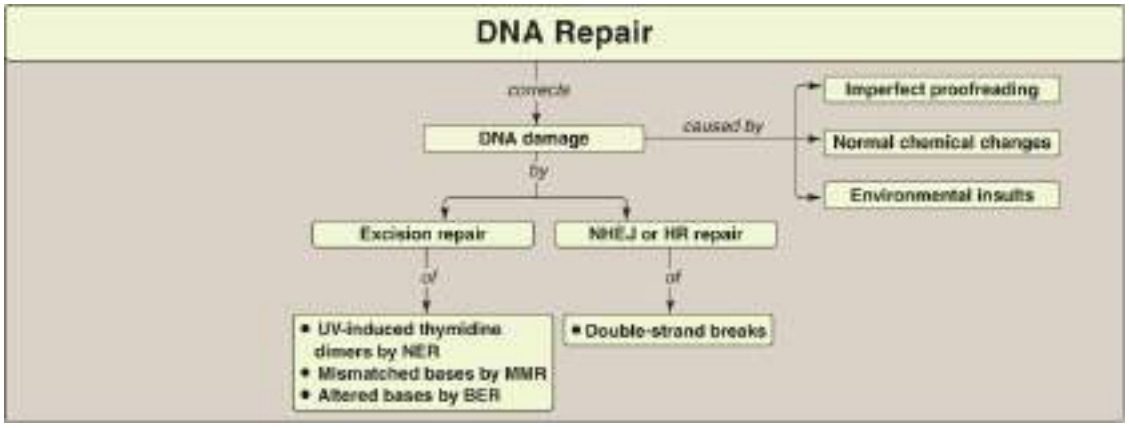


Figure 30.33
Key concept map for DNA repair. NHEJ = nonhomologous end joining; HR = homologous recombination; UV = ultraviolet; NER = nucleotide excision repair; MMR = mismatch repair; BER = base excision repair.

Study Questions

Choose the ONE best answer.

30.1 A 10-year-old female is brought by her parents to the dermatologist. She has many freckles on her face, neck, arms, and hands, and the parents report that she is unusually sensitive to sunlight. Two basal cell carcinomas are identified on her face. Based on the clinical picture, which of the following processes is most likely to be defective in this patient?

A. Repair of double-strand breaks by error-prone homologous recombination
B. Removal of mismatched bases from the 3′ end of Okazaki fragments by a methyl-directed process
C. Removal of pyrimidine dimers from DNA by nucleotide excision repair
D. Removal of uracil from DNA by base excision repair
E. Removal of incorrectly paired nucleotides by Pol ε 3′→5′ exonuclease activity

Correct answer = C. The sensitivity to sunlight, extensive freckling on parts of the body exposed to the sun, and presence of skin cancer at a young age indicate that the patient most likely suffers from xeroderma pigmentosum (XP). These patients are deficient in any one of several XP proteins required for nucleotide excision repair of pyrimidine dimers in ultraviolet radiation–damaged DNA. Double-strand breaks are repaired by nonhomologous end joining (error prone) or homologous recombination (“error free”). Methylation is not used for strand discrimination in eukaryotic mismatch repair. Uracil is removed from DNA molecules by a specific glycosylase in base excision repair, but a defect in this process does not cause XP.

30.2 Telomeres are complexes of DNA and protein that protect the ends of linear chromosomes. In most normal human somatic cells, telomeres shorten with each division. In stem cells and cancer cells, however, telomeric length is maintained. In the synthesis of telomeres:

A. telomerase, a ribonucleoprotein, provides both the RNA and the protein needed for synthesis.
B. the RNA of telomerase serves as a primer.
C. the RNA of telomerase is a ribozyme.
D. the protein of telomerase is a DNA-directed DNA polymerase.
E. the shorter C-rich strand gets extended.
F. the direction of synthesis is 3′→5′.

Correct answer = A. Telomerase is a ribonucleoprotein particle required for telomere maintenance. Telomerase contains an RNA that serves as the template, not the primer, for the synthesis of telomeric DNA by the reverse transcriptase of telomerase. Telomeric RNA has no catalytic activity. As a reverse transcriptase, telomerase synthesizes DNA using its RNA template and so is an RNA-directed DNA polymerase. The direction of synthesis,

as with all DNA synthesis, is 5′→3′, and it is the 3′ end of the already longer G-rich strand that gets extended.

30.3 While studying the structure of a small gene that was sequenced during the Human Genome Project, an investigator notices that one strand of the DNA molecule contains 20 A, 25 G, 30 C, and 22 T. How many of each base is found in the complete double-stranded molecule?

A. A = 40, G = 50, C = 60, T = 44
B. A = 42, G = 55, C = 55, T = 42
C. A = 44, G = 60, C = 50, T = 40
D. A = 45, G = 45, C = 52, T = 52
E. A = 50, G = 47, C = 50, T = 47

Correct answer = B. The two DNA strands are complementary to each other, with A base paired with T and G base paired with C. So, for example, the 20 A on the first strand would be paired with 20 T on the second strand, the 25 G on the first strand would be paired with 25 C on the second strand, and so forth. When these are all added together, the correct numbers of each base are indicated in choice B. Notice that, in the correct answer, A = T and G = C.

30.4 List the order in which the following enzymes participate in leading strand synthesis during prokaryotic replication.

A. Ligase
B. Polymerase I (3′→5′ exonuclease activity)
C. Polymerase I (5′→3′ exonuclease activity)
D. Polymerase I (5′→3′ polymerase activity)
E. Polymerase III
F. Primase

Correct answer: F, E, C, D, B, A. Primase makes the RNA primer; polymerase (pol) III extends the primer with DNA (and proofreads); pol I removes the primer with its 5′→3′ exonuclease activity, fills in the gap with its 5′→3′ polymerase activity, and removes errors with its 3′→5′ exonuclease activity; and ligase makes the 5′-to-3′ phosphodiester bond that links the DNA made by pols I and III.

30.5 Dideoxynucleotides lack a 3′-hydroxyl group. Why would incorporation of a dideoxynucleotide into DNA stop replication?

The lack of the 3′-OH group prevents formation of the 3′ hydroxyl-to-5′-phosphate bond that links one nucleotide to the next in DNA.

RNA Structure, Synthesis, and Processing 31

I. OVERVIEW

The genetic master plan of an organism is contained in the sequence of deoxyribonucleotides in its DNA. However, it is through ribonucleic acid (RNA), the "working copies" of DNA that the master plan is expressed (Fig. 31.1). The copying process, during which a DNA strand serves as a template for the synthesis of RNA, is called transcription. Transcription produces messenger RNA (mRNA), which is translated into sequences of amino acids (proteins), and ribosomal RNA (rRNA), transfer RNA (tRNA), and additional RNA molecules that perform specialized structural, catalytic, and regulatory functions and are not translated. That is, they are noncoding RNA (ncRNA). Therefore, the final product of gene expression can be RNA or protein, depending upon the gene. (Note: Only ~2% of the genome encodes proteins.) A central feature of transcription is that it is highly selective. For example, many transcripts are made of some regions of the DNA. In other regions, few or no transcripts are made. This selectivity is due, at least in part, to signals embedded in the nucleotide sequence of the DNA. These signals instruct the RNA polymerase (RNA pol) where to start, how often to start, and where to stop transcription. Several regulatory proteins are also involved in this selection process. The biochemical differentiation of an organism's tissues is ultimately a result of the selectivity of the transcription process. (Note: This selectivity of transcription is in contrast to the "all-or-none" nature of genomic replication.) Another important feature of transcription is that many RNA transcripts that initially are faithful copies of one of the two DNA strands may undergo various modifications, such as terminal additions, base modifications, trimming, and internal segment removal, which convert the inactive primary transcript into a functional molecule. The transcriptome is the complete set of RNA transcripts expressed by a genome.

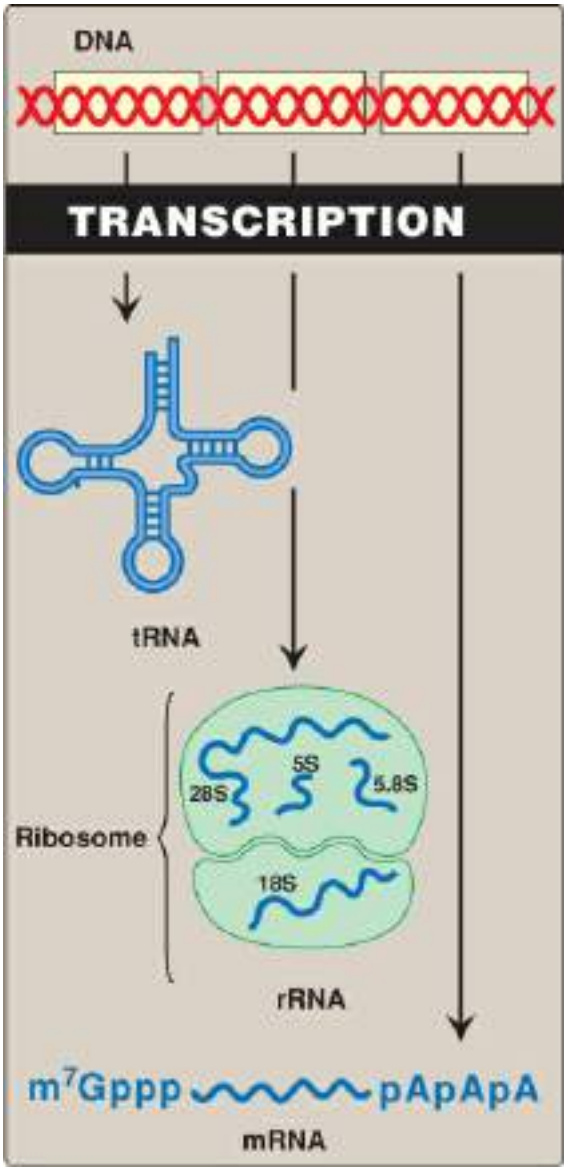


Figure 31.1
Expression of genetic information by transcription. (Note: RNA shown are eukaryotic.) tRNA = transfer RNA; rRNA = ribosomal RNA; mRNA = messenger RNA; m^7Gppp = 7-methylguanosine-triphosphate cap; pApApA = poly-A tail; p = phosphate.

II. RNA STRUCTURE

There are three major types of RNA that participate in the process of protein synthesis: rRNA, tRNA, and mRNA. Like DNA, these RNA are unbranched polymeric molecules composed of nucleoside monophosphates joined together by 3′-to-5′ phosphodiester bonds (see p. 460). However, they differ from DNA in several ways. For example, they are considerably smaller than DNA, contain ribose instead of deoxyribose and uracil (U) instead of thymine (T), and exist as single strands that are capable of folding into complex structures. The three major types of RNA also differ from each other in size,

function, and special structural modifications. (Note: In eukaryotes, additional small ncRNA molecules found in the nucleolus [small nucleolar RNA (snoRNA)], nucleus [small nuclear RNA (snRNA)], and cytoplasm [microRNA (miRNA)] perform specialized functions as described on pp. 490, 491 and 525.)

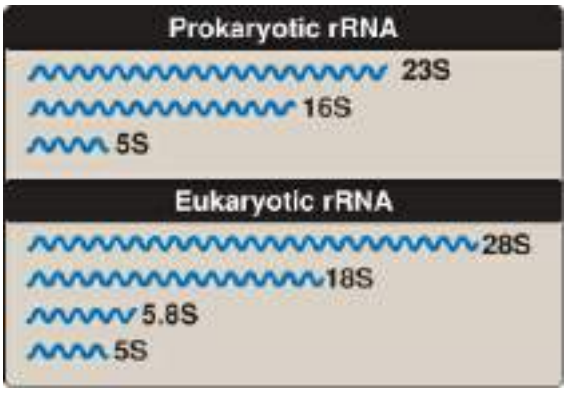


Figure 31.2
Prokaryotic and eukaryotic ribosomal RNA (rRNA). S = Svedberg unit.

A. Ribosomal RNA

rRNA are found in association with several proteins as components of the ribosomes, the complex structures that serve as the sites for protein synthesis (see p. 500). Prokaryotic cells contain three distinct size species of rRNA (23S, 16S, and 5S, where S is the Svedberg unit for sedimentation rate that is determined by the size and shape of the particle), as shown in Figure 31.2. Eukaryotic cells contain four nuclear rRNA species (28S, 18S, 5.8S, and 5S) and two rRNA species (12S and 16S) encoded by the mitochondrial DNA. Together, rRNA make up ~80% of the total RNA in the cell. (Note: Some RNA function as catalysts, e.g., an rRNA in protein synthesis [see p. 504]. RNA with catalytic activity is termed a ribozyme.)

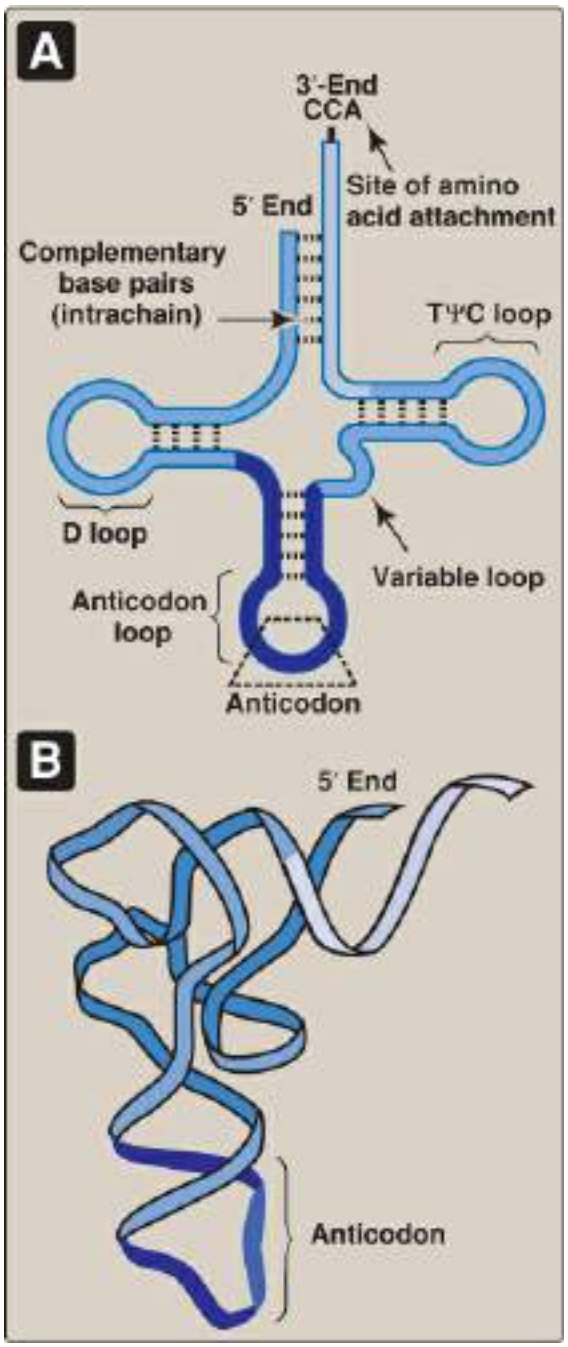


Figure 31.3
A: Characteristic transfer RNA (tRNA) secondary structure (cloverleaf). **B:** Folded (tertiary) tRNA structure found in cells. D = dihydrouracil; Ψ = pseudouracil; T = thymine; C = cytosine; A = adenine.

B. Transfer RNA

tRNA are the smallest (4S) of the three major types of RNA molecules. There is at least one specific type of tRNA molecule for each of the 20 amino acids commonly found in proteins. Together, tRNA make up ~15% of the total RNA in the cell. The tRNA molecules contain a high percentage of unusual (modified) bases, for

example, dihydrouracil (see Fig. 22.2, p. 325), and have extensive intrachain base pairing (Fig. 31.3) that leads to characteristic secondary cloverleaf structure and tertiary structure. Each tRNA serves as an adaptor molecule that carries its specific amino acid, covalently attached to its 3′ end, to the site of protein synthesis. There, it recognizes the genetic code sequence on an mRNA, which specifies the addition of that amino acid to the growing peptide chain (see p. 496). In eukaryotic cells, tRNA are encoded within both the nuclear and mitochondrial chromosomes.

> The human mitochondrial chromosome carries 22 tRNA genes. Mutations in these genes can cause human disease. Mutations in the mitochondrial gene for $tRNA^{Lys}$ are associated with myoclonic epilepsy (jerking muscle spasms) with ragged red fibers (MERRF), a disorder that affects skeletal muscle structure and function (myopathy), and with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), which affects the brain, nervous system, and muscles. MELAS is also caused by mutations in the mitochondrial $tRNA^{Leu}$ gene.

C. Messenger RNA

mRNA comprises only ~5% of the RNA in a cell, yet is by far the most heterogeneous type of RNA in size and base sequence. mRNA is coding RNA in that it carries genetic information from DNA for use in protein synthesis. In eukaryotes, this involves transport of mRNA out of the nucleus and into the cytosol. An mRNA carrying information from more than one gene is polycistronic (cistron = gene). Polycistronic mRNA is characteristic of prokaryotes, mitochondria, some viruses, and in chloroplast in plants. An mRNA carrying information from only one gene is monocistronic and is characteristic of eukaryotes. In addition to the protein-coding regions that can be translated, mRNA contains untranslated regions at its 5′- and 3′ ends (Fig. 31.4). Special structural characteristics of eukaryotic (but not prokaryotic) mRNA include a long sequence of adenine (A) nucleotides (a poly-A tail) on the 3′ end of the RNA, plus a cap on the 5′ end consisting of a molecule of 7-methylguanosine attached through an unusual (5′-to-5′) triphosphate linkage. The mechanisms for modifying mRNA to create these special structural characteristics are discussed on pp. 490 and 491.

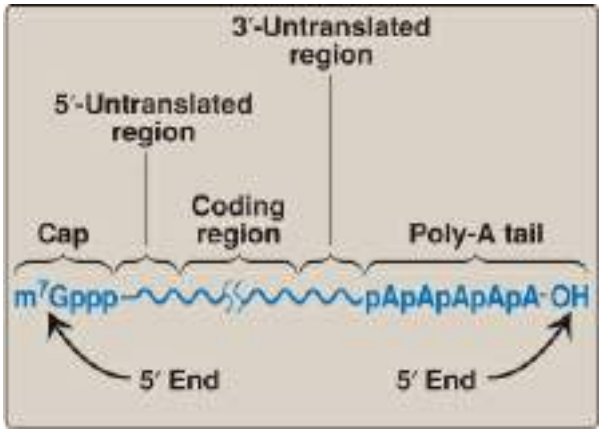


Figure 31.4
Structure of eukaryotic messenger RNA. G = guanine; A = adenine.

III. PROKARYOTIC GENE TRANSCRIPTION

The structure of magnesium-requiring RNA pol, the signals that control transcription, and the varieties of modification that RNA transcripts can undergo differ among organisms, particularly from prokaryotes to eukaryotes. Therefore, the discussions of prokaryotic and eukaryotic transcription are presented separately.

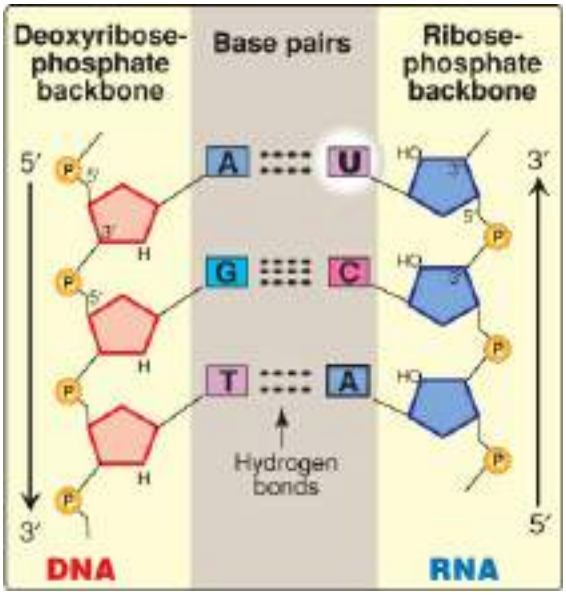


Figure 31.5
Antiparallel, complementary base pairs between DNA and RNA. T = thymine; A = adenine; C = cytosine; G = guanine; U = uracil.

A. Prokaryotic RNA polymerase

In bacteria, one species of RNA pol synthesizes all of the RNA except for the short RNA primers needed for DNA replication (Note: RNA primers are synthesized by the specialized, monomeric enzyme primase [see p. 466].) RNA pol is a multisubunit enzyme that recognizes a nucleotide sequence (the promoter region) at the beginning of a length of DNA that is to be transcribed. It next makes a complementary RNA copy of the DNA template strand and then recognizes the end of the DNA sequence to be transcribed (the termination region). RNA is synthesized from its 5′ end to its 3′ end, antiparallel to its DNA template strand (see p. 463). The template is copied as it is in DNA synthesis, in which a guanine (G) on the DNA specifies a cytosine (C) in the RNA, a C specifies a G, a T specifies an A, but an A specifies a U instead of a T (Fig. 31.5). The RNA, then, is complementary to the DNA template (antisense, minus) strand and identical to the coding (sense, plus) strand, with U replacing T. Within the DNA molecule, regions of both strands can serve as templates for transcription. For a given gene, however, only one of the two

DNA strands can be the template. Which strand is used is determined by the location of the promoter for that gene. Transcription by RNA pol involves a core enzyme and several auxiliary proteins.

1. Core enzyme: Five of the enzyme's peptide subunits, 2 α, 1 β, 1 β′, and 1 Ω, are required for enzyme assembly (α, Ω), template binding (β′), and the 5′→3′ polymerase activity (β) and together are referred to as the core enzyme (Fig. 31.6). However, this enzyme lacks specificity (i.e., it cannot recognize the promoter region on the DNA template).
2. Holoenzyme: The σ subunit (sigma factor) enables RNA pol to recognize promoter regions on the DNA. The σ subunit plus the core enzyme make up the holoenzyme. (Note: Different σ factors recognize different groups of genes, with σ^{70} predominating.)

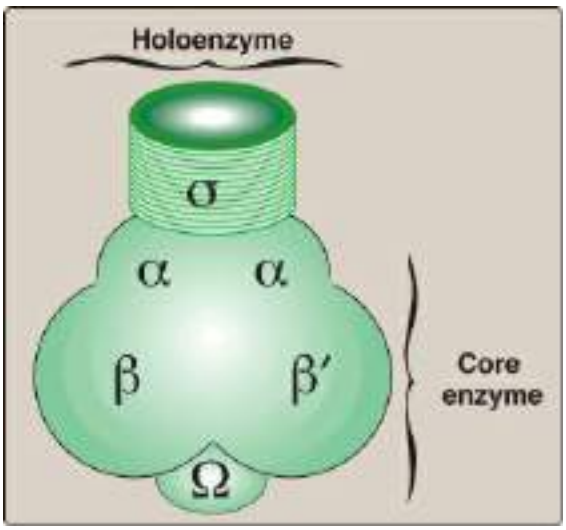


Figure 31.6
Components of prokaryotic RNA polymerase.

B. Steps in RNA synthesis

The process of transcription of a typical gene of *Escherichia coli (E. coli)* can be divided into three phases: initiation, elongation, and termination. A transcription unit extends from the promoter to the termination region, and the initial product of transcription by RNA pol is termed the primary transcript.

1. Initiation: Transcription begins with the binding of the RNA pol holoenzyme to a region of the DNA known as the promoter, which is not transcribed. The prokaryotic promoter contains characteristic consensus sequences (Fig. 31.7). (Note: Consensus sequences are idealized sequences in which the base shown at each position is the base most frequently [but not necessarily always] encountered at that position.) Those that are recognized by prokaryotic RNA pol σ factors include the following.

a. −35 Sequence: A consensus sequence (5′-TTGACA-3′), centered about 35 bases to the left of the transcription start site (see Fig. 31.7), is the initial point of contact for the holoenzyme, and a closed complex is formed. (Note: By convention, the regulatory sequences that control transcription are designated by the 5′→3′ nucleotide sequence on the coding strand. A base in the promoter region is assigned a negative number if it occurs prior to [to the left of, toward the 5′ end of, or "upstream" of] the transcription start site. Therefore, the TTGACA sequence is centered at approximately base −35. The first base at the transcription start site is assigned a position of +1. There is no base designated "0.")

b. Pribnow box: The holoenzyme moves and covers a second consensus sequence (5′-TATAAT-3′), centered at about −10 (see Fig. 31.7), which is the site of melting (unwinding) of a short stretch (~14 base pairs) of DNA. This initial melting converts the closed initiation complex to an open complex known as a transcription bubble. (Note: A mutation in either the −10 or the −35 sequence can affect the transcription of the gene controlled by the mutant promoter.)

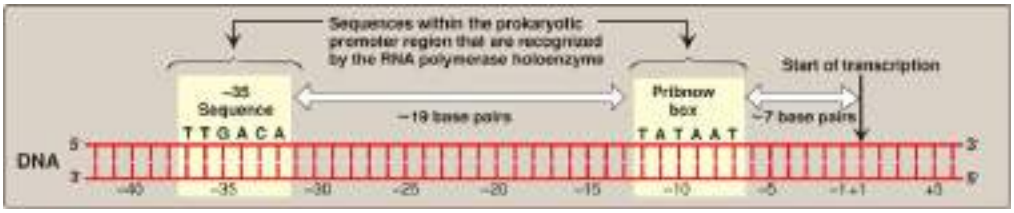


Figure 31.7
Structure of the prokaryotic promoter region. T = thymine; G = guanine; A = adenine; C = cytosine.

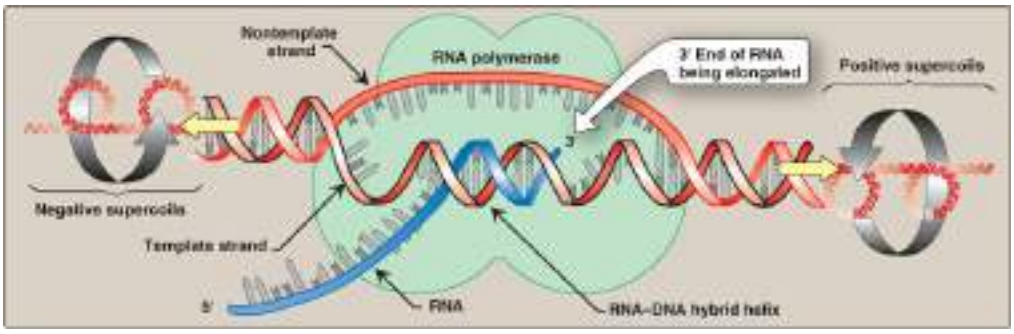


Figure 31.8
Local unwinding of DNA by RNA polymerase and formation of an open initiation complex (transcription bubble).

2. Elongation: Once the promoter has been recognized and bound by the holoenzyme, local unwinding of the DNA helix continues (Fig. 31.8), mediated by the polymerase. (Note: Unwinding generates supercoils in the DNA that can be relieved by DNA topoisomerases [see p. 465].) RNA pol begins to synthesize a transcript of the DNA sequence, and several short pieces of RNA are made

and discarded. The elongation phase begins when the transcript (typically starting with a purine) exceeds 10 nucleotides in length. Sigma factor is then released, and the core enzyme is able to leave (clear) the promoter and move along the template strand in a processive manner, serving as its own sliding clamp. During transcription, a short DNA–RNA hybrid helix is formed (see Fig. 31.8). Like DNA pol, RNA pol uses nucleoside triphosphates as substrates and releases pyrophosphate each time a nucleoside monophosphate is added to the growing chain. As with replication, transcription is always in the 5′→3′ direction. In contrast to DNA pol, RNA pol does not require a primer and does not have a 3′→5′ exonuclease domain for proofreading. (Note: Misincorporation of a ribonucleotide causes RNA pol to pause, backtrack, cleave the transcript, and restart. Nonetheless, transcription has a higher error rate than does replication.)

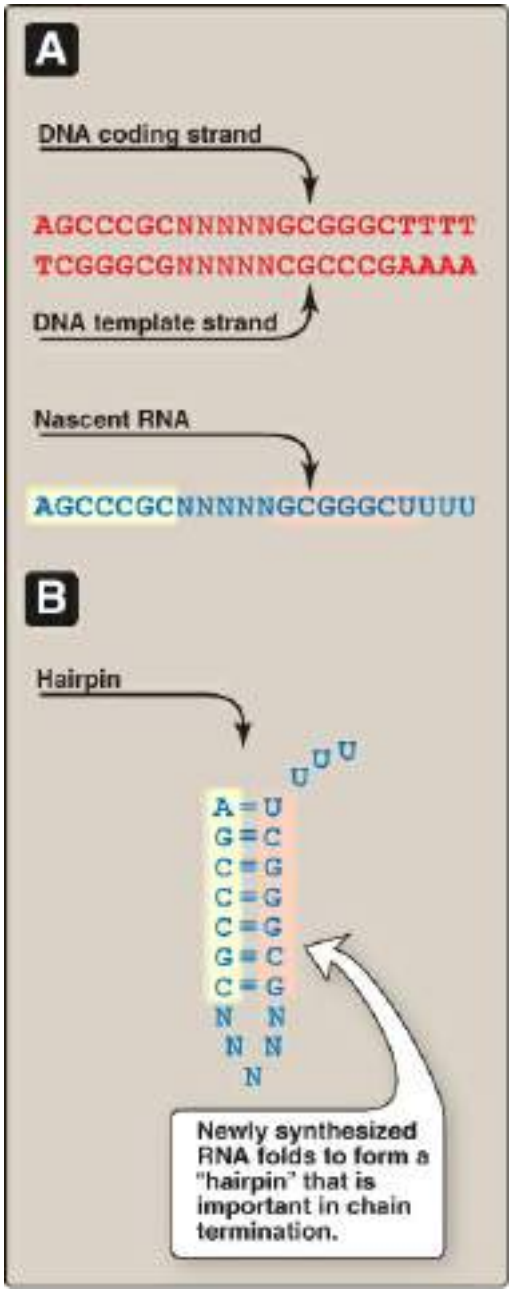


Figure 31.9
Rho-independent termination of prokaryotic transcription. A: DNA template sequence generates a self-complementary sequence in the nascent RNA. B: Hairpin structure formed by the RNA. N represents a noncomplementary base; A = adenine, T = thymine; G = guanine; C = cytosine; U = uracil.

3. Termination: The elongation of the single-stranded RNA chain continues until a termination signal is reached. Termination can be intrinsic (occur without additional proteins) or dependent upon the participation of a protein known as the ρ (rho) factor.

a. Rho Independent: For most prokaryotic genes, this type of termination

requires a sequence in the DNA template for generating a sequence in the nascent (newly made) RNA that is self-complementary (Fig. 31.9). This allows the RNA to fold back on itself, forming a GC-rich stem (stabilized by hydrogen bonds) plus a loop. This structure is known as a "hairpin." Additionally, just beyond the hairpin, the RNA transcript contains a string of uracil residues (Us) at the 3′ end. The bonding of these Us to the complementary As of the DNA template is weak. This facilitates the separation of the newly synthesized RNA from its DNA template, as the double helix "zips up" behind the RNA pol.

b. Rho Dependent: This requires the participation of the additional protein rho, which is a hexameric ATPase with helicase activity. Rho binds a C-rich rho utilization (rut) site near the 5′ end of the nascent RNA and, using its ATPase activity, moves along the RNA until it reaches the RNA pol paused at the termination site. The ATP-dependent helicase activity of rho separates the RNA–DNA hybrid helix, causing the release of the RNA.

4. Antibiotics: Some antibiotics prevent bacterial cell growth by inhibiting RNA synthesis. For example, rifampin (rifampicin) inhibits transcription initiation by binding to the β subunit of prokaryotic RNA pol and preventing chain growth beyond three nucleotides (Fig. 31.10). Rifampin is important in the treatment of tuberculosis. Dactinomycin (actinomycin D) was the first antibiotic to find therapeutic application in tumor chemotherapy. It inserts (intercalates) between the DNA bases and inhibits transcription initiation and elongation in tumor cells.

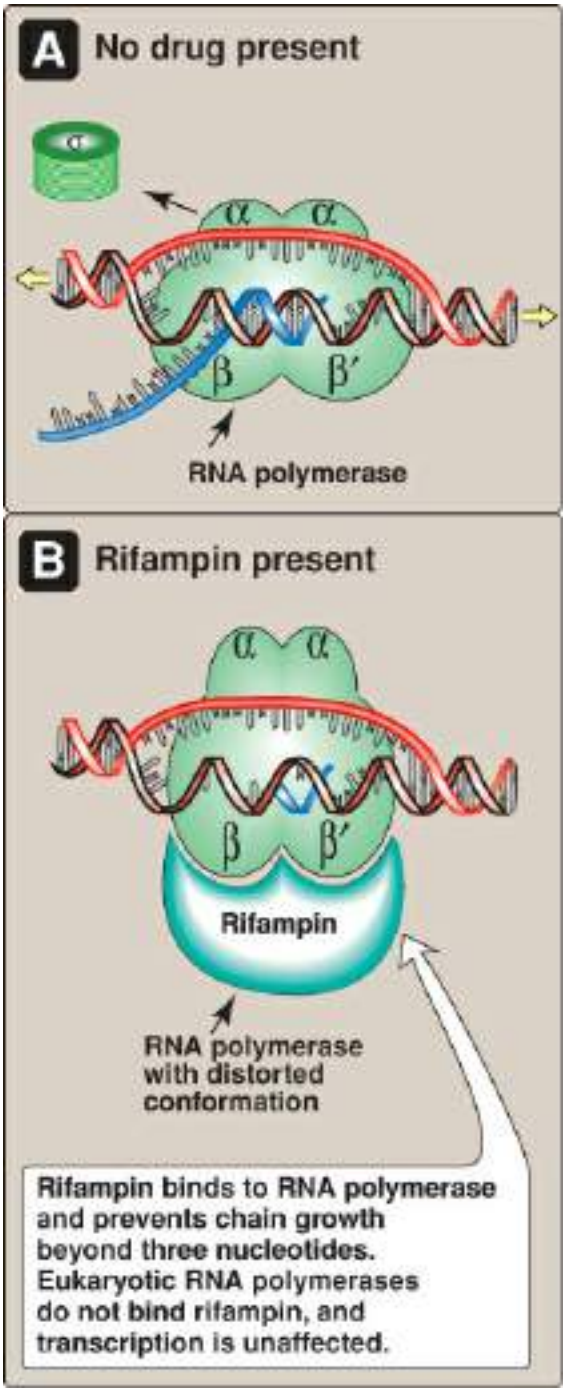


Figure 31.10
A: Prokaryotic transcript elongation by RNA polymerase with no drug present. **B:** Inhibition of prokaryotic RNA polymerase by rifampin (rifampicin).

IV. EUKARYOTIC GENE TRANSCRIPTION

The transcription of eukaryotic genes is a far more complicated process than transcription in prokaryotes. Eukaryotic transcription involves separate polymerases for

the synthesis of rRNA, tRNA, and mRNA. In addition, a large number of proteins called transcription factors (TFs) are involved. TFs bind to distinct sites on the DNA within the core promoter region, close (proximal) to it, or some distance away (distal). They are required for both the assembly of a transcription initiation complex at the promoter and the determination of which genes are to be transcribed. (Note: Each eukaryotic RNA pol has its own promoters and TFs that bind core promoter sequences.) For TF to recognize and bind to their specific DNA sequences, the chromatin structure in that region must be decondensed (relaxed) to allow access to the DNA. The role of transcription in the regulation of gene expression is discussed in Chapter 33.

A. Chromatin structure and gene expression

The association of DNA with histones to form nucleosomes (see p. 473) affects the ability of the transcription machinery to access the DNA to be transcribed. Most actively transcribed genes are found in a relatively decondensed form of chromatin called euchromatin, whereas most inactive segments of DNA are found in highly condensed heterochromatin. The interconversion of these forms is called chromatin remodeling. A major component of chromatin remodeling is the covalent modification of histones (e.g., the acetylation of lysine residues at the amino terminus of histone proteins), as shown in Figure 31.11. Acetylation, mediated by histone acetyltransferases (HATs), eliminates the positive charge on the lysine, thereby decreasing the interaction of the histone with the negatively charged DNA. Removal of the acetyl group by histone deacetylases (HDACs) restores the positive charge and fosters stronger interactions between histones and DNA. (Note: The ATP-dependent repositioning of nucleosomes is also required to access DNA.)

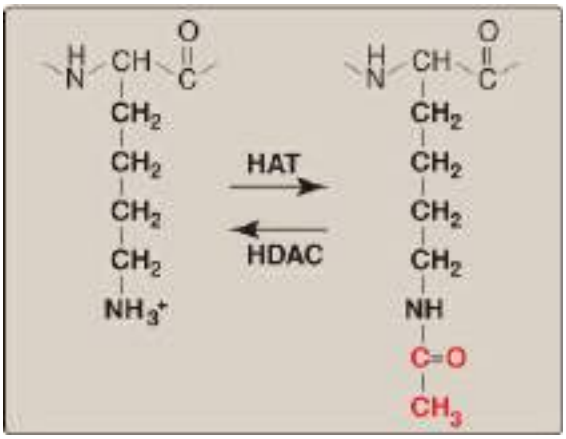


Figure 31.11
Acetylation/deacetylation of a lysine residue in a histone. Acetyl coenzyme A provides the acetyl group. HAT = histone acetyltransferase; HDAC = histone deacetylase.

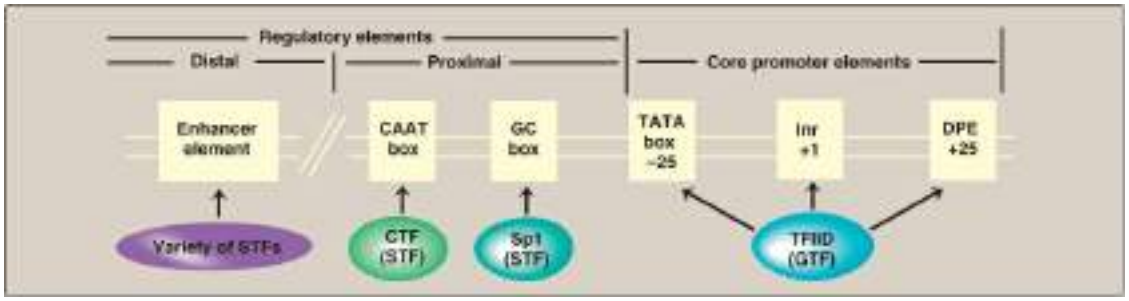


Figure 31.12
Eukaryotic gene cis-acting promoter and regulatory elements and their trans-acting general and specific transcription factors (GTFs and STFs, respectively). Inr = initiator; DPE = downstream promoter element.

B. Nuclear RNA polymerases

There are three distinct types of RNA pol in the nucleus of eukaryotic cells. All are large enzymes with multiple subunits. Each type of RNA pol recognizes particular genes. (Note: Mitochondria contain a single RNA pol that resembles the bacterial enzyme in its function.)

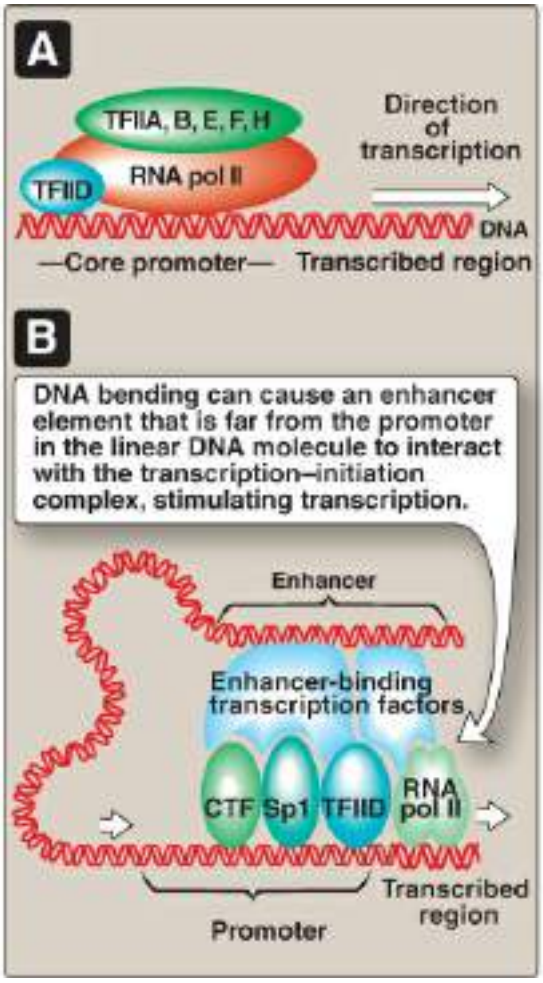


Figure 31.13

A: Association of the general transcription factors (TFII) and RNA polymerase II (RNA pol II) at the core promoter. (Note: The Roman numeral II denotes a TF for RNA pol II.) **B:** Enhancer stimulation of transcription. CTF = CAAT box transcription factor; Sp1 = specificity factor-1.

1. RNA polymerase I: This enzyme synthesizes the precursor of the 28S, 18S, and 5.8S rRNA in the nucleolus.

2. RNA polymerase II: This enzyme synthesizes the nuclear precursors of mRNA that are processed and then translated to proteins. RNA pol II also synthesizes certain small ncRNA, such as snoRNA, snRNA, and miRNA.

   a. Promoters for RNA polymerase II: In some genes transcribed by RNA pol II, a sequence of nucleotides (TATAAA) that is nearly identical to that of the Pribnow box (see p. 485) is found centered ~25 nucleotides upstream of the transcription start site. This core promoter consensus sequence is called the TATA, or Hogness, box. In the majority of genes, however, no TATA box is present. Instead, different core promoter elements such as initiator (Inr) or downstream promoter element (DPE) are present (Fig. 31.12). (Note: No one consensus sequence is found in all core promoters.) Because these sequences are on the same molecule of DNA as the gene being transcribed, they are cis-acting. The sequences serve as binding sites for proteins known as general transcription factors (GTFs), which in turn interact with each other and with RNA pol II.

   b. General transcription factors: GTFs are the minimal requirements for recognition of the promoter, recruitment of RNA pol II to the promoter, formation of the preinitiation complex, and initiation of transcription at a basal level (Fig. 31.13A). GTFs are encoded by different genes, synthesized in the cytosol, and diffuse (transit) to their sites of action, and so are trans-acting. (Note: In contrast to the prokaryotic holoenzyme, eukaryotic RNA pol II does not itself recognize and bind the promoter. Instead, transcription factor IID [TFIID], a GTF containing TATA-binding protein and TATA-associated factors, recognizes and binds the TATA box [and other core promoter elements]. TFIIF, another GTF, brings the polymerase to the promoter. The helicase activity of TFIIH melts the DNA, and its kinase activity phosphorylates polymerase, allowing it to clear the promoter.)

   c. Regulatory elements and transcriptional activators: Additional consensus sequences lie upstream of the core promoter (see Fig. 31.12). Those close to the core promoter (within ~200 nucleotides) are the proximal regulatory elements, such as the CAAT and GC boxes. Those farther away are the distal regulatory elements such as enhancers (see d. below). Proteins known as transcriptional activators or specific transcription factors (STFs) bind these regulatory elements. STFs bind to promoter proximal elements to regulate the frequency of transcription initiation and to distal elements to mediate the response to signals such as hormones (see p. 522) and regulate which genes are expressed at a given point in time. A typical

protein-coding eukaryotic gene has binding sites for many such factors. STFs have two binding domains. One is a DNA-binding domain, the other is a transcription activation domain that recruits the GTF to the core promoter as well as coactivator proteins such as the HAT enzymes involved in chromatin modification. (Note: The Mediator, a multisubunit coactivator of RNA pol II–catalyzed transcription, binds the polymerase, the GTF, and the STF and regulates transcription initiation.)

> Transcriptional activators bind DNA through a variety of motifs, such as the helix-loop-helix, zinc finger, and leucine zipper (see p. 18).

d. Role of enhancers: Enhancers are special DNA sequences that increase the rate of initiation of transcription by RNA pol II. Enhancers are typically on the same chromosome as the gene whose transcription they stimulate (Fig. 31.13B). However, they can (1) be located upstream (to the 5′ side) or downstream (to the 3′ side) of the transcription start site, (2) be close to or thousands of base pairs away from the promoter (Fig. 31.14), and (3) occur on either strand of the DNA. Enhancers contain DNA sequences called response elements that bind STFs. By bending or looping the DNA, STFs can interact with other TFs bound to a promoter and with RNA pol II, thereby stimulating transcription (see Fig. 31.13B). The Mediator also binds enhancers. (Note: Although silencers are similar to enhancers in that they also can act over long distances, they reduce gene expression.)

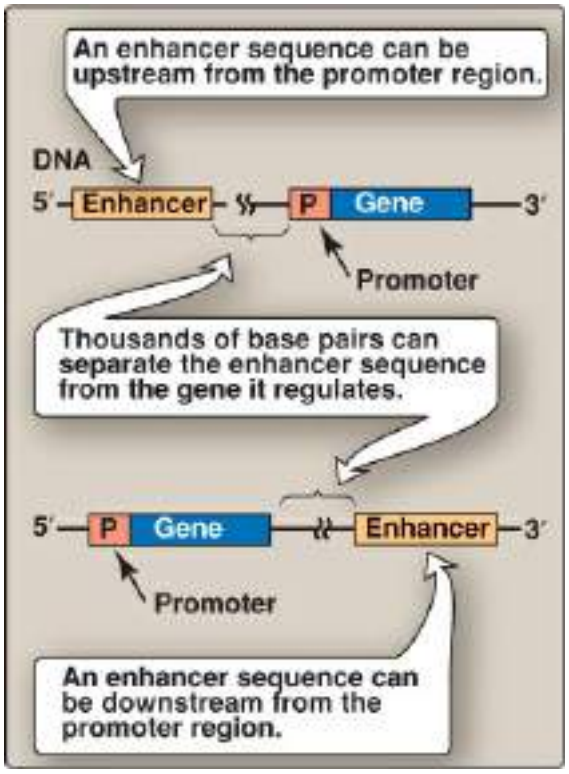


Figure 31.14
Some possible locations of enhancer sequences.

e. RNA polymerase II inhibitor: α-Amanitin, a potent toxin produced by the poisonous mushroom *Amanita phalloides* (sometimes called the "death cap"), binds RNA pol II tightly and slows its movement, thereby inhibiting mRNA synthesis.

3. RNA polymerase III: This enzyme synthesizes tRNA, 5S rRNA, and some snRNA and snoRNA.

V. POSTTRANSCRIPTIONAL MODIFICATION OF RNA

A primary transcript is the initial, linear, RNA copy of a transcription unit (the segment of DNA between specific initiation and termination sequences). The primary transcripts of both prokaryotic and eukaryotic tRNA and rRNA are posttranscriptionally modified by cleavage of the original transcripts by ribonucleases. tRNA are further modified to help give each species its unique identity. In contrast, prokaryotic mRNA is generally identical to its primary transcript, whereas eukaryotic mRNA is extensively modified both co- and posttranscriptionally.

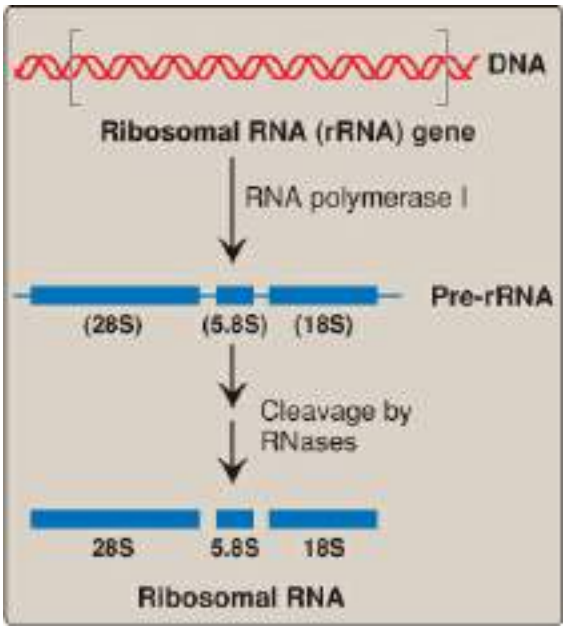


Figure 31.15
Posttranscriptional processing of eukaryotic ribosomal RNA by ribonucleases (RNases). S = Svedberg unit.

A. Ribosomal RNA

rRNA of both prokaryotic and eukaryotic cells are generated from long precursor molecules called pre-rRNA. The 23S, 16S, and 5S rRNA of prokaryotes are produced from a single pre-rRNA molecule, as are the 28S, 18S, and 5.8S rRNA of eukaryotes (Fig. 31.15). (Note: Eukaryotic 5S rRNA is synthesized by RNA pol III and modified separately.) The pre-rRNA are cleaved by ribonucleases to yield intermediate-sized pieces of rRNA, which are further processed (trimmed by exonucleases and modified at some bases and riboses) to produce the required RNA species. (Note: In eukaryotes, rRNA genes are found in long, tandem arrays. rRNA synthesis and processing occur in the nucleolus, with base and sugar modifications facilitated by snoRNA.)

B. Transfer RNA

Both eukaryotic and prokaryotic tRNA are also made from longer precursor molecules that must be modified (Fig. 31.16). Sequences at both ends of the molecule are removed, and, if present, an intervening sequence intron is removed from the anticodon loop by nucleases. Other posttranscriptional modifications include addition of a –CCA sequence by nucleotidyltransferase to the 3′ terminal end of tRNA and modification of bases at specific positions to produce the unusual bases characteristic of tRNA (see p. 324).

C. Eukaryotic messenger RNA

The collection of all the primary transcripts synthesized in the nucleus by RNA pol II

is known as heterogeneous nuclear RNA (hnRNA). The pre-mRNA components of hnRNA undergo extensive co- and posttranscriptional modification in the nucleus and become mature mRNA. These modifications usually include the following. (Note: Pol II itself recruits the proteins required for the modifications.)

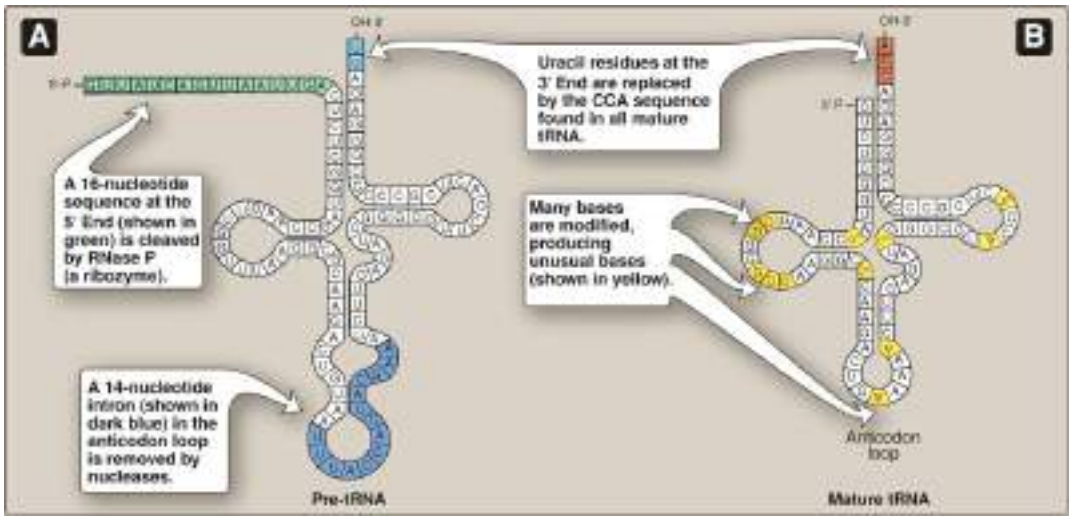


Figure 31.16
A: Precursor transfer RNA (pre-tRNA) transcript. **B:** Mature (functional) tRNA after posttranscriptional modification. Modified bases include D (dihydrouracil), ψ (pseudouracil), and m, which means that the base has been methylated.

1. Addition of a 5′ cap: This is the first of the processing reactions for pre-mRNA (Fig. 31.17). The cap is a 7-methylguanosine attached to the 5′ terminal end of the mRNA through an unusual 5′-to-5′ triphosphate linkage that is resistant to most nucleases. Creation of the cap requires removal of the γ-phosphoryl group from the 5′ triphosphate of the pre-mRNA, followed by addition of guanosine monophosphate (from guanosine triphosphate) by the nuclear enzyme guanylyltransferase. Methylation of this terminal G occurs in the cytosol and is catalyzed by guanine-7-methyltransferase. S-Adenosylmethionine (SAM) is the source of the methyl group (see p. 292). Additional methylation steps may occur. The addition of this 7-methylguanosine cap helps stabilize the mRNA and permits efficient initiation of translation (see p. 504).

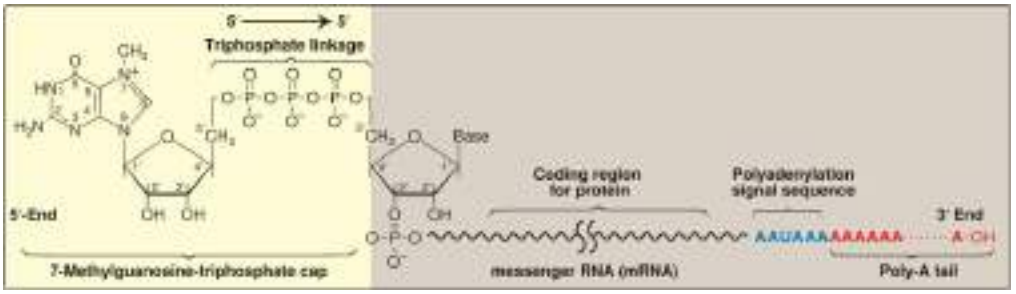


Figure 31.17
Posttranscriptional modification of mRNA showing the 7-methylguanosine cap and

polyadenylate (poly-A) tail.

2. Addition of a 3′-poly-A tail: Most eukaryotic mRNA (with several exceptions, including those for the histones) have a chain of 40 to 250 adenylates (adenosine monophosphates) attached to the 3′ end (see Fig. 31.17). This poly-A tail is not transcribed from the DNA but rather is added by the nuclear enzyme, polyadenylate polymerase, using ATP as the substrate. The pre-mRNA is cleaved downstream of a consensus sequence, called the polyadenylation signal sequence (AAUAAA), found near the 3′ end of the RNA, and the poly-A tail is added to the new 3′ end. The poly-A tail terminates eukaryotic transcription. In addition, it helps stabilize the mRNA, facilitates its exit from the nucleus, and aids in translation. After the mRNA enters the cytosol, the poly-A tail is gradually shortened.

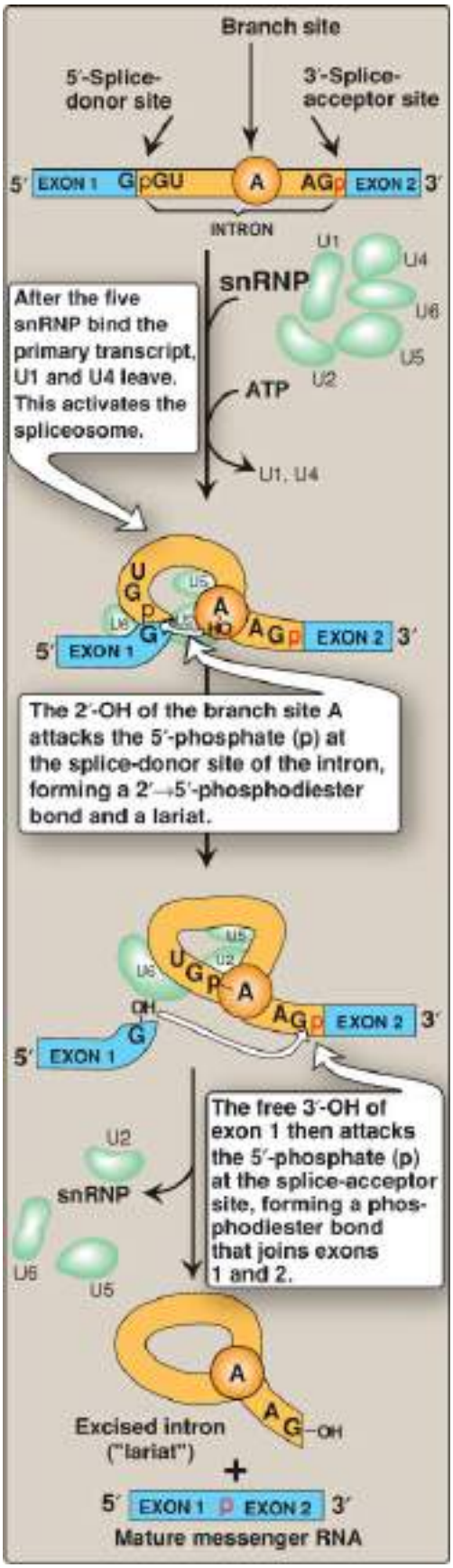


Figure 31.18
Splicing. (Note: U1 binds the 5′ donor site, and U2 binds the branch A and the 3′ acceptor site. Addition of U4–U6 completes the complex.) snRNP = small nuclear ribonucleoprotein particle.

3. Splicing: Maturation of eukaryotic mRNA usually involves removal from the primary transcript of RNA sequences (introns or intervening sequences) that do not code for protein. The remaining coding (expressed) sequences, the exons, are joined together to form the mature mRNA. The process of removing introns and joining exons is called splicing. The molecular complex that accomplishes these tasks is known as the spliceosome. A few eukaryotic primary transcripts contain no introns (e.g., those from histone genes). Others contain a few introns, whereas some, such as the primary transcripts for the α-chains of collagen, contain >50 introns that must be removed.

a. Role of small nuclear RNA: In association with multiple proteins, U-rich snRNA form five small nuclear ribonucleoprotein particles (snRNP, or "snurp") designated as U1, U2, U4, U5, and U6 that mediate splicing. They facilitate the removal of introns by forming base pairs with the consensus sequences at each end of the intron (Fig. 31.18). (Note: In systemic lupus erythematosus, an autoimmune disease, patients produce antibodies against their own nuclear proteins such as snRNP.)

b. Mechanism: The binding of snRNP brings the sequences of neighboring exons into the correct alignment for splicing, allowing two transesterification reactions (catalyzed by the RNA of U2, U5, and U6) to occur. The 2′-OH group of an A nucleotide (known as the branch site A) in the intron attacks the phosphate at the 5′ end of the intron (splice donor site), forming an unusual 2′→5′ phosphodiester bond and creating a "lariat" structure (see Fig. 31.18). The newly freed 3′-OH of exon 1 attacks the 5′ phosphate at the splice acceptor site, forming a phosphodiester bond that joins exons 1 and 2. The excised intron is released as a lariat, which is typically degraded but may be a precursor for ncRNA such as snoRNA. (Note: The GU and AG sequences at the beginning and end, respectively, of introns are invariant. However, additional sequences are critical for splice site recognition.) After introns have been removed and exons joined, the mature mRNA molecules pass into the cytosol through pores in the nuclear membrane. (Note: The introns in tRNA [see Fig. 31.16] are removed by a different mechanism.)

c. Effect of splice site mutations: Mutations at splice sites can lead to improper splicing and the production of aberrant proteins. It is estimated that at least 20% of all genetic diseases are a result of mutations that affect RNA splicing. For example, mutations that cause the incorrect splicing of β-globin mRNA are responsible for some cases of β-thalassemia, a disease in which the production of the β-globin protein is defective (see p. 39). Splice site mutations can result in exons being skipped (removed) or introns retained. They can also activate cryptic splice sites, which are sites that contain the 5′

or 3′ consensus sequence but are not normally used.

4. Alternative splicing: The pre-mRNA molecules from >90% of human genes can be spliced in alternative ways in different tissues. Because this produces multiple variations of the mRNA and, therefore, of its protein product (Fig. 31.19), it is a mechanism for producing a large, diverse set of proteins from a limited set of genes. For example, the mRNA for tropomyosin (TM), an actin filament–binding protein of the cytoskeleton (and of the contractile apparatus in muscle cells), undergoes extensive tissue-specific alternative splicing with production of multiple isoforms of the TM protein.

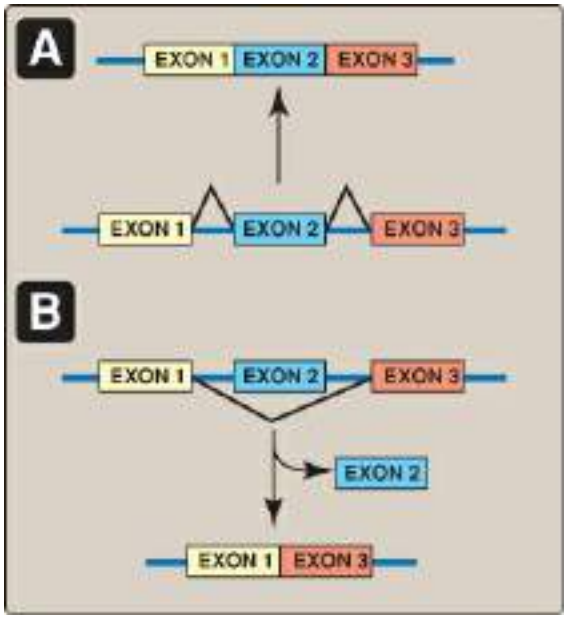


Figure 31.19
Alternative splicing patterns in eukaryotic messenger RNA (mRNA). The removal (skipping) of exon 2 from the mRNA in panel B results in a protein product that is different than the one made from the mRNA in panel A.

VI. Chapter Summary

- Three major types of RNA participate in the process of protein synthesis: **rRNA**, **tRNA**, and **mRNA**. RNA differ from DNA by containing **ribose** instead of deoxyribose and **U** instead of T. **rRNA** is a component of the **ribosomes**. **tRNA** serves as an **adaptor** molecule that carries a specific amino acid to the site of protein synthesis. **mRNA** (coding RNA) carries genetic information from DNA for use in protein synthesis.
- The process of RNA synthesis is called **transcription**. The enzyme that synthesizes RNA, **RNA pol**, uses **ribonucleoside triphosphates** as substrates for **5′→3′ polymerase activity**. In both prokaryotes and eukaryotes, RNA pol does not require a primer.
- In **prokaryotic** cells, the **core RNA pol enzyme** has five subunits (2 α, 1 β, 1 β′, and 1 Ω). The core enzyme requires an additional subunit, **sigma** (σ) **factor**, to recognize the nucleotide sequence (**promoter** region) in DNA. This region contains **consensus sequences** that are highly conserved and include the −**10 Pribnow box** and the −**35 sequence**. Another protein, **rho** (ρ), is required for **termination** of transcription of some genes.
- In the **eukaryotic** cell nucleus, there are three distinct types of RNA pol. **RNA pol I** synthesizes the precursor of rRNA in the nucleolus. **RNA pol II** synthesizes the precursors for mRNA and some ncRNA, and **RNA pol III** synthesizes the precursors of tRNA and 5S rRNA. Core **promoters** for genes transcribed by **RNA pol II** contain **cis-acting** consensus sequences, such as the **TATA** (**Hogness**) **box**, which serve as binding sites for **trans-acting GTFs**. Upstream of these are **proximal** regulatory elements, such as the CAAT and GC boxes, and **distal** regulatory elements, such as **enhancers**. **STFs** (transcriptional activators) and **mediator complex** bind these elements and regulate gene expression. Eukaryotic transcription requires that the **chromatin** be relaxed (decondensed) in a process known as **chromatin remodeling**.
- **A primary transcript** is a linear copy of a **transcription unit**, the segment of DNA between specific initiation and termination sequences. Prokaryotic mRNA is generally identical to its primary transcript, whereas eukaryotic **pre-mRNA** is extensively modified co- and posttranscriptionally. For example, a **7-methylguanosine cap** is attached to the 5′ end of the mRNA through a 5′-to-5′ linkage. A **long poly-A tail** is attached by polyadenylate polymerase to the 3′ end of most mRNA. Most eukaryotic mRNA also contains i**ntervening sequences** (**introns**) that must be removed for the mRNA to be functional. Their removal, as well as the joining of **expressed sequences** (**exons**), requires a **spliceosome** composed of **"snurps"** that mediate the process of **splicing**. Eukaryotic mRNA is **monocistronic**, containing information from just one gene, whereas prokaryotic mRNA is **polycistronic** (Fig. 31.20).

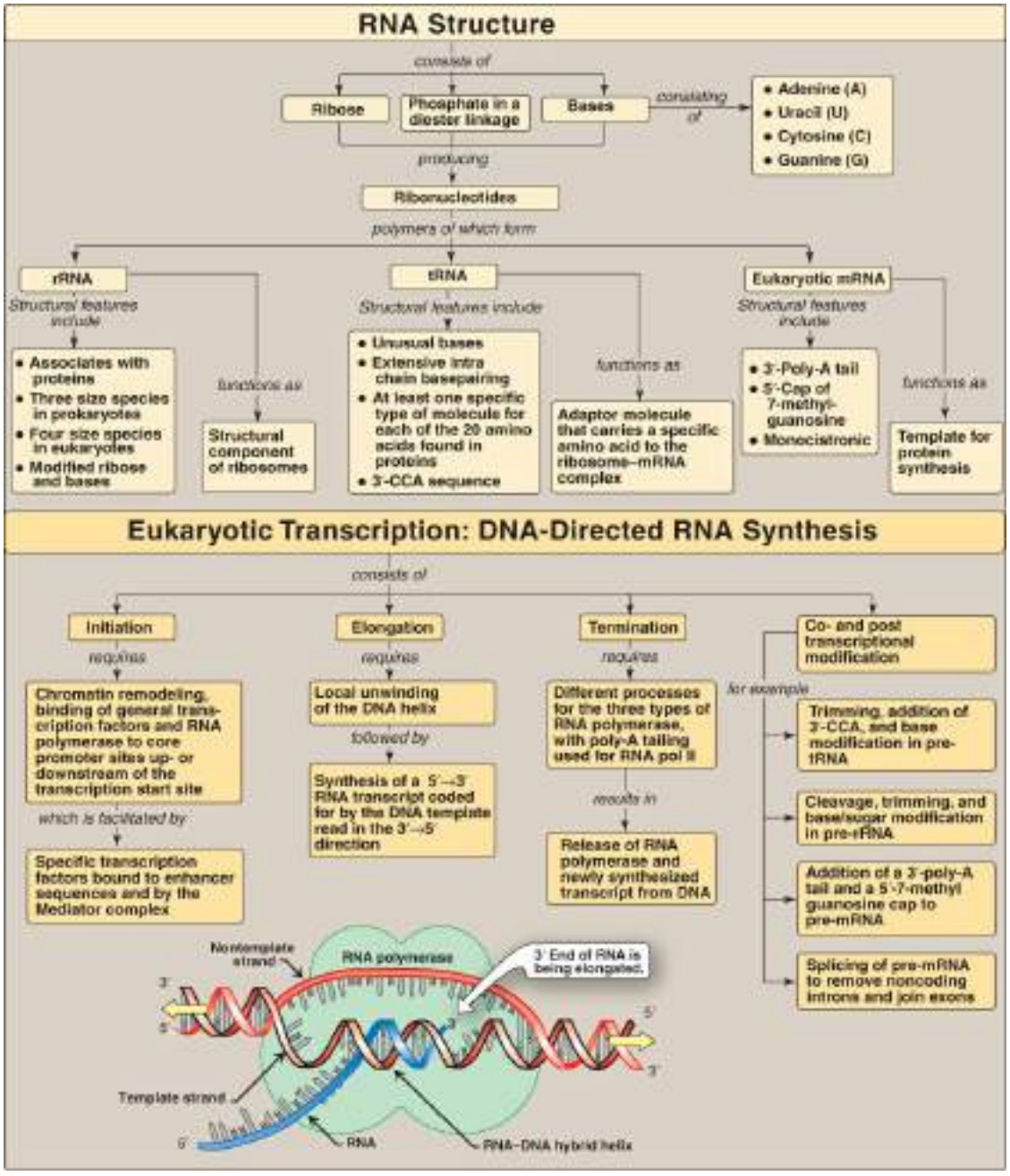


Figure 31.20
Key concept map for RNA structure and synthesis. rRNA = ribosomal RNA; tRNA = transfer RNA; mRNA = messenger RNA.

Study Questions

Choose the ONE best answer.

31.1 An 8-month-old male with severe anemia is found to have β-thalassemia. Genetic analysis shows that one of his β-globin genes has a mutation that creates a new splice acceptor site 19 nucleotides upstream of the normal splice acceptor site of the first intron. Which of the following best describes the new messenger RNA molecule that can be produced from this mutant gene?

A. Exon 1 will be too short.
B. Exon 1 will be too long.
C. Exon 2 will be too short.
D. Exon 2 will be too long.
E. Exon 2 will be missing.

Correct answer = D. Because the mutation creates an additional splice acceptor site (the 3′ end) upstream of the normal acceptor site of intron 1, the 19 nucleotides that are usually found at the 3′ end of the excised intron 1 lariat can remain behind as part of exon 2. The presence of these extra nucleotides in the coding region of the mutant messenger RNA (mRNA) molecule will prevent the ribosome from translating the message into a normal β-globin protein molecule. Those mRNA for which the normal splice site is used to remove the first intron will be normal, and their translation will produce normal β-globin protein.

31.2 A 4-year-old child who easily tires and has trouble walking is diagnosed with Duchenne muscular dystrophy, an X-linked recessive disorder. Genetic analysis shows that the patient's gene for the muscle protein dystrophin contains a mutation in its promoter region. Of the choices listed, which of the following would be the most likely to be defective due to this mutation?

A. Initiation of dystrophin transcription
B. Termination of dystrophin transcription
C. Capping of dystrophin messenger RNA
D. Splicing of dystrophin messenger RNA
E. Tailing of dystrophin messenger RNA

Correct answer = A. Mutations in the promoter typically prevent formation of the RNA polymerase II transcription initiation complex, resulting in a decrease in the initiation of messenger RNA (mRNA) synthesis. A deficiency of dystrophin mRNA will result in a deficiency in the production of the dystrophin protein. Capping, splicing, and tailing defects are not a consequence of promoter mutations. They can, however, result in mRNA with decreased stability (capping and tailing defects) or an mRNA in which exons have been skipped (lost) or introns retained (splicing defects).

31.3 A mutation to which of the following sequences in eukaryotic messenger RNA (mRNA) would most likely affect the process by which the 3′-end polyadenylate (poly-A) tail is added to the mRNA?

A. AAUAAA
B. CAAT
C. CCA
D. GU… A … AG
E. TATAAA

Correct answer = A. An endonuclease cleaves mRNA just downstream of this polyadenylation signal, creating a new 3′ end to which polyadenylate polymerase adds the poly-A tail using ATP as the substrate in a template-independent process. CAAT and TATAAA are sequences found in promoters for RNA polymerase II. CCA is added to the 3′ end of pre–transfer RNA by nucleotidyltransferase. GU…A…AG denotes an intron in eukaryotic pre-mRNA.

31.4 Which of the following protein factors identifies the promoter of protein-coding genes in eukaryotes?

A. Pribnow box
B. Rho
C. Sigma
D. TFIID
E. U1

Correct answer = D. The general transcription factor TFIID recognizes and binds core promoter elements such as the TATA-like box in eukaryotic protein-coding genes. These genes are transcribed by RNA polymerase II. The Pribnow box is a cis-acting element in prokaryotic promoters. Rho is involved in the termination of prokaryotic

transcription. Sigma is the subunit of prokaryotic RNA polymerase that recognizes and binds the prokaryotic promoter. U1 is a ribonucleoprotein involved in splicing of eukaryotic pre-mRNA.

31.5 What is the sequence (conventionally written) of the RNA product of the DNA template sequence, GATCTAC, also conventionally written?

Correct answer = 5′-GUAGAUC-3′. Nucleic acid sequences are conventionally written 5′ to 3′. The template strand (5′-GATCTAC-3′) is used as 3′-CATCTAG-5′. The RNA product is complementary to the template strand (and identical to the coding strand), with U replacing T.

Protein Synthesis 32

I. OVERVIEW

Genetic information, stored in the chromosomes and transmitted to daughter cells through DNA replication, is expressed through transcription to RNA and, in the case of messenger RNA (mRNA), subsequent translation into proteins (polypeptides) as shown in Figure 32.1. (Note: The proteome is the complete set of proteins expressed in a cell.) The process of protein synthesis is called translation because the “language” of the nucleotide sequence on the mRNA is translated into the language of an amino acid sequence. Translation requires a genetic code, through which the information contained in the nucleotide sequence is expressed to produce a specific amino acid sequence. Any alteration in the nucleotide sequence may result in an incorrect amino acid being inserted into the protein, potentially causing disease or even death of the organism. Newly made immature (nascent) proteins undergo a number of processes to achieve their functional form. They must fold properly, otherwise misfolding can result in aggregation or degradation of the protein. Many proteins are covalently modified to alter their activities. Lastly, proteins are targeted to their final intra- or extracellular destinations by signals present in the proteins themselves.

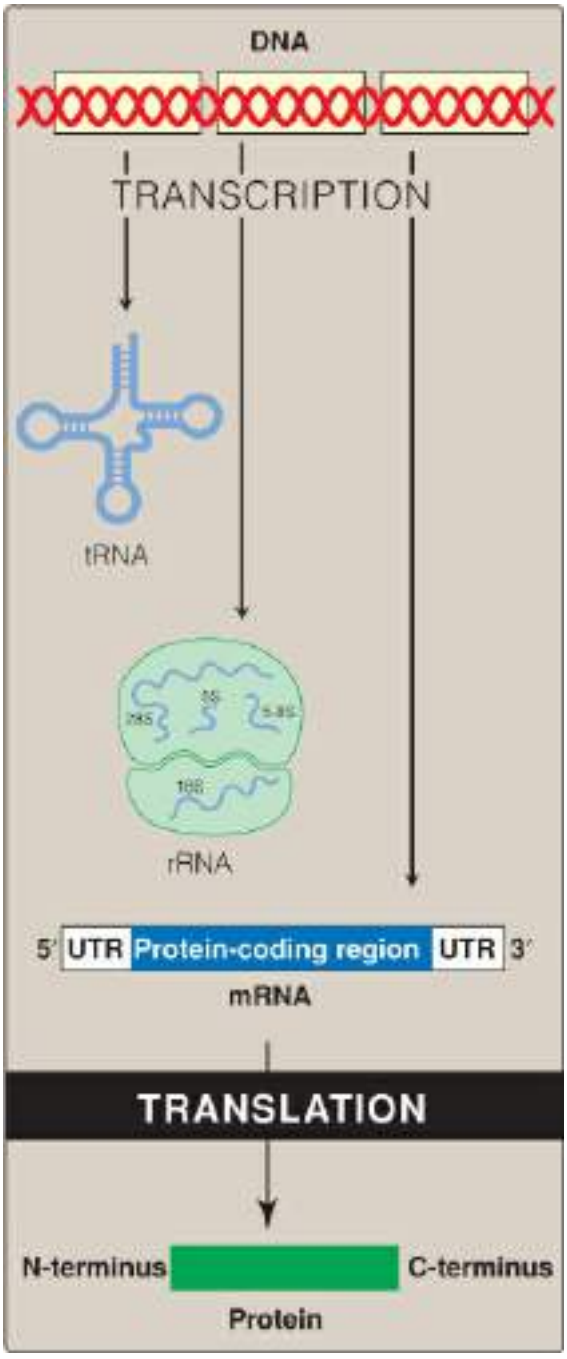


Figure 32.1
Protein synthesis or translation. tRNA = transfer RNA; rRNA = ribosomal RNA; mRNA = messenger RNA; UTR = untranslated region.

II. THE GENETIC CODE

The genetic code is a “dictionary” that identifies the correspondence between a sequence of nucleotide bases and a sequence of amino acids. Each individual “word” in the code is composed of three nucleotide bases. These genetic words are called

codons.

A. Codons

Codons are presented in the mRNA language of adenine (A), guanine (G), cytosine (C), and uracil (U). Their nucleotide sequences are always written from the 5′ end to the 3′ end. The four nucleotide bases are used to produce the three-base codons. Therefore, 64 different combinations of bases exist, taken three at a time (a triplet code), as shown in the table in Figure 32.2.

1. How to translate a codon: This table can be used to translate any codon and, thus, to determine which amino acids are coded for by an mRNA sequence. For example, the codon AUG codes for methionine ([Met], see Fig. 32.2). (Note: AUG is the initiation [start] codon for translation.) Sixty-one of the 64 codons code for the 20 standard amino acids (see p. 1).

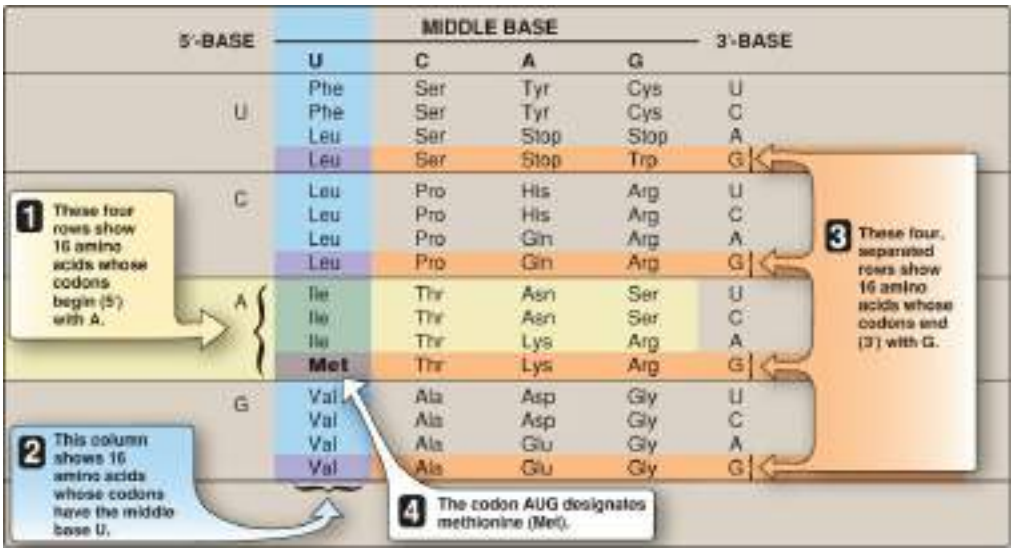


Figure 32.2
Use of the genetic code table to translate the codon AUG. A = adenine; G = guanine; C = cytosine; U = uracil. The three-letter abbreviations for many common amino acids are shown as examples.

2. Termination codons: Three of the codons, UAA, UAG, and UGA, do not code for amino acids but, rather, are termination (also called stop, or nonsense) codons. When one of these codons appears in an mRNA sequence, synthesis of the polypeptide coded for by that mRNA stops.

B. Characteristics

Usage of the genetic code is remarkably consistent throughout all living organisms. It is assumed that once the standard genetic code evolved in primitive organisms, any mutation (a permanent change in DNA sequence) that altered its meaning would have caused the alteration of most, if not all, protein sequences, resulting in

lethality. Characteristics of the genetic code include the following.

1. Specificity: The genetic code is specific (unambiguous), because a particular codon always codes for the same amino acid.
2. Universality: The genetic code is virtually universal insofar as its specificity has been conserved from very early stages of evolution, with only slight differences in the manner in which the code is translated. (Note: An exception occurs in mitochondria, in which a few codons have meanings different than those shown in Figure 32.2; e.g., UGA codes for tryptophan [Trp].)
3. Degeneracy: The genetic code is degenerate (sometimes called redundant). Although each codon corresponds to a single amino acid, a given amino acid may have more than one triplet coding for it. For example, arginine (Arg) is specified by six different codons (see Fig. 32.2). Only Met and Trp have just one coding triplet. Most codons that code for the same amino acid differ only in the last base of the triplet.
4. Nonoverlapping and commaless: The genetic code is nonoverlapping and commaless, meaning that the code is read from a fixed starting point as a continuous sequence of bases, taken three at a time without any punctuation between codons. For example, AGCUGGAUACAU is read as AGC UGG AUA CAU. The order of the codons that produces the correct sequence of amino acids in a protein is called the reading frame.

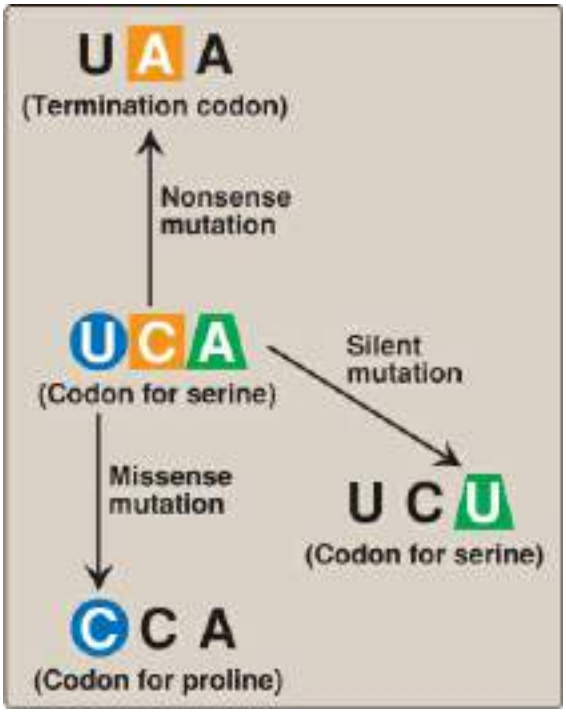


Figure 32.3
Possible effects of changing a single nucleotide base in the coding region of a messenger RNA. A = adenine; C = cytosine; U = uracil.

C. Consequences of altering the nucleotide sequence

Changing a single nucleotide base (a point mutation) in the coding region of an mRNA can lead to any one of three results (Fig. 32.3).

1. Silent mutation: The codon containing the changed base may code for the same amino acid. For example, if the serine (Ser) codon UCA is changed at the third base and becomes UCU, it still codes for Ser. This is termed a silent mutation.

2. Missense mutation: The codon containing the changed base may code for a different amino acid. For example, if the Ser codon UCA is changed at the first base and becomes CCA, it will code for a different amino acid (in this case, proline [Pro]). This is termed a missense mutation.

3. Nonsense mutation: The codon containing the changed base may become a termination codon. For example, if the Ser codon UCA is changed at the second base and becomes UAA, the new codon causes premature termination of translation at that point and the production of a shortened (truncated) protein. This is termed a nonsense mutation. (Note: The nonsense-mediated degradation pathway can degrade mRNA containing premature stops.)

4. Other mutations: These can alter the amount or structure of the protein produced by translation.

    a. Trinucleotide repeat expansion: Occasionally, a sequence of three bases that is repeated in tandem will become amplified in number so that too many copies of the triplet occur. If this happens within the coding region of a gene, the protein will contain many extra copies of one amino acid. For example, expansion of the CAG codon in exon 1 of the gene for huntingtin protein leads to the insertion of many extra glutamine residues in the protein, causing the neurodegenerative disorder Huntington disease (Fig. 32.4). The additional glutamines result in an abnormally long protein that is cleaved, producing toxic fragments that aggregate in neurons. If the trinucleotide repeat expansion occurs in an untranslated region (UTR) of a gene, the result can be a decrease in the amount of protein produced, as seen in fragile X syndrome and myotonic dystrophy. Over 20 triplet expansion diseases are known. (Note: In fragile X syndrome, the most common cause of intellectual disability in males, the expansion results in gene silencing through DNA hypermethylation [see p. 526].)

    b. Splice site mutations: Mutations at splice sites (see p. 492) can alter the way in which introns are removed from pre-mRNA molecules, producing aberrant proteins. (Note: In myotonic dystrophy, a muscle disorder, gene silencing is the result of splicing alterations due to triplet expansion.)

    c. Frameshift mutations: If one or two nucleotides are either deleted from or added to the coding region of an mRNA, a frameshift mutation occurs, altering the reading frame. This can result in a product with a radically

different amino acid sequence or a truncated product due to the eventual creation of a termination codon (Fig. 32.5). If three nucleotides are added or deleted, the effect on the protein depends on where the changes occur. If the three nucleotides are added within an existing codon sequence or are deleted from two adjacent codons, then a frameshift happens. If three nucleotides are added between two codons, either a new amino acid is added into the protein or a stop is generated that shortens the product. The deletion of a codon causes the loss of an amino acid. Loss or addition of three nucleotides may maintain the reading frame but can result in serious pathology. For example, cystic fibrosis (CF), a chronic, progressive, inherited disease that primarily affects the pulmonary and digestive systems, is most commonly caused by deletion of three nucleotides from the coding region of a gene, resulting in the loss of phenylalanine (Phe, or F; see p. 5) at the 508th position (ΔF508) in the CF transmembrane conductance regulator (CFTR) protein encoded by that gene. This ΔF508 mutation prevents normal folding of CFTR, leading to its destruction by the proteasome (see p. 273). CFTR normally functions as a chloride channel in epithelial cells, and its loss results in the production of thick, sticky secretions in the lungs and pancreas, leading to lung damage and a digestive deficiency known as pancreatic insufficiency (see p. 192). The incidence of CF is highest (1 in 3,300) in those of Northern European origin. In >70% of individuals with CF, the ΔF508 mutation is the cause of the disease.

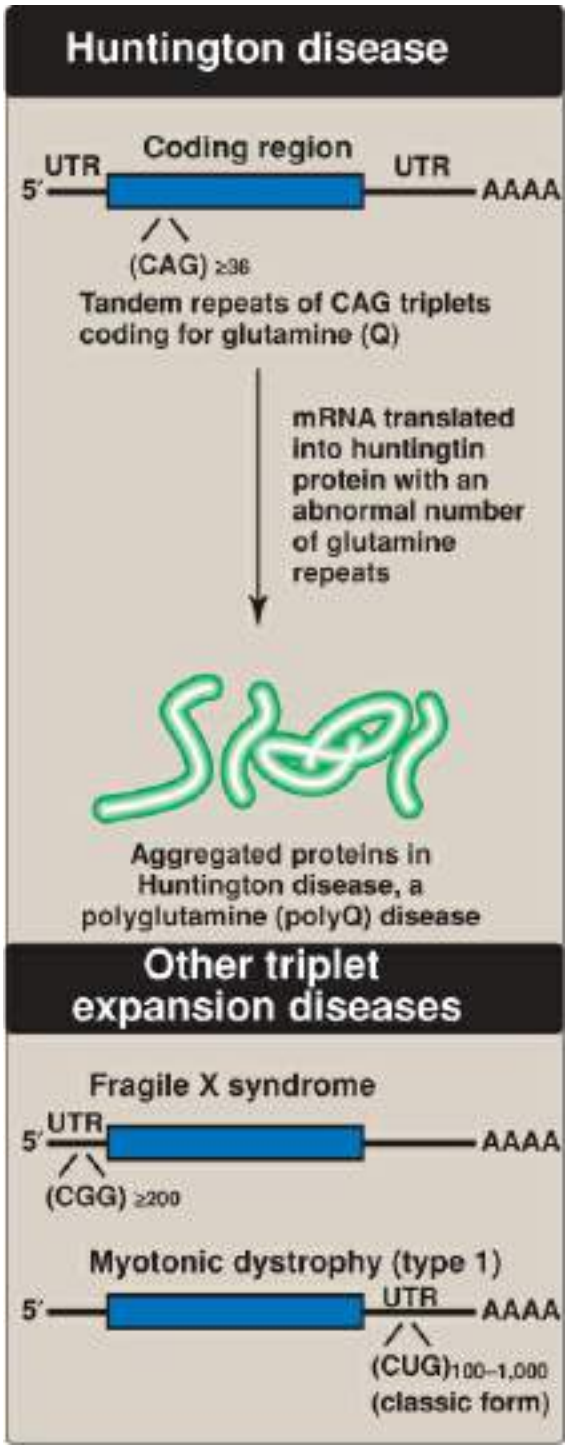


Figure 32.4
Tandem triplet repeats in messenger RNA (mRNA) causing Huntington disease and other triplet expansion diseases. (Note: In unaffected individuals, the number of repeats in the huntingtin protein is <27; in fragile X mental retardation protein, it is 5–44; and in myotonic dystrophy protein kinase, it is 5–34.) UTR = untranslated region; A = adenine; C = cytosine; G = guanine; U = uracil; Q = single-letter abbreviation for glutamine.

III. COMPONENTS REQUIRED FOR TRANSLATION

A large number of components are required for the synthesis of a protein. These include all the amino acids that are found in the finished product, the mRNA to be translated, transfer RNA (tRNA) for each of the amino acids, functional ribosomes, energy sources, and enzymes as well as noncatalytic protein factors needed for the initiation, elongation, and termination steps of polypeptide chain synthesis.

A. Amino acids

All the amino acids that eventually appear in the finished protein must be present at the time of protein synthesis. If one amino acid is missing, translation stops at the codon specifying that amino acid. (Note: This demonstrates the importance of having all the essential amino acids [see p. 291] in sufficient quantities in the diet to ensure continued protein synthesis.)

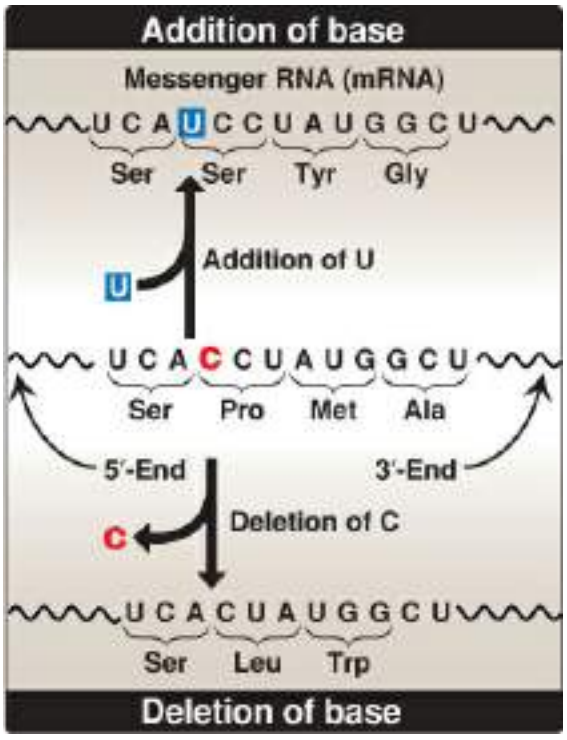


Figure 32.5
Frameshift mutations as a result of addition or deletion of a base can cause an alteration in the reading frame of mRNA. A = adenine; C = cytosine; G = guanine; U = uracil.

B. Transfer RNA

At least one specific type of tRNA is required for each amino acid. In humans, there are at least 50 species of tRNA, whereas bacteria contain at least 30 species. Because there are only 20 different amino acids commonly carried by tRNA, some amino acids have more than one specific tRNA molecule. This is particularly true of those amino acids that are coded for by several codons.

1. Amino acid attachment site: Each tRNA molecule has an attachment site for a

specific (cognate) amino acid at its 3′ end (Fig. 32.6). The carboxyl group of the amino acid is in an ester linkage with the 3′ hydroxyl of the ribose portion of the A nucleotide in the –CCA sequence at the 3′ end of the tRNA. (Note: A tRNA with a covalently attached [activated] amino acid is charged. Without an attached amino acid, it is uncharged.)

2. Anticodon: Each tRNA molecule also contains a three-base nucleotide sequence, the anticodon, which pairs with a specific codon on the mRNA (see Fig. 32.6). This codon specifies the insertion into the growing polypeptide chain of the amino acid carried by that tRNA.

C. Aminoacyl-tRNA synthetases

This family of 20 different enzymes is required for attachment of amino acids to their corresponding tRNA. Each member of this family recognizes a specific amino acid and all the tRNA that correspond to that amino acid (isoaccepting tRNA, up to five per amino acid). Aminoacyl-tRNA synthetases catalyze a two-step reaction that results in the covalent attachment of the α-carboxyl group of an amino acid to the A in the –CCA sequence at the 3′ end of its corresponding tRNA. The overall reaction requires ATP, which is cleaved to adenosine monophosphate and inorganic pyrophosphate (PP_i), as shown in Figure 32.7. The extreme specificity of the synthetases in recognizing both the amino acid and its cognate tRNA contributes to the high fidelity of translation of the genetic message. In addition to their synthetic activity, the aminoacyl-tRNA synthetases have a proofreading, or editing activity that can remove an incorrect amino acid from the enzyme or the tRNA molecule.

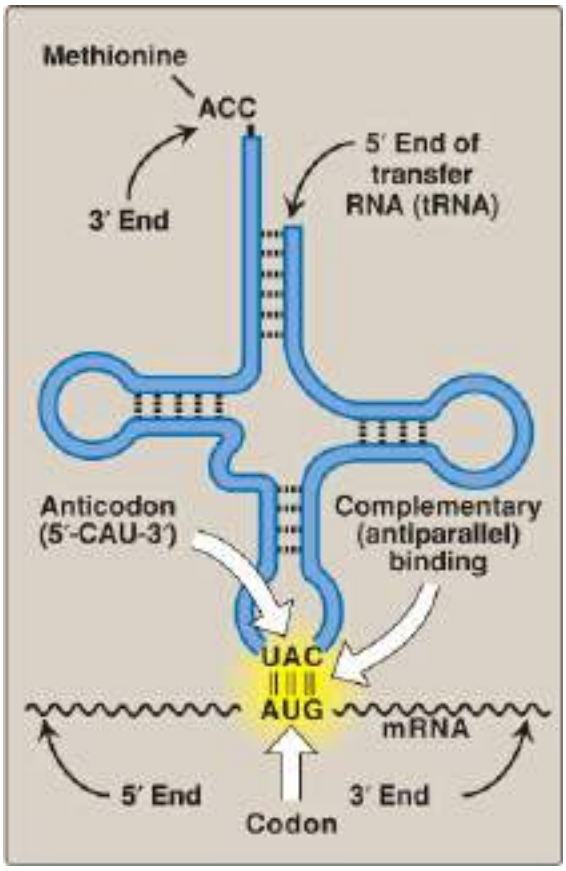


Figure 32.6
Complementary, antiparallel binding of the anticodon for methionyl-tRNA (CAU) to the messenger RNA (mRNA) codon for methionine (AUG), the initiation codon for translation.

D. Messenger RNA

The specific mRNA required as a template for the synthesis of the desired polypeptide must be present.

E. Functionally competent ribosomes

As shown in Figure 32.8, ribosomes are large complexes of protein and ribosomal RNA (rRNA), in which rRNA predominates. They consist of two subunits (one large and one small) whose relative sizes are given in terms of their sedimentation coefficients, or Svedberg (S) values. (Note: Because the S values are determined by both shape and size, their numeric values are not strictly additive; e.g., the prokaryotic 50S and 30S ribosomal subunits together form a 70S ribosome. The eukaryotic 60S and 40S subunits form an 80S ribosome.) Prokaryotic and eukaryotic ribosomes are similar in structure and serve the same function, namely, as the macromolecular complexes in which the synthesis of proteins occurs.

The small ribosomal subunit binds mRNA and determines the accuracy of translation by ensuring correct base pairing between the mRNA codon and the tRNA anticodon. The large ribosomal

subunit catalyzes formation of the peptide bonds that link amino acid residues in a protein.

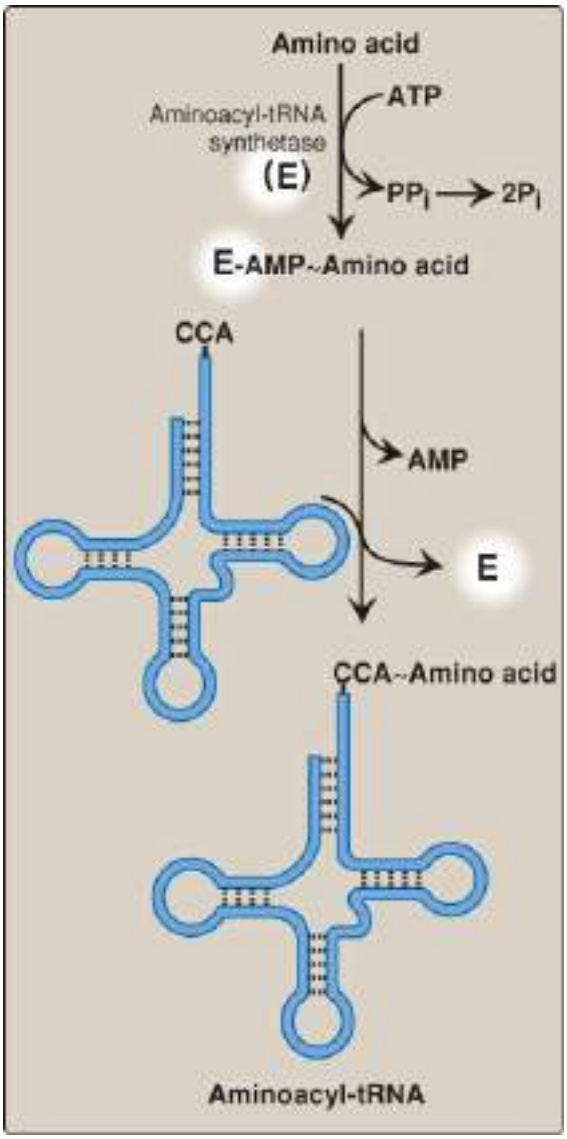


Figure 32.7
Attachment of a specific amino acid to its corresponding transfer RNA (tRNA) by an aminoacyl-tRNA synthetase. PP_i = pyrophosphate; P_i = inorganic phosphate; A = adenine; C = cytosine; AMP = adenosine monophosphate; ~ = high-energy bond.

1. Ribosomal RNA: As discussed on p. 483, prokaryotic ribosomes contain three size species of rRNA, whereas eukaryotic ribosomes contain four (see Fig. 32.8). The rRNA are generated from a single pre-rRNA by the action of ribonucleases, and some bases and ribose sugars are modified.

2. Ribosomal proteins: Ribosomal proteins are present in greater numbers in eukaryotic ribosomes than in prokaryotic ribosomes. These proteins play a variety of roles in the structure and function of the ribosome and its interactions

with other components of the translation system.

3. A, P, and E sites: The ribosome has three binding sites for tRNA molecules: the A, P, and E sites, each of which extends over both subunits. Together, they cover three neighboring codons. During translation, the A site binds an incoming aminoacyl-tRNA as directed by the codon currently occupying this site. This codon specifies the next amino acid to be added to the growing peptide chain. The P site is occupied by peptidyl-tRNA. This tRNA carries the chain of amino acids that has already been synthesized. The E site is occupied by the empty tRNA as it is about to exit the ribosome. (See Fig. 32.13 for an illustration of the role of the A, P, and E sites in translation.)

4. Cellular location: In eukaryotic cells, the ribosomes either are free in the cytosol or are in close association with the endoplasmic reticulum (which is then known as the rough endoplasmic reticulum or RER). RER-associated ribosomes are responsible for synthesizing proteins (including glycoproteins; see p. 182) that are to be exported from the cell, incorporated into membranes, or imported into lysosomes (see p. 185 for an overview of the latter process). Cytosolic ribosomes synthesize proteins required in the cytosol itself or destined for the nucleus, mitochondria, or peroxisomes. (Note: Mitochondria contain their own ribosomes [55S] and their own unique, circular DNA. Most mitochondrial proteins, however, are encoded by nuclear DNA, synthesized completely in the cytosol, and then targeted to mitochondria.)

F. Protein factors

Initiation, elongation, and termination (or, release) factors are required for polypeptide synthesis. Some of these protein factors perform a catalytic function, whereas others appear to stabilize the synthetic machinery. (Note: A number of the factors are small, cytosolic G proteins and thus are active when bound to guanosine triphosphate [GTP] and inactive when bound to guanosine diphosphate [GDP]. See p. 104 for a discussion of the membrane-associated G proteins.)

G. Energy sources

Cleavage of four high-energy bonds is required for the addition of one amino acid to the growing polypeptide chain: two from ATP in the aminoacyl-tRNA synthetase reaction, one in the removal of PP_i and one in the subsequent hydrolysis of the PP_i, to two P_i by pyrophosphatase, and two from GTP, one for binding the aminoacyl-tRNA to the A site and one for the translocation step (see Fig. 32.13). (Note: Additional ATP and GTP molecules are required for initiation in eukaryotes, whereas an additional GTP molecule is required for termination in both eukaryotes and prokaryotes.) Translation, then, is a major consumer of energy.

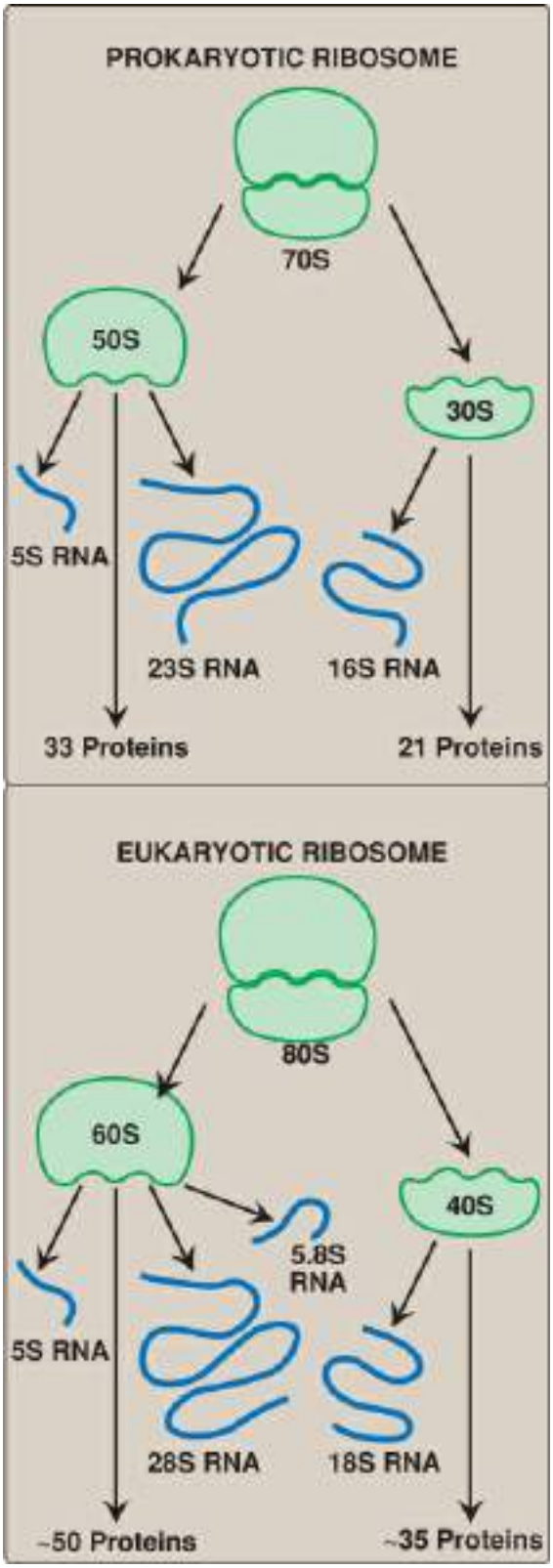


Figure 32.8
Ribosomal composition. (Note: The number of proteins in the eukaryotic ribosomal subunits varies somewhat from species to species.) S = Svedberg unit.

IV. CODON RECOGNITION BY TRANSFER RNA

Correct pairing of the codon in the mRNA with the anticodon of the tRNA is essential for accurate translation (see Fig. 32.6). Most tRNA (isoaccepting tRNA) recognize more than one codon for a given amino acid.

A. Antiparallel binding between codon and anticodon

Binding of the tRNA anticodon to the mRNA codon follows the rules of complementary and antiparallel binding, that is, the mRNA codon is read 5′→3′ by an anticodon pairing in the opposite (3′→5′) orientation (Fig. 32.9). (Note: Nucleotide sequences are always written in the 5′ to 3′ direction unless otherwise noted. Two nucleotide sequences orient in an antiparallel manner.)

B. Wobble hypothesis

The mechanism by which a tRNA can recognize more than one codon for a specific amino acid is described by the wobble hypothesis, which states that codon–anticodon pairing follows the traditional Watson–Crick rules (G pairs with C and A pairs with U) for the first two bases of the codon but can be less stringent for the last base. The base at the 5′ end of the anticodon (the first base of the anticodon) is not as spatially defined as the other two bases. Movement of that first base allows nontraditional base pairing with the 3′ base of the codon (the last base of the codon). This movement is called wobble and allows a single tRNA to recognize more than one codon. Examples of these flexible pairings are shown in Figure 32.9. The result of wobble is that 61 tRNA species are not required to read the 61 codons that code for amino acids.

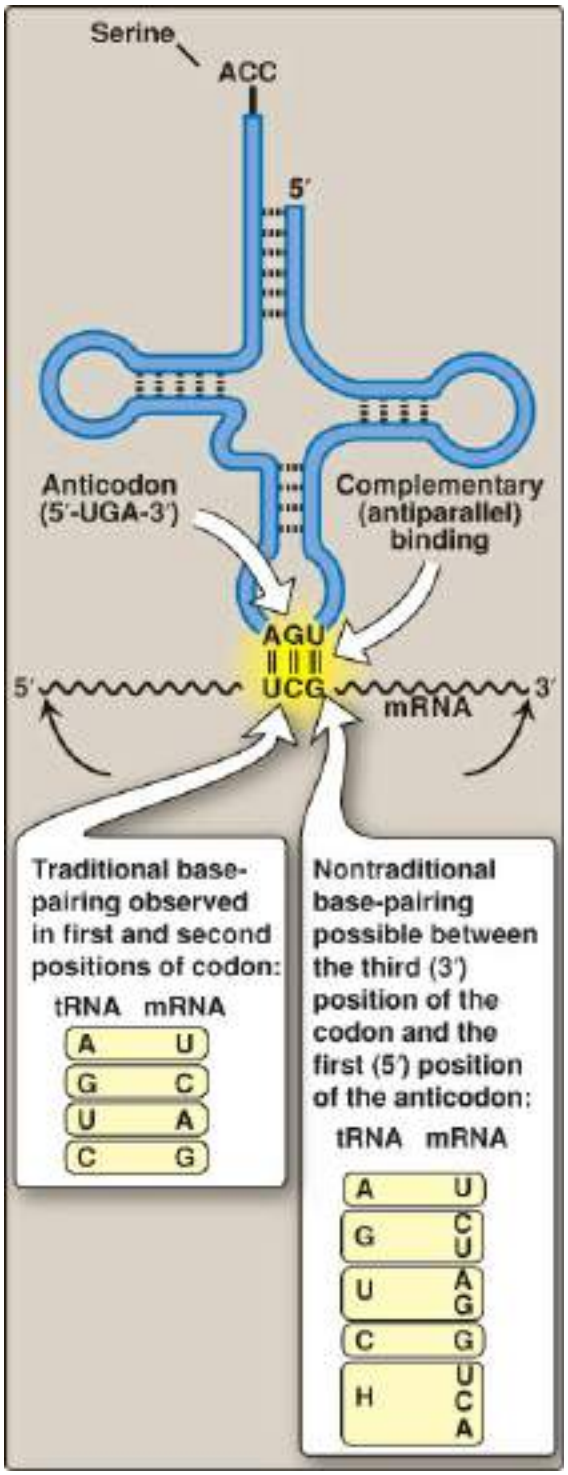


Figure 32.9
Wobble: Nontraditional base pairing between the 5′ nucleotide (first nucleotide) of the anticodon and the 3′ nucleotide (last nucleotide) of the codon. Hypoxanthine (H) is the product of adenine deamination and the base in the nucleotide inosine monophosphate (IMP). A = adenine; G = guanine; C = cytosine; U = uracil; tRNA = transfer RNA; mRNA = messenger RNA.

V. STEPS IN TRANSLATION

The process of protein synthesis translates the 3-letter alphabet of nucleotide sequences on mRNA into the 20-letter alphabet of amino acids that constitute proteins. The mRNA is translated from its 5′ end to its 3′ end, producing a protein synthesized from its amino (N)-terminal end to its carboxyl (C)-terminal end. Prokaryotic mRNA often have several coding regions (i.e., they are polycistronic). Each coding region has its own initiation and termination codon and produces a separate species of polypeptide. In contrast, each eukaryotic mRNA has only one coding region (i.e., it is monocistronic). The process of translation is divided into three separate steps: initiation, elongation, and termination. Eukaryotic translation resembles that of prokaryotes in most aspects. Individual differences are noted in the text.

> One important difference is that translation and transcription are temporally linked in prokaryotes, with translation starting before transcription is completed as a consequence of the lack of a nuclear membrane in prokaryotes.

A. Initiation

Initiation of protein synthesis involves the assembly of the components of the translation system before peptide-bond formation occurs. These components include the two ribosomal subunits, the mRNA to be translated, the aminoacyl-tRNA specified by the first codon in the message, GTP, and initiation factors (IFs) that facilitate the assembly of this initiation complex (see Fig. 32.13). (Note: In prokaryotes, three IFs are known [IF-1, IF-2, and IF-3], whereas in eukaryotes, there are many [designated eIF to indicate eukaryotic origin]. Eukaryotes also require ATP for initiation.) The following are two mechanisms by which the ribosome recognizes the nucleotide sequence (AUG) that initiates translation.

1. Shine–Dalgarno sequence: In *Escherichia coli (E. coli*), a purine-rich sequence of nucleotide bases, known as the Shine–Dalgarno (SD) sequence, is located 6 to 10 bases upstream of the initiating AUG codon on the mRNA molecule (i.e., near its 5′ end). The 16S rRNA component of the small (30S) ribosomal subunit has a nucleotide sequence near its 3′ end that is complementary to all or part of the SD sequence. Therefore, the 5′ end of the mRNA and the 3′ end of the 16S rRNA can form complementary base pairs, facilitating the positioning of the 30S subunit on the mRNA in close proximity to the initiating AUG codon (Fig. 32.10).

2. 5′ Cap: Eukaryotic mRNA do not have SD sequences. In eukaryotes, the small (40S) ribosomal subunit (aided by members of the eIF-4 family of proteins) binds close to the cap structure at the 5′ end of the mRNA and moves 5′→3′ along the mRNA until it encounters the initiator AUG. This scanning process requires ATP. Cap-independent initiation can occur if the 40S subunit binds to an internal ribosome entry site close to the start codon. (Note: Interactions between the cap-binding eIF-4 proteins and the poly-A tail–binding proteins on eukaryotic mRNA mediate circularization of the mRNA and likely prevent the use of incompletely processed mRNA in translation.)

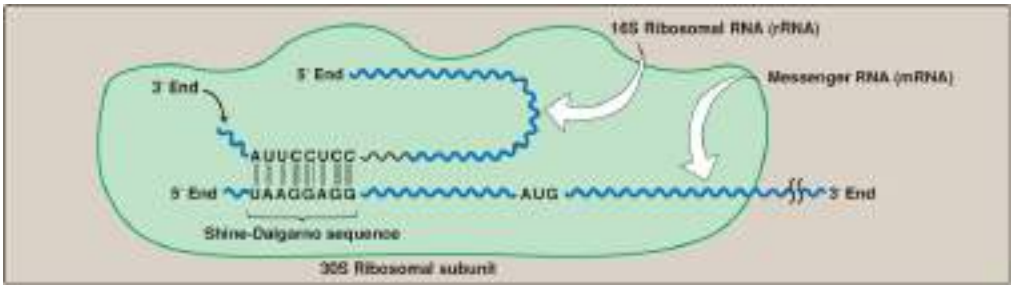


Figure 32.10
Complementary binding between prokaryotic mRNA Shine–Dalgarno sequence and 16S rRNA. S = Svedberg unit.

3. Initiation codon: The initiating AUG is recognized by a special initiator tRNA ($tRNA_i$). Recognition is facilitated by IF-2-GTP in prokaryotes and eIF-2-GTP (plus additional eIF) in eukaryotes. The charged $tRNA_i$ is the only tRNA recognized by (e)IF-2 and the only tRNA to go directly to the P site on the small subunit. (Note: Base modifications distinguish $tRNA_i$ from the tRNA used for internal AUG codons.) In bacteria and mitochondria, $tRNA_i$ carries an N-formylated methionine (fMet), as shown in Figure 32.11. After Met is attached to $tRNA_i$, the formyl group is added by the enzyme transformylase, which uses N^{10}-formyl tetrahydrofolate (see p. 296) as the carbon donor. In eukaryotes, the cytosolic $tRNA_i$ carries a Met that is not formylated. In both prokaryotic and eukaryotic cells, this N-terminal Met is usually removed before translation is completed. The large ribosomal subunit then joins the complex, and a functional ribosome is formed with the charged $tRNA_i$ in the P site. The A site is empty. (Note: Specific [e]IF function as antiassociation factors and prevent premature addition of the large subunit.) The GTP on (e)IF-2 gets hydrolyzed to GDP. In eukaryotes, the G nucleotide exchange factor eIF-2B facilitates the reactivation of eIF-2-GDP through replacement of GDP by GTP.

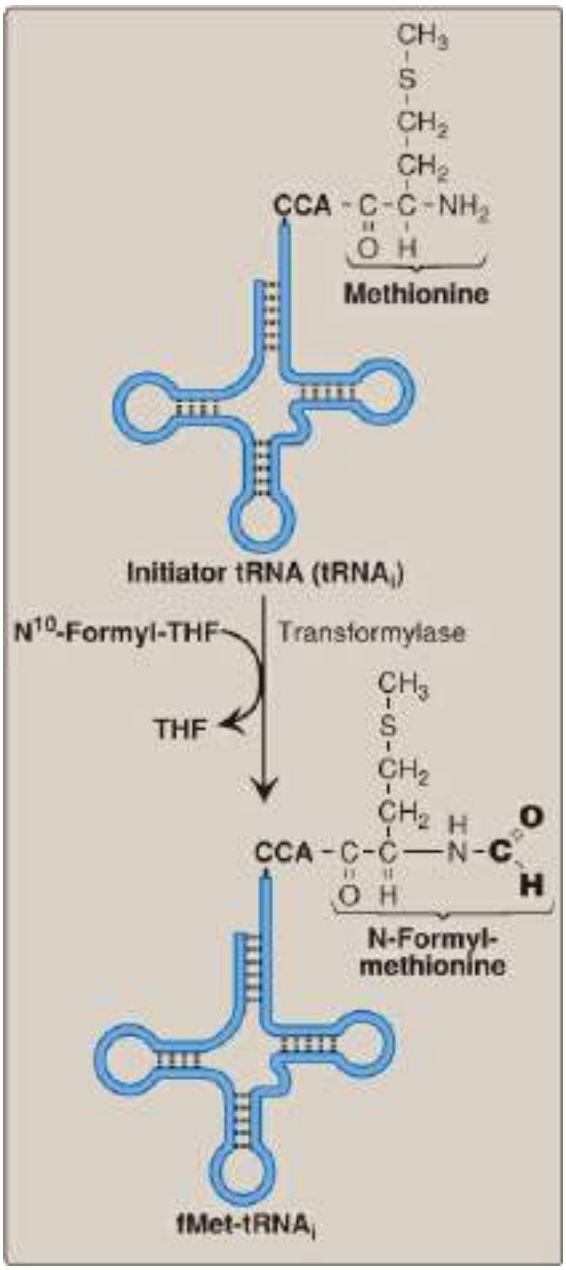


Figure 32.11
Generation of the initiator N-formylmethionyl-transfer RNA (fMet-tRNA$_i$). THF = tetrahydrofolate; C = cytosine; A = adenine.

B. Elongation

Elongation of the polypeptide involves the addition of amino acids to the carboxyl end of the growing chain. Delivery of the aminoacyl-tRNA whose codon appears next on the mRNA template in the ribosomal A site (a process known as decoding) is facilitated in *E. coli* by elongation factors EF-Tu-GTP and EF-Ts and requires GTP hydrolysis. (Note: In eukaryotes, comparable elongation factors are EF-1α-GTP and EF-1γ. Both EF-Ts and EF-1γ function in guanine nucleotide exchange.)

Peptide bond formation between the α-carboxyl group of the amino acid in the P site and the α-amino group of the amino acid in the A site is catalyzed by peptidyl transferase, an activity intrinsic to an rRNA of the large subunit (Fig. 32.12). (Note: Because this rRNA catalyzes the reaction, it is a ribozyme.) After the peptide bond has been formed, the peptide on the tRNA at the P site is transferred to the amino acid on the tRNA at the A site, a process known as transpeptidation. The ribosome then advances three nucleotides toward the 3′ end of the mRNA. This process is known as translocation and, in prokaryotes, requires the participation of EF-G-GTP (eukaryotes use EF-2-GTP) and GTP hydrolysis. Translocation causes movement of the uncharged tRNA from the P to the E site for release and movement of the peptidyl-tRNA from the A to the P site. The process is repeated until a termination codon is encountered. (Note: Because of the length of most mRNA, more than one ribosome at a time can translate a message. Such a complex of one mRNA and a number of ribosomes is called a polysome, or polyribosome.)

C. Termination

Termination occurs when one of the three termination codons moves into the A site. These codons are recognized in *E. coli* by release factors: RF-1, which recognizes UAA and UAG, and RF-2, which recognizes UGA and UAA. The binding of these release factors results in hydrolysis of the bond linking the peptide to the tRNA at the P site, causing the nascent protein to be released from the ribosome. A third release factor, RF-3-GTP, then causes the release of RF-1 or RF-2 as GTP is hydrolyzed (see Fig. 32.13). (Note: Eukaryotes have a single release factor, eRF, which recognizes all three termination codons. A second factor, eRF-3, functions like the prokaryotic RF-3. See Figure 32.14 for a summary of the factors used in translation.) The steps in prokaryotic protein synthesis, as well as some antibiotic inhibitors of the process, are summarized in Figure 32.13. The newly synthesized polypeptide may undergo further modification as described below, and the ribosomal subunits, mRNA, tRNA, and protein factors can be recycled and used to synthesize another polypeptide. (Note: In prokaryotes, ribosome recycling factors mediate separation of the subunits. In eukaryotes, eRF and ATP hydrolysis are required.)

D. Translation regulation

Gene expression is most commonly regulated at the transcriptional level, but translation may also be regulated. An important mechanism by which this is achieved in eukaryotes is by covalent modification of eIF-2: Phosphorylated eIF-2 is inactive (see p. 526). In both eukaryotes and prokaryotes, regulation can also be achieved through proteins that bind mRNA and inhibit its use by blocking translation.

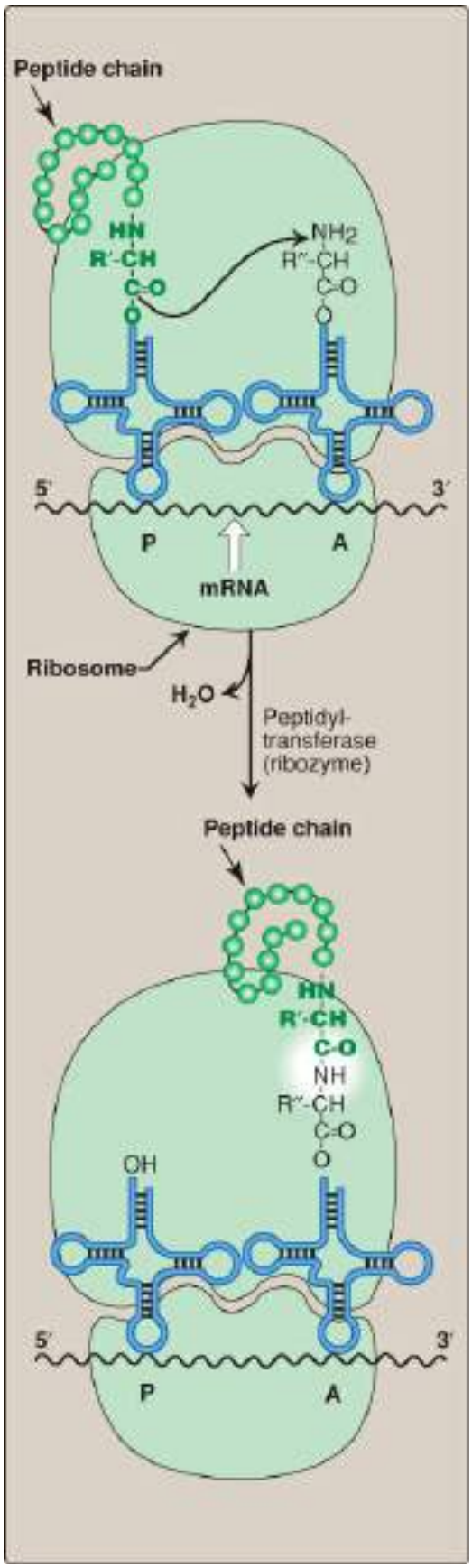
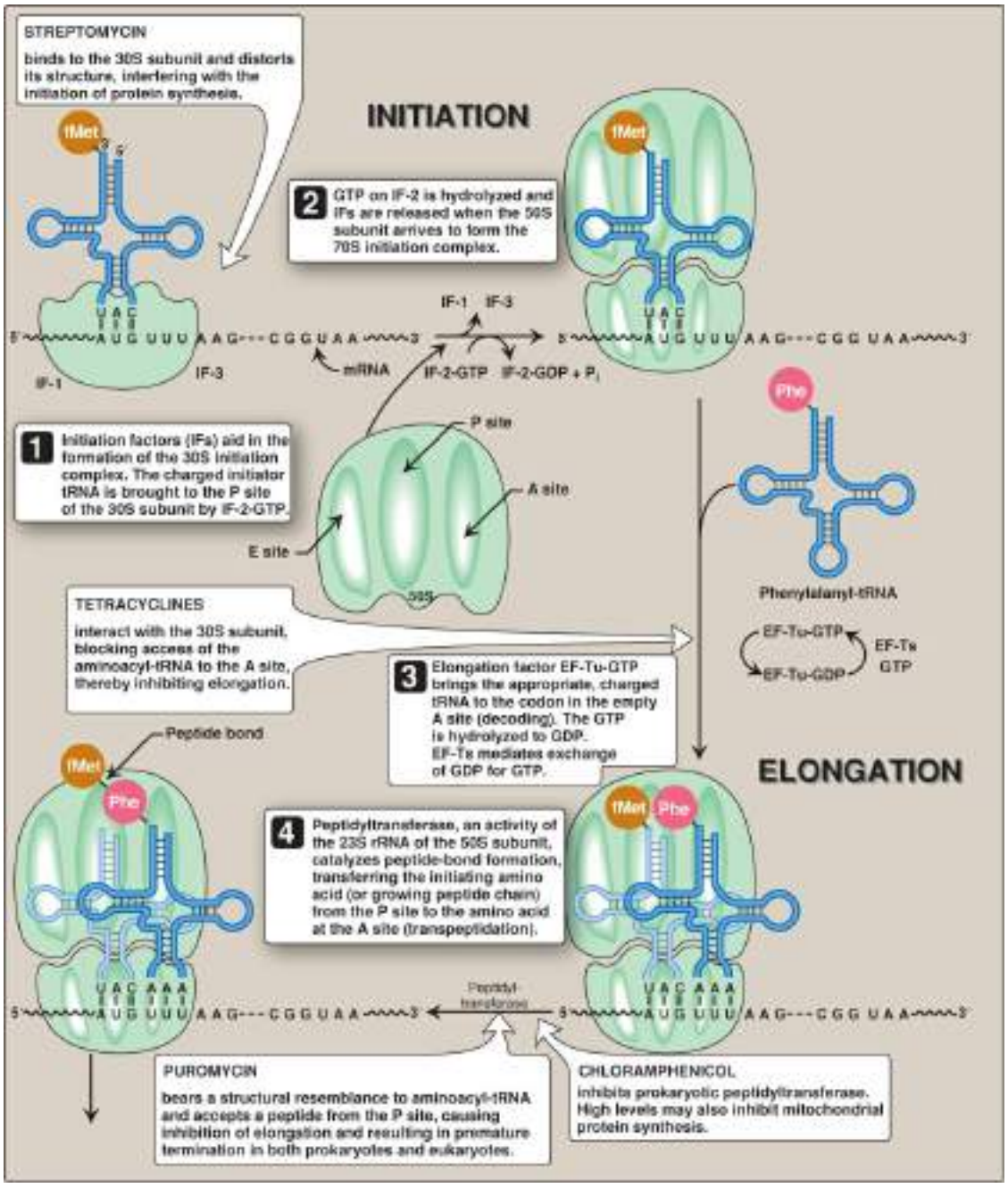


Figure 32.12
Formation of a peptide bond. Peptide bond formation results in transfer of the peptide on the transfer RNA (tRNA) in the P site to the amino acid on the tRNA in the A site (transpeptidation). mRNA = messenger RNA; R′, R″ = different amino acid side chains.

E. Protein folding

Proteins must fold to assume their functional, native state. Folding can be spontaneous (as a result of the primary structure) or facilitated by proteins known as chaperones (see p. 21).



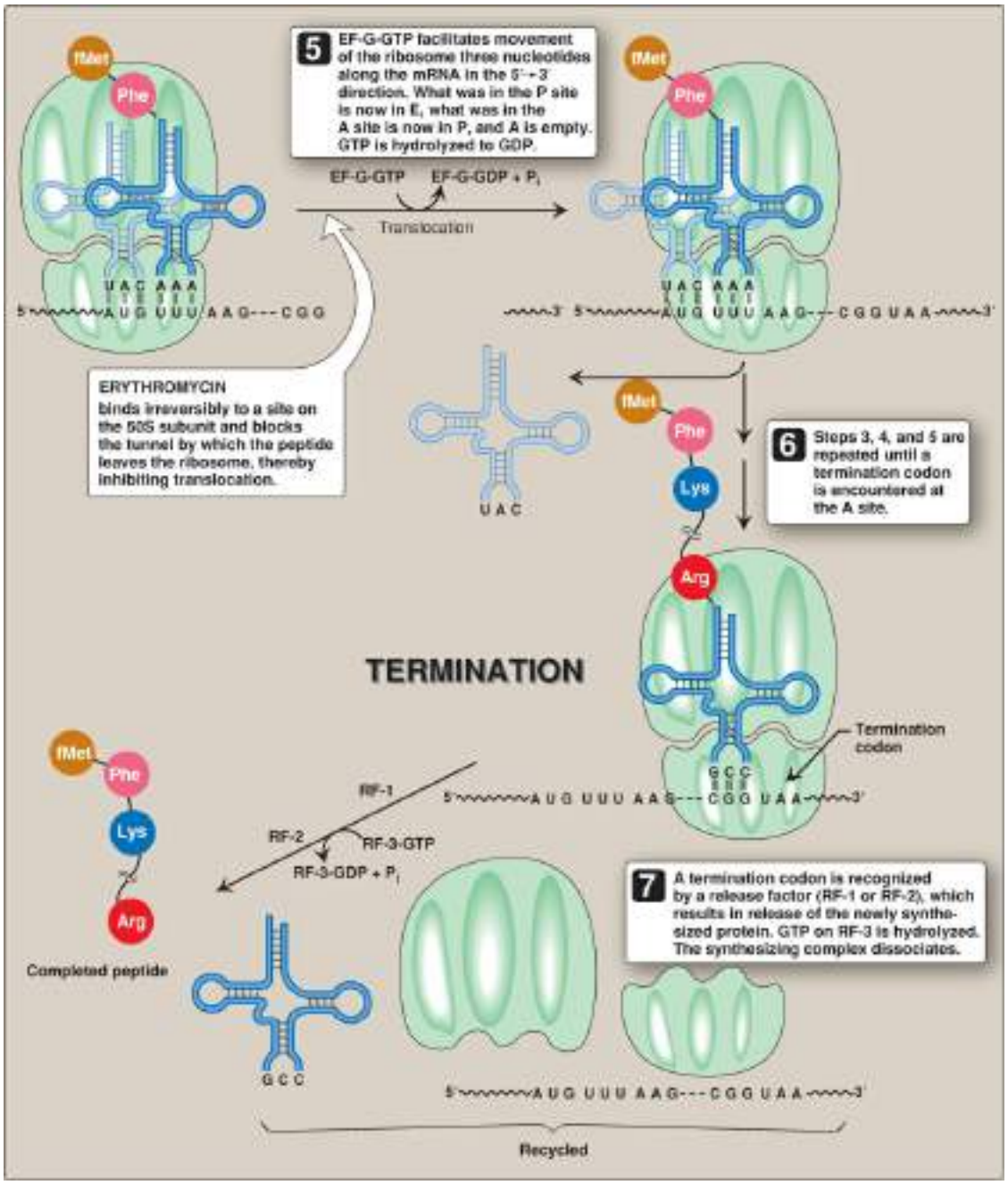


Figure 32.13
Steps in prokaryotic protein synthesis (translation), and their inhibition by antibiotics. (Note: EF-Ts is a guanine nucleotide exchange factor. It facilitates the removal of guanosine diphosphate (GDP) from EF-Tu, allowing its replacement by guanosine triphosphate [GTP]. The eukaryotic equivalent is EF-1βγ.) fMet = formylated methionine; S = Svedberg unit; Phe = phenylalanine; Lys = lysine; Arg = arginine; tRNA = transfer RNA; mRNA = messenger RNA. (Note: In eukaryotes, diphtheria toxin inactivates EF-2 [the equivalent of prokaryotic EF-G], thereby inhibiting the translocation phase of elongation. Ricin, a toxin from castor beans, removes a specific A from the 28S ribosomal RNA [rRNA] in the large subunit of eukaryotic ribosomes, thereby inhibiting ribosomal function.)

F. Protein targeting

Although most protein synthesis in eukaryotes is initiated in the cytoplasm, many proteins perform their functions within subcellular organelles or outside of the cell. Such proteins normally contain amino acid sequences that direct the proteins to their final locations. For example, secreted proteins are targeted during synthesis (cotranslational targeting) to the RER by the presence of an N-terminal hydrophobic signal sequence. The sequence is recognized by the signal recognition particle (SRP), a ribonucleoprotein that binds the ribosome, halts elongation, and delivers the ribosome–peptide complex to an RER membrane channel (the translocon) via interaction with the SRP receptor. Translation resumes, the protein enters the RER lumen, and its signal sequence is cleaved (Fig. 32.15). The protein moves through the RER and the Golgi, is processed, packaged into vesicles, and secreted. Proteins targeted after synthesis (posttranslational) include nuclear proteins that contain an internal, short, basic nuclear localization signal; mitochondrial matrix proteins that contain an N-terminal, amphipathic, α-helical mitochondrial entry sequence; and peroxisomal proteins that contain a C-terminal tripeptide signal.

Cell	Factor	Function
Initiation		
Prok Euk	IF-2-GTP eIF-2-GTP	Bring charged initiating tRNA to P site
Prok Euk	IF-3 eIF-3	Prevent association of subunits
Elongation		
Prok Euk	EF-Tu-GTP EF1α-GTP	Bring all other charged tRNA to A site
Prok Euk	EF-Ts EF-1βγ	Guanine nucleotide exchange factors
Prok Euk	EF-G-GTP EF-2-GTP	Translocation
Termination		
Prok Euk	RF-1, 2 eRF	Recognize stop codons
Prok Euk	RF-3-GTP eRF-3-GTP	Release of other RF

Figure 32.14
Protein factors in the three stages of translation. Prok = prokaryotes; Euk = eukaryotes; tRNA = transfer RNA; IF = initiation factor; EF = elongation factor; RF = release factor; GTP = guanosine triphosphate.

VI. CO- AND POSTTRANSLATIONAL MODIFICATIONS

Many polypeptides are covalently modified, either while they are still attached to the

ribosome (cotranslational) or after their synthesis has been completed (posttranslational). These modifications may include removal of part of the translated sequence or the covalent addition of one or more chemical groups required for protein activity.

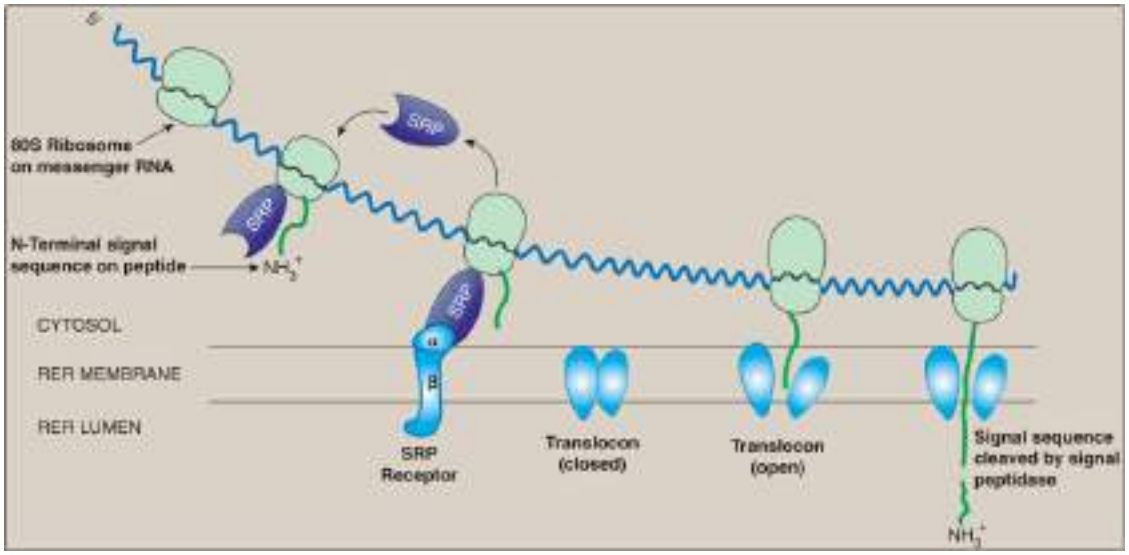


Figure 32.15
Cotranslational targeting of proteins to the rough endoplasmic reticulum (RER). SRP = signal recognition particle.

A. Trimming

Many proteins destined for secretion are initially made as large, precursor molecules that are not functionally active. Portions of the protein must be removed by specialized endoproteases, resulting in the release of an active molecule. The cellular site of the cleavage reaction depends on the protein to be modified. Some precursor proteins are cleaved in the RER or the Golgi; others are cleaved in developing secretory vesicles (e.g., insulin; see Fig. 23.4, p. 343); and still others, such as collagen (see p. 49), are cleaved after secretion.

B. Covalent attachments

Protein function can be affected by the covalent attachment of a variety of chemical groups (Fig. 32.16). Examples include the following.

1. Phosphorylation: Phosphorylation occurs on the hydroxyl groups of Ser, threonine, or, less frequently, tyrosine residues in a protein. It is catalyzed by one of a family of protein kinases and may be reversed by the action of protein phosphatases. The phosphorylation may increase or decrease the functional activity of the protein. Several examples of phosphorylation reactions have been previously discussed (e.g., see Chapter 11, p. 144, for the regulation of glycogen synthesis and degradation).

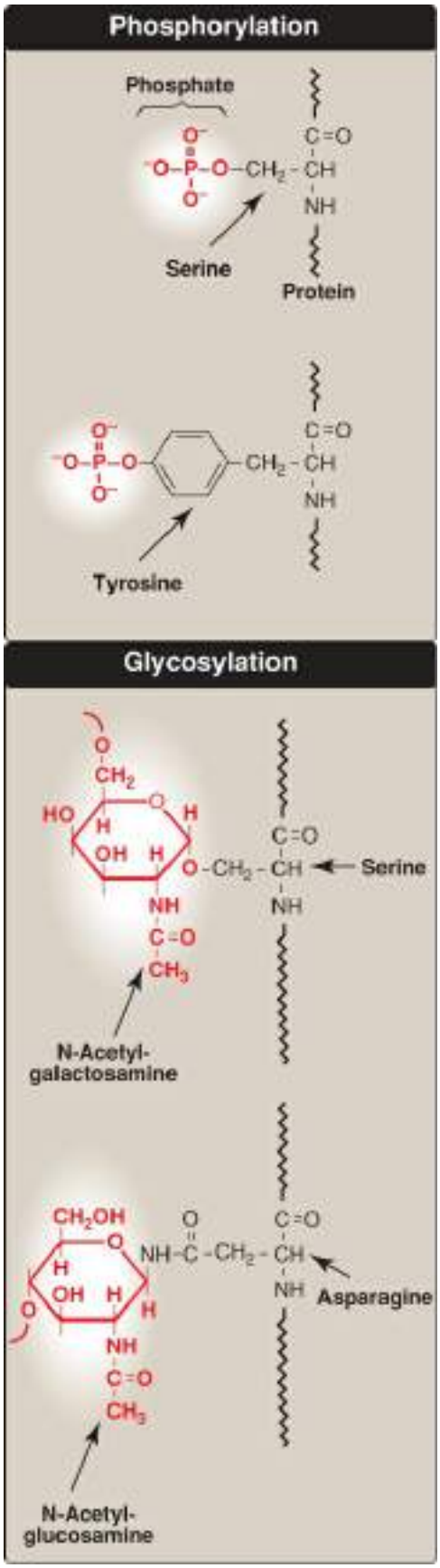
2. Glycosylation: Many of the proteins that are destined to become part of a membrane or to be secreted from a cell have carbohydrate chains added *en bloc* to the amide nitrogen of an asparagine (N linked) or built sequentially on the hydroxyl groups of a Ser, threonine, or hydroxylysine (O linked). N-glycosylation occurs in the RER and O-glycosylation in the Golgi. (The process of producing such glycoproteins was discussed on p. 181.) N-glycosylated acid hydrolases are targeted to the matrix of lysosomes by the phosphorylation of mannose residues at carbon 6 (see p. 185).

3. Hydroxylation: Pro and lysine residues of the α-chains of collagen are extensively hydroxylated by vitamin C–dependent hydroxylases in the RER (see p. 49).

4. Other covalent modifications: These may be required for the functional activity of a protein. For example, additional carboxyl groups can be added to glutamate residues by vitamin K–dependent carboxylation (see p. 440). The resulting γ-carboxyglutamate (Gla) residues are essential for the activity of several of the blood-clotting proteins (see Chapter 35). Biotin is covalently bound to the ε-amino groups of lysine residues of biotin-dependent enzymes that catalyze carboxylation reactions such as pyruvate carboxylase (see Fig. 10.3 on p. 130). Attachment of lipids, such as farnesyl groups, can help anchor proteins to membranes (see p. 221). Many eukaryotic proteins are cotranslationally acetylated at the N end. (Note: Reversible acetylation of histone proteins influences gene expression [see p. 526].)

C. Protein degradation

Proteins that are defective (e.g., misfolded) or destined for rapid turnover are often marked for destruction by ubiquitination, the covalent attachment of chains of a small, highly conserved protein called ubiquitin (see Fig. 19.3 on p. 273). Proteins marked in this way are rapidly degraded by the proteasome, which is a macromolecular, ATP-dependent, proteolytic system located in the cytosol. For example, misfolding of the CFTR protein (see p. 499) results in its proteasomal degradation. (Note: If folding is impeded, unfolded proteins accumulate in the RER causing stress that triggers the unfolded protein response, in which the expression of chaperones is increased; global translation is decreased by eIF-2 phosphorylation; and the unfolded proteins are sent to the cytosol, ubiquitinated, and degraded in the proteasome by a process called ER-associated degradation.)



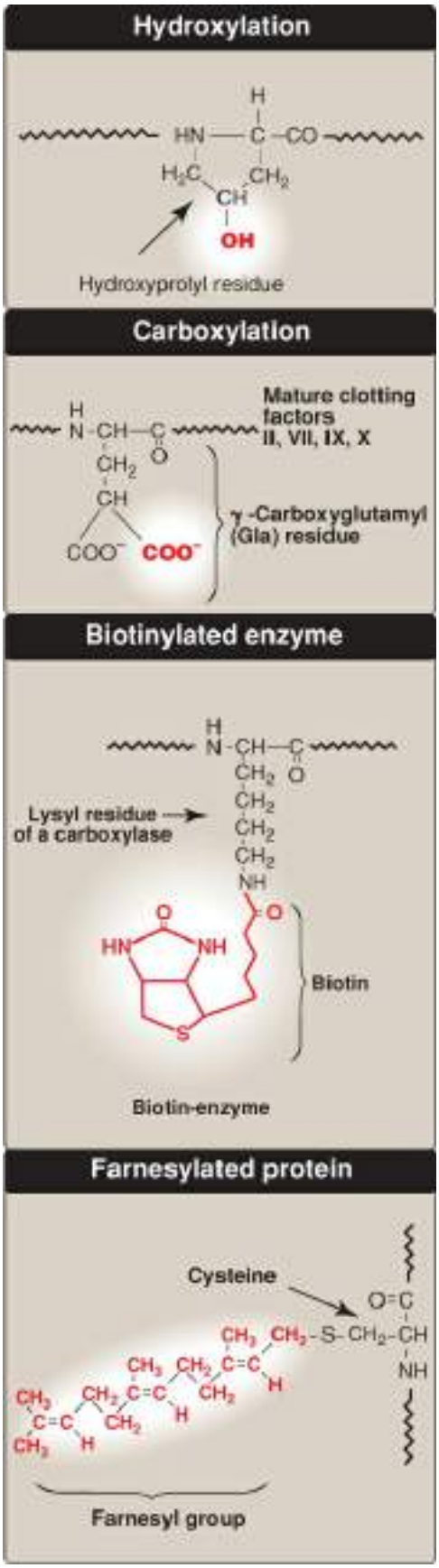


Figure 32.16
Covalent modification of some amino acid residues.

VII. Chapter Summary

- **Codons** in are composed of three nucleotides in mRNA, which contains the bases **A**, **G**, **C**, and **U**. Codons are always written **5′→3′**.
- Of the 64 possible three-base combinations, 61 code for the 20 standard amino acids and three signal for the termination of protein synthesis (**translation**). In an organism, the genetic code is specific (each codon produces one amino acid) and degenerate (more than one codon can code for each amino acid).
- Altering the nucleotide sequence in a codon can cause **silent mutations** (the altered codon codes for the original amino acid), **missense mutations** (the altered codon codes for a different amino acid), or **nonsense mutations** (the altered codon is a termination codon). Frameshift mutations that result from the addition or deletion of a base can cause an alteration in the reading frame of mRNA.
- **Translation** of a protein requires all of the **amino acids** in the protein; the **tRNA** and **aminoacyl-tRNA synthetase** for each amino acid; the **mRNA** coding for the protein; fully competent **ribosomes** (70S in prokaryotes, 80S in eukaryotes); **protein factors** needed for initiation, elongation, and termination of protein synthesis; and **ATP** and **GTP** as energy sources.
- Ribosomes are large complexes of **protein** and **rRNA**. They consist of **two subunits**, 30S and 50S in prokaryotes and 40S and 60S in eukaryotes. Each ribosome has three tRNA binding sites: the A, P, and E sites that cover three neighboring codons. The **A site** binds an **incoming aminoacyl-tRNA**, the **P site** is occupied by **peptidyl-tRNA**, and the **E site** is occupied by the **empty tRNA**.
- An mRNA codon is recognized by a tRNA **anticodon** following the rules of **complementarity** and **antiparallel** binding. The **wobble hypothesis** states that the first (5′) base of the anticodon is not as spatially constrained as the other two bases. Nontraditional base pairing may occur between the first (5′) anticodon base and the last (3′) base of the codon, thus allowing a single tRNA to recognize more than one codon for a specific amino acid.
- For the initiation of **translation**, an mRNA must associate with the small ribosomal subunit. The process requires **IFs**. In **prokaryotes**, a purine-rich region of the mRNA (the **SD sequence**) base pairs with a complementary sequence on 16S rRNA, resulting in the positioning of the small subunit on the mRNA. In eukaryotes, this positioning is guided by the **5′ cap** of the mRNA, which is bound by proteins of the eIF-4 family. The **initiation codon** is **AUG**. **N-formylmethionine** is the initiating amino acid in prokaryotes, whereas **Met** is used in eukaryotes. The charged initiator tRNA ($tRNA_i$) is brought to the P site by (**e)IF-2**.
- **Elongation** (lengthening) of the polypeptide chain occurs by the addition of amino acids to its carboxyl end. **Elongation factors** facilitate the binding of the aminoacyl-tRNA to the A site as well as the movement of the ribosome along the mRNA. The formation of the peptide bond is catalyzed by **peptidyl transferase**, which is an activity intrinsic to the rRNA of the large subunit and, therefore, is a **ribozyme**. Following peptide bond formation, the ribosome advances along the mRNA in the **5′→3′ direction** to the next codon (**translocation**). Because of the length of most mRNA, more than one ribosome at a time can translate a message, forming a **polysome.**
- **Termination** begins when a termination codon moves into the A site and is recognized by **release factors**. The newly synthesized protein is released from the ribosomal complex, and the ribosome is dissociated from the mRNA.
- Numerous **antibiotics** interfere with the process of protein synthesis in prokaryotes.
- Polypeptide chains may be covalently modified during or after translation. Such modifications include amino acid **removal**; **phosphorylation,** which may activate or inactivate the protein; **glycosylation**, which plays a role in **protein targeting**; and **hydroxylation,** such as that seen in collagen.
- Protein targeting can be either **cotranslational** (as with secreted proteins) or **posttranslational** (as with mitochondrial matrix proteins).
- Proteins must **fold** to achieve their functional form. Folding can be spontaneous or facilitated by **chaperones**. Proteins that are defective (e.g., misfolded) or destined for rapid turnover are marked for destruction by the attachment of chains of a small, highly conserved protein called **ubiquitin**. Ubiquitinated proteins are rapidly degraded by a cytosolic complex known as the **proteasome** (Fig. 32.17).

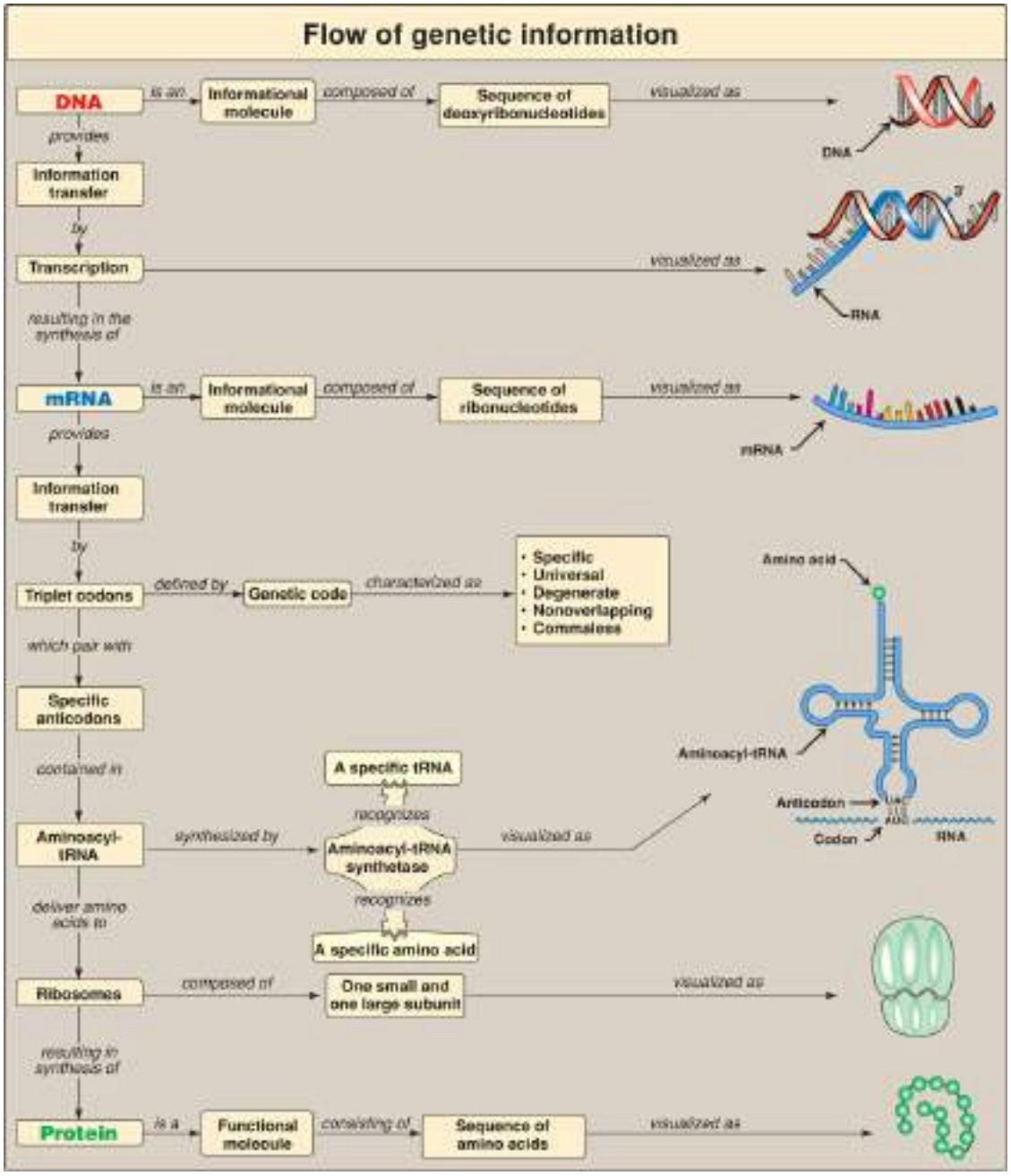


Figure 32.17
Key concept map for protein synthesis. mRNA = messenger RNA; tRNA = transfer RNA; A = adenine; G = guanine; C = cytosine; U = uracil.

Study Questions

Choose the ONE best answer.

32.1 A 20-year-old male with a microcytic anemia is found to have an abnormal form of β-globin (Hemoglobin Constant Spring) that is 172 amino acids long, rather than the 141 found in the normal protein. Which of the following point mutations is consistent with this abnormality? Use Figure 32.2 to answer the question.

A. CGA → UGA

B. GAU → GAC
C. GCA → GAA
D. UAA → CAA
E. UAA → UAG

Correct answer = D. Mutating the normal termination (stop) codon from UAA to CAA in β-globin messenger RNA causes the ribosome to insert a glutamine at that point. It will continue extending the protein chain until it comes upon the next stop codon farther down the message, resulting in an abnormally long protein. The replacement of CGA (arginine) with UGA (stop) would cause the protein to be too short. GAU and GAC both code for aspartate and would cause no change in the protein. Changing GCA (alanine) to GAA (glutamate) would not change the size of the protein product. A change from UAA to UAG would simply change one termination codon for another and would have no effect on the protein.

32.2 A pharmaceutical company is studying a new antibiotic that inhibits bacterial protein synthesis. When this antibiotic is added to an *in vitro* protein synthesis system that is translating mRNA sequence AUGUUUUUUUAG, the only product formed is the dipeptide fMet-Phe. What step in protein synthesis is most likely inhibited by the antibiotic?

A. Initiation
B. Binding of tRNA to the ribosomal A site
C. Peptidyl transferase activity
D. Ribosomal translocation
E. Termination

Correct answer = D. Because fMet-Phe (formylated methionyl-phenylalanine) is made, the ribosomes must be able to complete initiation, bind Phe-tRNA to the A site, and use peptidyl transferase activity to form the first peptide bond. Because the ribosome is not able to proceed any further, ribosomal movement (translocation) is most likely the inhibited step. Therefore, the ribosome is stopped before it reaches the termination codon of this message.

32.3 A tRNA molecule that is supposed to carry cysteine ($tRNA^{Cys}$) is mischarged, so that it actually carries alanine (Ala-$tRNA^{Cys}$). Assuming no correction occurs, what would be the most likely fate of this alanine residue during protein synthesis?

A. Alanine is incorporated into a protein.
B. Cysteine is incorporated into a protein.
C. Alanine is transferred to a $tRNA^{Ala}$ in the E site of the ribosome.
D. No protein synthesis occurs as alanine remains attached to the tRNA.
E. Alanine is chemically converted to cysteine by cellular enzymes.

Correct answer = A. Once an amino acid is attached to a tRNA molecule, only the anticodon of that tRNA determines the specificity of incorporation. Therefore, the incorrectly activated alanine will be incorporated into the protein at a position determined by a cysteine codon. A mischarged tRNA will cause a change in the protein that is not due to a mutation in the DNA.

32.4 In a patient with cystic fibrosis (CF) caused by the ΔF508 mutation, the mutant CF transmembrane conductance regulator (CFTR) protein folds incorrectly. The patient's cells modify this abnormal protein by attaching ubiquitin molecules to it. What is the fate of this modified CFTR protein?

A. It is degraded by the proteasome.
B. It is placed into storage vesicles.
C. It is repaired by cellular enzymes.
D. It is targeted to the lysosome.
E. It is secreted from the cell.

Correct answer = A. Ubiquitination usually marks old, damaged, or misfolded proteins for destruction by the

cytosolic proteasome. There is no known cellular mechanism for repair of damaged proteins. Proteins are targeted to the matrix of the lysosome by a mannose 6-phosphate residue.

32.5 Many antimicrobials inhibit translation. Which of the following antimicrobials is correctly paired with its mechanism of action?

A. Chloramphenicol inhibits transformylase.
B. Erythromycin binds to the 60S ribosomal subunit.
C. Puromycin inactivates elongation factor-2.
D. Streptomycin binds to the 30S ribosomal subunit.
E. Tetracyclines inhibit peptidyl transferase.

Correct answer = D. Streptomycin binds the 30S subunit and inhibits translation initiation. Chloramphenicol inhibits the peptidyl transferase activity of the 23S rRNA (ribozyme) of the 50S subunit. Erythromycin binds the 50S ribosomal subunit (60S denotes a eukaryote) and blocks the tunnel through which the peptide leaves the ribosome. Puromycin has structural similarity to aminoacyl-transfer RNA. It is incorporated into the growing chain, inhibits elongation, and results in premature termination in both prokaryotes and eukaryotes. Tetracyclines bind the 30S ribosomal subunit and block access to the A site, inhibiting elongation.

32.6 Translation of a synthetic polyribonucleotide containing the repeating sequence CAA in a cell-free protein-synthesizing system produces three homopolypeptides: polyglutamine, polyasparagine, and polythreonine. If the codons for glutamine and asparagine are CAA and AAC, respectively, which of the following triplets is the codon for threonine?

A. AAC
B. ACA
C. CAA
D. CAC
E. CCA

Correct answer = B. The synthetic polynucleotide sequence of CAACAACAACAA … could be read by the *in vitro* protein-synthesizing system starting at the first C, the first A, or the second A (i.e., in any one of three reading frames). In the first case, the first triplet codon would be CAA, which codes glutamine; in the second case, the first triplet codon would be AAC, which codes for asparagine; in the last case, the first triplet codon would be ACA, which codes for threonine.

32.7 Which of the following is required for both prokaryotic and eukaryotic protein synthesis?

A. Binding of the small ribosomal subunit to the Shine–Dalgarno sequence
B. Formylated methionyl-transfer (t)RNA
C. Movement of the messenger RNA out of the nucleus and into the cytoplasm
D. Recognition of the 5′ cap by initiation factors
E. Translocation of the peptidyl-tRNA from the A site to the P site

Correct answer = E. In both prokaryotes and eukaryotes, continued translation (elongation) requires movement of the peptidyl-tRNA from the A to the P site to allow the next aminoacyl-tRNA to enter the A site. Only prokaryotes have a Shine–Dalgarno sequence and use formylated methionine and only eukaryotes have a nucleus and co- and posttranscriptionally process their mRNA.

32.8 α1-Antitrypsin (AAT) deficiency can result in emphysema, a lung pathology, because the action of elastase, a serine protease, is unopposed. Deficiency of AAT in the lungs is the consequence of impaired secretion from the liver, the site of its synthesis. Proteins such as AAT that are destined to be secreted are best characterized by which of the following statements?

A. Their synthesis is initiated on the smooth endoplasmic reticulum.
B. They contain a mannose 6-phosphate targeting signal.
C. They always contain methionine as the N-terminal amino acid.

D. They are produced from translation products that have an N-terminal hydrophobic signal sequence.
E. They contain no sugars with O-glycosidic linkages because their synthesis does not involve the Golgi.

Correct answer = D. Synthesis of secreted proteins is begun on free (cytosolic) ribosomes. As the N-terminal signal sequence of the peptide emerges from the ribosome, it is bound by the signal recognition particle, taken to the rough endoplasmic reticulum (RER), threaded into the lumen, and cleaved as translation continues. The proteins move through the RER and the Golgi and undergo processing such as N-glycosylation (RER) and O-glycosylation (Golgi). In the Golgi, they are packaged in secretory vesicles and released from the cell. The smooth endoplasmic reticulum is associated with synthesis of lipids, not proteins, and has no ribosomes attached. Phosphorylation at carbon 6 of terminal mannose residues in glycoproteins targets these proteins (acid hydrolases) to lysosomes. The N-terminal methionine is removed from most proteins during processing.

32.9 Why is the genetic code described as both degenerate and unambiguous?

A given amino acid can be coded for by more than one codon (degenerate code), but a given codon codes for just one particular amino acid (unambiguous code).

Regulation of Gene Expression 33

I. OVERVIEW

Gene expression refers to the multistep process that ultimately results in the production of a functional gene product, either ribonucleic acid (RNA) or protein. The first step in gene expression, the use of deoxyribonucleic acid (DNA) for the synthesis of RNA (transcription), is the primary site of regulation in both prokaryotes and eukaryotes. In eukaryotes, however, gene expression also involves extensive posttranscriptional and posttranslational processes as well as actions that influence access to particular regions of the DNA. Each of these steps can be regulated to provide additional control over the kinds and amounts of functional products that are produced.

Not all genes are tightly regulated. For example, genes described as ‘constitutive’ encode products required for basic cellular functions and so are expressed at essentially a constant level. They are also known as “housekeeping” genes. Regulated genes, however, are expressed only under certain conditions. They may be expressed in all cells of the body or in only a subset of cells, for example, the gene for fibrinogen alpha chain, which is expressed only in hepatocytes. The ability to regulate gene expression (i.e., to determine if, how much, and when particular gene products will be made) gives the cell control over structure and function. It is the basis for cellular differentiation, morphogenesis, and adaptability of any organism. Control of gene expression is best understood in prokaryotes, but many themes are repeated in eukaryotes. Figure 33.1 shows some of the sites where gene expression can be controlled.

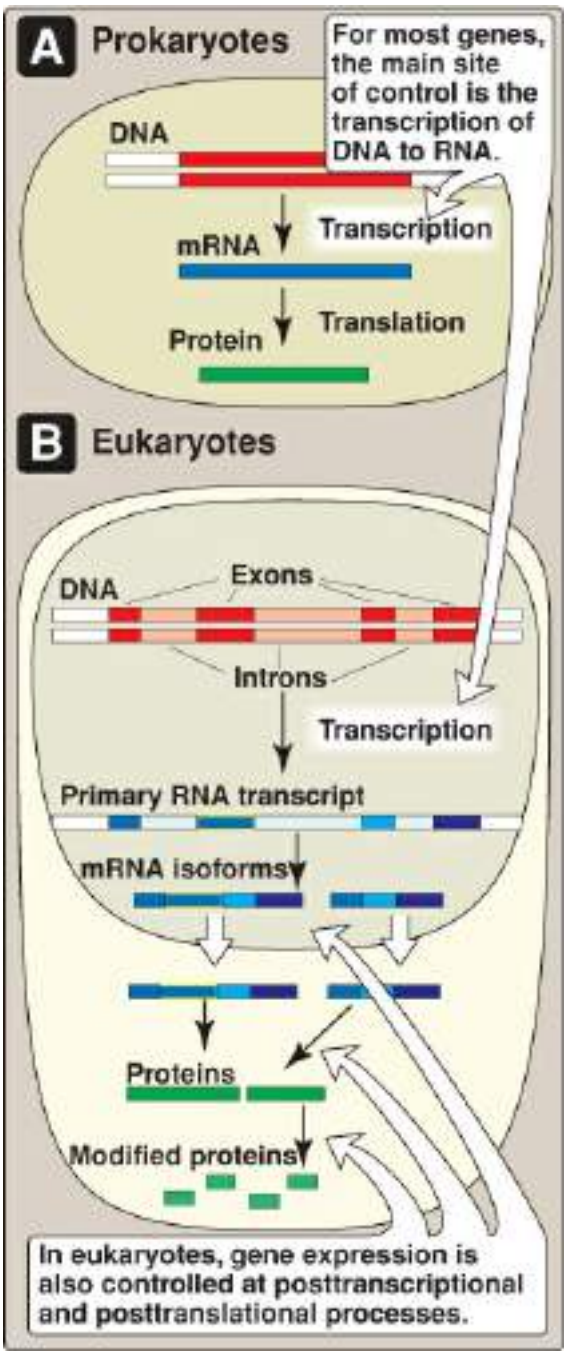


Figure 33.1
Control of gene expression. mRNA = messenger RNA.

II. REGULATORY SEQUENCES AND MOLECULES

Regulation of transcription, the initial step in all gene expression, is controlled by regulatory sequences of DNA that are usually embedded in the noncoding regions of the genome. The interaction between these DNA sequences and regulatory molecules, such as transcription factors, can induce or repress the transcriptional machinery,

influencing the kinds and amounts of products that are produced. The regulatory DNA sequences are called cis-acting because they influence expression of genes on the same chromosome as the regulatory sequence (see p. 488). The regulatory molecules are called trans-acting because they can diffuse (transit) through the cell from their site of synthesis to their DNA-binding sites (Fig. 33.2). For example, a protein transcription factor (a trans-acting molecule) that regulates a gene on chromosome 6 might itself have been encoded by a gene on chromosome 11. The binding of proteins to DNA is through structural motifs such as the zinc finger (Fig. 33.3), leucine zipper, or helix-turn-helix in the protein.

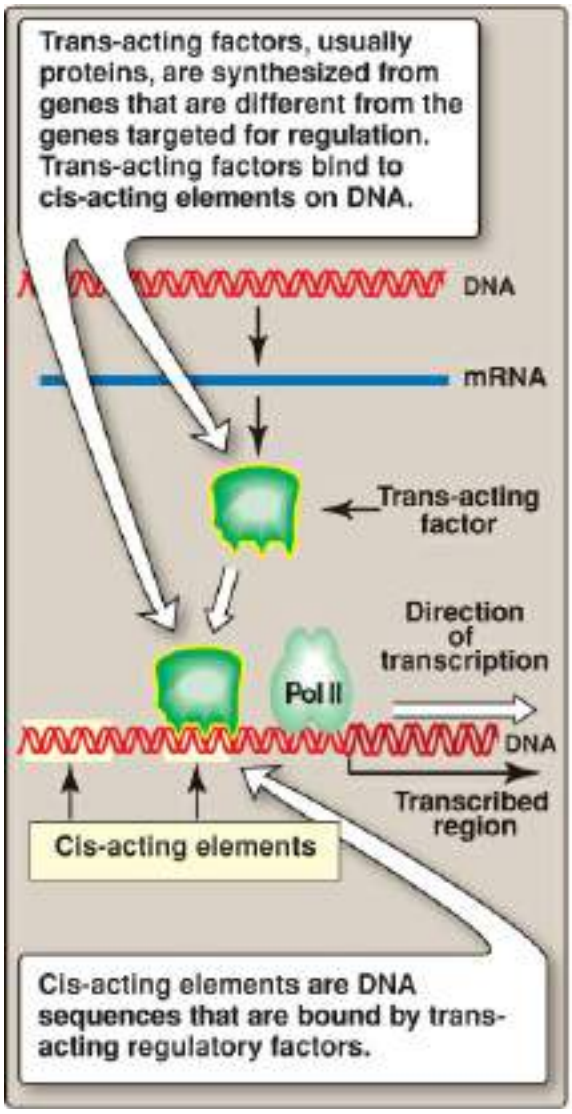


Figure 33.2
Cis-acting elements and trans-acting factors. mRNA = messenger RNA; Pol II = RNA polymerase II.

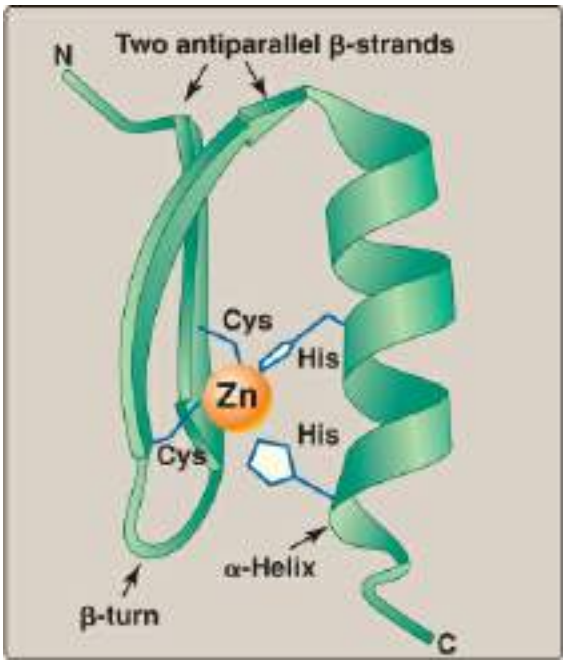


Figure 33.3
Zinc (Zn) finger is a common motif in proteins that bind DNA. Cys = cysteine; His = histidine.

III. REGULATION OF PROKARYOTIC GENE EXPRESSION

In prokaryotes such as the bacterium *Escherichia coli (E. coli*), regulation of gene expression occurs primarily at the level of transcription and, in general, is mediated by the binding of trans-acting proteins to cis-acting regulatory elements on their single DNA molecule (chromosome). (Note: Regulating the first step in the expression of a gene is an efficient approach, insofar as energy is not wasted making unneeded gene products.) Transcriptional control in prokaryotes can involve the initiation or premature termination of transcription.

A. Messenger RNA transcription from bacterial operons

In bacteria, the structural genes that encode proteins involved in a particular metabolic pathway are often found sequentially grouped on the chromosome along with the cis-acting elements that regulate the transcription of these genes. The transcription product is a single polycistronic messenger RNA ([mRNA], see p. 483). The genes are, thus, coordinately regulated (i.e., turned on or off as a unit). This entire package is referred to as an operon.

B. Operators in bacterial operons

Bacterial operons contain an operator, a segment of DNA that regulates the activity of the structural genes of the operon by reversibly binding a protein known as the repressor. If the operator is not bound by the repressor, RNA polymerase (RNA pol) binds the promoter, passes over the operator, and reaches the protein-coding

genes that it transcribes to mRNA. If the repressor is bound to the operator, the polymerase is blocked and does not produce mRNA. As long as the repressor is bound to the operator, no mRNA (and, therefore, no proteins) are made. However, when an inducer molecule is present, it binds to the repressor, causing the repressor to change shape so that it no longer binds the operator. When this happens, RNA pol can initiate transcription. One of the best-understood examples is the inducible lactose *(lac)* operon of *E. coli* that illustrates both positive and negative regulation (Fig. 33.4).

C. Lactose operon

The *lac* operon contains the genes that code for three proteins involved in the catabolism of the disaccharide lactose: the *lacZ* gene codes for β-galactosidase, which hydrolyzes lactose to galactose and glucose; the *lacY* gene codes for a permease, which facilitates the movement of lactose into the cell; and the *lacA* gene codes for thiogalactosidetransacetylase, which acetylates lactose. (Note: The physiologic function of this acetylation is unknown.) All of these proteins are maximally produced only when lactose is available to the cell and glucose is not. (Note: Bacteria use glucose, if available, as a fuel in preference to any other sugar.) The regulatory portion of the operon is upstream of the three structural genes and consists of the promoter region where RNA pol binds and two additional sites, the operator (O) and the catabolite activator protein (CAP) sites, where regulatory proteins bind. The *lacZ*, *lacY*, and *lacA* genes are maximally expressed only when the O site is empty and the CAP site is bound by a complex of cyclic adenosine monophosphate ([cAMP], see p. 103) and the CAP, sometimes called the cAMP regulatory protein (CRP). A regulatory gene, the *lacI* gene, codes for the repressor protein (a trans-acting factor) that binds to the O site with high affinity. (Note: The *lacI* gene has its own promoter and is not part of the *lac* operon.)

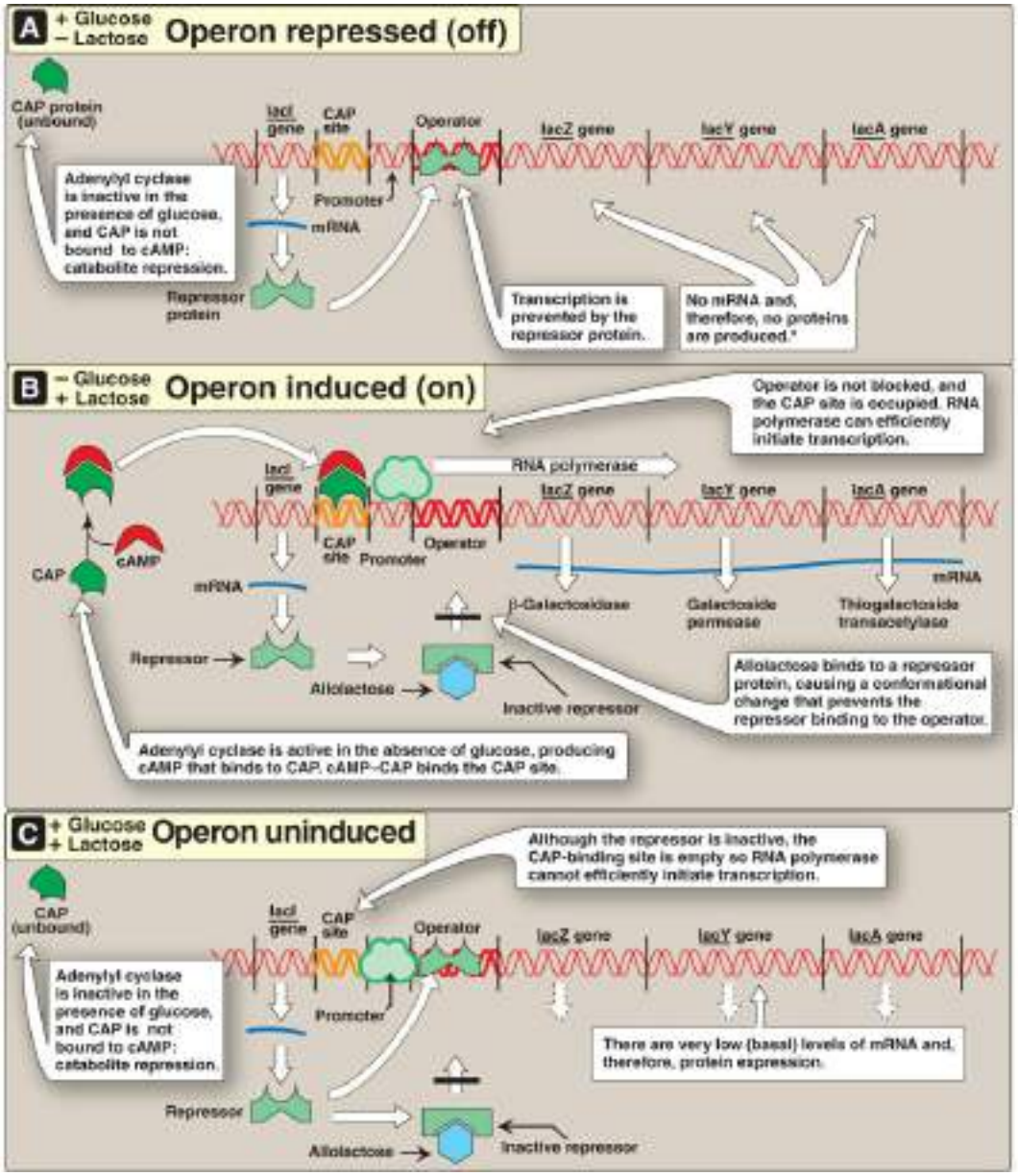


Figure 33.4
The lactose operon of *Escherichia coli* in the presence of (**A**) only glucose, (**B**) only lactose, and (**C**) both sugars. *(Note: Even when the operon has been turned off, the repressor transiently dissociates from the operator at a slow rate, allowing a very low level of expression. The synthesis of a few molecules of permease (and β-galactosidase) allows the organism to respond rapidly should glucose become unavailable.) CAP = catabolite activator protein; cAMP = cyclic adenosine monophosphate; mRNA = messenger RNA.

1. When only glucose is available: In this case, the *lac* operon is repressed (turned off). Repression is mediated by the repressor protein binding via a helix-turn-helix motif (Fig. 33.5) to the O site, which is downstream of the promoter (see Fig. 33.4A). Binding of the repressor interferes with the binding of RNA pol to the promoter, thereby inhibiting transcription of the structural genes. This is an example of negative regulation.

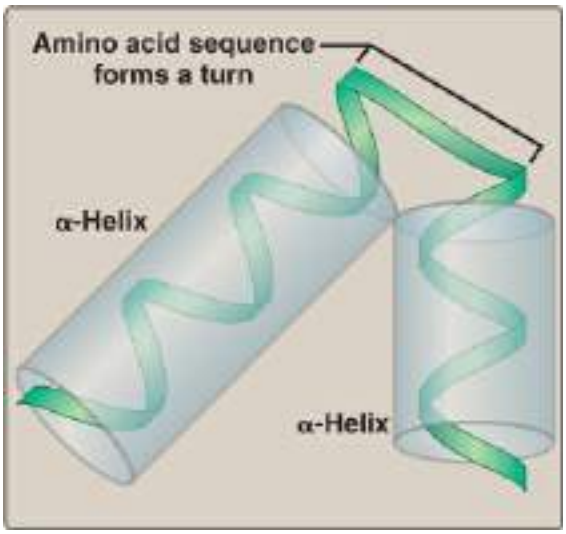


Figure 33.5
Helix-turn-helix motif of the *lac* repressor protein.

2. When only lactose is available: In this case, the *lac* operon is induced (maximally expressed, or turned on). A small amount of lactose is converted to an isomer, allolactose. This compound is an inducer that binds to the repressor protein, changing its conformation so that it can no longer bind to the O site. In the absence of glucose, adenylyl cyclase is active, and cAMP is made and binds to the CAP. The cAMP–CAP trans-acting complex binds to the CAP site, causing RNA pol to initiate transcription with high efficiency at the promoter site (see Fig. 33.4B). This is an example of positive regulation. The transcript is a single polycistronic mRNA molecule that contains three sets of start and stop codons. Translation of the mRNA produces the three proteins that allow lactose to be used for energy production by the cell. (Note: In contrast to the inducible *lacZ*, *lacY*, and *lacA* genes, whose expression is regulated, the *lacI* gene is constitutive. Its gene product, the repressor protein, is always made and is active unless the inducer is present.)

3. When both glucose and lactose are available: In this case, the *lac* operon is uninduced, and transcription is negligible, even if lactose is present at a high concentration. Adenylyl cyclase is inhibited in the presence of glucose (a process known as catabolite repression) so no cAMP–CAP complex forms, and the CAP site remains empty. Therefore, the RNA pol is unable to effectively initiate transcription, even though the repressor is not bound to the O site. Consequently, the three structural genes of the operon are expressed only at a very low (basal) level (see Fig. 33.4C). (Note: Induction causes a 50-fold enhancement over basal expression.)

D. Tryptophan operon

The tryptophan *(trp)* operon contains five structural genes that code for enzymes required for the synthesis of the amino acid tryptophan (Trp). As with the *lac* operon,

the *trp* operon is subject to negative control. However, for the repressible trp operon, negative control includes Trp itself binding to a repressor protein and facilitating the binding of the repressor to the operator: Trp is a corepressor. Because repression by Trp is not always complete, the *trp* operon, unlike the *lac* operon, is also regulated by a process known as attenuation. With attenuation, transcription is initiated but is terminated well before completion (Fig. 33.6). If Trp is plentiful, transcription initiation that escaped repression by Trp is attenuated (stopped) by the formation of an attenuator, a hairpin (stem-loop) structure in the mRNA similar to that seen in rho-independent termination (see p. 486). (Note: Because transcription and translation are temporally linked in prokaryotes [see p. 503], attenuation also results in the formation of a truncated, nonfunctional peptide product that is rapidly degraded.) If Trp becomes scarce, the operon is expressed. The 5′ end of the mRNA contains two adjacent codons for Trp. The lack of Trp causes ribosomes to stall at these codons, covering regions of the mRNA required for formation of the attenuation hairpin. This prevents attenuation and allows transcription to continue.

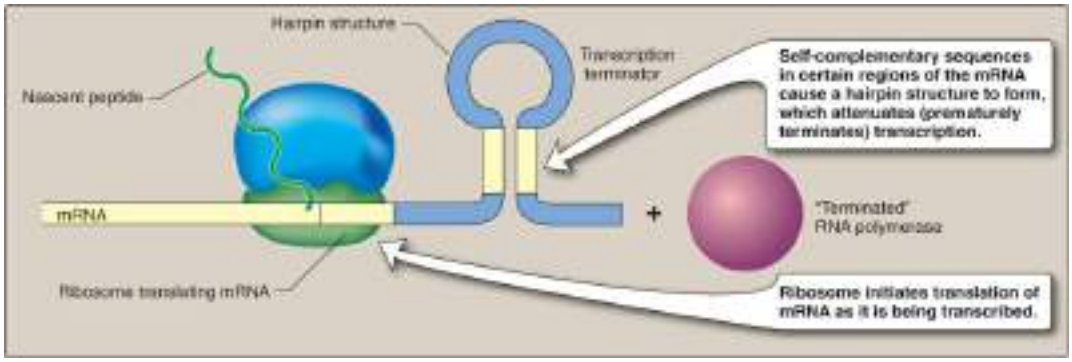


Figure 33.6
Attenuation of transcription of the *trp* operon when tryptophan is plentiful. mRNA = messenger RNA.

Transcriptional attenuation can occur in prokaryotes because translation of an mRNA begins before its synthesis is complete. This does not occur in eukaryotes because the presence of a membrane-bound nucleus spatially and temporally separates transcription and translation.

E. Coordination of transcription and translation

Although transcriptional regulation of mRNA production is primary in bacteria, regulation of ribosomal RNA (rRNA) and protein synthesis plays an important role in adaptation to environmental stress.

1. Stringent response: *E. coli* has seven operons that synthesize the rRNA needed for ribosome assembly, and each is regulated in response to changes in environmental conditions. Regulation in response to amino acid starvation is known as the stringent response. The binding of an uncharged transfer RNA (tRNA) to the A site of a ribosome (see p. 501) triggers a series of events that

leads to the production of the alarmone, guanosine 5′-diphosphate, 3′-diphosphate (ppGpp). The synthesis of this unusual derivative of guanosine diphosphate (GDP) is catalyzed by stringent factor (RelA), an enzyme physically associated with ribosomes. Elevated levels of ppGpp result in inhibition of rRNA synthesis (Fig. 33.7). ppGpp binds RNA pol and alters promoter selection through the use of different sigma factors for the polymerase (see p. 484). In addition to rRNA synthesis, tRNA synthesis and some mRNA synthesis (e.g., for ribosomal proteins [r-proteins]) are also inhibited. However, synthesis of mRNA for enzymes required for amino acid biosynthesis is not inhibited. The stringent response prevents the wasteful production of more ribosomes and promotes the production of needed amino acids when amino acids are scarce.

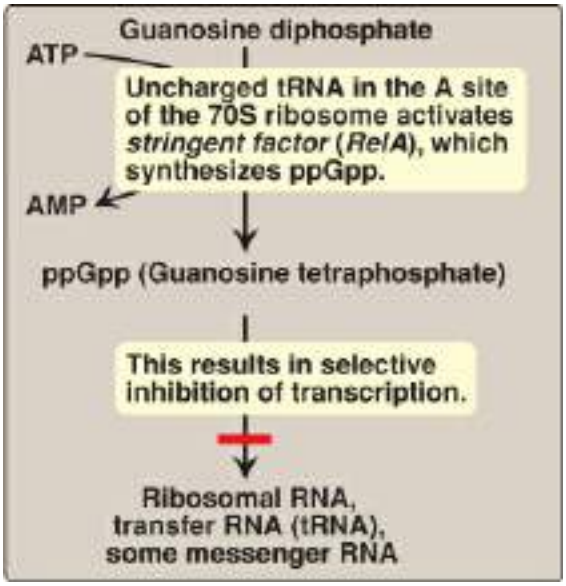


Figure 33.7
Regulation of transcription by the stringent response to amino acid starvation. S = Svedberg unit.

2. Regulatory ribosomal proteins: Operons for r-proteins can be inhibited by an excess of their own protein products. For each operon, one specific r-protein functions in the repression of translation of the polycistronic mRNA from that operon (Fig. 33.8). The r-protein does so by binding to the Shine–Dalgarno (SD) sequence located on the mRNA just upstream of the first initiating AUG codon (see p. 497) and acting as a physical impediment to the binding of the small ribosomal subunit to the SD sequence. Thus, one r-protein inhibits synthesis of all the r-proteins of the operon. This same r-protein also binds to rRNA and with a higher affinity than for mRNA. If the concentration of rRNA falls, the r-protein then is available to bind its own mRNA and inhibit its translation. This coordinated regulation keeps the synthesis of r-proteins in balance with the transcription of rRNA, so that each is present in appropriate amounts for the formation of ribosomes.

IV. REGULATION OF EUKARYOTIC GENE EXPRESSION

The higher degree of complexity of eukaryotic genomes, as well as the presence of a nuclear membrane, necessitates a wider range of regulatory processes. As with the prokaryotes, transcription is the primary site of regulation. Again, the theme of trans-acting factors binding to cis-acting elements is seen. Operons, however, are not found in eukaryotes, which must use alternate strategies to solve the problem of how to coordinately regulate all the genes required for a specific response. In eukaryotes, gene expression is also regulated at multiple levels other than transcription. For example, the major modes of posttranscriptional regulation at the mRNA level are alternative mRNA splicing and polyadenylation, control of mRNA stability, and control of translational efficiency. Additional regulation at the protein level occurs by mechanisms that modulate stability, processing, or targeting of the protein.

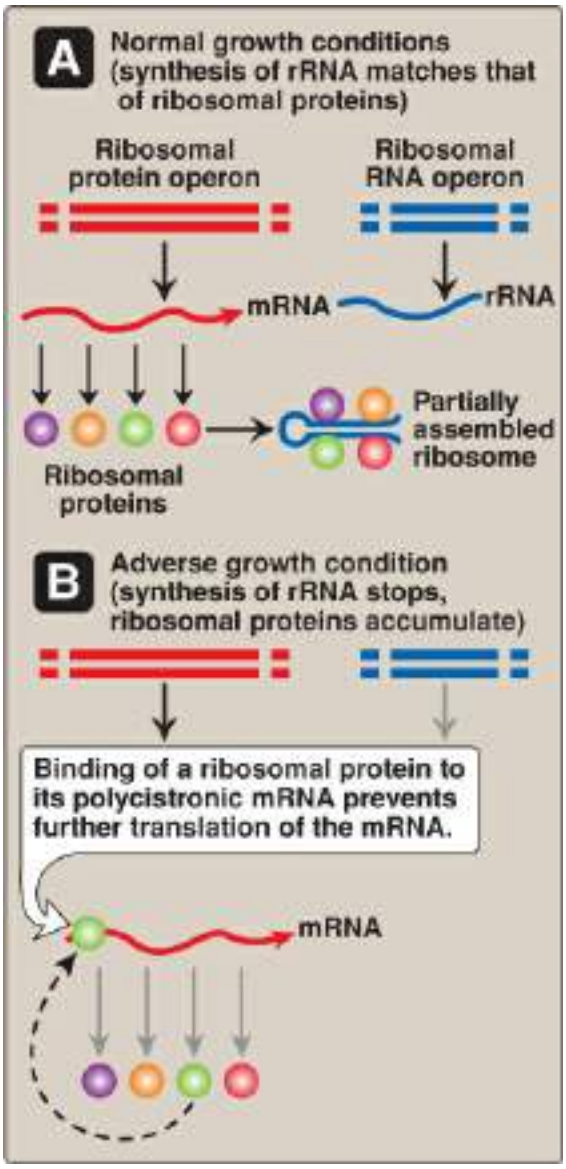


Figure 33.8
Regulation of translation by an excess of ribosomal proteins. mRNA = messenger RNA; rRNA = ribosomal RNA.

A. Coordinate regulation

The need to coordinately regulate a group of genes to cause a particular response is of key importance in organisms with more than one chromosome. An underlying theme occurs repeatedly: A trans-acting protein functions as a specific transcription factor (STF) that binds to a cis-acting regulatory consensus sequence (see p. 463) on each of the genes in the group even if they are on different chromosomes. (Note: The STF has a DNA-binding domain [DBD] and a transcription-activation domain [TAD]. The TAD recruits coactivators, such as histone acetyltransferases [see p. 487], and the general transcription factors [see p. 488] that, along with RNA pol, are required for formation of the transcription initiation complex at the promoter. Although the TAD recruits a variety of proteins, the specific effect of any one of them is dependent upon the protein composition of the complex. This is known as combinatorial control.) Examples of coordinate regulation in eukaryotes include the galactose circuit and the hormone response system.

1. Galactose circuit: This regulatory scheme allows for the use of galactose when glucose is not available. In yeast, a unicellular organism, the genes required to metabolize galactose are on different chromosomes. Coordinated expression is mediated by the protein Gal4 (Gal = galactose), a STF that binds to a short regulatory DNA sequence upstream of each of the genes. The sequence is called the upstream activating sequence Gal (UAS_{Gal}). Binding of Gal4 to UAS_{Gal} through zinc fingers in its DBD occurs in both the absence and presence of galactose. When the sugar is absent, the regulatory protein Gal80 binds Gal4 at its TAD, thereby inhibiting gene transcription (Fig. 33.9A). When present, galactose activates the Gal3 protein. Gal3 binds Gal80, thereby allowing Gal4 to activate transcription (Fig. 33.9B). (Note: Glucose prevents the use of galactose by inhibiting expression of Gal4 protein.)

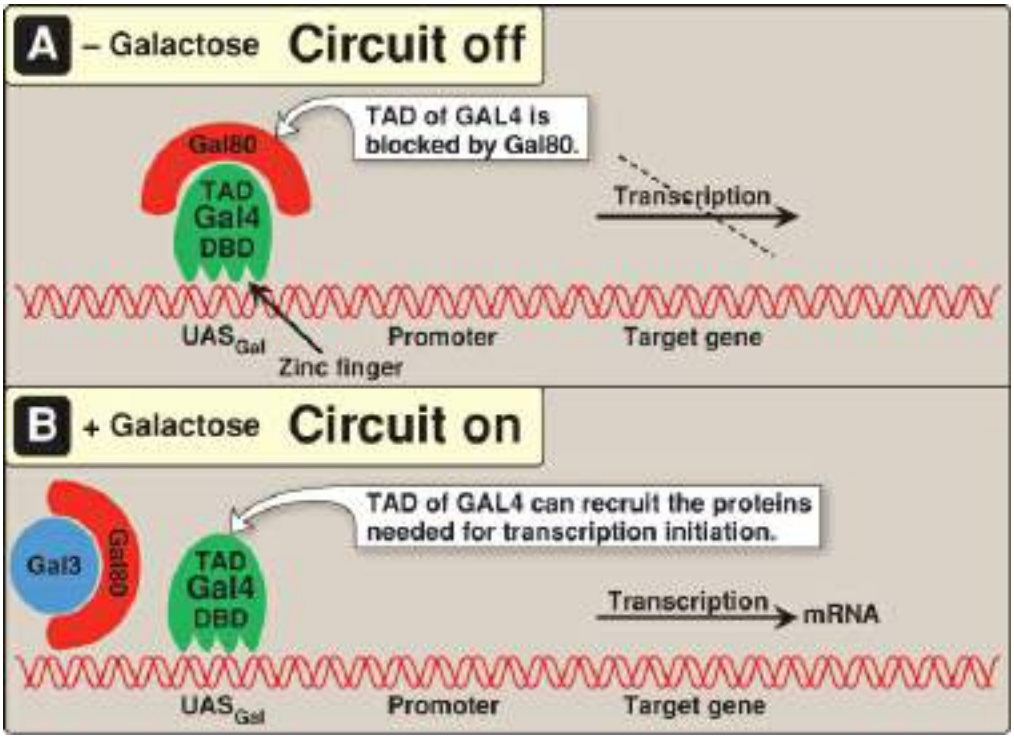


Figure 33.9
Regulation of galactose circuit in yeast in the (A) absence and (B) presence of galactose. (Note: Target genes, whether on the same or a different chromosome, each have an upstream activating sequence galactose [UAS_{Gal}].) TAD = transcription-activation domain; DBD = DNA-binding domain; mRNA = messenger RNA.

2. Hormone response system: Hormone response elements (HREs) are DNA sequences that bind trans-acting proteins and regulate gene expression in response to hormonal signals in multicellular organisms. Hormones bind to either intracellular (nuclear) receptors (e.g., steroid hormones; see Figure 18.28) or cell-surface receptors (e.g., the peptide hormone glucagon; see Figure 23.12).

a. Intracellular receptors: Members of the nuclear receptor superfamily, which includes the steroid hormone (glucocorticoids, mineralocorticoids, androgens, and estrogens), vitamin D, retinoic acid, and thyroid hormone receptors, function as STF. In addition to domains for DNA-binding and transcriptional activation, these receptors also contain a ligand-binding domain. For example, the steroid hormone cortisol (a glucocorticoid) binds intracellular receptors at the ligand-binding domain (Fig. 33.10). Binding causes a conformational change in the receptor that activates it. The receptor–hormone complex enters the nucleus, dimerizes, and binds via a zinc finger motif to DNA at a regulatory element, the glucocorticoid response element (GRE) that is an example of a HRE. Binding allows recruitment of coactivators to the TAD of the receptor and results in expression of cortisol-responsive genes, each of which is under the control of its own GRE. Binding of the receptor–hormone complex to the GRE allows coordinate

expression of a group of target genes, even though these genes are on different chromosomes. The GRE can be located upstream or downstream of the genes it regulates and at great distances from them. The GRE, then, can function as a true enhancer (see p. 489). (Note: If associated with repressors, hormone–receptor complexes inhibit transcription.)

b. Cell-surface receptors: These receptors include those for insulin, epinephrine, and glucagon. Glucagon, for example, is a peptide hormone that binds its G protein–coupled plasma membrane receptor on glucagon-responsive cells. This extracellular signal is then transduced to intracellular cAMP, a second messenger (Fig. 33.11; also see Fig. 8.7), which can affect protein expression (and activity) through protein kinase A–mediated phosphorylation. In response to a rise in cAMP, a trans-acting factor (cAMP response element–binding [CREB] protein) is phosphorylated and activated. Active CREB protein binds via a leucine zipper motif to a cis-acting regulatory element, the cAMP response element (CRE) resulting in transcription of target genes with CRE in their promoters. (Note: The genes for phosphoenolpyruvate carboxykinase and glucose 6-phosphatase, key enzymes of gluconeogenesis [see p. 133] are examples of genes upregulated by the cAMP/CRE/CREB system.)

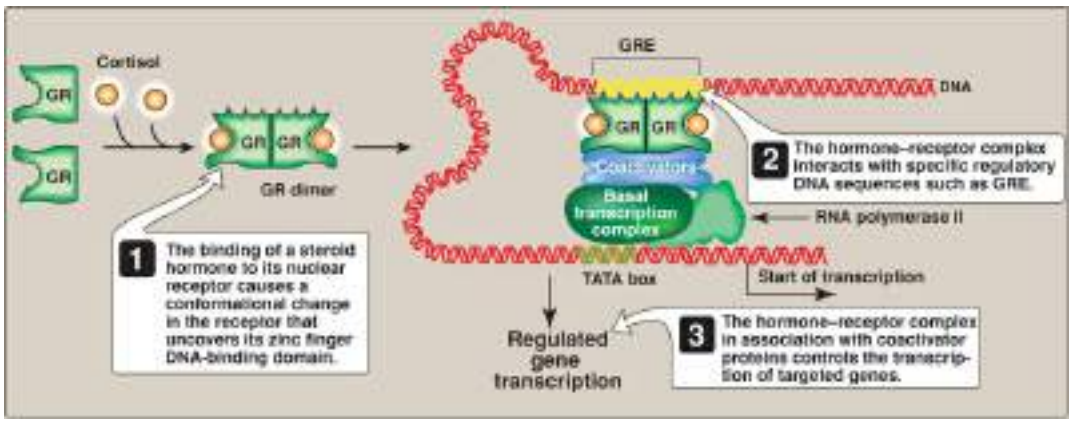


Figure 33.10
Transcriptional regulation by intracellular steroid hormone receptors. GRE = glucocorticoid response element; GR = glucocorticoid receptor.

B. Messenger RNA processing and use

Eukaryotic mRNA undergoes several processing events before it is exported from the nucleus to the cytoplasm for use in protein synthesis. Capping at the 5′ end (see p. 490), polyadenylation at the 3′ end (see p. 491), and splicing (see p. 491) are essential for the production of a functional eukaryotic messenger from most pre-mRNA. Variations in splicing and polyadenylation can affect gene expression. In addition, messenger stability also affects gene expression.

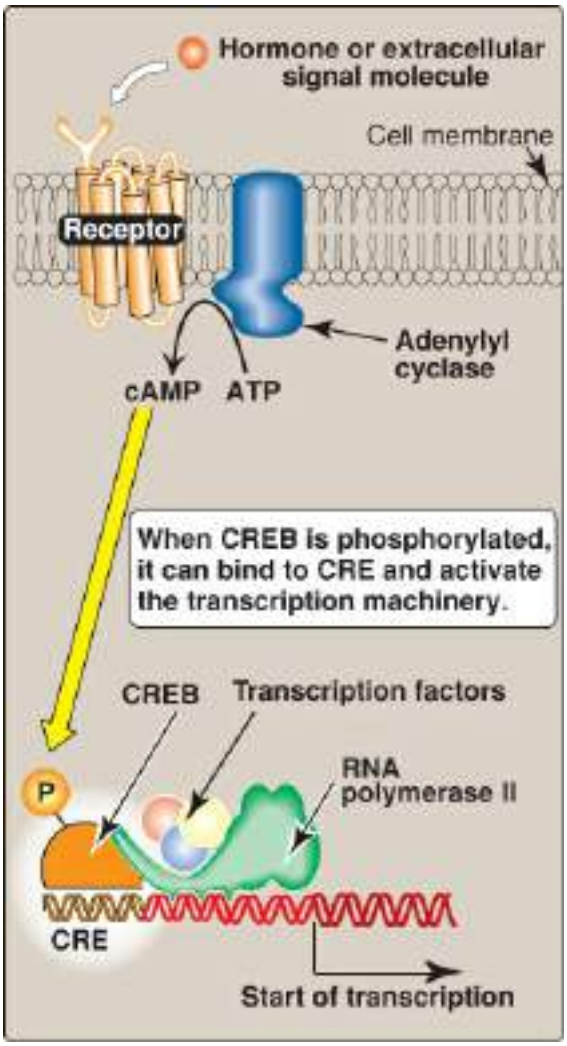


Figure 33.11
Transcriptional regulation by receptors located in the cell membrane. (Note: Cyclic adenosine monophosphate [cAMP] activates protein kinase A that phosphorylates cAMP response element–binding [CREB] protein.) CRE = cAMP response element.

1. Alternative splicing: Tissue-specific protein isoforms can be made from the same pre-mRNA through alternative splicing, which can involve exon skipping (loss), intron retention, and use of alternative splicedonor or acceptor sites (Fig. 33.12). For example, the pre-mRNA for tropomyosin (TM) undergoes tissue-specific alternative splicing to yield a number of TM isoforms (see p. 492). (Note: Over 90% of all human genes undergo alternative splicing.)

2. Alternative polyadenylation: Some pre-mRNA transcripts have more than one site for cleavage and polyadenylation. Alternative polyadenylation (APA) generates mRNA with different 3′ends, altering the untranslated region (UTR) or the coding (translated) sequence. (Note: APA is involved in the production of the membrane-bound and secreted forms of immunoglobulin M.)

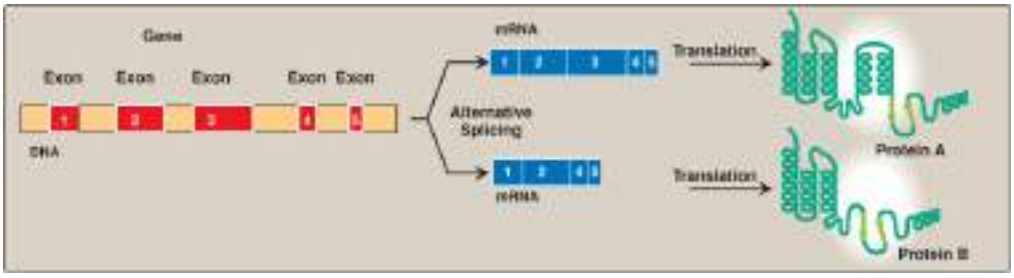


Figure 33.12
Tissue-specific alternative splicing produces different proteins, or isoforms, from a single gene. mRNA = messenger RNA.

> The use of alternative splicing and polyadenylation sites, as well as alternative transcription start sites explains, at least in part, how the ~20,000 to 25,000 genes in the human genome can give rise to well over 100,000 proteins.

3. Messenger RNA editing: Even after mRNA has been fully processed, it may undergo an additional posttranscriptional modification in which a base in the mRNA is altered. This is known as RNA editing. An important example in humans occurs with the transcript for apolipoprotein (apo) B, an essential component of chylomicrons (see p. 254) and very–low-density lipoproteins ([VLDLs], see p. 256). Apo B mRNA is made in the liver and the small intestine. However, in the intestine only, the cytosine (C) base in the CAA codon for glutamine is enzymatically deaminated to uracil (U), changing the sense codon to the nonsense or stop codon UAA, as shown in Figure 33.13. This results in a shorter protein (apo B-48, representing 48% of the message) being made in the intestine (and incorporated into chylomicrons) than is made in the liver (apo B-100, full-length, incorporated into VLDL).

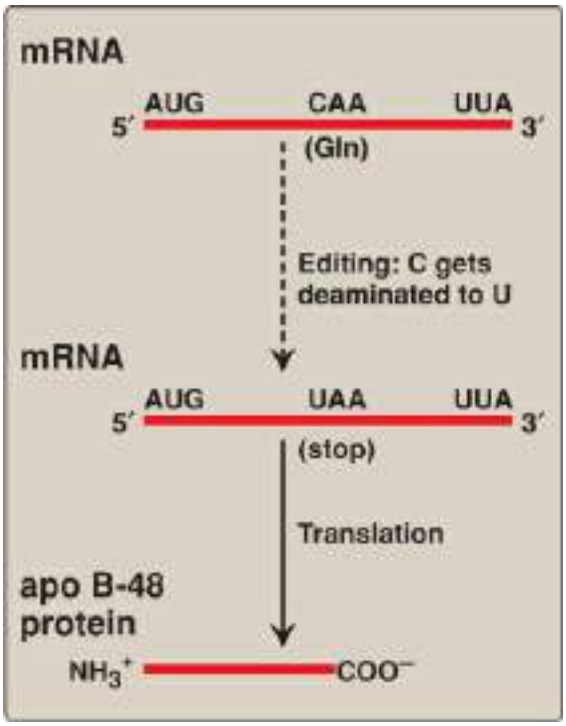


Figure 33.13
Editing of apolipoprotein (apo) B mRNA in the intestine and generation of the apo B-48 protein needed for chylomicron synthesis. Gln = glutamine; mRNA = messenger RNA; A = adenine; C = cytosine; G = guanine; U = uracil.

4. Messenger RNA stability: How long an mRNA remains in the cytosol before it is degraded influences how much protein product can be produced from it. Regulation of iron metabolism and the gene-silencing process of RNA interference (RNAi) illustrate the importance of mRNA stability in the regulation of gene expression.

a. Iron metabolism: Transferrin (Tf) is a plasma protein that transports iron. Tf binds to cell-surface receptors (transferrin receptors [TfRs]) that get internalized and provide cells, such as erythroblasts, with iron. The mRNA for the TfR has several cis-acting iron-responsive elements (IREs) in its 3′-UTR. IREs have a short stem-loop structure that can be bound by trans-acting iron regulatory proteins (IRPs), as shown in Figure 33.14. When the iron concentration in the cell is low, the IRPs bind to the 3′-IRE and stabilize the mRNA for TfR, allowing TfR synthesis. When intracellular iron levels are high, the IRPs dissociate. The lack of IRP bound to the mRNA hastens its destruction, resulting in decreased TfR synthesis. (Note: The mRNA for ferritin, an intracellular protein of iron storage, has a single IRE in its 5′-UTR. When iron levels in the cell are low, IRPs bind the 5′-IRE and prevent the use of the mRNA, and less ferritin is made. When iron accumulates in the cell, the IRPs dissociate, allowing synthesis of ferritin molecules to store the excess iron. Aminolevulinic acid synthase 2, the regulated enzyme of heme synthesis [see p. 309] in erythroblasts, also contains a 5′-IRE.) (See Chapter

21 for a discussion of heme synthesis.)

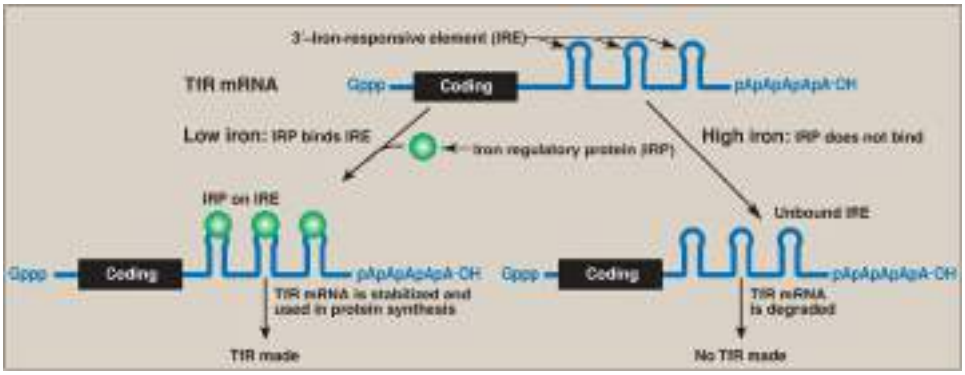


Figure 33.14
Regulation of transferrin receptor (TfR) synthesis. (Note: The IREs are located in the 3′-UTR [untranslated region] of TfR messenger RNA [mRNA].) Gppp = 7-methylguanosine cap; $p(Ap)_nA$-OH = polyadenylate tail.

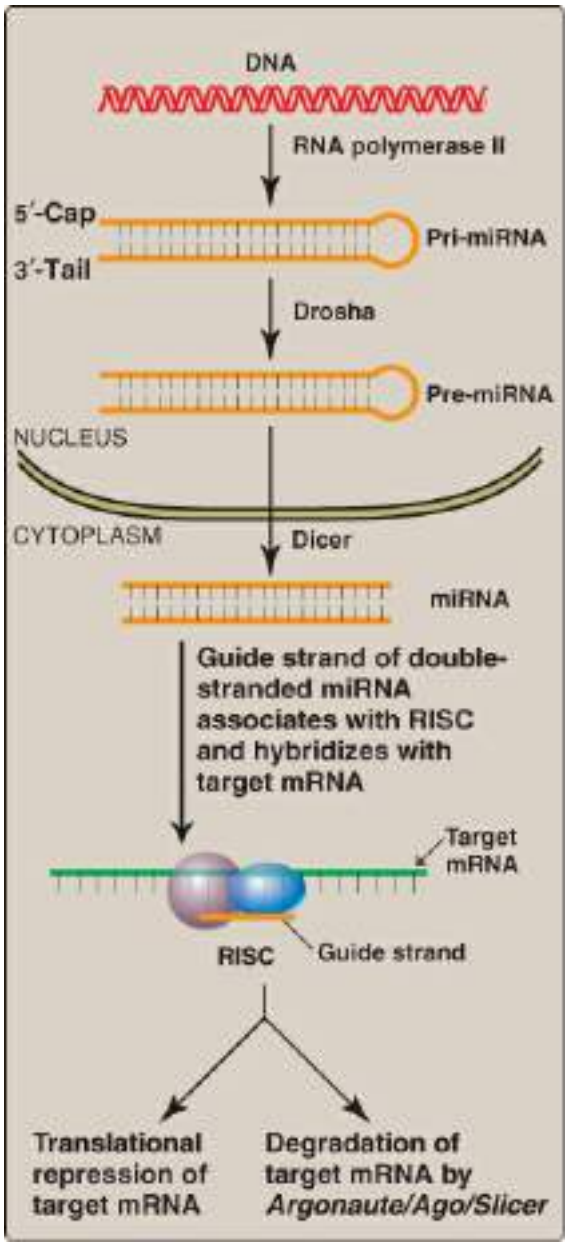


Figure 33.15
Biogenesis and actions of microRNA (miRNA). (Note: The extent of complementarity between the target messenger RNA [mRNA] and the miRNA determines the final outcome, with perfect complementarity resulting in mRNA degradation.) Pri = primary; RISC = RNA-induced silencing complex.

b. RNA interference: RNAi is a mechanism of gene silencing through decreased expression of mRNA, either by repression of translation or by increased degradation. It plays a key role in such fundamental processes as cell proliferation, differentiation, and apoptosis. RNAi is mediated by short (~22 nucleotides), noncoding RNA called microRNA (miRNA). The miRNA arise from far longer, genomically encoded nuclear transcripts, primary miRNA (pri-miRNA) that are partially processed in the nucleus to pre-miRNA

by an endonuclease (Drosha), then transported to the cytoplasm. There, another endonuclease (Dicer) completes the processing and generates short, double-stranded miRNA. A single strand (the guide or antisense strand) of the miRNA associates with a cytosolic protein complex known as the RNA-induced silencing complex (RISC). The guide strand hybridizes with a complementary sequence in the 3′-UTR of a full-length target mRNA, bringing RISC to the mRNA. This can result in repression of translation of the mRNA or its degradation by an endonuclease (Argonaute/Ago/Slicer) of the RISC. The extent of complementarity appears to be the determining factor (Fig. 33.15). RNAi can also be triggered by the introduction of exogenous double-stranded short interfering RNA (siRNA) into a cell, a process that has enormous therapeutic potential.

1) RNA interference–based therapeutics: In 2018, the first RNAi-based therapy was approved to treat peripheral nerve disease (polyneuropathy) in patients with hereditary transthyretin-mediated amyloidosis (hATTR) caused by a mutation in the gene encoding transthyretin (TTR). The siRNA-based drug, patisiran, prevents the production of abnormal TTR protein and reduces the buildup of amyloid deposits containing TTR that form in peripheral nerves and in the heart. Several other RNAi therapeutics are undergoing clinical trials.

5. Messenger RNA translation: Regulation of gene expression can also occur at the level of mRNA translation. One mechanism by which translation is regulated is through phosphorylation of the eukaryotic translation initiation factor, eIF-2 (Fig. 33.16). Phosphorylation of eIF-2 inhibits its function and so inhibits translation at the initiation step (see p. 508). (Note: Phosphorylation of eIF-2 prevents its reactivation by inhibiting GDP–GTP exchange.) Phosphorylation is catalyzed by kinases that are activated in response to environmental conditions, such as amino acid starvation, heme deficiency in erythroblasts, the presence of double-stranded RNA (signaling viral infection), and the accumulation of misfolded proteins in the rough endoplasmic reticulum (see p. 509).

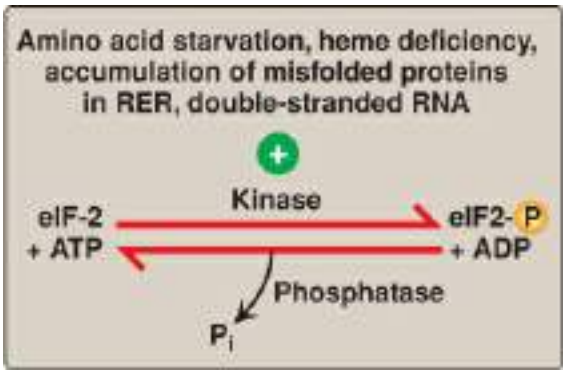


Figure 33.16
Regulation of translation initiation in eukaryotes by phosphorylation of eukaryotic translation initiation factor, eIF-2. RER = rough endoplasmic reticulum; ADP = adenosine diphosphate; P_i

= inorganic phosphate; Ⓟ = phosphate.

C. Regulation through variations in DNA

Gene expression in eukaryotes is also influenced by the accessibility of DNA to the transcriptional apparatus, the number of copies of genes, and the arrangement of DNA. (Note: Localized transitions between the B and Z forms of DNA [see p. 462] can also affect gene expression.)

1. Access to DNA: In eukaryotes, DNA is found complexed with histone and nonhistone proteins to form chromatin (see p. 473). Transcriptionally active, decondensed chromatin (euchromatin) differs from the more condensed, inactive form (heterochromatin) in a number of ways. Active chromatin contains histone proteins that have been covalently modified at their amino terminal ends by reversible methylation, acetylation, or phosphorylation (see p. 487 for a discussion of histone acetylation/deacetylation by histone acetyltransferase and histone deacetylase). Such modifications decrease the positive charge of these basic proteins, thereby decreasing the strength of their association with negatively charged DNA. This relaxes the nucleosome (see p. 473), allowing transcription factors access to specific regions on the DNA. Nucleosomes can also be repositioned, an ATP-requiring process that is part of chromatin remodeling. Another difference between transcriptionally active and inactive chromatin is the extent of methylation of C bases in CG-rich regions (CpG islands) in the promoter region of many genes. Methylation is by methyltransferases that use S-adenosylmethionine as the methyl donor (Fig. 33.17). Transcriptionally active genes are less methylated (hypomethylated) than their inactive counterparts, suggesting that DNA hypermethylation silences gene expression. Modification of histones and methylation of DNA are epigenetic in that they are heritable changes in DNA that alter gene expression without altering the base sequence.

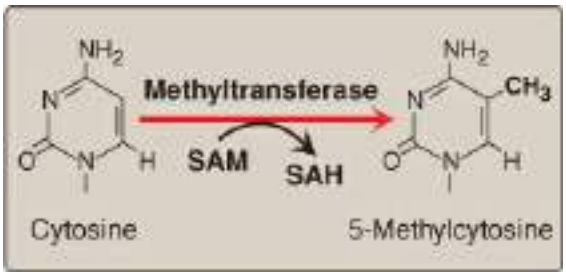


Figure 33.17
The methylation of cytosine in eukaryotic DNA. SAM = S-adenosylmethionine; SAH = S-adenosylhomocysteine.

2. Gene copy number: A change up or down in the number of copies of a gene can affect the amount of gene product produced. An increase in copy number (gene amplification) has contributed to increased genomic complexity and is still a normal developmental process in certain nonmammalian species. In mammals,

however, gene amplification is associated with some diseases and is involved in the mechanism by which cells develop resistance to particular chemotherapeutic drugs. One example is methotrexate, an inhibitor of the enzyme dihydrofolate reductase (DHFR), required for the synthesis of thymidine triphosphate (TTP) in the pyrimidine biosynthetic pathway (see p. 336 and Figure 28.2). TTP is essential for DNA synthesis. The amplification of the DHFR gene results in the expression of more DHFR enzyme, which enables the cells exposed to methotrexate to survive because TTP production can continue in the presence of the drug.

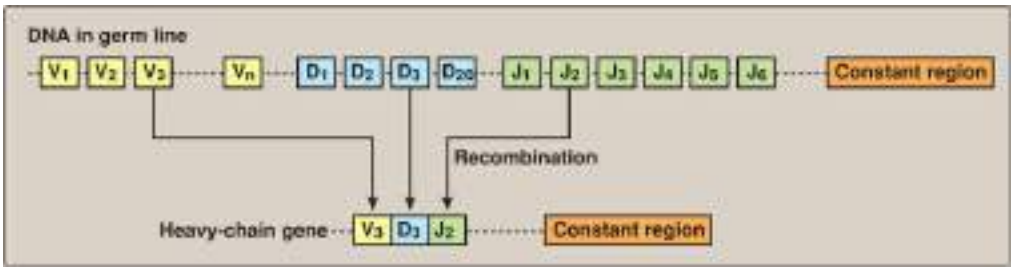


Figure 33.18
DNA rearrangements in the generation of immunoglobulins. V = variable; D = diversity; J = joining.

3. Arrangement of DNA: The process by which immunoglobulins (antibodies) are produced by B lymphocytes involves permanent rearrangements of the DNA in these cells. The immunoglobulins (e.g., IgG) consist of two light and two heavy chains, with each chain containing regions of variable and constant amino acid sequence. The variable region is the result of somatic recombination of segments within both the light- and the heavy-chain genes. During B-lymphocyte development, single variable (V), diversity (D), and joining (J) gene segments are randomly selected and brought together through gene rearrangement to form a unique variable region (Fig. 33.18). This process allows the generation of 10^9 to 10^{11} different immunoglobulins from a single gene, providing the diversity needed for the recognition of an enormous number of antigens. (Note: Pathologic DNA rearrangement is seen with translocation, a process by which two different chromosomes exchange DNA segments.)

4. Mobile DNA elements: Transposons (Tns) are mobile segments of DNA that move in an essentially random manner from one site to another on the same or a different chromosome. Movement is mediated by transposase, an enzyme encoded by the Tn itself. Movement can be direct, in which transposase cuts out and then inserts the Tn at a new site, or replicative, in which the Tn is copied and the copy inserted elsewhere while the original remains in place. In eukaryotes, including humans, replicative transposition frequently involves an RNA intermediate made by a reverse transcriptase (see p. 472), in which case the Tn is called a retrotransposon. Transposition has contributed to structural variation in the genome but also has the potential to alter gene expression and

even to cause disease. Tns comprise ~50% of the human genome, with retrotransposons accounting for 90% of Tns. Although the vast majority of these retrotransposons have lost the ability to move, some are still active. Their transposition is thought to be the basis for some rare cases of hemophilia A and Duchenne muscular dystrophy. (Note: The growing problem of antibiotic-resistant bacteria is a consequence, at least in part, of the exchange of plasmids among bacterial cells. If the plasmids contain Tn-carrying antibiotic resistance genes, then these genes can move from the plasmid to the bacterial chromosome so that the bacterium is resistant to one or more antimicrobial drugs even if the plasmid is lost from the cell.)

V. Chapter Summary

- **Gene expression** produces a functional gene product (either RNA or protein).
- **Genes** can be either **constitutive** (always expressed) or **regulated** (expressed only under certain conditions).
- Regulation of gene expression occurs primarily at **transcription** in both prokaryotes and eukaryotes and is mediated through **trans-acting proteins** binding to **cis-acting regulatory DNA elements** (Fig. 33.19).
- In **eukaryotes**, regulation also occurs through DNA **modifications** and through **posttranscriptional** and **posttranslational processing**.
- In **prokaryotes**, the coordinate regulation of genes whose protein products are required for a particular process is achieved through **operons** (groups of functionally related genes sequentially arranged on the chromosome along with the regulatory elements that determine their transcription). Examples from *E. coli* are the ***lac* operon** containing the *Z, Y*, and *A* structural genes involved in the catabolism of lactose, and the ***trp* operon,** which contains genes needed for the synthesis of Trp. The trp operon is also regulated by attenuation, in which mRNA synthesis that escaped repression by Trp is terminated before completion.
- In prokaryotes, transcription of **rRNA** and **tRNA** is selectively inhibited by the **stringent response** to amino acid starvation. **Translation** is also a site of prokaryotic gene regulation: Excess r-proteins bind the **SD sequence** on their own polycistronic mRNA, preventing ribosomes from binding.
- In eukaryotes, hormones coordinate the expression of groups of genes by binding to an intracellular receptor that acts as a trans-acting protein (as with steroid hormones) or to a cell surface receptor that initiates **second messenger** signaling to activate a trans-acting protein (as with peptide hormones). In each case, the protein recognizes a specific response element and binds to the DNA sequence using structural motifs such as a **zinc finger** or a **leucine zipper**.
- **Co-** and **posttranscriptional regulation** is also seen in eukaryotes and includes **alternative mRNA splicing** and **polyadenylation**, mRNA **editing**, and variations in mRNA **stability. Transferrin receptor** synthesis is enhanced by mRNA stability when iron concentrations are low. **RNA interference** is used to control mRNA stability and translation and is the basis for a new class of therapeutic agents.
- Regulation at the **translational level** can be caused by the **phosphorylation** and inhibition of **eukaryotic initiation factor-2**. Gene expression in eukaryotes is also influenced by **accessibility** of DNA to the transcriptional apparatus (as seen with **epigenetic** changes to histone proteins), the gene copy number, and the **arrangement** of the DNA.

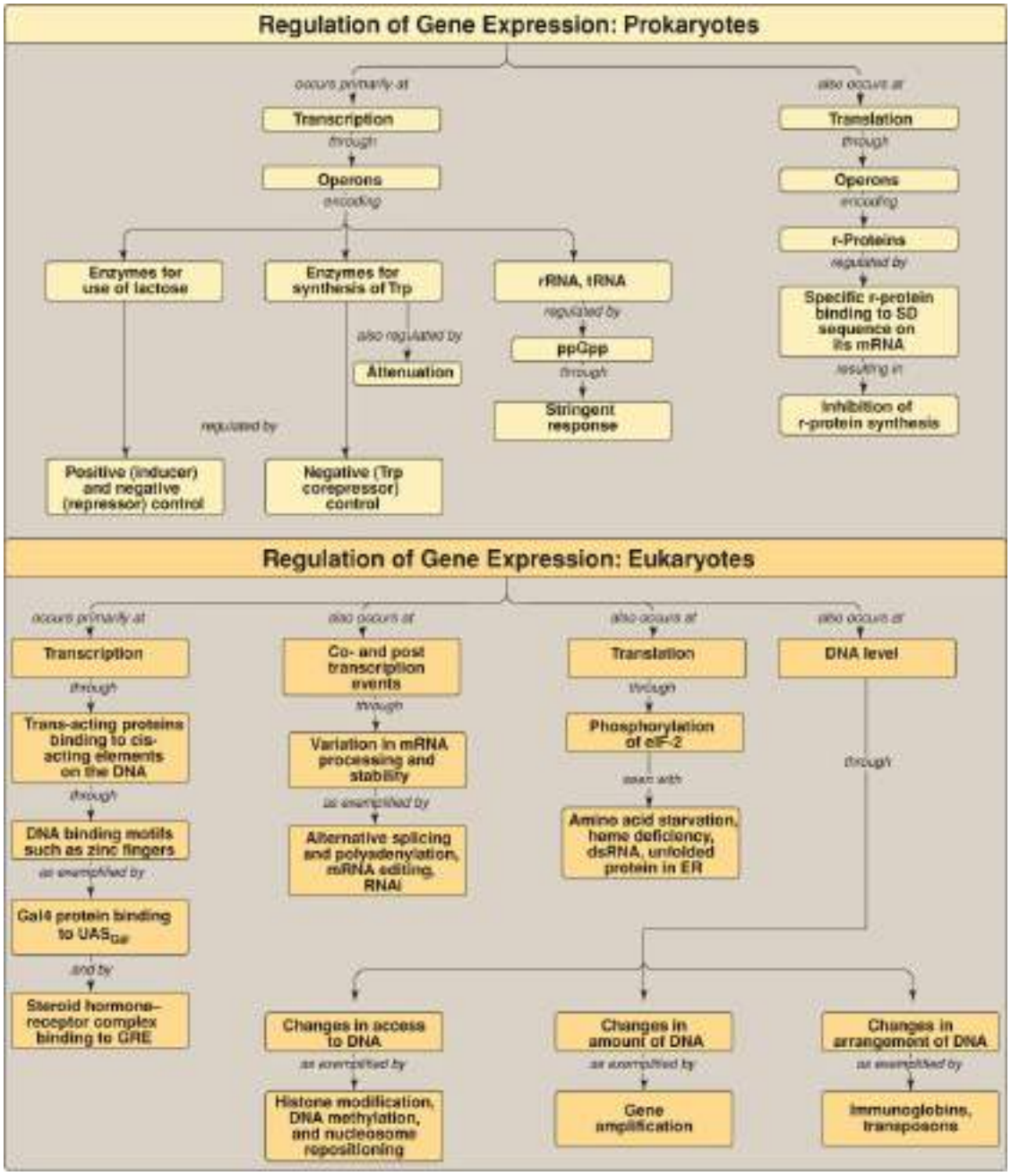


Figure 33.19
Summary of key concepts for the regulation of gene expression. Trp = tryptophan; rRNA, tRNA, mRNA = ribosomal, transfer, and messenger RNA, respectively; ppGpp = guanosine tetraphosphate; r-protein = ribosomal protein; SD = Shine–Dalgarno; Gal = galactose; UAS = upstream activating sequence; GRE = glucocorticoid response element; RNAi = RNA interference; eIF = eukaryotic initiation factor; ds = double stranded; ER = endoplasmic reticulum.

Study Questions

Choose the ONE best answer.

33.1 Which of the following mutations is most likely to result in reduced expression of the *lac* operon?

A. cya^{-} (no adenylyl cyclase made)
B. i^{-} (no repressor protein made)
C. O^{c} (operator cannot bind repressor protein)
D. One resulting in impaired glucose uptake
E. relA^{-} (no stringent response occurs)

Correct answer = A. In the absence of glucose, adenylyl cyclase makes cyclic adenosine monophosphate (cAMP), which forms a complex with the catabolite activator protein (CAP). The cAMP–CAP complex binds the CAP site on the DNA, causing RNA polymerase to bind more efficiently to the *lac* operon promoter, thereby increasing expression of the operon. With cya^{-} mutations, adenylyl cyclase is not made, and so the operon is unable to be maximally expressed even when glucose is absent and lactose is present. The absence of a repressor protein or decreased ability of the repressor to bind the operator results in constitutive (essentially constant) expression of the *lac* operon.

33.2 Which of the following is best described as cis-acting?

A. Cyclic adenosine monophosphate response element–binding protein
B. Operator
C. Repressor protein
D. Thyroid hormone nuclear receptor
E. Histone modification

Correct answer = B. The operator is part of the DNA itself, and so is cis-acting. The cyclic adenosine monophosphate response element–binding protein, repressor protein, and thyroid hormone nuclear receptor protein are molecules that diffuse (transit) to the DNA, bind, and affect the expression of that DNA and so are trans-acting.

33.3 Which of the following is the basis for the intestine-specific expression of apolipoprotein B-48?

A. DNA rearrangement and loss
B. DNA transposition
C. RNA alternative splicing
D. RNA editing
E. RNA interference

Correct answer = D. The production of apolipoprotein (apo) B-48 in the intestine and apo B-100 in liver is the result of RNA editing in the intestine, where a sense codon is changed to a nonsense codon by posttranscriptional deamination of cytosine to uracil. DNA rearrangement and transposition, as well as RNA interference and alternative splicing, do alter gene expression but are not the basis of apo B-48 tissue-specific production.

33.4 Which of the following is a likely consequence of the increased iron accumulation seen in patients with the disease hemochromatosis?

A. The messenger RNA for the transferrin receptor is stabilized by the binding of iron regulatory proteins to its 3′–iron-responsive elements.
B. The messenger RNA for the transferrin receptor is not bound by iron regulatory proteins and is degraded.
C. The messenger RNA for ferritin is not bound by iron regulatory proteins at its 5′–iron-responsive element and is translated.
D. The messenger RNA for ferritin is bound by iron regulatory proteins and is not translated.
E. Both B and C are correct.

Correct answer = E. When iron levels in the body are high, as is seen with hemochromatosis, there is increased synthesis of the iron-storage molecule, ferritin, and decreased synthesis of the transferrin receptor (TfR) that mediates iron uptake by cells. These effects are the result of cis-acting iron-responsive elements not being bound by trans-acting iron regulatory proteins, resulting in degradation of the messenger RNA (mRNA) for TfR and

increased translation of the mRNA for ferritin.

33.5 Patients with estrogen receptor–positive (hormone responsive) breast cancer may be treated with the drug tamoxifen, which binds the estrogen nuclear receptor without activating it. Which of the following is the most logical outcome of tamoxifen use?

A. Increased acetylation of estrogen-responsive genes
B. Increased growth of estrogen receptor–positive breast cancer cells
C. Increased production of cyclic adenosine monophosphate
D. Inhibition of the estrogen operon
E. Inhibition of transcription of estrogen-responsive genes

Correct answer = E. Tamoxifen competes with estrogen for binding to the estrogen nuclear receptor. Tamoxifen fails to activate the receptor, preventing its binding to DNA sequences that upregulate expression of estrogen-responsive genes. Tamoxifen, then, blocks the growth-promoting effects of these genes and results in growth inhibition of estrogen-dependent breast cancer cells. Acetylation increases transcription by relaxing the nucleosome. Cyclic adenosine monophosphate is a regulatory signal mediated by cell-surface rather than nuclear receptors. Mammalian cells do not have operons.

33.6 The *ZYA* region of the *lac* operon will be maximally expressed if:

A. cyclic adenosine monophosphate levels are low.
B. glucose and lactose are both available.
C. the attenuation stemloop is able to form.
D. the CAP site is occupied.
E. the Shine–Dalgarno sequence is not accessible.

Correct answer = D. It is only when glucose is gone, cyclic adenosine monophosphate (cAMP) levels are increased, the cAMP–catabolite activator protein (CAP) complex is bound to the CAP site, and lactose is available that the operon is maximally expressed (induced). If glucose is present, the operon is off as a result of catabolite repression. The *lac* operon is not regulated by attenuation, a mechanism for stopping transcription in some operons such as the *trp* operon.

33.7 X chromosome inactivation is a process by which one of two X chromosomes in mammalian females is condensed and inactivated to prevent overexpression of X-linked genes. What would most likely be true about the degree of DNA methylation and histone acetylation on the inactivated X chromosome?

Cytosines in CpG islands would be hypermethylated, and histone proteins would be deacetylated. Both conditions are associated with decreased gene expression, and both are important in maintaining X inactivation.

Biotechnology and Human Disease 34

I. OVERVIEW

In the past, efforts to understand genes and their expression have been confounded by the immense size and complexity of human deoxyribonucleic acid (DNA). The human genome contains ~3 billion (10^9) base pairs (bps) that encode 20,000 to 25,000 protein-coding genes located on 23 chromosomes in the haploid genome. It is now possible to determine the nucleotide sequence of long stretches of DNA, and the entire human genome has been sequenced. This effort (called the Human Genome Project and completed in 2003) was made possible by several tools that have already contributed to our understanding of many genetic diseases (Fig. 34.1). These tools include (1) restriction endonucleases that permit the cleavage of huge DNA molecules into defined fragments, (2) cloning techniques that provide a mechanism for amplification of specific nucleotide sequences, and (3) the ability to synthesize specific probes, which has allowed the identification and manipulation of nucleotide sequences of interest. These and other experimental approaches have permitted the identification of both normal and mutant nucleotide sequences in DNA. This knowledge has led to the development of methods for the diagnosis of genetic diseases and some successes in the treatment of patients by gene therapy. (Note: The genomes of several viruses, prokaryotes, and nonhuman eukaryotes have also been sequenced.)

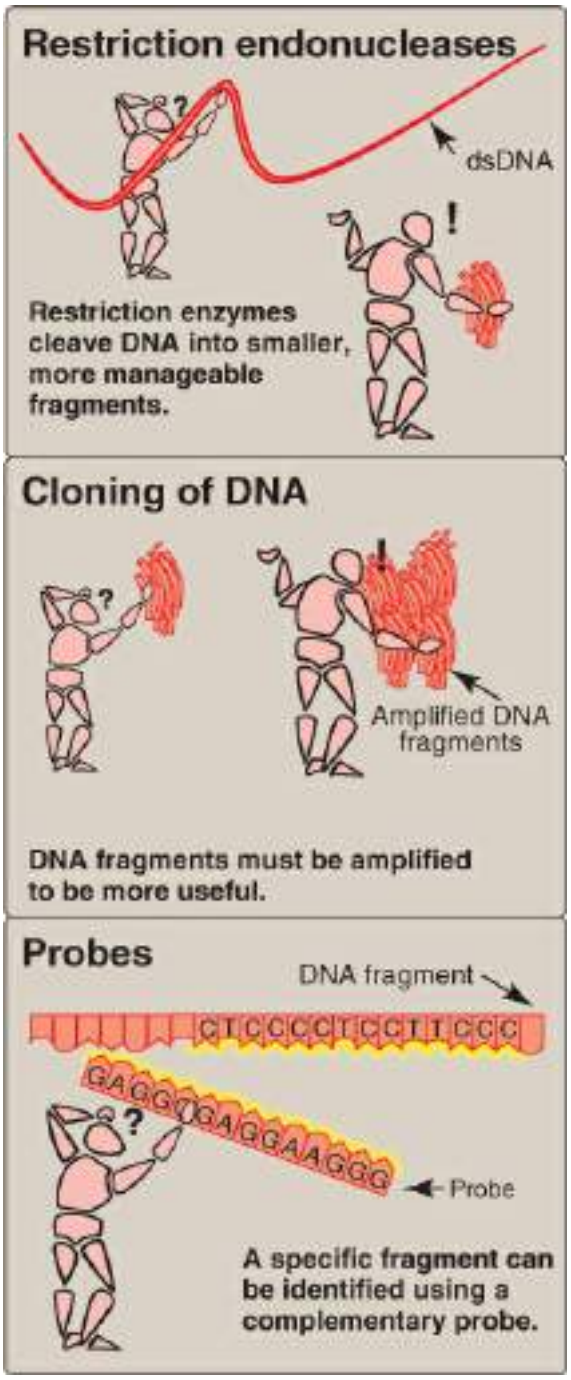


Figure 34.1
Three tools that facilitate analysis of human DNA. dsDNA = double-stranded DNA.

II. RESTRICTION ENDONUCLEASES

One of the major obstacles to molecular analysis of genomic DNA is the immense size of the molecules involved. The discovery of a special group of bacterial enzymes, called restriction endonucleases (restriction enzymes), which cleave double-stranded DNA

(dsDNA) into smaller, more manageable fragments, opened the way for DNA analysis. Because each enzyme cleaves dsDNA at a specific nucleotide sequence (restriction site), restriction enzymes are used experimentally to obtain precisely defined DNA segments called restriction fragments.

A. Specificity

Restriction endonucleases recognize restriction sites, which are short stretches of dsDNA (4 to 8 bps) that contain specific nucleotide sequences. These sequences, which differ for each restriction enzyme, are palindromes, that is, they exhibit twofold rotational symmetry (Fig. 34.2). This means that, within the restriction site, the nucleotide sequence on the two strands of DNA is identical if each is read in the 5′→3′ direction. Therefore, if you turn the page upside down (i.e., rotate it 180 degrees around its axis of symmetry) the sequence remains the same.

> In bacteria, restriction endonucleases limit (restrict) the expression of nonbacterial (foreign) DNA through cleavage. Bacterial DNA is methylated at adenine bases, which protects the DNA from being recognized and cleaved by the endonucleases at their restriction site sequences.

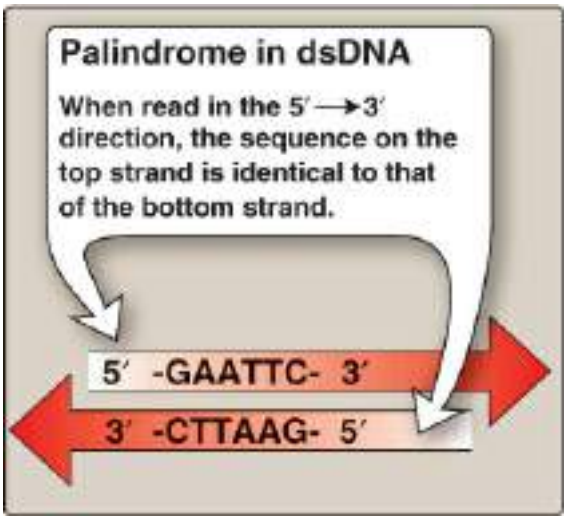


Figure 34.2
Recognition sequence of restriction endonuclease EcoRI shows twofold rotational symmetry. dsDNA = double-stranded DNA; A = adenine; C = cytosine; G = guanine; T = thymine.

B. Nomenclature

A restriction enzyme is named according to the organism from which it was isolated. The first letter of the name is from the genus of the bacterium. The next two letters are from the name of the species. An additional letter indicates the type or strain (as needed), and a number (Roman numeral) is appended to indicate the order in which the enzyme was discovered in that particular organism. For example, HaeIII is the third restriction endonuclease isolated from the bacterium *Haemophilus aegyptius*.

C. Sticky and blunt ends

Restriction enzymes cleave dsDNA so as to produce a 3′-hydroxyl group on one end and a 5′-phosphate group on the other. Some restriction endonucleases, such as TaqI, form staggered cuts that produce sticky or cohesive ends (i.e., the resulting DNA fragments have single-stranded regions that are complementary to each other), as shown in Figure 34.3. Other restriction endonucleases, such as HaeIII, produce fragments that have blunt ends that are entirely double stranded and, therefore, do not form hydrogen bonds with each other. Using the enzyme DNA ligase (see p. 466), sticky ends of a DNA fragment of interest can be covalently joined with other DNA fragments that have sticky ends produced by cleavage with the same restriction endonuclease (Fig. 34.4). (Note: A ligase encoded by bacteriophage T4 can covalently join blunt-ended fragments.)

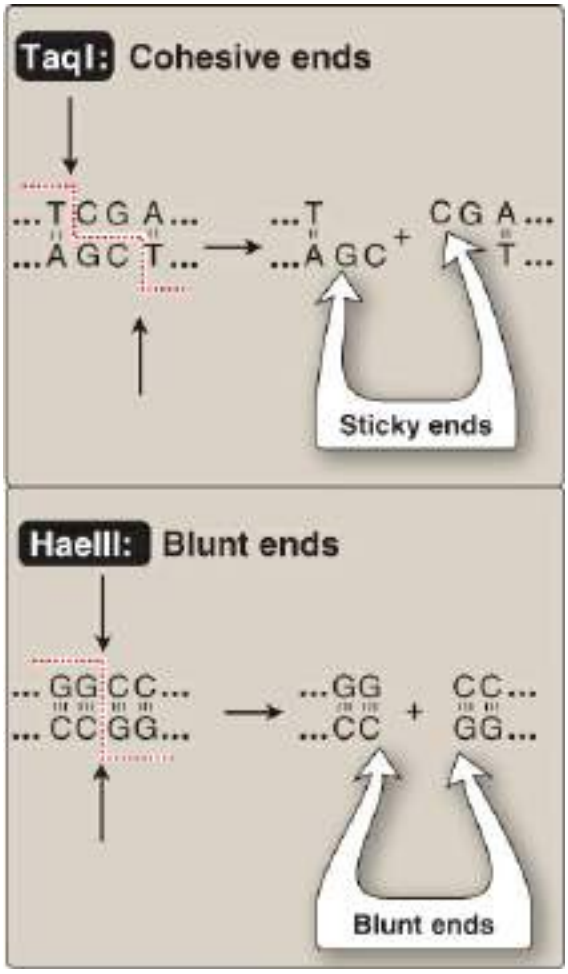


Figure 34.3
Specificity of TaqI and HaeIII restriction endonucleases. A = adenine; C = cytosine; G = guanine; T = thymine.

D. Restriction fragments

Restriction endonucleases cleave dsDNA into fragments of different sizes (restriction fragments) depending upon the size of the restriction site sequence. For example, an enzyme that recognizes a specific 4-bp sequence produces many cuts in the DNA molecule, one every 4^4 bps. In contrast, an enzyme requiring a unique sequence of 6 bps makes fewer cuts (one every 4^6 bps) and, therefore, produces longer pieces of DNA. Hundreds of these enzymes, each having different cleavage specificities (varying in both nucleotide sequences and length of recognition sites) are commercially available.

III. DNA CLONING

Introduction of a foreign DNA molecule into a replicating cell permits the cloning or, amplification (the production of many identical copies) of that DNA. In some cases, a single DNA fragment can be isolated and purified prior to cloning. More commonly, to clone a nucleotide sequence of interest, the total cellular DNA is first cleaved with a specific restriction enzyme, creating hundreds of thousands of fragments. Each of the resulting DNA fragments is joined to a DNA vector molecule (referred to as a cloning vector) to form a hybrid, or recombinant, DNA molecule. Each recombinant molecule is introduced into a single host cell (e.g., a bacterium), where it is replicated. (Note: The process of introducing foreign DNA into a cell is called transformation for bacteria and yeast and transfection for higher eukaryotes.) As the host cell multiplies, it creates a colony of cells in which every bacterium contains copies of the same inserted DNA fragment and is a “clone” of the original cell. The recombinant molecules can be released from the host cells by disruption of the cell membranes. The cloned DNA fragments can be cleaved from their vectors using the appropriate restriction endonuclease and then isolated by purification techniques. By this mechanism, many identical copies of the DNA of interest can be produced. An alternative to amplification by biologic cloning, the polymerase chain reaction (PCR), is described in Section VII. PCR is the preferred amplification technique in medicine when genetic analysis is needed to detect a prenatal disorder or to diagnose a patient with an inherited disease. Human DNA for cloning or PCR amplification can be obtained from blood, saliva, and solid tissue.

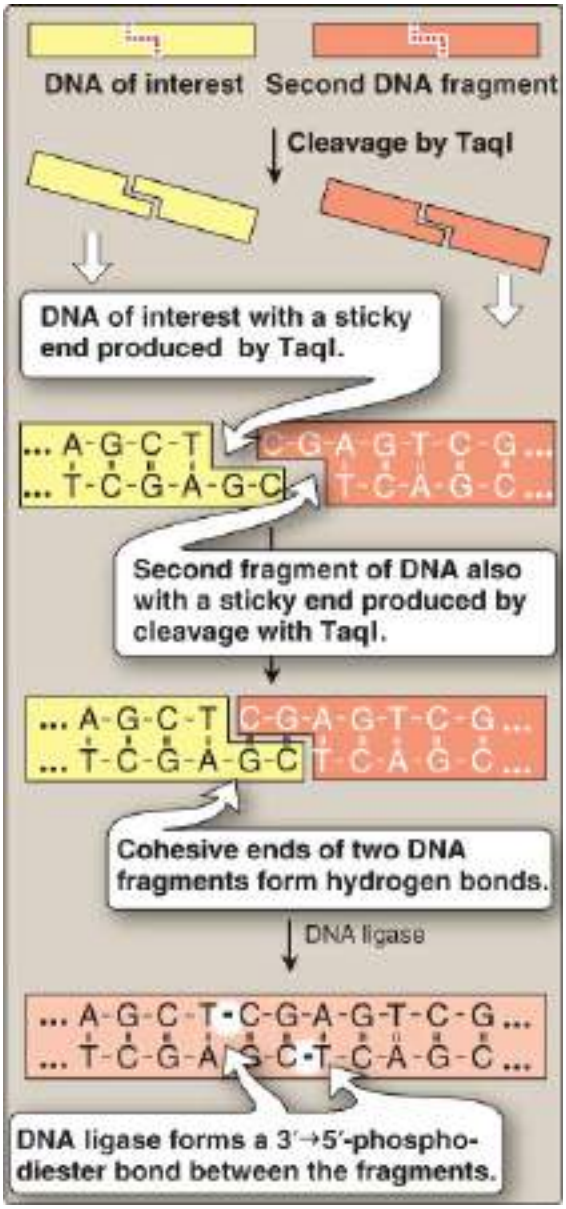


Figure 34.4
Formation of recombinant DNA from restriction fragments with sticky ends. A = adenine; C = cytosine; G = guanine; T = thymine.

A. Vectors

A vector is a molecule of DNA to which the fragment of DNA to be cloned is joined. Essential properties of a vector include the (1) capacity for autonomous replication within a host cell, (2) presence of at least one specific nucleotide sequence recognized by a restriction endonuclease, and (3) presence of at least one gene (such as an antibiotic resistance gene) that confers survival to the host cells and allows for host cell selection. Commonly used vectors include plasmids and viruses. If a viral vector is used to deliver DNA into human cells, the process of introducing the DNA into the cells is called transduction.

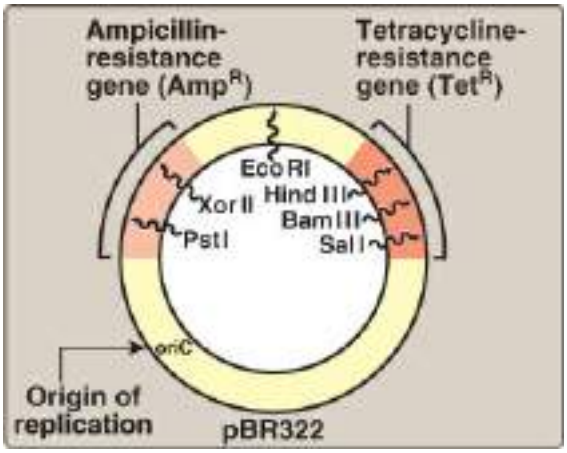


Figure 34.5
A partial map of pBR322 indicating the positions of its antibiotic resistance genes and 6 of the >40 unique sites recognized by specific restriction endonucleases. p = plasmid.

1. Prokaryotic plasmids: Prokaryotic organisms typically contain single, large, circular chromosomes. In addition, most species of bacteria also normally contain small, circular, extrachromosomal DNA molecules called plasmids (Fig. 34.5). Plasmid DNA undergoes replication that may or may not be synchronized to chromosomal division. Plasmids may carry genes that convey antibiotic resistance to the host bacterium and may facilitate the transfer of genetic information from one bacterium to another. They can be readily isolated from bacterial cells and can be cleaved at specific sites by restriction endonucleases to allow for the insertion of up to 15 kilobases (kb) of foreign DNA (cut with the same restriction enzyme). The recombinant DNA molecule (hybrid plasmid) can be introduced into a bacterium, which can survive growth challenges, multiply, and produce numerous copies of the plasmid. If the plasmid vector provides antibiotic resistance, the bacteria will grow in the presence of antibiotics, thus selecting for cells containing the hybrid plasmids (Fig. 34.6). Artificial plasmids are routinely constructed. An example is the classic pBR322 (see Fig. 34.5), which contains an origin of replication, two antibiotic resistance genes, and >40 unique restriction sites. Use of plasmids is limited by the size of the DNA that can be inserted.

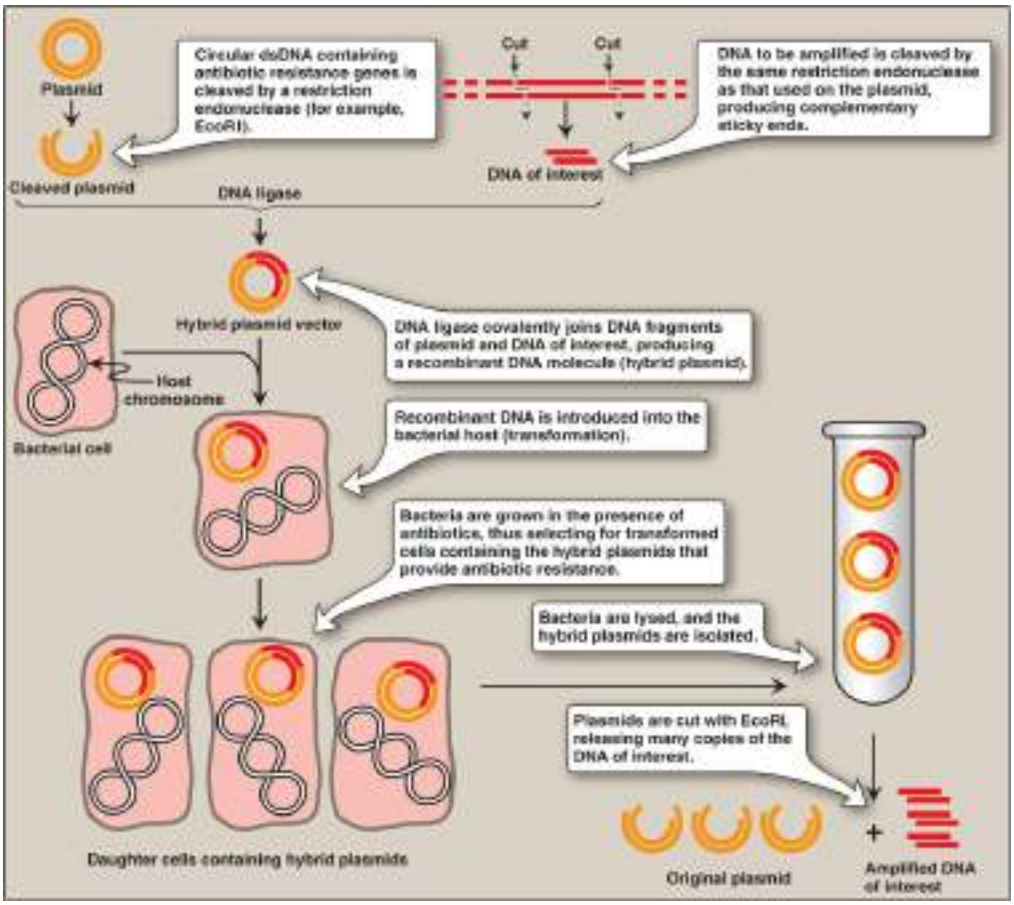


Figure 34.6
Summary of biologic gene cloning. (Note: Transformation is inefficient in that only a small percentage of the cells will contain the recombinant plasmid.) dsDNA = double-stranded DNA.

2. Other vectors: The development of improved vectors that can more efficiently accommodate larger DNA segments, or that can express genes from inserted DNA sequences in different cell types (expression vectors), has aided molecular genetics' research and therapeutics. In addition to the prokaryotic plasmids described above, naturally occurring viruses that infect bacteria (bacteriophage λ, for example) or mammalian cells (retroviruses, for example), as well as artificial constructs such as cosmids and bacterial or yeast artificial chromosomes (BAC or YAC, respectively), are currently used as cloning vectors. (Note: BAC and YAC can accept DNA inserts of 100 to 300 kb and 250 to 1,000 kb, respectively.)

B. DNA libraries

A DNA library is a collection of cloned restriction fragments of the DNA of an organism. Two kinds of libraries are commonly used: genomic libraries and complementary DNA (cDNA) libraries. Genomic libraries ideally contain a copy of every DNA nucleotide sequence in the genome. In contrast, cDNA libraries contain those DNA sequences that only appear as processed messenger RNA (mRNA)

molecules, and these differ according to cell type and environmental conditions. This means that cDNA lacks introns and the control regions of genes, which are present in genomic DNA.

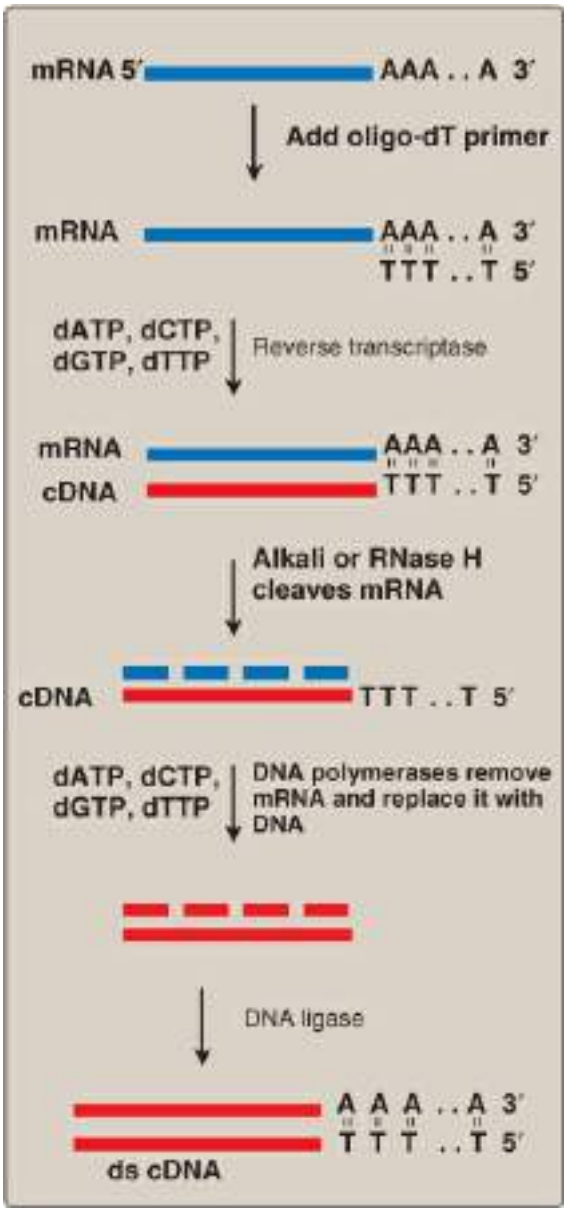


Figure 34.7
Synthesis of complementary DNA (cDNA) from messenger RNA (mRNA) using reverse transcriptase. Ligation of double-stranded (ds) DNA sequences containing a restriction site to each end allows biologic cloning of cDNA. (Note: DNA is resistant to alkaline hydrolysis.) dATP, dCTP, dGTP, dTTP = deoxyadenosine, deoxycytidine, deoxyguanosine, and deoxythymidine triphosphates.

1. Genomic DNA libraries: A genomic library is created by digestion of the total DNA of an organism with a restriction endonuclease and subsequent ligation of the restriction fragments into an appropriate vector. The recombinant DNA

molecules are replicated by the host bacteria. Thus, the amplified DNA fragments collectively represent the entire genome of the organism and are called a genomic library. Regardless of the restriction enzyme used, the chances are good that the gene of interest contains more than one restriction site recognized by that enzyme. If this is the case, and if the digestion is allowed to go to completion, the gene of interest is fragmented (i.e., it is not contained in any one clone in the library). To avoid this usually undesirable result, a partial digestion is performed in which either the amount or the time of action of the enzyme is limited. This results in cleavage occurring at only a fraction of the restriction sites on any one DNA molecule, thus producing fragments of ~20 kb. Enzymes that cut very frequently (i.e., those that recognize 4-bp sequences) are generally used for this purpose so that the result is an almost random collection of fragments. This ensures a high degree of probability that the gene of interest is contained, intact, in some fragment.

2. Complementary DNA libraries: If a protein-coding gene of interest is expressed at a high level in a particular tissue, the mRNA transcribed from that gene is likely also present at high concentrations in the cells of that tissue. For example, reticulocyte mRNA is composed largely of molecules that code for the α-globin and β-globin chains of hemoglobin A (HbA). This mRNA can be used as a template to make a cDNA molecule using the enzyme reverse transcriptase (Fig. 34.7). Therefore, the resulting cDNA is a double-stranded copy of mRNA. (Note: The template mRNA is isolated from transfer RNA and ribosomal RNA by the presence of its poly-A tail.) cDNA can be amplified by biologic cloning or by PCR. It can be used as a probe to locate the gene that encodes the original mRNA (or fragments of the gene) in mixtures containing many unrelated DNA fragments. If the mRNA used as a template is a mixture of many different species, the resulting cDNA is heterogeneous. These mixtures can be cloned to form a cDNA library. Because cDNA lacks introns, it can be cloned into an expression vector for the synthesis of eukaryotic proteins by bacteria (Fig. 34.8). These special plasmids contain a bacterial promoter for transcription of the cDNA and a Shine–Dalgarno (SD) sequence (see p. 503) that allows the bacterial ribosome to initiate translation of the resulting mRNA molecule. The cDNA is inserted downstream of the promoter and within a gene for a protein that is expressed in the bacterium (e.g., *lacZ*; see p. 516), such that the mRNA produced contains an SD sequence, a few codons for the bacterial protein, and all of the codons for the eukaryotic protein. This allows for more efficient expression and results in the production of a fusion protein. (Note: Therapeutic human insulin is made in bacteria through this technology. However, the extensive co- and posttranslational modifications required for most other human proteins [e.g., blood clotting factors] necessitates the use of eukaryotic, even mammalian, hosts.)

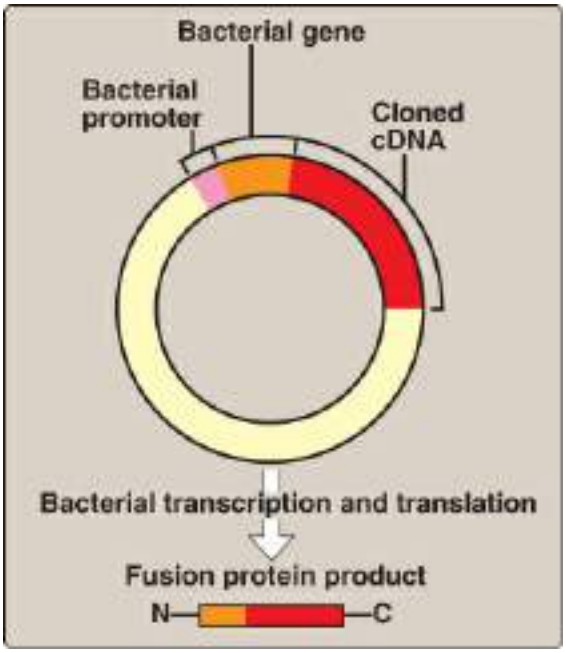


Figure 34.8
An expression vector. The product is a fusion protein that contains some amino acids of the bacterial protein ■ and all the amino acids of the complementary DNA (cDNA)-encoded protein ■. (Note: Proteins are written from the amino [N]-terminus to the carboxy [C]-terminus.)

C. Sequencing cloned DNA fragments

The base sequence of DNA fragments that have been cloned can be determined. The original procedure for this purpose was the Sanger dideoxynucleotide chain termination method illustrated in Figure 34.9. In this method, the single-stranded DNA (ssDNA) to be sequenced is used as the template for DNA synthesis by DNA polymerase (DNA pol). A radiolabeled primer complementary to the 3′ end of the target DNA is added to the sample, along with the four deoxyribonucleoside triphosphates (dNTPs). The sample is divided into four reaction tubes, and a small amount of one of the four dideoxyribonucleoside triphosphates (ddNTPs) is added to each tube. Because it contains no 3′-hydroxyl group, incorporation of a ddNMP terminates elongation at that point. The products of this reaction, then, consist of a mixture of DNA strands of different lengths, each terminating at a specific base. Separation of the various DNA products by size in an electric field using polyacrylamide gel electrophoresis, followed by autoradiography, yields a pattern of bands from which the DNA base sequence can be read. The shorter the fragment, the farther it travels on the gel, with the shortest fragment representing that which was made first (i.e., the 5′ end). In a more modern approach to the Sanger method, the four ddNTPs, each linked to a different fluorescent dye, are mixed with the ssDNA in a single reaction tube. The sample is separated by capillary electrophoresis, the fluorescent labels are detected, and a color readout of the sequence is generated (Fig. 34.10). The Human Genome Project, requiring nearly 13 years and finishing in 2003, used variations of this technique to sequence the

human genome. Advances in sequencing technology, so-called next generation, or high-throughput sequencing, now allow the rapid sequencing of an entire genome with increased fidelity and decreased cost through the simultaneous (parallel) sequencing of many DNA pieces. Today, selective sequencing of the exome, that portion of a genome that encodes proteins, is possible.

IV. PROBES

Cleavage of large DNA molecules by restriction enzymes produces an enormous array of fragments. How can the DNA sequence of interest be picked out of such a mixture? The answer lies in the use of a probe, a short piece of ssDNA or RNA, labeled with a radioisotope, such as ^{32}P, or with a nonradioactive molecule, such as biotin or a fluorescent dye. The sequence of a probe is complementary to a sequence in the DNA of interest, called the target DNA. Probes are used in a process called screening to identify which band on a gel or which clone in a library contains the target DNA.

A. Hybridization to DNA

The utility of probes hinges on the process of hybridization (or annealing) in which a probe containing a complementary sequence binds a single-stranded sequence of a target DNA. ssDNA, produced by alkaline denaturation of dsDNA, is first bound to a solid support, such as a nitrocellulose membrane. The immobilized DNA strands are prevented from self-annealing but are available for hybridization to the exogenous, radiolabeled, single-stranded probe. The extent of hybridization is measured by the retention of radioactivity on the membrane. Excess probe molecules that do not hybridize are removed by washing the membrane.

B. Synthetic oligonucleotide probes

If the sequence of all or part of the target DNA is known, short, single-stranded oligonucleotide probes can be synthesized that are complementary to a small region of the gene of interest. If the sequence of the gene is unknown, the amino acid sequence of the protein, the final gene product, may be used to construct a nucleic acid probe using the genetic code as a guide. Because of the degeneracy of the genetic code (see p. 498), it is necessary to synthesize several oligonucleotides. In contrast, cDNA probes contain many thousands of bases, and their binding to a target DNA with a single-base change is unaffected.

1. Detecting the βS-globin mutation: Oligonucleotides can be used to detect single-base changes in the sequence to which they are complementary. For example, a synthetic allele-specific oligonucleotide (ASO) probe can be used to detect the presence of the sickle cell mutation in the β-globin gene (Fig. 34.11). DNA, isolated from white blood cells (WBC) and amplified, is denatured and applied to a membrane. A radiolabeled oligonucleotide probe, complementary to the point mutation (GAG → GTG) at codon 6 in patients with the β^S gene, is

applied to the membrane. DNA isolated from a heterozygous individual (sickle cell trait) or a homozygous patient (sickle cell anemia) contains a sequence that is complementary to the probe and a double-stranded hybrid form can be detected. In contrast, DNA obtained from individuals not affected by sickle cell is not complementary at this position and, therefore, does not form a hybrid with the probe (see Fig. 34.11). Use of a pair of such ASO probes (one specific for the normal allele and one specific for the mutant allele) allows all three possible genotypes (homozygous normal, heterozygous, and homozygous mutant) to be distinguished (Fig. 34.12). (Note: ASO probes are useful only if the mutation and its location are known.)

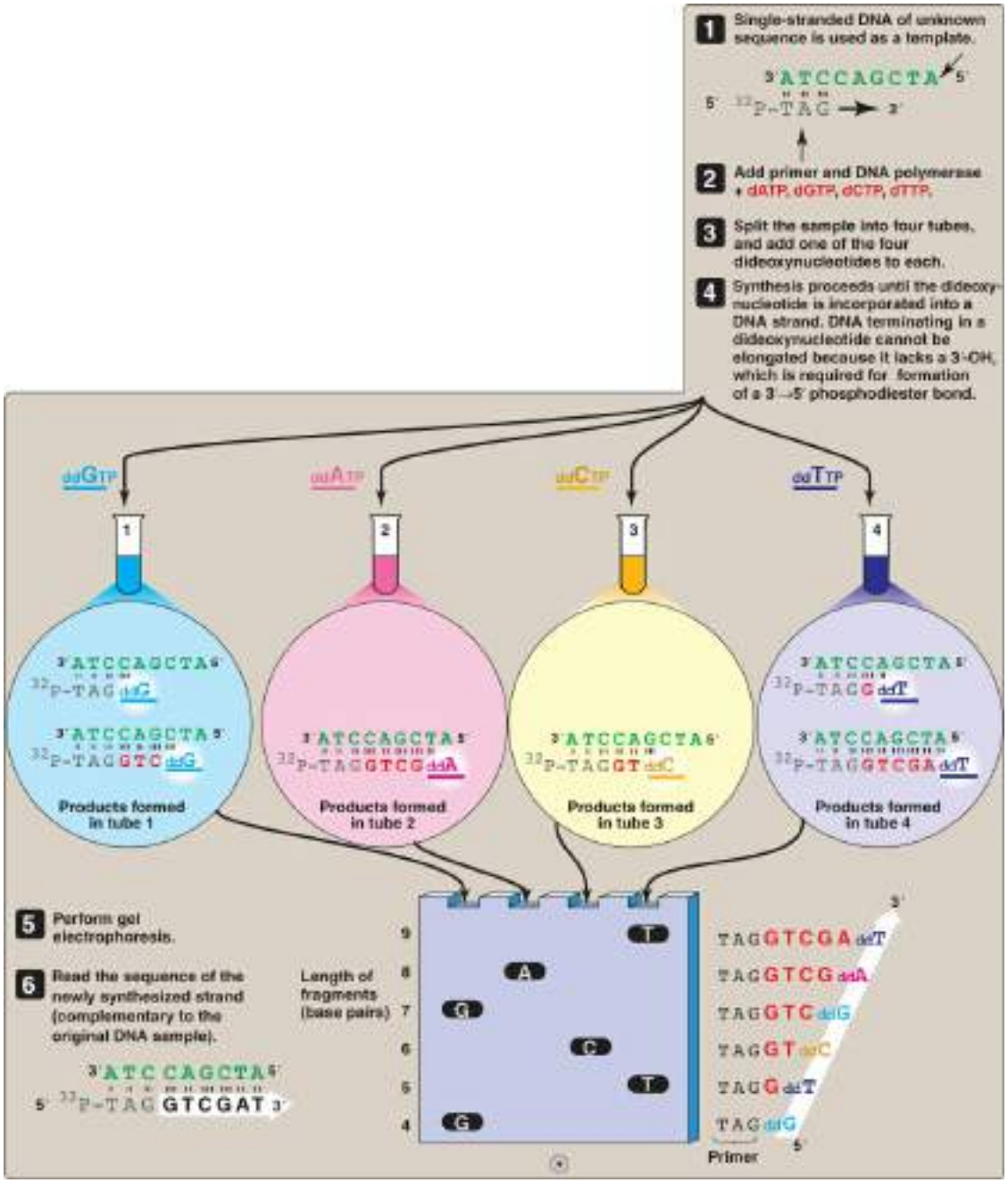


Figure 34.9
DNA sequencing by the Sanger dideoxynucleotide method. (Note: The original method utilized a radiolabeled primer. Fluorescent dye-labeled dideoxyribonucleoside triphosphates are now commonly used.) A = adenine; C = cytosine; G = guanine; T = thymine; d = deoxy; dd = dideoxy.

C. Biotinylated probes

Because the disposal of radioactive waste is becoming increasingly expensive, nonradiolabeled probes have been developed. One of the most successful is based on the vitamin biotin (see p. 431), which can be chemically linked to the nucleotides used to synthesize the probe. Biotin was chosen because it binds very tenaciously

to avidin, a readily available protein contained in chicken egg whites. Avidin can be attached to a fluorescent dye that is detectable optically with great sensitivity. Thus, a DNA fragment (displayed, for example, by gel electrophoresis) that hybridizes with the biotinylated probe can be made visible by immersing the gel in a solution of dye-coupled avidin. After washing away the excess avidin, the DNA fragment that bound the probe is fluorescent. (Note: Labeled probes can allow detection and localization of DNA or RNA sequences in cell or tissue preparations, a process called *in situ* hybridization [ISH]. If the probe is fluorescent [F], the technique is called FISH.)

D. Antibodies

If no amino acid sequence information is available to guide the synthesis of a probe for direct detection of the DNA of interest, a gene can be identified indirectly by cloning cDNA in an expression vector that allows the cloned cDNA to be transcribed and translated. A labeled antibody (Ab) is used to identify which bacterial colony produces the protein and, therefore, contains the cDNA of interest.

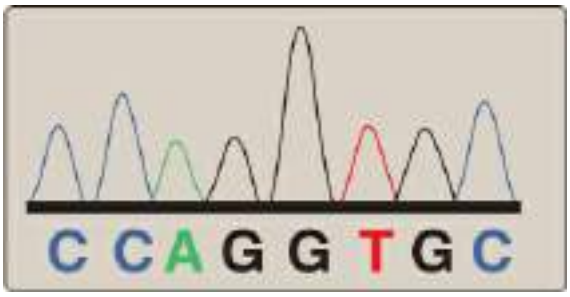


Figure 34.10
Color readout of a DNA sequence.

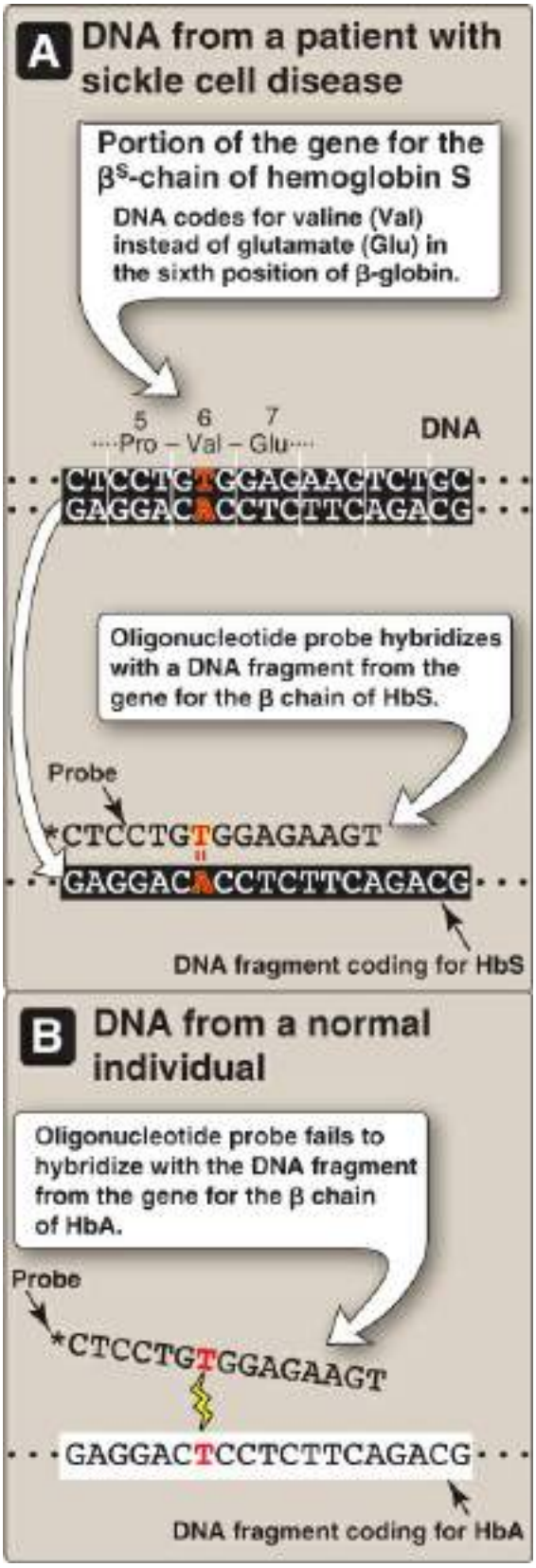


Figure 34.11

Allele-specific oligonucleotide probe detects hemoglobin (Hb) S allele. (Note: The * indicates ^{32}P

radiolabel.)

V. SOUTHERN BLOTTING

Southern blotting is a technique that combines the use of restriction enzymes, electrophoresis, and DNA probes to generate, separate, and detect pieces of DNA.

A. Procedure

This method, named after its inventor, Edward Southern, involves the following steps (Fig. 34.13). First, DNA is extracted from cells, for example, a patient's WBC. Second, the DNA is cleaved into many fragments using a restriction enzyme. Third, the resulting fragments (all of which are negatively charged) are separated on the basis of size by electrophoresis. (Note: Because the large fragments move more slowly than the smaller ones, the lengths of the fragments, usually expressed as the number of bps, can be calculated from comparison of the positions of the fragments relative to standard fragments of known size in a DNA ladder.) The DNA fragments in the gel are denatured and transferred (blotted) to a nitrocellulose membrane for analysis. If the original DNA consists of the individual's entire genome, the enzymic digest contains $\geq 10^6$ fragments. The gene of interest is on only one (or a few if the gene itself was fragmented) of these pieces of DNA. If all the DNA fragments were visualized by a nonspecific technique, they would appear as an unresolved blur of overlapping bands. To avoid this, the last step in Southern blotting uses a probe to identify the DNA fragments of interest. The patterns observed on Southern blot analysis depend both on the specific restriction endonuclease and on the probe used to visualize the restriction fragments. (Note: Variants of the Southern blot have been facetiously named northern if RNA is being studied [see p. 550] and western if protein is being studied [see p. 551], neither of which relates to anyone's name or to points of the compass.)

B. Mutation detection

Southern blotting can detect DNA mutations such as large insertions or deletions, trinucleotide repeat expansions, and rearrangements of nucleotides. It can also detect point mutations (replacement of one nucleotide by another; see p. 498) that cause the loss or gain of restriction sites. Such mutations cause the pattern of bands to differ from those seen with a normal gene. Longer fragments are generated if a restriction site is lost. For example, in Figure 34.13, person 2 lacks a restriction site present in person 1. Alternatively, the point mutation may create a new cleavage site with the production of shorter fragments. (Note: Most sequence differences at restriction sites are harmless variations in the DNA.)

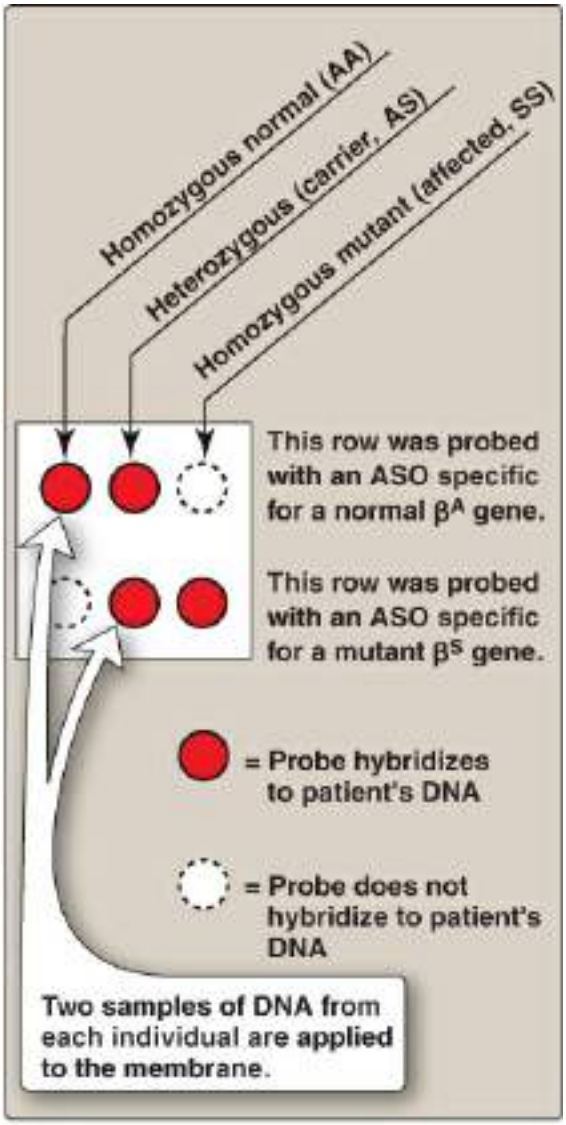


Figure 34.12
Allele-specific oligonucleotide (ASO) probes used to detect the sickle cell mutation and differentiate between sickle cell trait and disease.

VI. RESTRICTION FRAGMENT LENGTH POLYMORPHISM

It has been estimated that the genomes of any two unrelated people are 99.5% identical. With 6 billion bps in the diploid human genome, that represents variation in ~30 million bps. These genome variations are the result of mutations that lead to polymorphisms. A polymorphism is traditionally defined as a sequence variation at a given locus (allele) in >1% of a population. The change in genotype results in either no change in phenotype or a harmless change in phenotype, causes increased susceptibility to a disease, or, rarely, causes a disease. Polymorphisms primarily occur in the 98% of the genome that does not encode proteins (i.e., in introns and intergenic regions). A restriction fragment length polymorphism (RFLP) is a genetic variant that

can be observed by cleaving the DNA into fragments (restriction fragments) with a restriction endonuclease. The length of the restriction fragments is altered if the variant alters the DNA sequence so as to create or abolish a restriction site. RFLP can be used to detect human genetic variations, for example, in prospective parents or in fetal tissue.

A. DNA variations resulting in RFLP

Two types of DNA variations commonly result in RFLP: single-base changes in the DNA sequence and tandem repeats of DNA sequences.

1. Single-base changes: About 90% of human genome variation comes in the form of single nucleotide polymorphisms (SNPs, pronounced “snips”), that is, variations that involve just one base (Fig. 34.14). The substitution of one nucleotide at a restriction site can render the site unrecognizable by a particular restriction endonuclease. A new restriction site can also be created by the same mechanism. In either case, cleavage with an endonuclease results in fragments of lengths that differ from the normal and can be detected by DNA hybridization (see Fig. 34.13). The altered restriction site can be either at the site of a disease-causing mutation (rare) or at a site some distance from the mutation. (Note: The HapMap, developed by The International Haplotype Map Project, is a catalog of common SNP in the human genome. The data are being used in genome-wide association studies [GWASs] to identify those alleles that affect health and disease.)

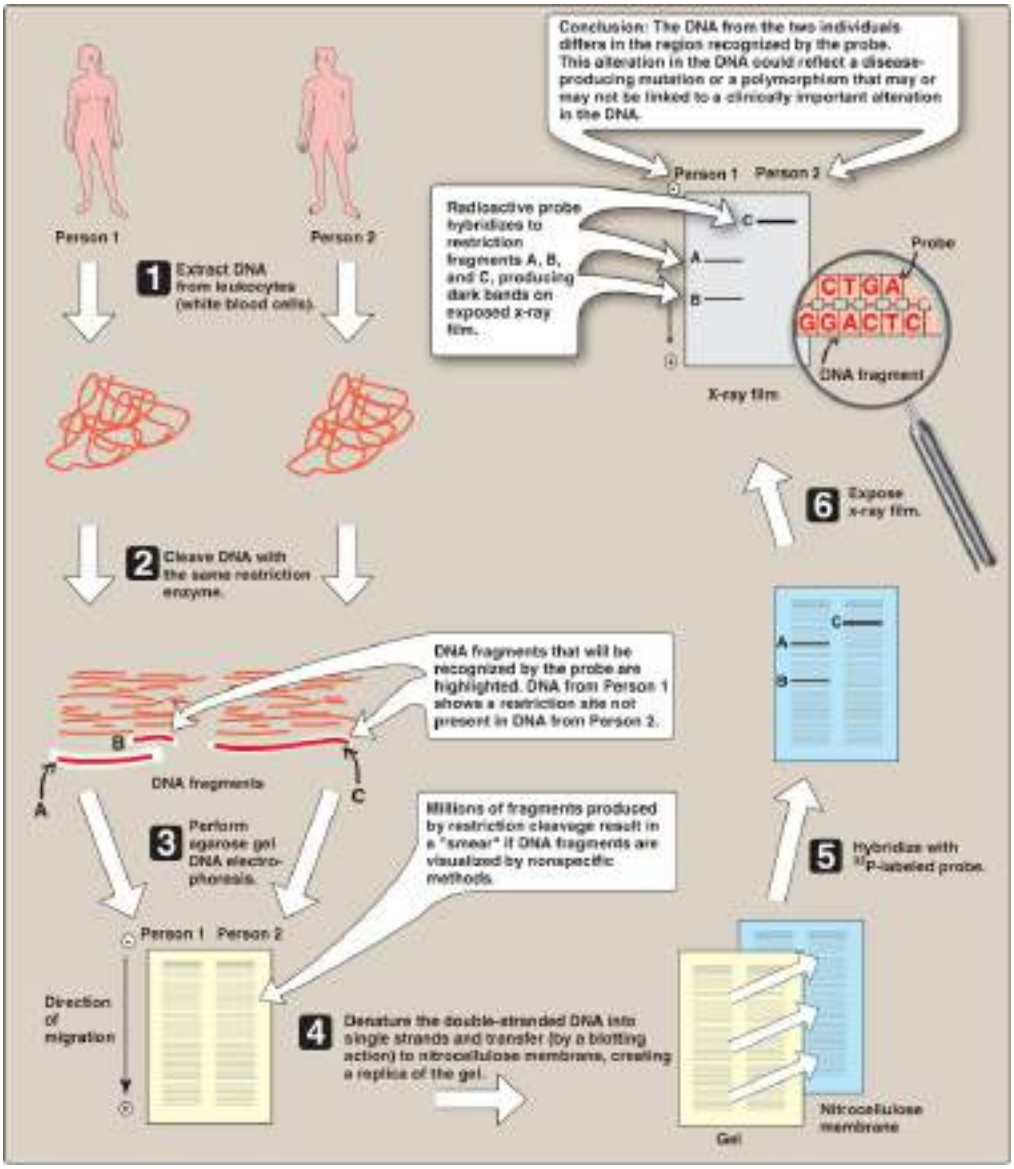


Figure 34.13
Southern blotting procedure. (Note: Nonradiolabeled probes are now commonly used.)

2. Tandem repeats: Polymorphisms in chromosomal DNA can also arise from the presence of a variable number of tandem repeats (VNTRs), as shown in Figure 34.14. These are short sequences of DNA at scattered locations in the genome, repeated in tandem (one after another). The number of these repeat units varies from person to person but is unique for any given individual and, therefore, serves as a molecular "fingerprint." Cleavage by restriction enzymes yields fragments that vary in length depending on how many repeated segments are contained in the fragment (see Fig. 34.15). Many different VNTR loci have been identified and are extremely useful for DNA fingerprint analysis, such as in forensic and paternity cases. It is important to emphasize that these polymorphisms, whether SNP or VNTR, are simply markers, which, in most

cases, have no known effect on the structure, function, or rate of production of any particular protein.

B. Tracing chromosomes from parent to offspring

If the DNA of an individual has gained a restriction site by base substitution, then enzymatic cleavage yields at least one additional fragment. Conversely, if a mutation results in loss of a restriction site, fewer fragments are produced by enzymatic cleavage. An individual who is heterozygous for a polymorphism has a sequence variation in the DNA of one chromosome and not in the homologous chromosome (i.e., in the chromosome from the mother and not from the father, or *vice versa*). In such individuals, each chromosome can be traced from parent to offspring by determining the presence or absence of the polymorphism.

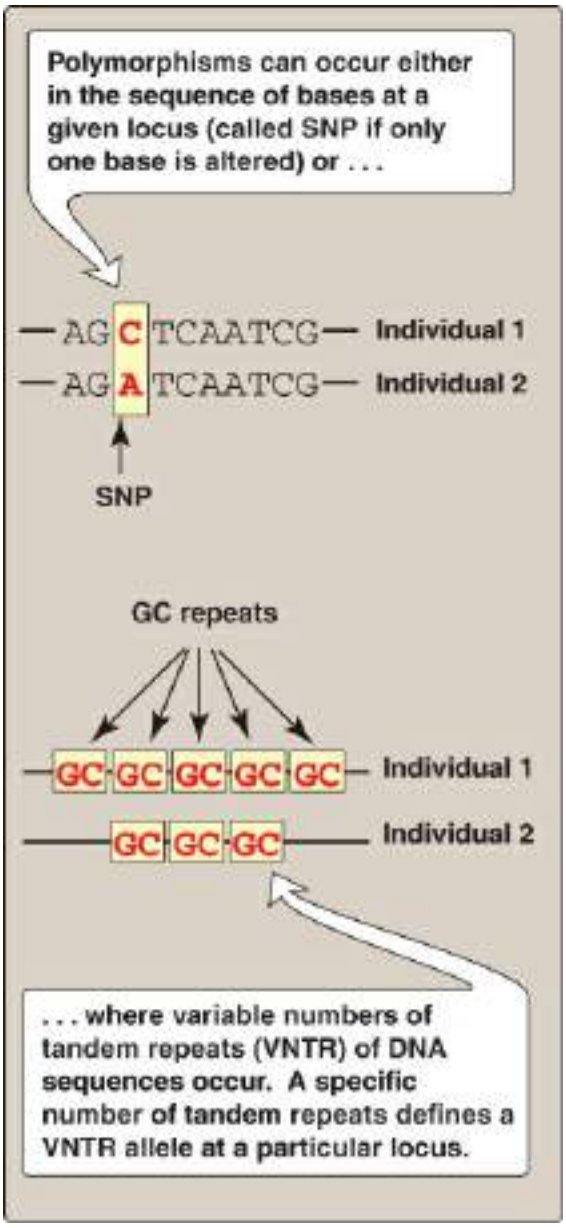


Figure 34.14
Common forms of genetic polymorphism. SNP = single nucleotide polymorphism; A = adenine; C = cytosine; G = guanine; T = thymine.

C. Prenatal diagnosis

Families with a history of severe genetic disease, such as an affected previous child or near relative, may wish to determine the presence of the disorder in a developing fetus. Genetic testing of a fetus is common when the mother is over the age of 35, because the chance of specific chromosomal abnormalities increases with maternal age. Prenatal diagnosis, in association with genetic counseling, allows for an informed reproductive decision if the fetus is affected.

1. Diagnostic methods: The available prenatal diagnostic methods vary in

sensitivity and specificity. Visualization of the fetus, for example, by ultrasound or fiberoptic devices (fetoscopy), is useful only if the genetic abnormality results in gross anatomic defects (e.g., neural tube defects [NTDs]). The chemical composition of the amniotic fluid can also provide diagnostic clues. For example, the presence of high levels of α-fetoprotein is associated with NTD. Fetal DNA in a mother's blood can be isolated and used for detecting specific fetal chromosomal problems, such as trisomy 21 (Down syndrome). Fetal cells obtained from amniotic fluid or from biopsy of the chorionic villi can be used for karyotyping, which assesses the morphology of metaphase chromosomes. Staining and cell sorting techniques permit the rapid identification of trisomies and translocations that produce an extra chromosome or chromosomes of abnormal lengths. However, molecular analysis of fetal DNA provides the most detailed genetic picture.

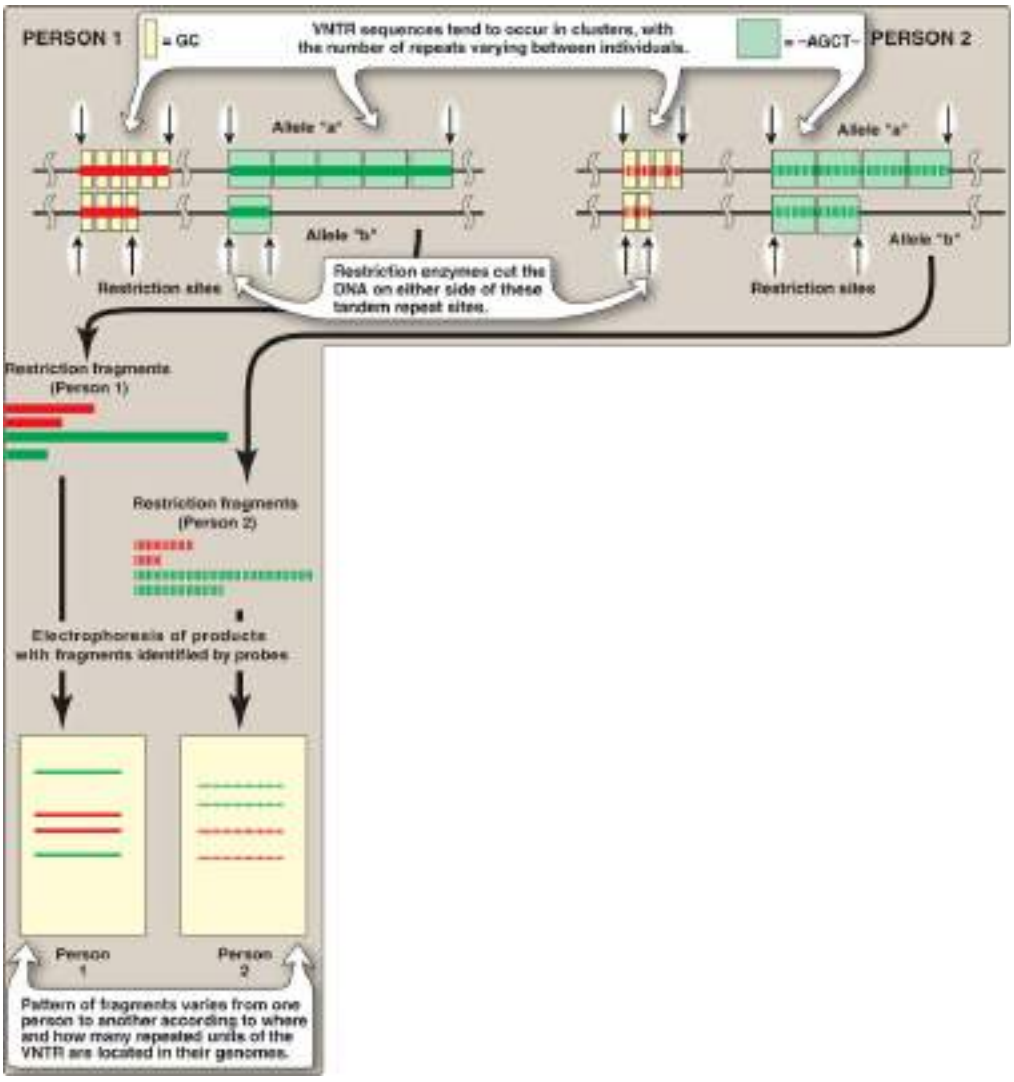


Figure 34.15
Restriction fragment length polymorphism of variable number tandem repeats (VNTRs). For each person, a pair of homologous chromosomes is shown.

2. DNA sources: During pregnancy, fetal DNA may be obtained from maternal blood (cell-free fetal DNA), fetal blood cells, or fetal cells in the amniotic fluid or from the chorionic villi of the placenta (Fig. 34.16). For amniotic fluid, it was formerly necessary to grow cells in culture for 2 to 3 weeks in order to have sufficient DNA for analysis. The ability to amplify DNA by PCR has dramatically shortened the time needed for a DNA analysis.

3. Direct diagnosis of sickle cell anemia using RFLP: The genetic disorders of Hb are the most common genetic diseases in humans. In the case of sickle cell anemia (Fig. 34.17), the point mutation that gives rise to the disease (see p. 36) is actually one and the same mutation that gives rise to the polymorphism. However, direct detection by RFLP of diseases that result from point mutations is limited to only a few genetic diseases.

   a. Early diagnostic efforts: In the past, prenatal diagnosis of sickle cell anemia involved the determination of the amount and kind of Hb synthesized in the nucleated red cells obtained from fetal blood. However, the invasive procedures to obtain fetal blood have a high mortality rate (~5%), and analysis cannot be carried out until late in the second trimester of pregnancy when HbA (and its HbS variant) begins to be produced.

   b. RFLP analysis: In sickle cell anemia, the sequence alteration caused by the point mutation abolishes the recognition site of the restriction endonuclease MstII: CCTNAGG (where N is any nucleotide; see Fig. 34.17). Thus, the A-to-T mutation in codon 6 of the β^S-globin gene eliminates a cleavage site for the enzyme. Normal DNA digested with MstII yields a 1.15-kb fragment, whereas a 1.35-kb fragment is generated from the β^S gene as a result of the loss of one MstII cleavage site. Diagnostic techniques that allow analysis of fetal DNA from amniotic cells or chorionic villus sampling rather than fetal blood have proved valuable because they provide safe, early detection of sickle cell anemia as well as other genetic diseases. (Note: Genetic disorders caused by insertions or deletions between two restriction sites, rather than by the creation or loss of cleavage sites, will also display RFLP.)

4. Indirect diagnosis of phenylketonuria using RFLP: The gene for phenylalanine hydroxylase (PAH), the enzyme deficient in phenylketonuria ([PKU], see p. 298), is located on chromosome 12. It spans ~90 kb of genomic DNA and contains 13 exons separated by introns (Fig. 34.18; see p. 492 for a description of exons and introns). Mutations in the *PAH* gene usually do not directly affect any restriction endonuclease recognition site. To establish a diagnostic protocol for PKU, DNA from family members of the affected individual must be analyzed. The goal is to identify genetic markers (RFLP) that are tightly linked to the disease trait. Once these markers are identified, RFLP analysis can be used to carry out prenatal diagnosis.

   a. Mutant gene identification: Determining the presence of the mutant gene by

identifying the polymorphism marker can be done if two conditions are satisfied. First, if the polymorphism is closely linked to a disease-producing mutation, the defective gene can be traced by detection of the RFLP. For example, if DNA from a family carrying a disease-causing gene is examined by restriction enzyme cleavage and Southern blotting, it is sometimes possible to find an RFLP that is consistently associated with that gene (i.e., they show close linkage and are coinherited). It is then possible to trace the inheritance of the gene within a family without knowledge of the nature of the genetic defect or its precise location in the genome. (Note: The polymorphism may be known from the study of other families with the disorder or may be discovered to be unique in the family under investigation.) Second, for autosomal recessive disorders, such as PKU, the presence of an affected individual in the family would aid in the diagnosis. This individual would have the mutation present on both chromosomes, allowing identification of the RFLP associated with the genetic disorder.

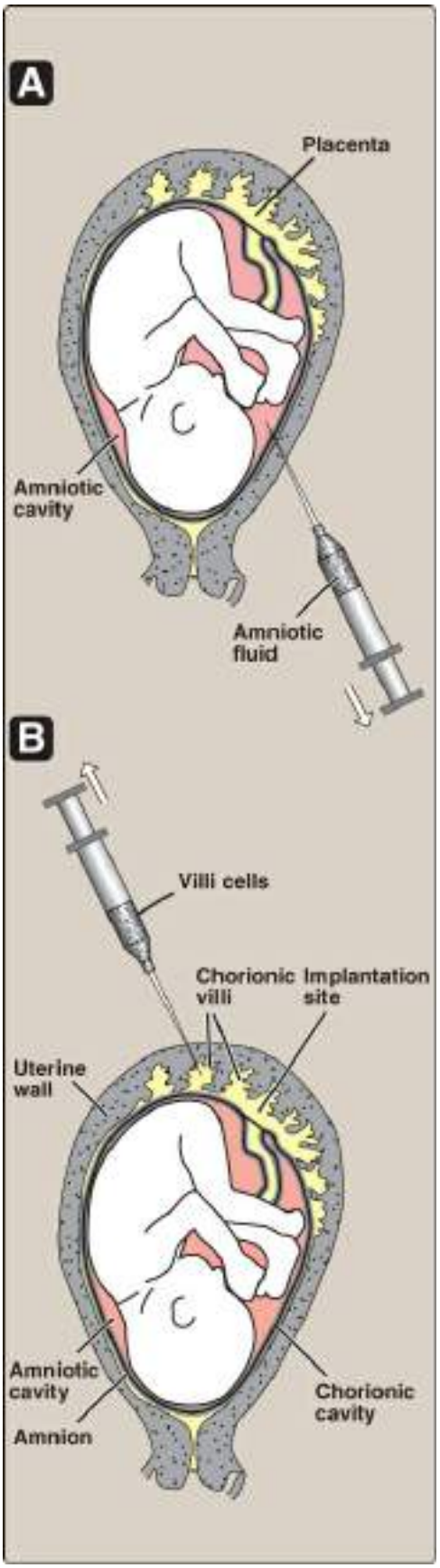


Figure 34.16
Sampling of fetal cells. A: Amniotic fluid. B: Chorionic villus.

b. RFLP analysis: The presence of abnormal genes for PAH can be shown using DNA polymorphisms as markers to distinguish between normal and mutant genes. For example, Figure 34.19 shows a typical pattern obtained when DNA from members of an affected family is cleaved with an appropriate restriction enzyme and subjected to electrophoresis. The vertical arrows represent the cleavage sites for the restriction enzyme used. The presence of a polymorphic site creates fragment “b” in the autoradiogram (after hybridization with a labeled *PAH*-cDNA probe), whereas the absence of this site yields only fragment “a.” Note that subject II-2 demonstrates that the polymorphism, as shown by the presence of fragment “b,” is associated with the mutant gene. Therefore, in this particular family, the appearance of fragment “b” corresponds to the presence of a polymorphic site that marks the abnormal gene for PAH. The absence of fragment “b” corresponds to having only the normal gene. In Figure 34.19, examination of fetal DNA shows that the fetus (subject II-4) inherited two abnormal genes from the parents and, therefore, has PKU.

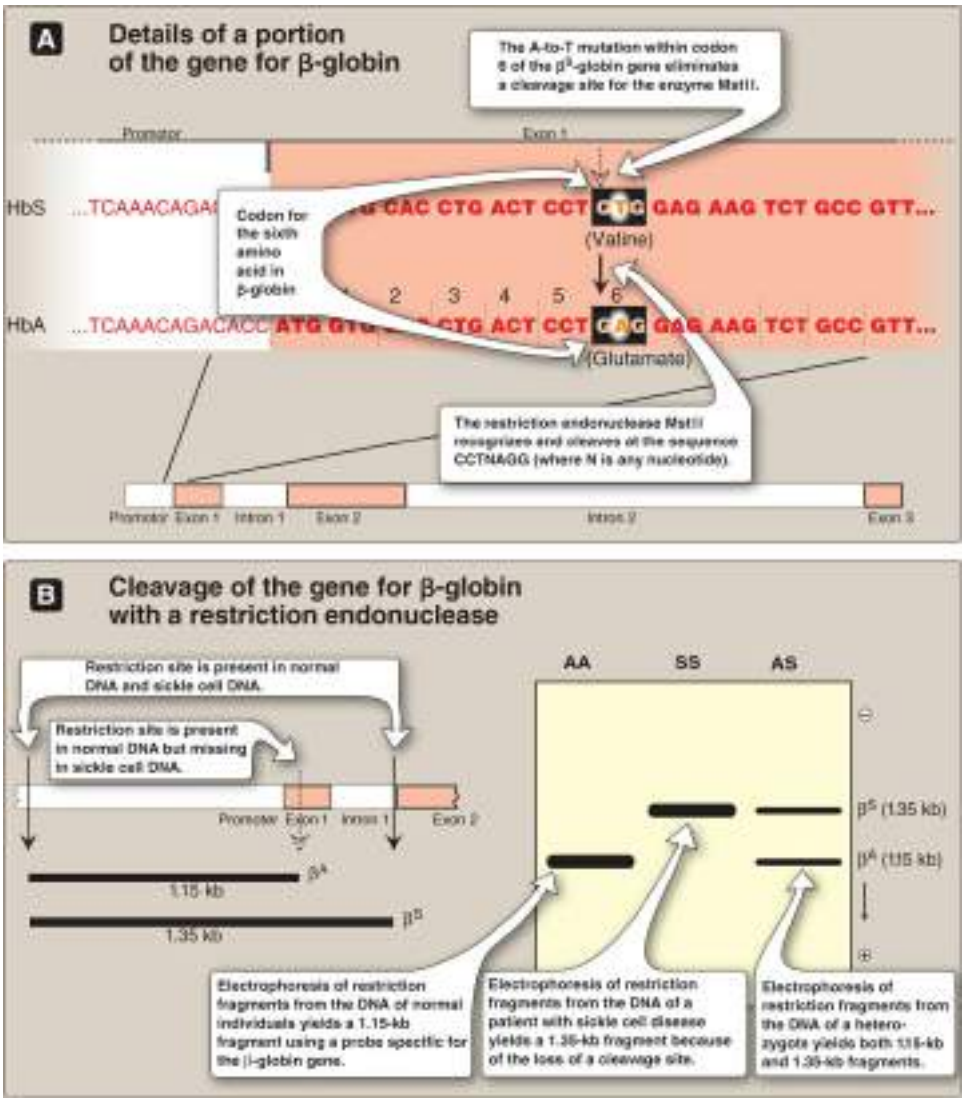


Figure 34.17
Detection of β^S-globin mutation. kb = kilobase (1 kb = 1,000 bps in double-stranded DNA); Hb = hemoglobin.

c. Value of DNA testing: DNA-based testing is useful not only in determining if an unborn fetus is affected by PKU but also in detecting unaffected carriers of the mutated gene to aid in family planning. (Note: PKU is treatable by dietary restriction of phenylalanine. Early diagnosis and treatment are essential in preventing severe neurologic damage in affected individuals.)

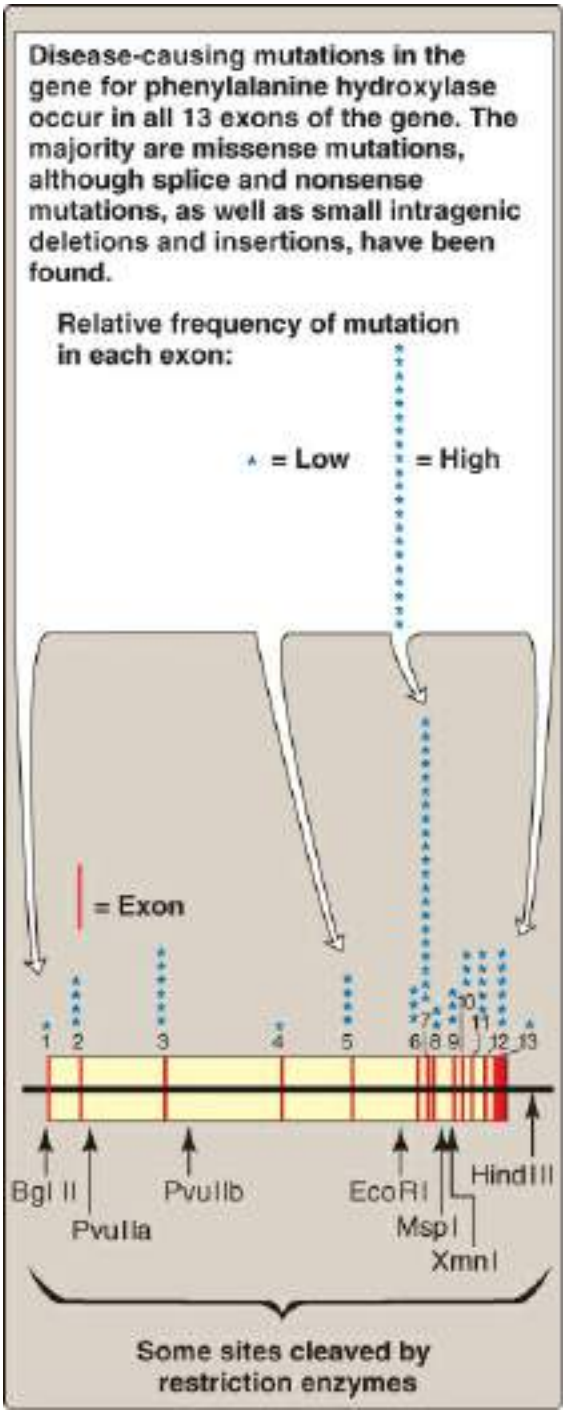


Figure 34.18
The gene for phenylalanine hydroxylase showing 13 exons, restriction sites, and some of the >500 mutations causing phenylketonuria.

VII. POLYMERASE CHAIN REACTION

PCR is an *in vitro* method for amplifying a selected DNA sequence that does not rely on the biologic (*in vivo*) cloning method described on p. 534. PCR permits the synthesis of

millions of copies of a specific nucleotide sequence in a few hours. It can amplify the sequence, even when the targeted sequence makes up less than one part in a million of the total initial sample. The method can be used to amplify DNA sequences from any source, including viral, bacterial, plant, or animal. The steps in PCR are summarized in Figures 34.20 and 34.21.

A. Procedure

PCR uses DNA pol to repetitively amplify targeted portions of genomic or cDNA. Each cycle of amplification doubles the amount of DNA in the sample, leading to an exponential increase (2^n, where n = cycle number) in DNA with repeated cycles of amplification. The amplified DNA products can then be separated by gel electrophoresis, detected by Southern blotting and hybridization, and sequenced.

1. Constructing primer: It is not necessary to know the nucleotide sequence of the target DNA in the PCR method. However, it is necessary to know the nucleotide sequence of short segments on each side of the target DNA. These stretches, called flanking sequences, bracket the DNA sequence of interest. The nucleotide sequences of the flanking regions are used to construct two, single-stranded oligonucleotides, usually 20 to 35 nucleotides long, which are complementary to the respective flanking sequences. The 3′-hydroxyl end of each oligonucleotide points toward the target sequence (see Fig. 34.20). These synthetic oligonucleotides function as primers in PCR.
2. Sample Preparation: A sample for analysis is prepared by adding the DNA (either genomic DNA or cDNA), the primers, an excess of dNTPs, and a heat-stable DNA pol to an appropriate buffer solution.
3. Denaturing DNA: The sample is heated to ~95°C to separate the DNA into single strands (ssDNAs).

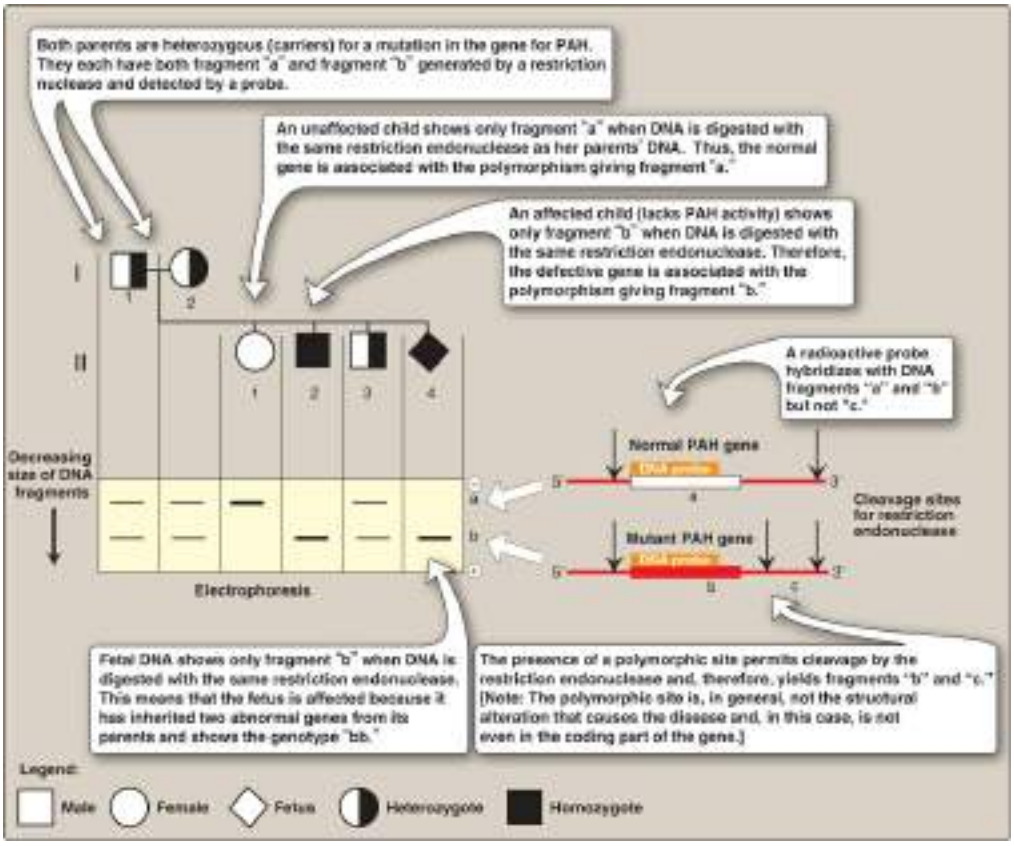


Figure 34.19
Analysis of restriction fragment length polymorphism in a family with a child affected by phenylketonuria (PKU), an autosomal-recessive disease. The molecular defect in the gene for phenylalanine hydroxylase (PAH) in the family is not known. The family wanted to know if the current pregnancy would be affected by PKU.

4. Annealing primers: The sample is cooled to ~50°C, and the two primers (one for each strand) anneal to a complementary sequence on the ssDNA.

5. Extending primers: The sample temperature is raised to ~72°C to initiate the synthesis of two new strands complementary to the original DNA strands. DNA pol adds nucleotides to the 3′-hydroxyl end of the primer, and strand growth extends in the 5′→3′ direction across the target DNA, making complementary copies of the target. The PCR products from this extension can be several thousand bps long. At the completion of one cycle of replication, the reaction mixture is heated again to separate the strands (of which there are now four). Each strand anneals to the complementary primer, and the step of primer extension is repeated. By using a heat-stable DNA pol (e.g., Taq from the bacterium *Thermus aquaticus* that normally lives at high temperatures), the polymerase is not denatured and, therefore, does not have to be added at each successive cycle. However, Taq lacks proofreading activity. Typically, 20 to 30 cycles are run during this process, amplifying the DNA by a million fold (2^{20}) to a billion fold (2^{30}). (Note: Each extension product includes a sequence at its 5′ end that is complementary to the primer [see Fig. 34.20]. Thus, each newly

synthesized strand can act as a template for the successive cycles [see Fig. 34.21]. This leads to an exponential increase in the amount of target DNA with each cycle, hence, the name “polymerase chain reaction.”) Probes can be made during PCR by adding labeled nucleotides to the samples before the last few cycles.

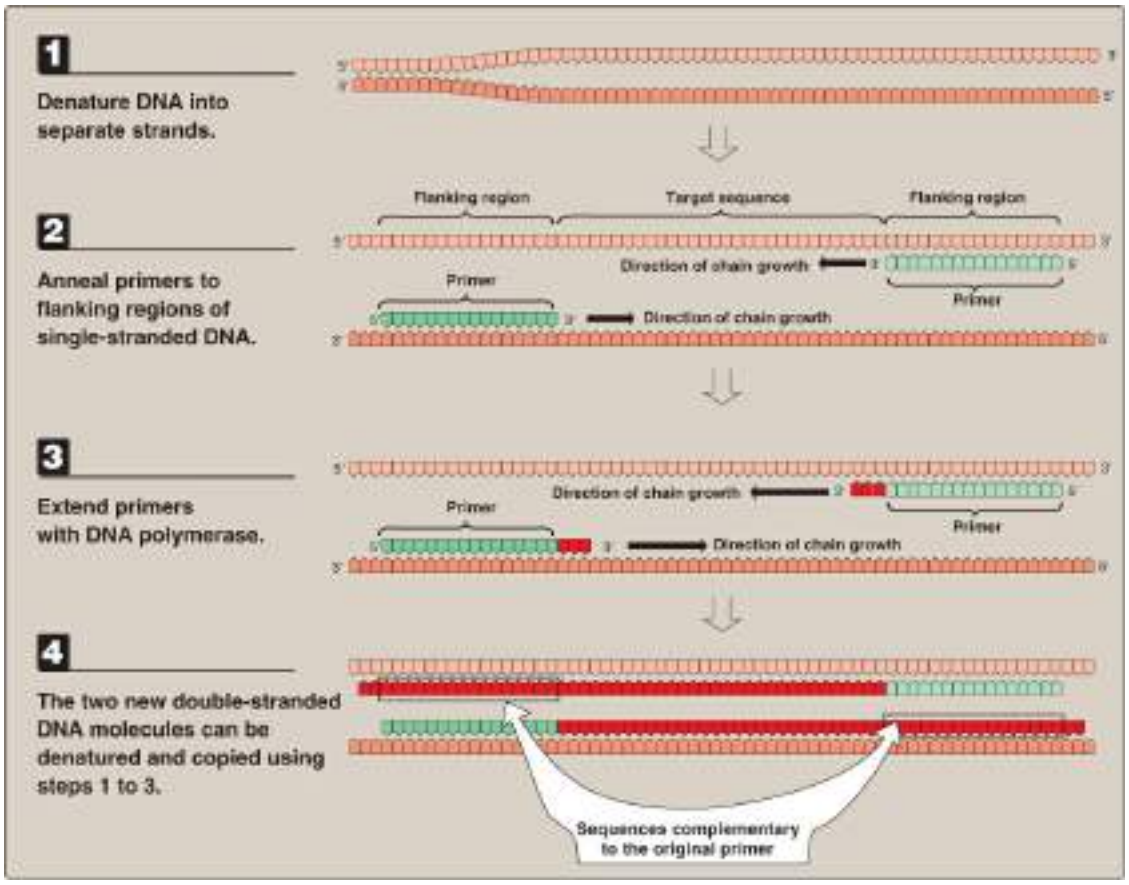


Figure 34.20
Steps (denature, anneal, extend) in one cycle of the polymerase chain reaction.

B. Advantages

The major advantages of PCR over biologic cloning as a mechanism for amplifying a specific DNA sequence are sensitivity and speed. DNA sequences present in only trace amounts can be amplified to become the predominant sequence. PCR is so sensitive that DNA sequences present in an individual cell can be amplified and studied. Isolating and amplifying a specific DNA sequence by PCR is faster and less technically difficult than traditional cloning methods that utilize recombinant DNA techniques.

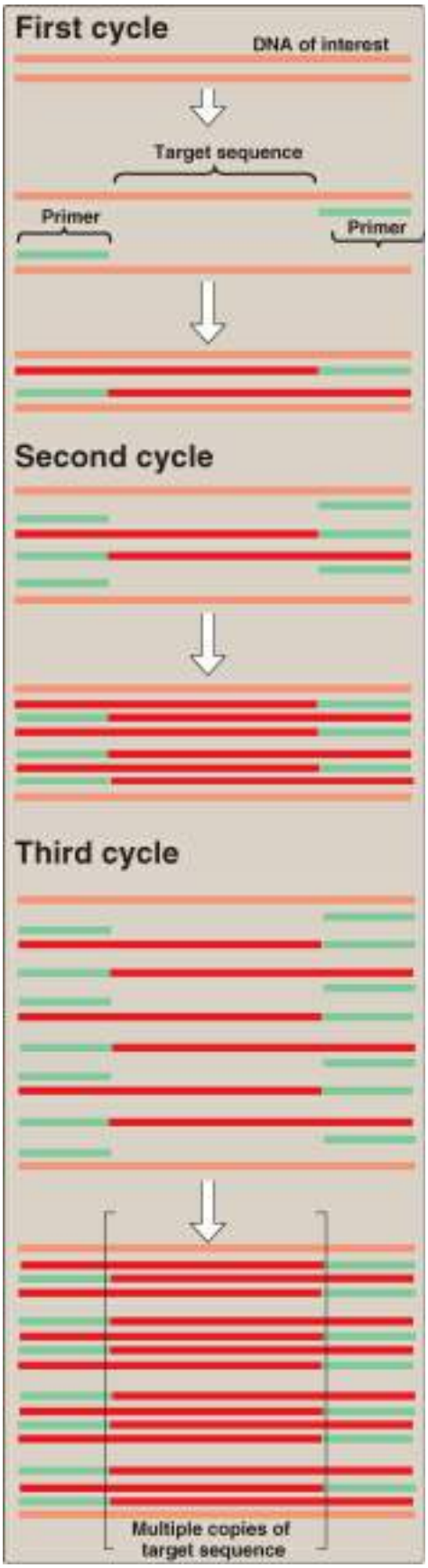


Figure 34.21
Multiple cycles of the polymerase chain reaction.

C. Applications

PCR has become a very common tool in research, forensics, and clinical diagnostics.

1. Comparison of a normal gene to its mutant form: PCR allows the synthesis of mutant DNA in sufficient quantities for a sequencing protocol without laborious biologic cloning of the DNA.

2. Forensic analysis of DNA samples: DNA fingerprinting by means of PCR has revolutionized the analysis of evidence from crime scenes. DNA isolated from a single human hair, a tiny spot of blood, or a sample of semen is sufficient to determine whether the sample comes from a specific individual. The DNA markers analyzed for such fingerprinting are most commonly a type of polymorphism known as short tandem repeats. These are very similar to the VNTR described previously (see p. 541) but are smaller in size. (Note: Paternity testing uses the same techniques.)

3. Detection of low-abundance nucleic acid sequences: Viruses that have a long latency period, such as human immunodeficiency virus (HIV), are difficult to detect at the early stage of infection using conventional methods. PCR offers a rapid and sensitive method for detecting viral DNA sequences even when only a small proportion of cells harbors the virus. (Note: Quantitative PCR [qPCR], also known as real-time PCR, allows quantification of the amount [copy number] of the target nucleic acid after each cycle of amplification [i.e., in real time] rather than at the end and is useful in determining viral load [the amount of virus].)

4. Prenatal diagnosis and carrier detection of cystic fibrosis: Cystic fibrosis is an autosomal recessive genetic disease resulting from mutations in the gene for the cystic fibrosis transmembrane conductance regulator (CFTR) protein. The most common mutation is a three-base deletion that results in the loss of a phenylalanine residue from the CFTR protein (see p. 499). Because the mutant allele is three bases shorter than the normal allele, it is possible to distinguish them from each other by the size of the PCR products obtained by amplifying that portion of the DNA. Figure 34.22 illustrates how the results of such a PCR test can distinguish between homozygous normal, heterozygous (carriers), and homozygous mutant (affected) individuals.

The simultaneous amplification of multiple regions of a target DNA using multiple primer pairs is known as multiplex PCR. It allows detection of the loss of ≥1 exons in a gene with many exons such as the gene for CFTR, which has 27 exons.

VIII. GENE EXPRESSION ANALYSIS

The tools of biotechnology not only allow the study of gene structure, but also provide ways of analyzing the mRNA and protein products of gene expression.

A. Determining messenger RNA levels

mRNA levels are usually determined by the hybridization of labeled probes to either mRNA itself or to cDNA produced from mRNA. (Note: Amplification by PCR of cDNA made from mRNA by retroviral reverse transcriptase [RT] is referred to as RT-PCR.)

1. Northern blots: Northern blots are similar to Southern blots (see Fig. 34.13), except that the sample contains a mixture of mRNA molecules that are separated by electrophoresis, then transferred to a membrane and hybridized with a radiolabeled probe. The bands obtained by autoradiography give a measure of the amount and size of the mRNA molecules in the sample.

2. Microarrays: DNA microarrays contain thousands of immobilized ssDNA sequences organized in an area no larger than a microscope slide. These microarrays are used to analyze a sample for the presence of gene variations or mutations (genotyping) or to determine the patterns of mRNA production (gene expression analysis), analyzing thousands of genes at the same time. For genotyping analysis, the sample is from genomic DNA. For expression analysis, the population of mRNA molecules from a particular cell type is converted to cDNA and labeled with a fluorescent tag (Fig. 34.23). This mixture is then exposed to a gene (or, DNA) chip, which is a glass slide or membrane containing thousands of tiny spots of DNA, each corresponding to a different gene. The amount of fluorescence bound to each spot is a measure of the amount of that particular mRNA in the sample. DNA microarrays are used to determine the differing patterns of gene expression in two different types of cells (e.g., normal and cancer cells; see Fig. 34.23). They can also be used to subclassify cancers, such as breast cancer, to optimize treatment. (Note: Microarrays involving proteins and the Ab or other proteins that recognize them are being used to identify biomarkers to aid in the diagnosis, prognosis, and treatment of disease based on a patient's protein expression profile. Protein [and DNA] microarrays are important tools in the development of personalized [precision] medicine in which the treatment and/or prevention strategies consider the genetic, environmental, and lifestyle variations among individuals.)

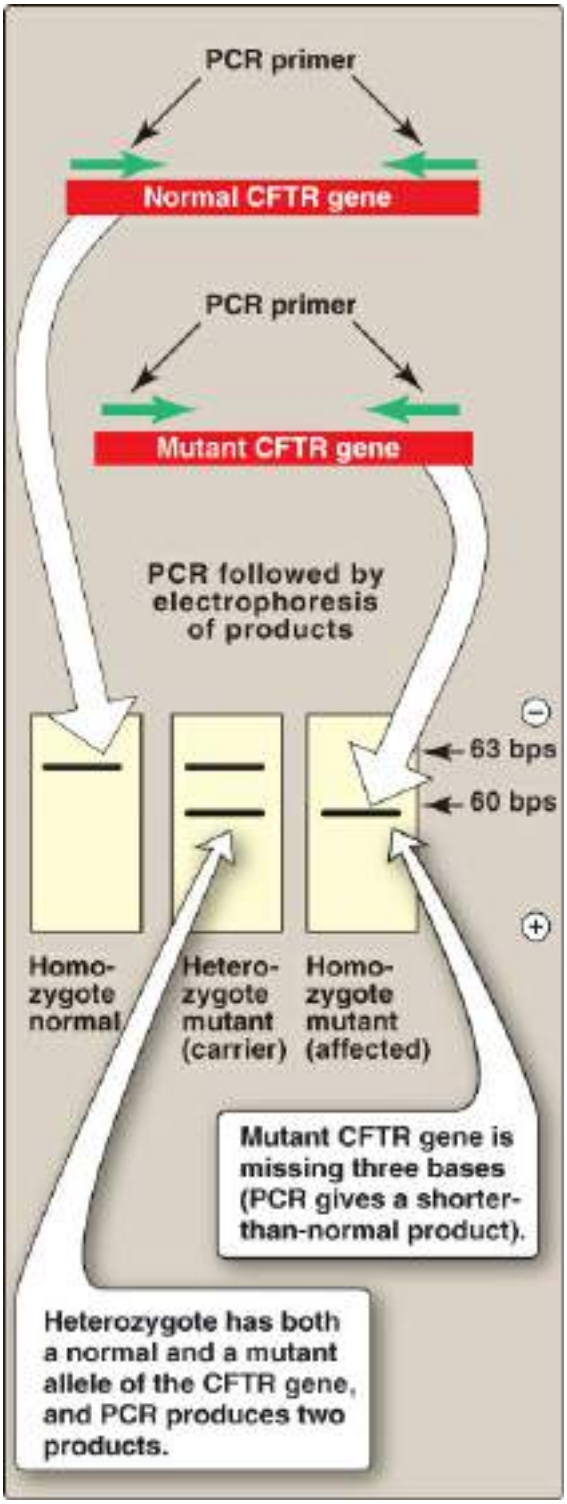


Figure 34.22
Genetic testing for cystic fibrosis (CF) using the polymerase chain reaction (PCR). (Note: CF is also diagnosed using allele-specific oligonucleotide analysis [see p. 539].) CFTR = cystic fibrosis transmembrane conductance regulator; bps = base pairs.

B. Protein analysis

The kinds and amounts of proteins in cells do not always directly correspond to the amounts of mRNA present. Some mRNA are translated more efficiently than others, and some proteins undergo posttranslational modification. When analyzing the abundance and interactions of a large number of proteins, automated methods involving a variety of techniques, such as mass spectrometry and two-dimensional electrophoresis, are used. When investigating one, or a limited number of proteins, labeled Ab are used to detect and quantify specific proteins and to determine posttranslational modifications.

1. Enzyme-linked immunosorbent assays: These assays (known as ELISAs) are performed in the wells of a microtiter dish. The antigen (protein) is bound to the plastic of the dish. The probe used consists of an Ab specific for the protein (such as troponin, see p. 71) to be measured. The Ab is covalently bound to an enzyme, which will produce a colored product when exposed to its substrate. The amount of color produced is proportional to the amount of Ab present and, indirectly, to the amount of protein in a test sample.

2. Western blots: Western blots (also called immunoblots) are similar to Southern blots, except that proteins, instead of nucleic acid molecules, are separated by electrophoresis and blotted (transferred) to a membrane. The probe is a labeled Ab, which produces a band on the membrane at the location of its antigen.

3. Detecting exposure to human immunodeficiency virus: ELISAs and western blots are commonly used to detect exposure to HIV by measuring the amount of anti-HIV Ab present in a patient's blood sample. ELISAs are used as the primary screening tool because they are very sensitive. Because these assays sometimes give false positives, however, western blots, which are more specific, are often used as a confirmatory test (Fig. 34.24). (Note: ELISA and western blots can only detect HIV exposure after anti-HIV Ab appear in the bloodstream following their production by the immune system. PCR-based testing for HIV is more useful in the first few months after exposure, because it directly detects the viral nucleic acid.)

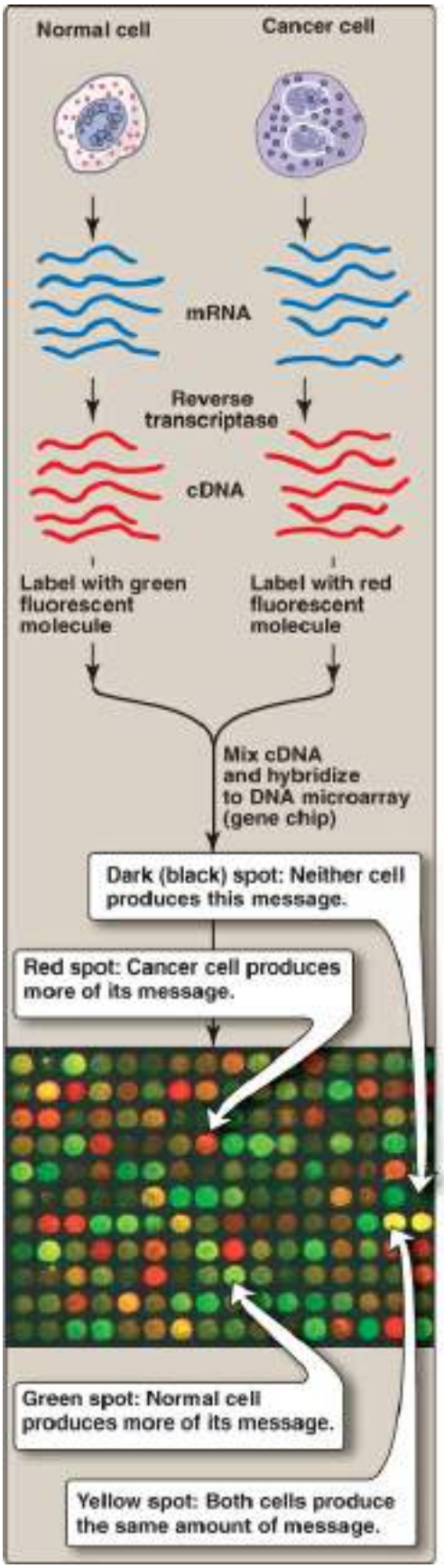


Figure 34.23
Microarray analysis of gene expression using DNA (gene) chips. (Note: Protein chips are also used.) mRNA = messenger RNA; cDNA = complementary DNA.

C. Proteomics

The study of the proteome, or all the proteins expressed by a genome, including their relative abundance, distribution, posttranslational modifications, functions, and interactions with other macromolecules, is known as proteomics. The 20,000 to 25,000 protein-coding genes of the human genome translate into well over 100,000 proteins when posttranscriptional and posttranslational modifications are considered. Although a genome remains essentially unchanged, the amounts and types of proteins in any particular cell change dramatically as genes are turned on and off. (Note: Proteomics [and genomics] required the parallel development of bioinformatics, the computer-based organization, storage, and analysis of biologic data.) Figure 34.25 compares some of the analytic techniques discussed in this chapter.

IX. GENE THERAPY

The goal of gene therapy is to treat disease through delivery of a functional gene (typically a clone of the normal DNA sequence for the gene) into the somatic cells of a patient who has a defect in that gene as a result of a disease-causing mutation. Because somatic gene therapy changes only the targeted somatic cells, the change is not passed on to the next generation. (Note: In germline gene therapy, the germ cells are modified, and so the change is passed on. A long-standing moratorium on germline gene therapy is in effect worldwide.) There are two types of gene transfer: (1) *ex vivo*, in which cells from the patient are removed, transduced, and returned, and (2) *in vivo*, in which the cells are directly transduced. Both types require use of a viral vector to deliver the DNA. Challenges of gene therapy include development of vectors, achievement of long-lived expression, and prevention of side effects such as an immune response. The first successful gene therapy involved two patients with severe combined immunodeficiency disease (SCID) caused by mutations to the gene for adenosine deaminase (ADA, see p. 334). It utilized mature T lymphocytes transduced *ex vivo* with a retroviral vector (Fig. 34.26). (Note: Human *ADA* cDNA is now used.) Since 1990, only a small number of patients (with a variety of disorders, such as hemophilia, cancers, and certain types of blindness) have been treated with gene therapy, with varying degrees of success.

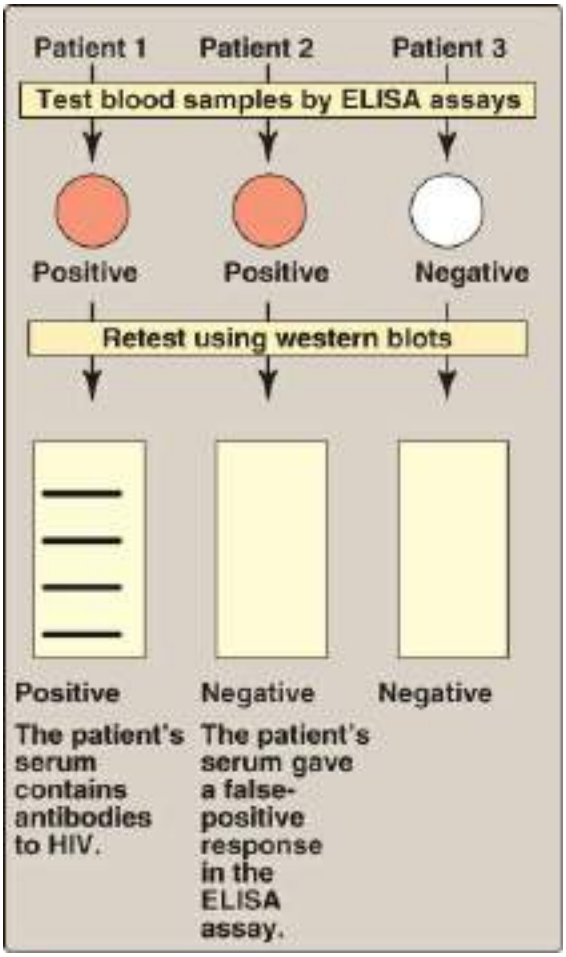


Figure 34.24
Testing for human immunodeficiency virus (HIV) exposure by enzyme-linked immunosorbent assays (ELISA) and western blots.

Gene editing, as opposed to gene transfer, allows a mutated gene to be repaired. Combinations of DNA-binding molecules (proteins or RNA) and endonucleases are used to identify and cleave the mutated sequence. Cleavage activates homologous recombination repair of dsDNA breaks (see p. 477) that integrates DNA containing the correct sequence into the gene. An endonuclease guided to a specific DNA sequence by a custom-designed RNA has been used in gene editing in human cells. The technique is based on (and named for) the prokaryotic CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats [CRISPR]-associated protein) system that identifies and cleaves foreign DNA in bacterial cells. CRISPR-Cas9 technology for gene editing in human cells (depicted in Fig. 34.27) is currently in clinical trials for modifying globin gene expression in hematopoietic stem cells to treat patients with sickle cell anemia.

TECHNIQUE	SAMPLE ANALYZED
Southern blot	DNA
Northern blot	RNA
Western blot	Protein
ASO	DNA
Microarray	cDNA or genomic DNA
	Protein
ELISA	Protein

Figure 34.25
Techniques used to analyze DNA, RNA, and proteins. (Note: The three blotting techniques involve the use of a gel.) ASO = allele-specific oligonucleotide. ELISA = enzyme-linked immunosorbent assay; cDNA = complementary DNA.

X. TRANSGENIC ANIMALS

Transgenic animals can be produced by injecting a cloned foreign gene (a transgene) into a fertilized egg. If the gene randomly and stably integrates into a chromosome, it will be present in the germline of the resulting animal and can be passed from generation to generation. A giant mouse called “Supermouse” was produced in this way by injecting the gene for rat growth hormone into a fertilized mouse egg. Transgenic animals have been designed that produce therapeutic human proteins in their milk, a process called “pharming.” Antithrombin, an anticlotting protein, was produced by transgenic goats and approved for clinical use in 2009. If the functional transgene undergoes targeted (not random) insertion, a knockin (KI) animal that expresses the gene is created. Targeted insertion of a nonfunctional version of the transgene creates a knockout (KO) animal that does not express the gene. Such genetically engineered animals can serve as models for the study of a corresponding human disease.

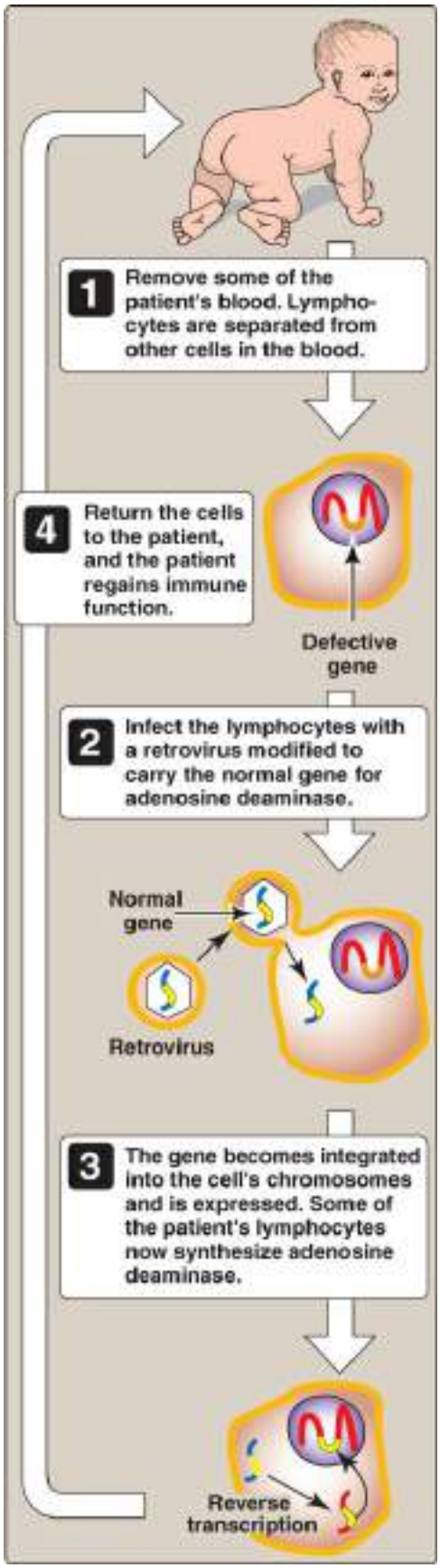


Figure 34.26
Gene therapy for severe combined immunodeficiency disease caused by adenosine deaminase deficiency. (Note: Bone marrow stem cells and a modified retroviral vector are now used.)

XI. Chapter Summary

- **Restriction endonucleases** cleave dsDNA at specific **palindromic** sequences (**restriction sites**). DNA fragments produced by cuts at the same **restriction site** can be ligated to form a **recombinant DNA molecule**.
- DNA cloning, which is the **amplification** (production of many copies) of DNA, requires a **recombinant DNA molecule** produced from a **vector** and a smaller DNA fragment of interest.
- Vectors are capable of **autonomous replication** within the host cell, contain at least one specific restriction site, and carry at least one gene, such as an **antibiotic resistance gene**, that confers the ability to select for host cells containing the vector. Prokaryotic **plasmids** can serve as vectors.
- A **genomic DNA library** contains the total DNA of an organism and ideally includes a copy of every nucleotide sequence in the organism's genome. In contrast, a **cDNA library** contains only those DNA sequences that have been produced from the mRNA molecules present in a cell. The cDNA for human genes can be cloned into an **expression vector** for the synthesis of proteins by bacteria or eukaryotes.
- **Southern blotting** can be used to detect specific sequences in DNA. The DNA is cleaved using a restriction endonuclease, after which the fragments are separated by **gel electrophoresis**, denatured within the gel, and transferred (blotted) onto a **nitrocellulose membrane** for analysis. The fragment of interest is detected using specific ssDNA or RNA probes.
- **Polymorphisms** (DNA sequence variations) in the human genome can arise from single-base changes or from tandem repeats. A polymorphism can serve as a genetic marker that can be followed through families.
- An **RFLP** is a genetic variant that can be observed by cleaving the DNA of chromosomes with restriction endonucleases and separating the fragments by gel electrophoresis. A base substitution in one or more nucleotides at a restriction site can render the site unrecognizable by a particular restriction endonuclease or can create a new restriction site to produce DNA fragments of lengths differing from the expected fragments. **RFLP analysis** can be used to diagnose genetic diseases.
- The **PCR** is a rapid method for **amplifying** a DNA sequence selected by the use of specific DNA primers that pair with flanking sequences. Some applications of the PCR technique include (1) comparison of a normal gene with a mutant form of the gene, (2) forensic analysis of DNA samples, (3) detection of low-abundance nucleic acid sequences, and (4) prenatal diagnosis and carrier detection of genetic disorders.
- The mRNA products of gene expression can be measured by **northern blots** and **microarrays.** In northern blot analysis, a sample containing a mixture of mRNA molecules are separated by electrophoresis, transferred to a membrane, and then hybridized to a radiolabeled probe for detection. In a **microarray**, the differing patterns of gene expression between two types of cells (e.g., normal and cancer cells) can be analyzed.
- **ELISAs** and **western blots** (**immunoblots**) are used to detect specific proteins using Ab.
- **Proteomics** is the study of all proteins expressed by a genome.
- The goal of **gene therapy** is to replace a defective gene in a **somatic cell** with a normal cloned gene, whereas the goal of **gene editing** is to repair a mutated gene or to modify gene expression. CRISPR-Cas9 technology for gene editing is currently in clinical trials for modifying globin gene expression in hematopoietic stem cells to treat patients with sickle cell anemia.
- Insertion of a foreign gene (transgene) into the germline of an animal creates a **transgenic animal** that can produce therapeutic proteins or serve as gene **KI** or **KO** models for human diseases.

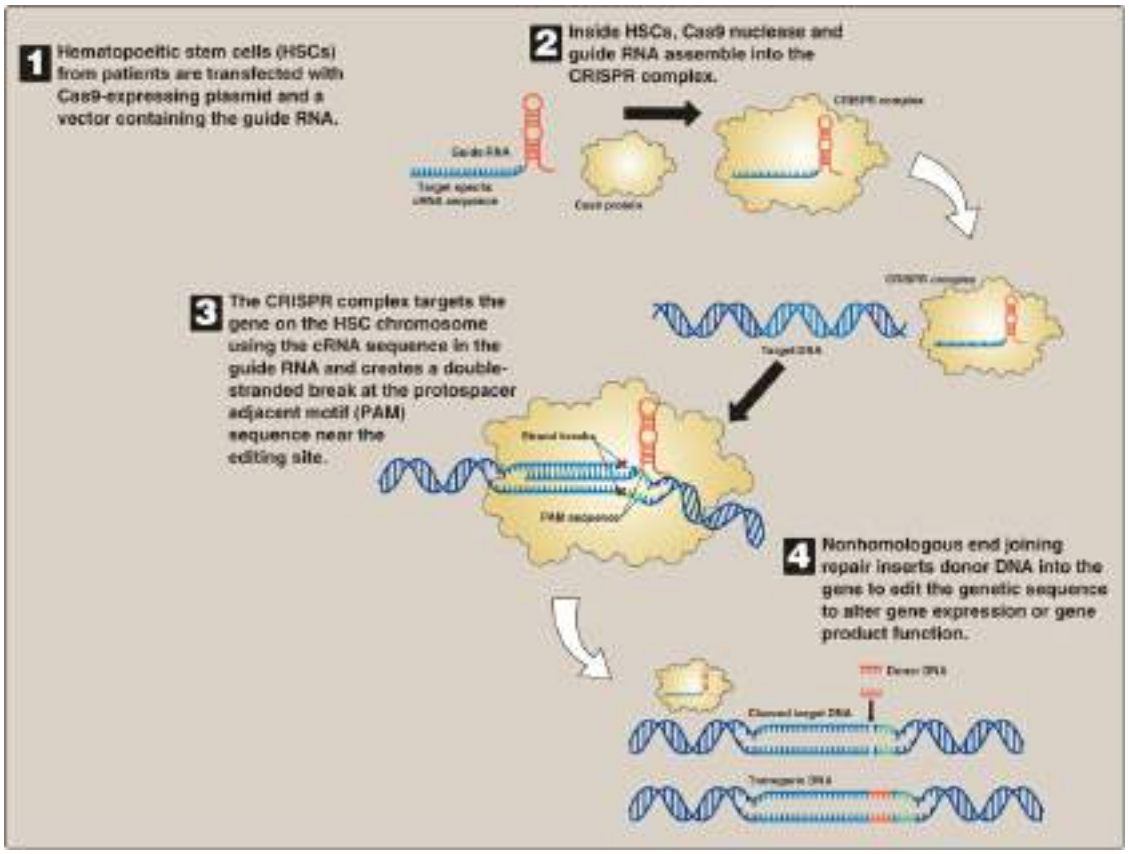


Figure 34.27
CRISPR-Cas9 gene editing mechanism in transfected human cells.

Study Questions

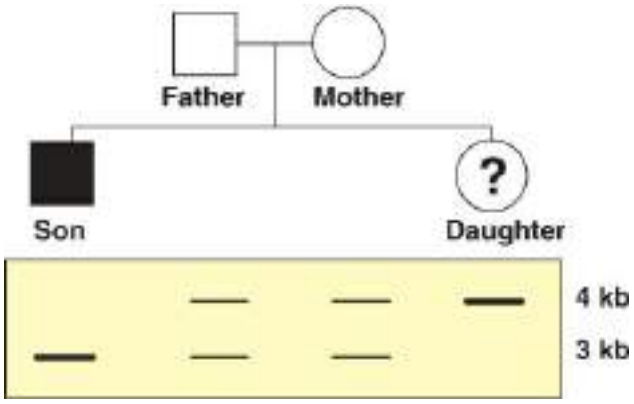
Choose the ONE best answer.

34.1 HindIII is a restriction endonuclease. Which of the following is most likely to be the recognition sequence for this enzyme?

A. AAGAAG
B. AAGAGA
C. AAGCTT
D. AAGGAA
E. AAGTTC

Correct answer = C. The vast majority of restriction endonucleases recognize palindromes in double-stranded DNA, and AAGCTT is the only palindrome among the choices. Because the sequence of only one DNA strand is given, the base sequence of the complementary strand must be determined. To be a palindrome, both strands must have the same sequence when read in the 5′→3′ direction. Thus, the complement of 5′-AAGCTT-3′ is also 5′-AAGCTT-3′.

34.2 An Ashkenazi Jewish couple has their 6-month-old son evaluated for listlessness, poor head control, and a fixed gaze. Tay–Sachs disease, an autosomal-recessive disease of lipid degradation, is diagnosed. The couple also has a daughter. The family's pedigree is shown below, along with Southern blots of a restriction fragment length polymorphism very closely linked to the gene for hexosaminidase A, which is defective in Tay–Sachs disease. Which of the statements below is most accurate with respect to the daughter?



A. She has a 25% chance of having Tay–Sachs disease.
B. She has a 50% chance of having Tay–Sachs disease.
C. She has Tay–Sachs disease.
D. She is a carrier for Tay–Sachs disease.
E. She is homozygous normal.

Correct answer = E. Because they have an affected son, both the biologic father and mother must be carriers for this disease. The affected son must have inherited a mutant allele from each parent. Because he shows only the 3-kilobase (kb) band on the Southern blot, the mutant allele for this disease must be linked to the 3-kb band. The normal allele must be linked to the 4-kb band, and because the daughter inherited only the 4-kb band, she must be homozygous normal for the hexosaminidase A gene.

34.3 A physician would like to determine the global patterns of gene expression in two different types of tumor cells in order to develop the most appropriate form of chemotherapy for each patient. Which of the following techniques would be most appropriate for this purpose?

A. Enzyme-linked immunosorbent assay
B. Microarray
C. Northern blot
D. Southern blot
E. Western blot

Correct answer = B. Microarray analysis allows the determination of messenger RNA (mRNA) production (gene expression) from thousands of genes at once. A northern blot only measures mRNA production from one gene at a time. Western blots and enzyme-linked immunosorbent assay measure protein production (also gene expression) but only from one gene at a time. Southern blots are used to analyze DNA, not the products of DNA expression.

34.4 A 2-week-old infant is diagnosed with a urea cycle defect. Enzymic analysis showed no activity for ornithine transcarbamoylase (OTC), an enzyme of the cycle. Molecular analysis revealed that the messenger RNA (mRNA) product of the gene for OTC was identical in length to that of a control. Which of the techniques listed below was most likely used to analyze mRNA?

A. Dideoxy chain termination
B. Northern blot
C. Polymerase chain reaction
D. Southern blot
E. Western blot

Correct answer = B. Northern blot allows analysis of the messenger RNA present (expressed) in a particular cell or tissue. Southern blot is used for DNA analysis, whereas western blot is used for protein analysis. Dideoxy chain termination is used to sequence DNA. Polymerase chain reaction is used to generate multiple, identical

copies of a DNA sequence *in vitro*.

34.5 For the patient above, which phase of the central dogma was most likely affected?

Correct answer = Translation. The gene is present and is able to be expressed as evidenced by normal production of messenger RNA. The lack of enzymic activity means that some aspect of protein synthesis is affected.

Blood Clotting 35

I. OVERVIEW

Blood clotting (coagulation) is designed to rapidly stop bleeding from a damaged blood vessel in order to maintain a constant blood volume (hemostasis). Coagulation is accomplished through vasoconstriction and the formation of a clot (thrombus) that consists of a plug of platelets (primary hemostasis) and a meshwork of the protein fibrin (secondary hemostasis) that stabilizes the platelet plug. Clotting occurs in association with membranes on the surface of platelets and damaged blood vessels (Fig. 35.1). (Note: If clotting occurs within an intact vessel such that the lumen is occluded and blood flow is impeded, a condition known as thrombosis, serious tissue damage, and even death can occur. This is what happens, for example, during a myocardial infarction [MI].) Processes to limit clot formation to the area of damage and remove the clot once vessel repair is underway also play essential roles in hemostasis. (Note: Separate discussions of the formation of the platelet plug and the fibrin meshwork facilitate presentation of these multistep, multicomponent processes. However, the two work together to maintain hemostasis.)

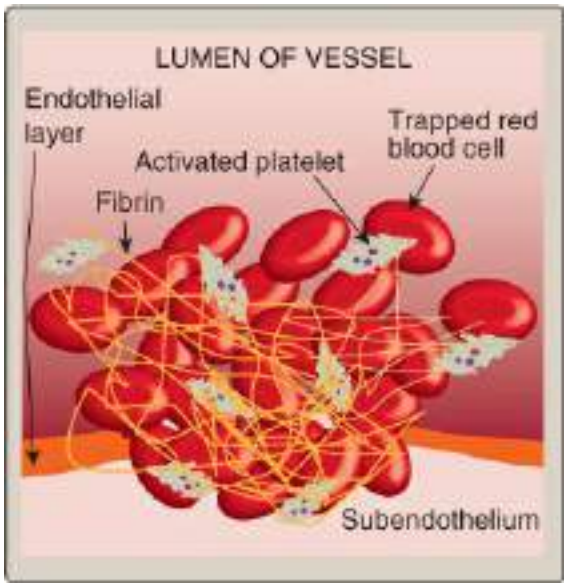


Figure 35.1
A blood clot formed by a plug of activated platelets and a meshwork of fibrin at the site of vessel injury.

II. SECONDARY HEMOSTASIS—FIBRIN MESHWORK FORMATION

The formation of the fibrin meshwork requires participation of platelets and involves two

unique pathways, the extrinsic and intrinsic pathways that converge to form a common pathway (Fig. 35.2). In each pathway, the major components are proteins (called factors [F]) designated by Roman numerals. A few factors have additional names. For example, factor I (FI) is fibrinogen and factor II (FII) is prothrombin. The factors are glycoproteins that are synthesized and secreted primarily by the liver.

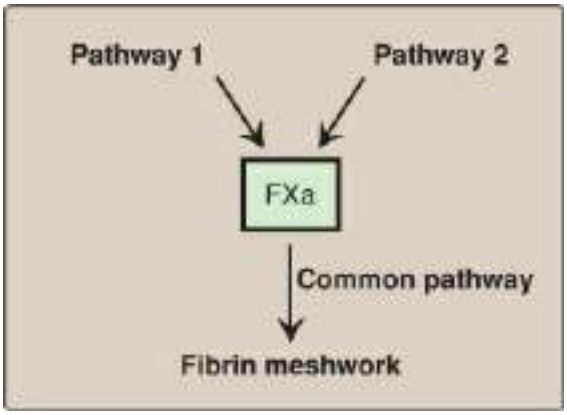


Figure 35.2
Three pathways involved in formation of the fibrin meshwork. F = factor; a = active.

A. Proteolytic cascade

In response to a vascular injury, the factors, which are inactive zymogen proteases, are converted sequentially to an active form by proteolytic cleavage. The protein product of one activation reaction initiates the next cleavage event in a cascade. The active form of an F is denoted by a lowercase “a” after the numeral. The active proteins FIIa (also called thrombin), FVIIa, FIXa, FXa, and FXIa, are enzymes of the serine protease family and cleave a peptide bond on the carboxyl side of an arginine or lysine residue in a polypeptide. For example, FIX is activated through cleavage at arginine 145 and arginine 180 by FXIa (Fig. 35.3). The proteolytic cascade results in enormous rate acceleration, because one active protease can produce many molecules of active product each of which, in turn, can activate many molecules of the next protein in the cascade. In some cases, activation can be caused by a conformational change in the protein in the absence of proteolysis. Nonproteolytic proteins also play a role as accessory proteins (cofactors) in the pathways. FIII (also called tissue factor [TF]), FV, and FVIII are the accessory proteins.

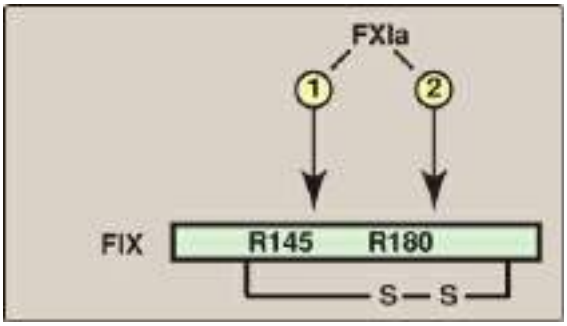


Figure 35.3
Activation of FIX via proteolysis by the serine protease FXIa. (Note: Activation can occur by conformational change for some of the factors.) F = factor; a = active; R = arginine.

B. Role of phosphatidylserine and calcium

The presence of the negatively charged phospholipid phosphatidylserine (PS) and positively charged calcium ions (Ca^{2+}) accelerates the rate of some steps in the clotting cascade.

1. Phosphatidylserine: PS is located primarily on the intracellular (cytosolic) face of the plasma membrane. Its exposure signals injury to the endothelial cells that line blood vessels. PS is also exposed on the surface of activated platelets.

2. Calcium ions: Ca^{2+} binds the negatively charged γ-carboxyglutamate (Gla) residues present in four of the serine proteases of clotting (FII, FVII, FIX, and FX), facilitating the binding of these proteins to exposed phospholipids (Fig. 35.4). The Gla residues are good chelators of Ca^{2+} because of their two adjacent negatively charged carboxylate groups (Fig. 35.5). (Note: The use of chelating agents, such as sodium citrate to bind Ca^{2+} in blood-collecting tubes or bags, prevents the blood from clotting.)

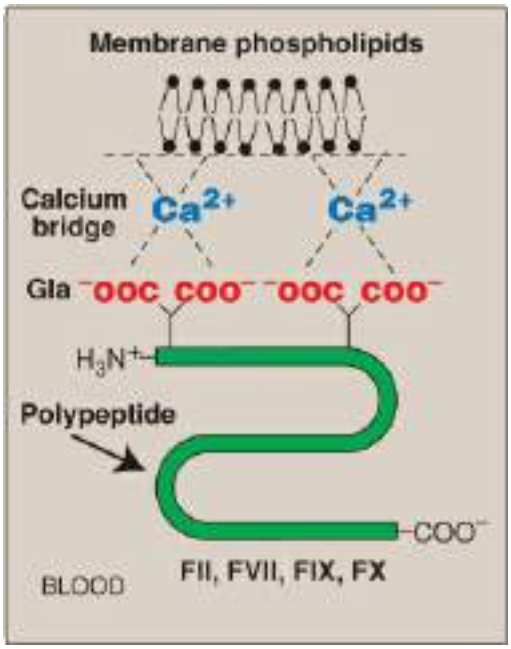


Figure 35.4
Ca^{2+} facilitates the binding of γ-carboxyglutamate (Gla)-containing factors to membrane phospholipids. F = factor.

C. Formation of γ-carboxyglutamate residues

γ-Carboxylation is a posttranslational modification in which 9 to 12 glutamate residues (at the amino [N]-terminus of the target protein) become carboxylated at the γ carbon, thereby forming Gla residues. The process occurs within the rough endoplasmic reticulum (RER) of hepatocytes.

1. γ-Carboxylation: This carboxylation reaction requires a protein substrate, oxygen (O_2), carbon dioxide (CO_2), γ-glutamyl carboxylase, and the hydroquinone form of vitamin K as a coenzyme (Fig. 35.6). In the reaction, the hydroquinone form of vitamin K is oxidized to its epoxide form as O_2 is reduced to water. (Note: Dietary vitamin K, a fat-soluble vitamin [see p. 424], is reduced from the quinone form to the hydroquinone coenzyme form by vitamin K reductase [Fig. 35.7].)

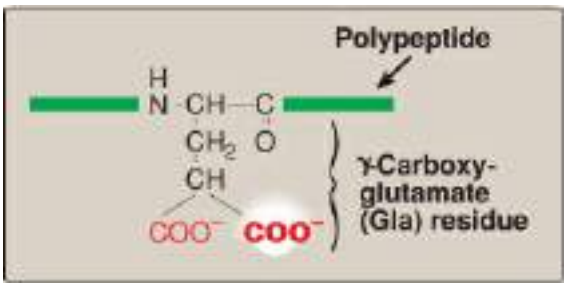


Figure 35.5
Gla residue.

2. Inhibition by warfarin: The formation of Gla residues is sensitive to inhibition by warfarin, a synthetic analog of vitamin K that inhibits the enzyme vitamin K epoxide reductase (VKOR). The reductase, an integral protein of the RER membrane, is required to regenerate the functional hydroquinone form of vitamin K from the epoxide form generated in the γ-carboxylation reaction. Thus, warfarin and related drugs act as anticoagulants that inhibit clotting by functioning as vitamin K antagonists. Warfarin salts are used therapeutically to limit clot formation. (Note: Warfarin is also used commercially in rat poison. It was developed by the Wisconsin Alumni Research Foundation, hence the name.)

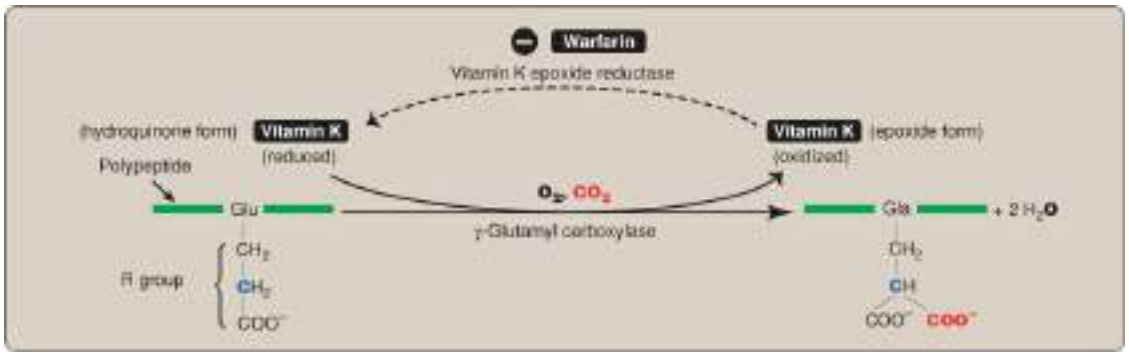


Figure 35.6
γ-Carboxylation of a glutamate (Glu) residue to γ-carboxyglutamate (Gla) by vitamin K–requiring γ-glutamyl carboxylase. The γ carbon is shown in *blue*. O_2 = oxygen; CO_2 = carbon dioxide.

D. Pathways

Two pathways may initiate the formation of the fibrin meshwork: the extrinsic pathway and the intrinsic pathway, which converge on the common pathway to create the fibrin clot. Production of FXa by both the extrinsic and intrinsic pathways triggers the common pathway (see Fig. 35.2).

1. Extrinsic pathway: This pathway involves a protein, TF that is not ordinarily in the blood but becomes exposed when blood vessels are injured. TF (or, FIII) is a transmembrane glycoprotein abundant in vascular subendothelium. It is an extravascular accessory protein and not a protease. Any injury that exposes TF to blood rapidly (within seconds) initiates the extrinsic or TF pathway. Once exposed, TF binds a circulating Gla-containing protein, FVII, activating it through conformational change. (Note: FVII also may be activated proteolytically by thrombin [see 3. below], or by several other serine proteases.) FVII–TF complex activation requires the presence of Ca^{2+} and phospholipids. The TF–FVIIa complex then binds and activates FX by proteolysis (Fig. 35.8). Therefore, activation of FX by the extrinsic pathway occurs in association with the cell membrane. FXa goes on to promote the common pathway activation of FII (prothrombin) to generate FIIa (thrombin). The extrinsic pathway is quickly inactivated by TF pathway inhibitor (TFPI) that, in an FXa-dependent process,

binds to the TF–FVIIa complex and prevents further production of FXa.

Clinical Application 35.1: Warfarin Response

Genetic differences (genotypes) in the gene for catalytic subunit 1 of VKOR (VKORC1) influence patient response to warfarin. For example, a polymorphism (see p. 541) in the promoter region of the gene decreases gene expression, resulting in less VKOR being made, thereby necessitating a lower dose of warfarin to achieve a therapeutic level. Polymorphisms in the cytochrome P450 enzyme (CYP2C9) that metabolizes warfarin are also known. In 2010, the U.S. Food and Drug Administration added a genotype-based dose table to the warfarin label. The influence of genetics on an individual's response to drugs is known as pharmacogenetics.

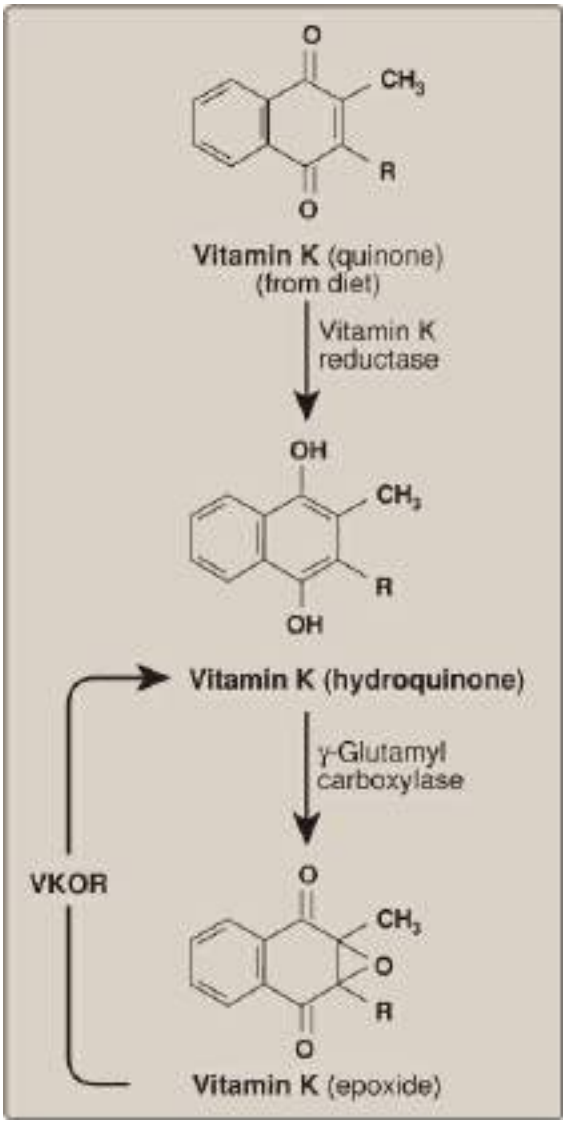


Figure 35.7
The vitamin K cycle. VKOR = vitamin K epoxide reductase.

2. Intrinsic pathway: All of the protein factors involved in the intrinsic pathway are

present in the blood and are, therefore, intravascular. The sequence of events leading to the activation of FX to FXa by the intrinsic pathway is initiated by thrombin. Thrombin converts FXI to FXIa, which in turn activates FIX, a Gla-containing serine protease. FIXa combines with FVIIIa (a blood-borne accessory protein), and this complex activates FX, another Gla-containing serine protease (Fig. 35.9). (Note: The complex containing FIXa, FVIIIa, and FX forms on exposed negatively charged membrane regions, where FX is activated to FXa. This complex is sometimes referred to as Xase. Binding of the complex to membrane phospholipids requires Ca^{2+}.)

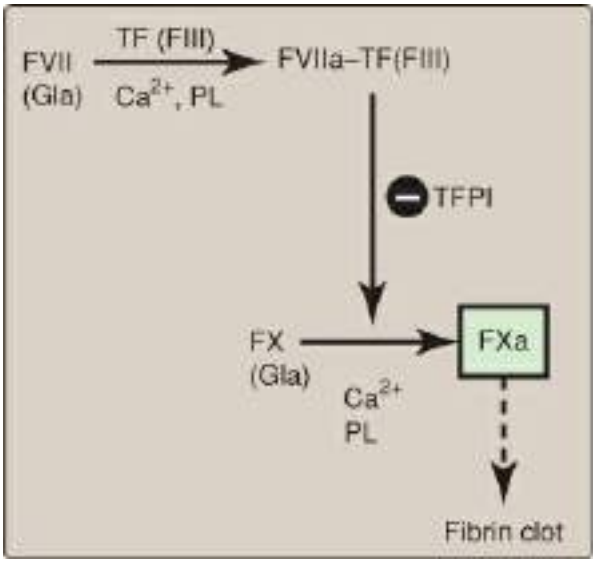


Figure 35.8
The extrinsic or tissue factor (TF) pathway. Binding of FVII to exposed TF (FIII) activates FVII. (Note: The pathway is quickly inhibited by tissue factor pathway inhibitor [TFPI].) F = factor; Gla = γ-carboxyglutamate; Ca^{2+} = calcium; PL = phospholipid; a = active.

The inactivation of the extrinsic pathway by TFPI results in dependence on the intrinsic pathway for continued production of FXa. This explains why individuals with hemophilia bleed even though they have an intact extrinsic pathway.

3. Common pathway: FXa produced by both the intrinsic and the extrinsic paths initiates the common pathway, a sequence of reactions that results in the generation of fibrin (FIa), as shown in Figure 35.11. FXa associates with FVa (a blood-borne accessory protein) and, in the presence of Ca^{2+} and phospholipids, forms a membrane-bound complex referred to as prothrombinase. The complex cleaves prothrombin (FII) to thrombin (FIIa). (Note: FVa potentiates the proteolytic activity of FXa.) The binding of Ca^{2+} to the Gla residues in FII facilitates the binding of FII to the membrane and to the prothrombinase complex, with subsequent cleavage to FIIa. Cleavage excises the Gla-containing region, releasing FIIa from the membrane and, thereby, freeing it to

activate fibrinogen (FI) in the blood. (Note: This is the only example of cleavage of a Gla protein that results in the release of a Gla-containing peptide. The peptide travels to the liver where it is thought to act as a signal for increased production of clotting proteins.) Oral, direct inhibitors of FXa have been approved for clinical use as anticoagulants. In contrast to warfarin, they have a more rapid onset and shorter half-life and do not require routine monitoring.

Clinical Application 35.2: Hemophilia

Hemophilia is a coagulopathy—a defect in the ability to clot. Hemophilia A, which accounts for 80% of all hemophilia, results from deficiency of FVIII, whereas deficiency of FIX causes hemophilia B. Each deficiency is characterized by decreased and delayed ability to clot and/or formation of abnormally friable (easily disrupted) clots. This can be manifested, for example, by bleeding into the joints (Fig. 35.10). The extent of the factor deficiency determines the severity of the disease. Current treatment for hemophilia is factor replacement therapy using FVIII or FIX obtained from pooled human blood or from recombinant DNA technology. However, antibodies to the factors can develop. Gene therapy is a goal. Because the genes for both proteins are on the X chromosome, hemophilia is an X-linked disorder. (Note: Deficiency of FXI results in a bleeding disorder that sometimes is referred to as hemophilia C.)

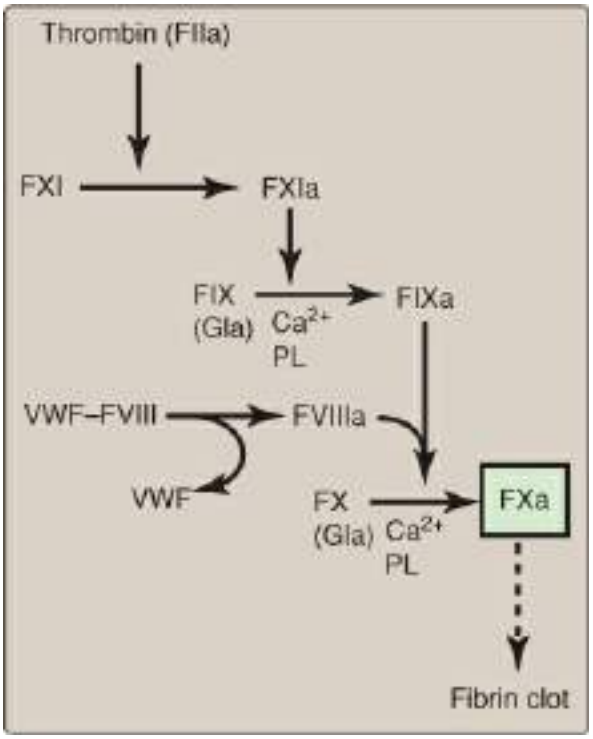


Figure 35.9
FX-activation phase of the intrinsic pathway. (Note: von Willebrand factor [VWF] stabilizes FVIII in the circulation.) Gla = γ-carboxyglutamate; PL = phospholipid; a = active; F = factor; Ca^{2+} = calcium.

A common point mutation (G20210A) in which an adenine (A) replaces a guanine (G) at

nucleotide 20210 in the 3′ untranslated region of the gene for FII leads to increased levels of FII in the blood. This results in one type of thrombophilia, a condition characterized by an increased tendency for blood to clot.

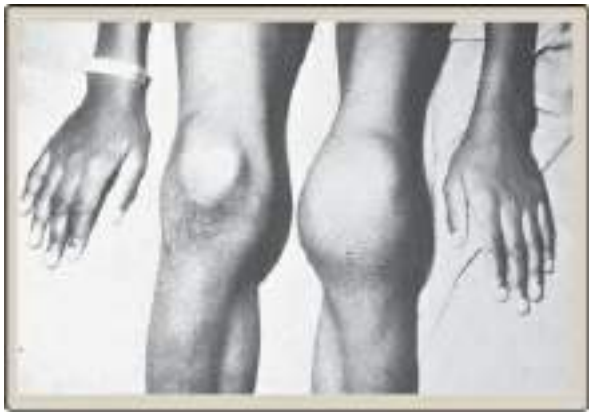


Figure 35.10
Acute bleeding into joint spaces (hemarthrosis) in an individual with hemophilia.

a. Fibrinogen cleavage to fibrin: Fibrinogen (sometimes referred to as FI) is a soluble glycoprotein made by the liver. It consists of dimers of three different polypeptide chains ($[\alpha\beta\gamma]_2$) held together at the N termini by disulfide bonds. The N termini of the α and β chains form "tufts" on the central of three globular domains (Fig. 35.12). The tufts are negatively charged and result in repulsion between fibrinogen molecules. Thrombin (FIIa) cleaves the charged tufts (releasing fibrinopeptides A and B), and FI becomes FIa (fibrin). As a result of the loss of charge, the fibrin monomers are able to noncovalently associate in a staggered array, and a soft (soluble) fibrin clot is formed.

b. Fibrin cross-linking: The associated fibrin molecules become covalently cross-linked, converting the soft clot to a hard (insoluble) clot. FXIIIa, a transglutaminase, covalently links the γ-carboxamide of a glutamine residue in one fibrin molecule to the ε-amino of a lysine residue in another through formation of an isopeptide bond and release of ammonia (Fig. 35.13). (Note: FXIII is also activated by thrombin.)

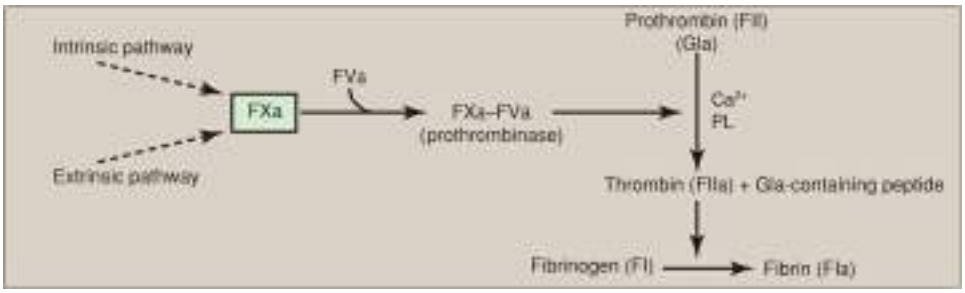


Figure 35.11

Generation of fibrin by FXa and the common pathway. F = factor; Gla = γ-carboxyglutamate; PL = phospholipid; a = active; Ca^{2+} = calcium.

c. Importance of thrombin: The activation of FX by the extrinsic pathway provides the "spark" of FXa that results in the initial activation of thrombin. Thrombin (FIIa) then activates factors of the common (FV, FI, FXIII), intrinsic (FXI, FVIII), and extrinsic (FVII) pathways (Fig. 35.14). The extrinsic pathway, then, initiates clotting by the generation of FXa, and the intrinsic pathway amplifies and sustains clotting after the extrinsic pathway has been inhibited by TFPI. (Note: Hirudin, a peptide secreted from the salivary gland of medicinal leeches, is a potent direct thrombin inhibitor [DTI]. Injectable recombinant hirudin has been approved for clinical use. Dabigatran is an oral DTI.) Additional cross talk between the pathways of clotting is achieved by the FVIIa–TF-mediated activation of the intrinsic pathway and the FXIIa-mediated activation of the extrinsic pathway. The complete picture of physiologic blood clotting via the formation of a hard fibrin clot is shown in Figure 35.15. The factors of the clotting cascade are shown organized by function in Figure 35.16.

Clinical laboratory tests are available to evaluate the function of the extrinsic through common pathways (prothrombin time [PT] using thromboplastin and expressed as the international normalized ratio [INR]) and the intrinsic through common pathways (activated partial thromboplastin time [aPTT]). Thromboplastin is a combination of phospholipids + FIII. A derivative, partial thromboplastin, contains just the phospholipid portion because FIII is not needed to activate the intrinsic pathway.

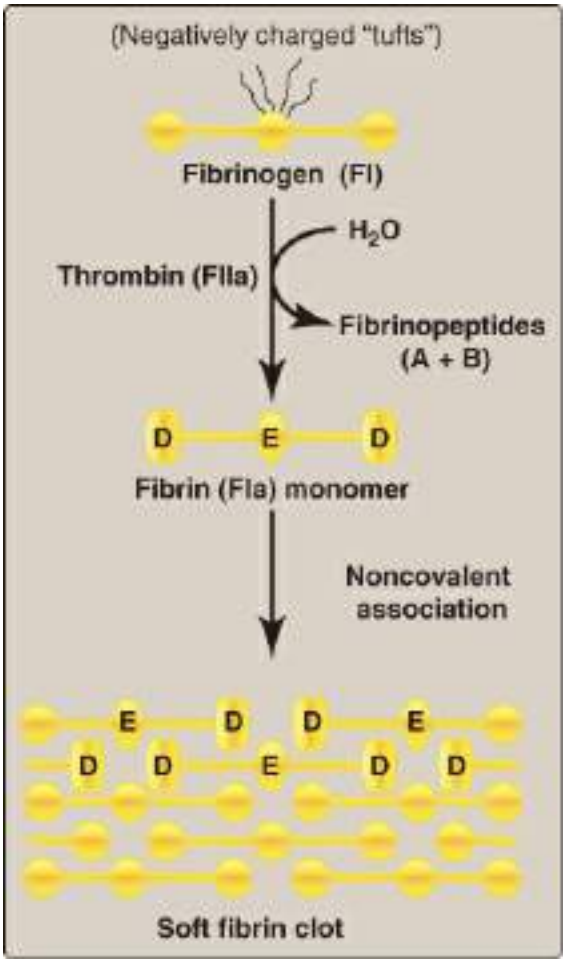


Figure 35.12
Conversion of fibrinogen to fibrin and formation of the soft fibrin clot. (Note: D and E refer to nodular domains on the protein.)

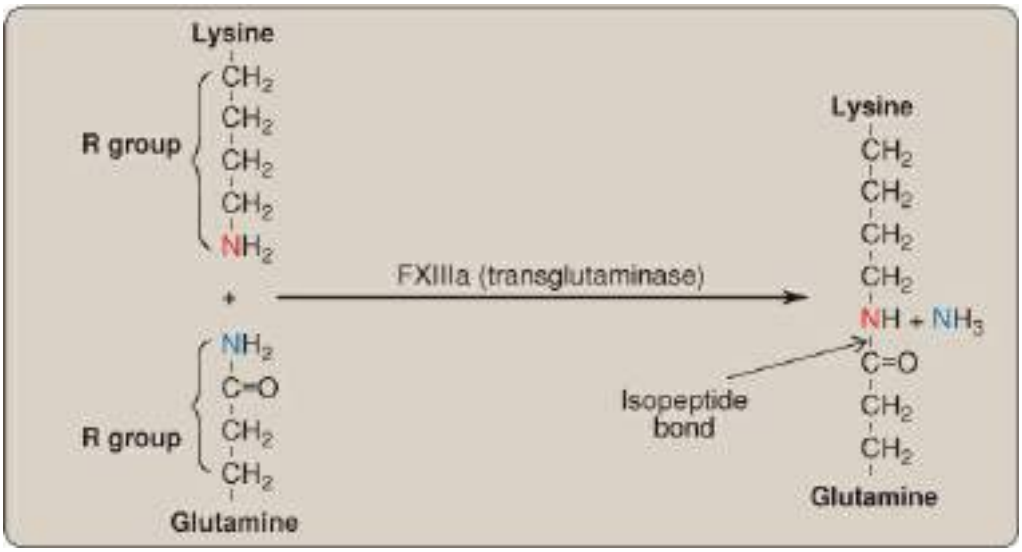


Figure 35.13
Cross-linking of fibrin. FXIIIa forms a covalent isopeptide bond between lysine and glutamine residues. F

= factor; NH_3 = ammonia.

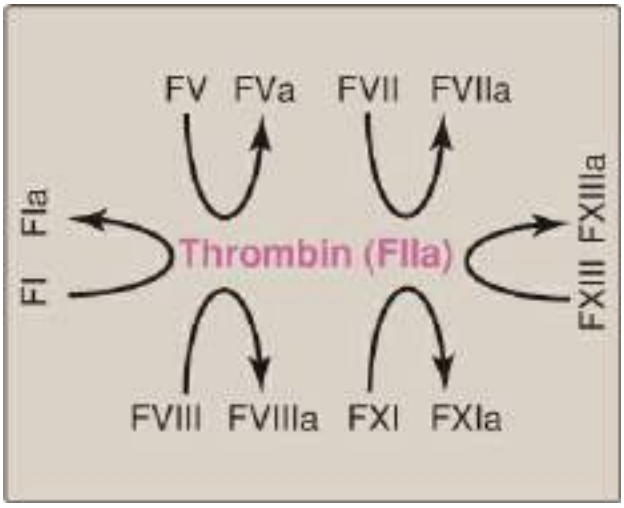


Figure 35.14
The importance of thrombin in formation of the fibrin clot. a = active; F = factor.

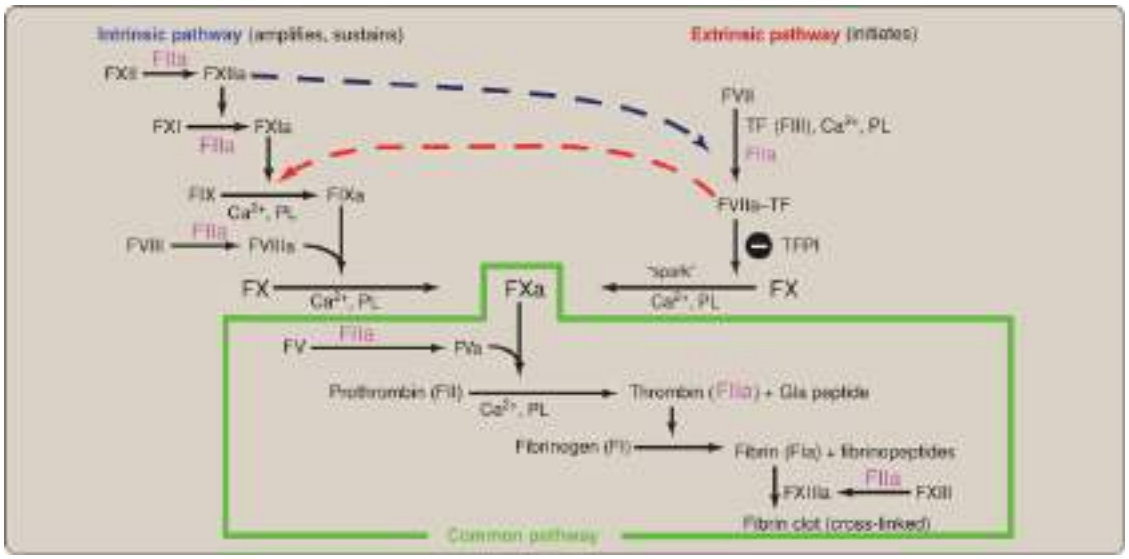


Figure 35.15
The complete picture of physiologic blood clotting via the formation of a cross-linked (hard) fibrin clot. a = active; F = factor; TF = tissue factor; TFPI = tissue factor pathway inhibitor; PL = phospholipid; Ca^{2+} = calcium; Gla = γ-carboxyglutamate.

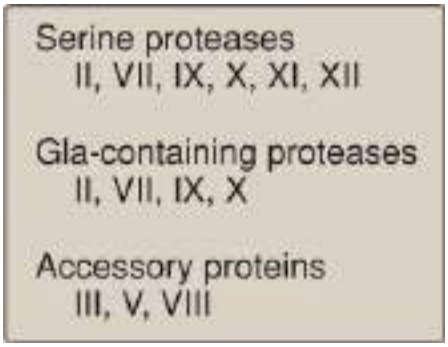


Figure 35.16
Protein factors of the clotting cascade organized by function. The activated form would be denoted by an "a" after the numeral. (Note: Calcium is IV. There is no VI. I (fibrin) is neither a protease nor an accessory protein. XIII is a transglutaminase.) Gla = γ-carboxyglutamate.

III. LIMITING CLOTTING

The ability to limit clotting to areas of damage (anticoagulation) and remove clots once repair processes are underway (fibrinolysis) are exceedingly important aspects of hemostasis. These actions are performed by proteins that inactivate clotting factors either by binding to them and removing them from the blood or by degrading them and also by proteins that degrade the fibrin meshwork.

A. Inactivating proteins

Proteins synthesized by the liver and by the blood vessels themselves balance the need to form clots at sites of vessel injury with the need to limit their formation beyond the injured area.

1. Antithrombin: Antithrombin III (ATIII; also referred to as antithrombin, AT), is a hepatic protein that circulates in the blood. It inactivates free thrombin by binding to it and carrying it to the liver (Fig. 35.17), preventing it from participating in coagulation. (Note: ATIII is a serine protease inhibitor or "serpin." A serpin contains a reactive loop to which a specific protease binds. Once bound, the protease cleaves a peptide bond in the serpin causing a conformational change that traps the enzyme in a covalent complex. α_1-Antitrypsin [see p. 52] is also a serpin.) The affinity of ATIII for thrombin is greatly increased when ATIII is bound to heparan sulfate, an intracellular glycosaminoglycan (see p. 173) released in response to injury by mast cells associated with blood vessels. Heparin, an anticoagulant, is used therapeutically to limit clot formation. (Note: In contrast to the anticoagulant warfarin, which has a slow onset and a long half-life and is administered orally, heparin has a rapid onset and a short half-life and requires intravenous administration. The two drugs are commonly used in an overlapping manner in the treatment [and prevention] of thrombosis.) ATIII also inactivates FXa and the other serine proteases of clotting, FIXa, FXIa, FXIIa, and the FVIIa–TF complex.

(Note: ATIII binds to a specific pentasaccharide within the oligosaccharide form of heparin. Inhibition of FIIa requires the oligosaccharide form, whereas inhibition of FXa requires only the pentasaccharide form. Fondaparinux, a synthetic version of the pentasaccharide, is used clinically to inhibit FXa.)

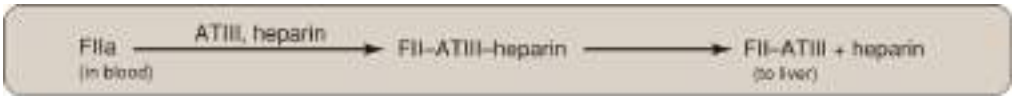


Figure 35.17
Inactivation of FIIa (thrombin) by binding of antithrombin III (ATIII) and transport to the liver. (Note: Heparin increases the affinity of ATIII for FIIa.) a = active; F = factor.

2. Protein C–protein S complex: Protein C, a circulating Gla-containing protein made in the liver, is activated by thrombin complexed with thrombomodulin. Thrombomodulin, an integral membrane glycoprotein of endothelial cells, binds thrombin, thereby decreasing thrombin's affinity for fibrinogen and increasing its affinity for protein C. Protein C in complex with protein S, also a Gla-containing protein, forms the activated protein C (APC) complex that cleaves the accessory proteins FVa and FVIIIa, which are required for maximal activity of FXa (Fig. 35.18). Protein S helps anchor APC to the clot. Thrombomodulin, then, modulates the activity of thrombin, converting it from a protein of coagulation to a protein of anticoagulation, thereby limiting the extent of clotting. Factor V Leiden is a mutant form of FV with a glutamine substituted for arginine at position 506 and a resistance to APC. It is the most common inherited cause of thrombophilia in the United States, with highest frequency in the Caucasian population. Heterozygotes are estimated to have a 7-fold increase in the risk for venous thrombosis, and homozygotes have up to a 50-fold increase. (Note: Women with FV Leiden are at even greater risk of thrombosis during pregnancy or when taking estrogen.)

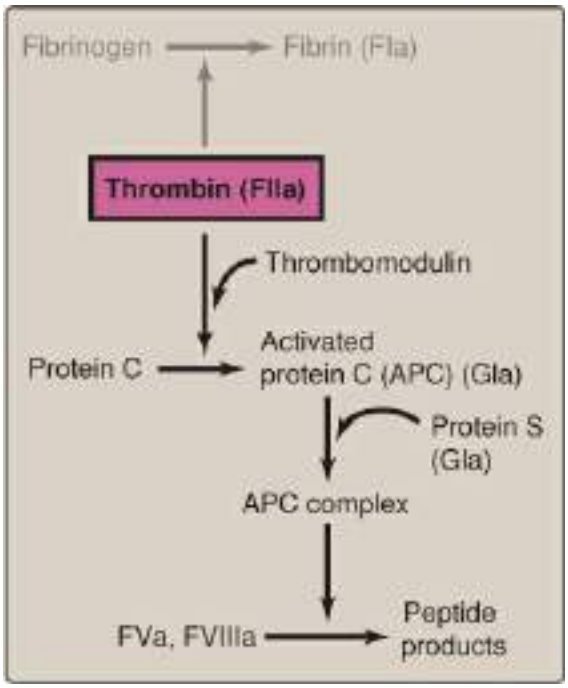


Figure 35.18
Formation and action of the APC complex. Gla = γ-carboxyglutamate; a = active; F = factor.

Clinical Application 35.3: Thrombophilia

Thrombophilia (hypercoagulability) can also result from deficiencies of proteins C, S, and ATIII; as well as from the presence of FV Leiden and from excess production of thrombin (G20210A mutation). Another form of thrombophilia is caused by antiphospholipid antibodies, which may be present in persons with autoimmune disorders, such as lupus. (Note: A thrombus that forms in the deep veins of the leg [deep venous thrombosis, or DVT] can cause a pulmonary embolism [PE] if the clot [or a piece of it] breaks off, travels to the lungs, and blocks circulation.)

B. Fibrinolysis

Clots are temporary patches that must be removed once wound repair has begun. The fibrin clot is cleaved by the protease plasmin to fibrin degradation products (Fig. 35.19). (Note: Measurement of D-dimer, a fibrin degradation product containing two cross-linked D domains released by the action of plasmin, can be used to assess the extent of clotting.) Plasmin is a serine protease that is generated from plasminogen by plasminogen activators. Plasminogen, secreted by the liver into the circulation, binds to fibrin and is incorporated into clots as they form. Tissue plasminogen activator (TPA, t-PA), made by vascular endothelial cells and secreted in an inactive form in response to thrombin, becomes active when bound to fibrin–plasminogen. Bound plasmin and TPA_a are protected from their inhibitors, α_2-antiplasmin and plasminogen activator inhibitors, respectively. Once the fibrin clot is dissolved, plasmin and TPA_a become available to their inhibitors. Therapeutic fibrinolysis can sometimes be achieved by treatment with commercially available

TPA made by recombinant DNA techniques. Its use is now mostly for ischemic stroke. Mechanical clot removal (thrombectomy) is more commonly used for treatment of MI. (Note: Urokinase is a plasminogen activator [u-PA] made in a variety of tissues and originally isolated from urine. Streptokinase [from bacteria] activates both free and fibrin-bound plasminogen.)

Plasminogen contains structural motifs known as "kringle domains" that mediate protein–protein interactions. Because lipoprotein (a) (Lp[a]) also contains kringle domains, it competes with plasminogen for binding to FIa. The potential to inhibit fibrinolysis may be the basis for the association of elevated Lp(a) with increased risk for cardiovascular disease (see p. 253).

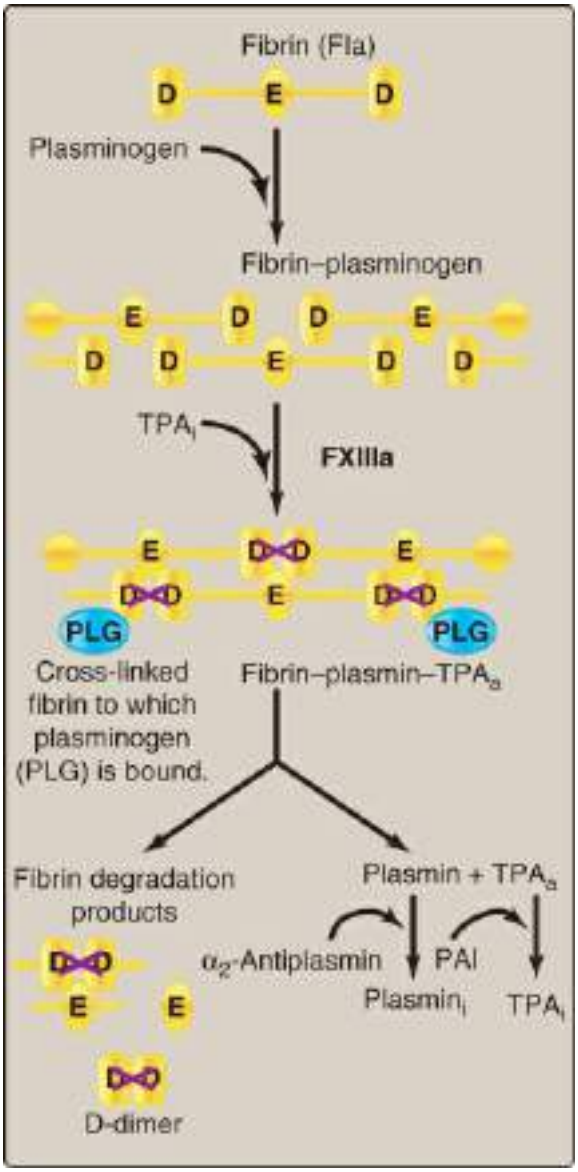


Figure 35.19
Fibrinolysis. Plasmin cleaves cross-linked fibrin into fibrin degradation products. Plasmin and TPA are released from the clot. TPA = tissue plasminogen activator; i = inactive; a = active; PAI = plasminogen

activator inhibitor.

IV. PRIMARY HEMOSTASIS—PLATELET PLUG FORMATION

Platelets (thrombocytes) are small, anucleate fragments of megakaryocytes that adhere to exposed collagen of damaged endothelium, get activated, and aggregate to form a platelet plug (Fig. 35.20; also see Fig. 35.1). Formation of the platelet plug is referred to as primary hemostasis because it is the first response to bleeding. In a healthy adult, there are 150,000 to 450,000 platelets per µL of blood. They have a life span of up to 10 days, after which they are taken up by the liver and spleen and destroyed. Clinical laboratory tests to measure platelet number and activity are available.

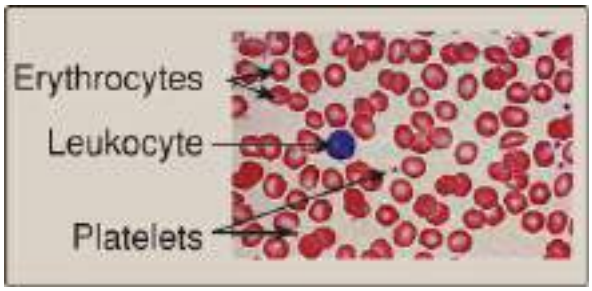


Figure 35.20
Size comparison of platelets, erythrocytes, and a leukocyte.

A. Adhesion

Adhesion of platelets to exposed collagen at the site of vessel injury is mediated by the protein von Willebrand factor (VWF). VWF binds to collagen, and platelets bind to VWF via glycoprotein Ib (GPIb), a component of a membrane receptor complex (GPIb–V–IX) on the platelet surface (Fig. 35.21). Binding to VWF stops the forward movement of platelets. (Note: Deficiency in the receptor for VWF results in Bernard–Soulier syndrome, a disorder of decreased platelet adhesion.) VWF is a glycoprotein that is released from platelets. It also is made and secreted by endothelial cells. In addition to mediating the binding of platelets to collagen, VWF also binds to and stabilizes FVIII in the blood. Deficiency of VWF results in von Willebrand disease (VWD), the most common inherited coagulopathy. VWD results from decreased binding of platelets to collagen and a deficiency in FVIII (due to increased degradation). Platelets can also bind directly to collagen via the membrane receptor glycoprotein VI (GPVI). Once adhered, platelets get activated. (Note: Damage to the endothelium also exposes TF, initiating the extrinsic pathway of blood clotting and activation of FX [see Fig. 35.8].)

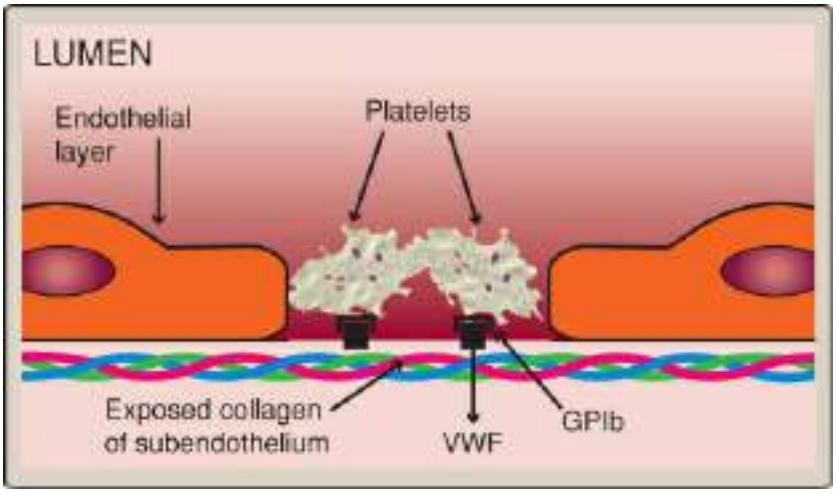


Figure 35.21
Binding of platelets via the glycoprotein Ib receptor (GPIb) to von Willebrand factor (VWF). VWF is bound to the exposed collagen at a site of injury.

B. Activation

Once adhered to areas of injury, platelets become activated. Platelet activation involves morphologic (shape) changes and degranulation, the process by which platelets secrete the contents of their α and δ (or, dense) storage granules. Activated platelets also expose PS on their surface. The externalization of PS is mediated by a Ca^{2+}-activated enzyme known as scramblase that disrupts the membrane asymmetry created by flippases (see p. 227). Thrombin is the most potent platelet activator. Thrombin binds to and activates protease-activated receptors, a type of G protein–coupled receptor (GPCR), on the surface of platelets (Fig. 35.22). Thrombin is primarily associated with G_q proteins (see p. 227), resulting in activation of phospholipase C and a rise in diacylglycerol (DAG) and inositol trisphosphate (IP_3). (Note: Thrombomodulin, through its binding of thrombin, decreases the availability of thrombin for platelet activation [see Fig. 35.18].)

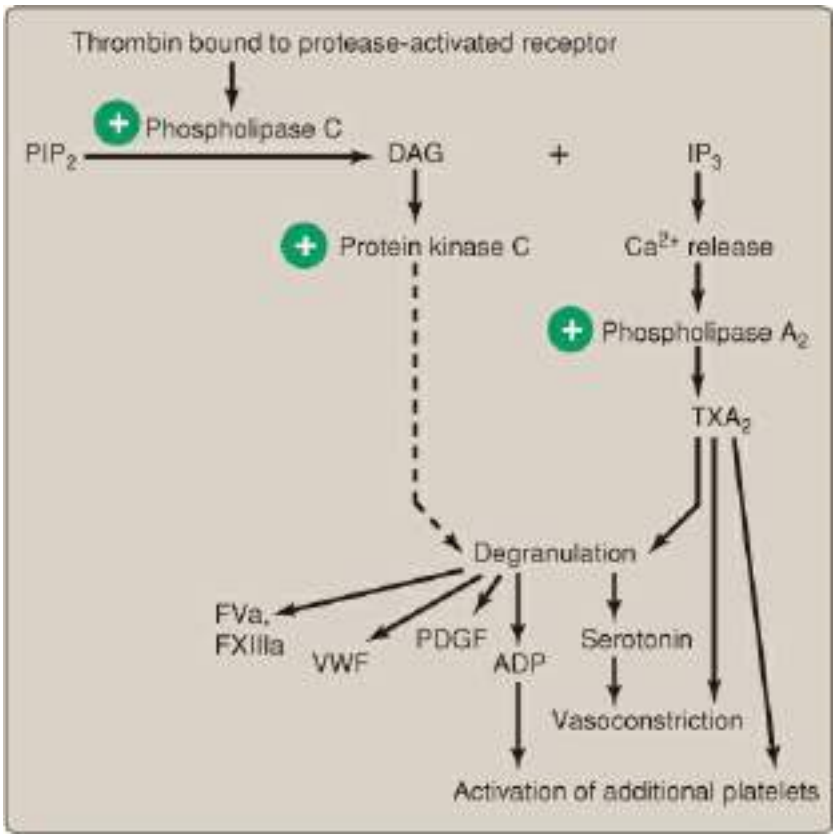


Figure 35.22
Platelet activation by thrombin. (Note: Protease-activated receptors are a type of G protein–coupled receptor.) PIP_2 = phosphoinositol bisphosphate; DAG = diacylglycerol; IP_3 = inositol trisphosphate; Ca^{2+} = calcium; TXA_2 = thromboxane A_2; ADP = adenosine diphosphate; PDGF = platelet-derived growth factor; VWF = von Willebrand factor; F = factor.

1. Degranulation: DAG activates protein kinase C, a key event for degranulation. IP_3 causes the release of Ca^{2+} (from dense granules). The Ca^{2+} activates phospholipase A_2, which cleaves membrane phospholipids to release arachidonic acid, the substrate for the synthesis of thromboxane A_2 (TXA_2) in activated platelets by cyclooxygenase-1 (COX-1) (see p. 236). TXA_2 causes vasoconstriction, augments degranulation, and binds to platelet GPCR, causing activation of additional platelets. Recall that aspirin irreversibly inhibits COX and, consequently, TXA_2 synthesis and is referred to as an antiplatelet drug. Degranulation also results in release of serotonin and adenosine diphosphate (ADP) from dense granules. Serotonin causes vasoconstriction. ADP binds to GPCR on the surface of platelets, activating additional platelets. (Note: Some antiplatelet drugs, such as clopidogrel, are ADP-receptor antagonists.) Platelet-derived growth factor (involved in wound healing), VWF, FV, FXIII, and fibrinogen are among other proteins released from α-granules. (Note: Platelet-activating factor [PAF], an ether phospholipid [see p. 225] synthesized by a variety of cell types including endothelial cells and platelets, binds PAF

receptors [GPCR] on the surface of platelets and activates them.)

2. Morphologic change: The change in shape of activated platelets from discoidal to spherical with pseudopod-like processes that facilitate platelet–platelet and platelet–surface interactions (Fig. 35.23) is initiated by the release of Ca^{2+} from dense granules. Ca^{2+} bound to calmodulin (see p. 144) mediates the activation of myosin light chain kinase that phosphorylates the myosin light chain, resulting in a major reorganization of the platelet cytoskeleton.

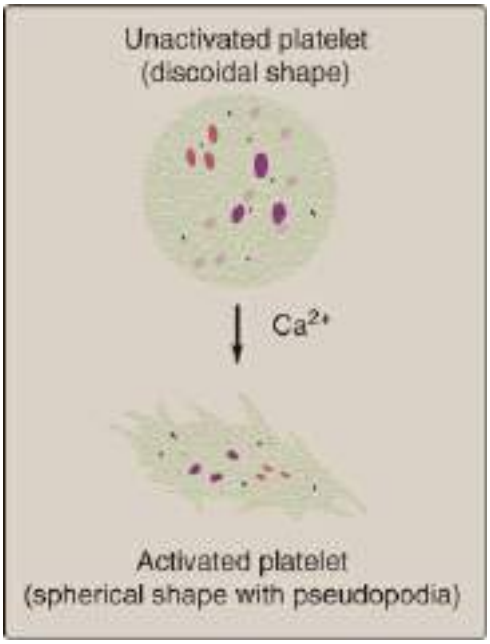


Figure 35.23
Activated platelets undergo calcium (Ca^{2+})-initiated shape change.

C. Aggregation

Activation causes dramatic changes in platelets that lead to their aggregation. Structural changes in a surface receptor (GPIIb/IIIa) expose binding sites for fibrinogen. Bound fibrinogen molecules link activated platelets to one another (Fig. 35.24), with a single fibrinogen able to bind two platelets. Fibrinogen is converted to fibrin by thrombin and then covalently cross-linked by FXIIIa coming from both the blood and the platelets. (Note: The exposure of PS on the surface of activated platelets allows formation of the Xase complex [VIIIa, IXa, X, and Ca^{2+}] with subsequent formation of FXa and generation of FIIa.) Fibrin formation (secondary hemostasis) strengthens the platelet plug. (Note: Rare defects in the platelet receptor for fibrinogen result in Glanzmann thrombasthenia [decreased platelet function], whereas autoantibodies to this receptor are a cause of immune thrombocytopenia [decreased platelet number].)

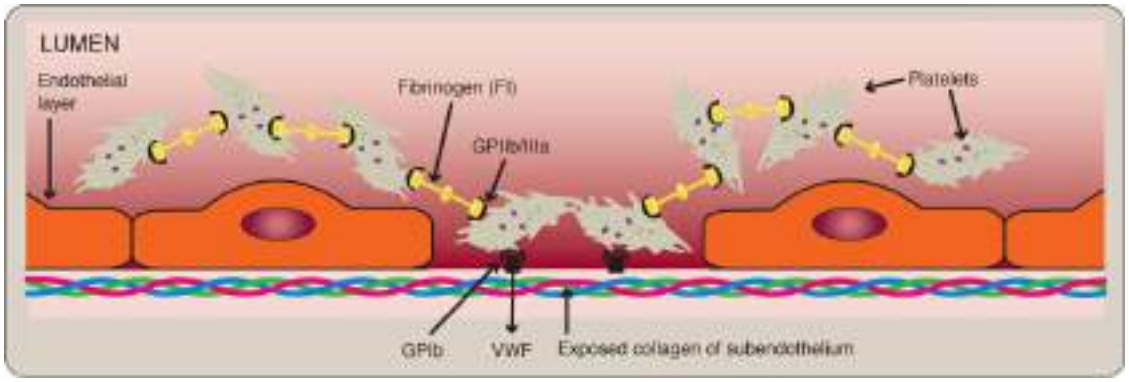


Figure 35.24
Linking of platelets by fibrinogen via the glycoprotein (GP) IIb/IIIa receptor. (Note: The shapes in the fibrinogen molecule represent the two D and one E domains.) GPIb = glycoprotein Ib receptor; VWF = von Willebrand factor.

Unnecessary activation of platelets is prevented because (1) an intact vascular wall is separated from the blood by a monolayer of endothelial cells, preventing the contact of platelets with collagen; (2) endothelial cells synthesize prostaglandin I_2 (PGI_2, or prostacyclin) and nitric oxide, each of which causes vasodilation; and (3) endothelial cells have a cell surface ADPase that converts ADP to adenosine monophosphate.

V. Chapter Summary

- **Blood clotting (coagulation)** rapidly stops bleeding from a damaged blood vessel in order to maintain a constant blood volume **(hemostasis)**. Coagulation is accomplished through formation of a **clot** (**thrombus**) consisting of a plug of **platelets** and a meshwork of the protein **fibrin** (Fig. 35.25).
- The formation of the **fibrin meshwork** by the **clotting cascade** involves the **extrinsic** and **intrinsic pathways**, which include protein factors (F) that converge at **FXa** to form the **common pathway**. Many of the protein factors are **serine proteases.**
- **γ-Glutamyl carboxylase** and its coenzyme, the hydroquinone form of **vitamin K**, are required for the formation of γ-carboxyglutamate (Gla) residues in the clotting proteases **FII**, **FVII**, **FIX**, and **FX**. Calcium and Gla residues facilitate the binding of these proteins to negatively charged **PS** at the site of vessel damage and on the surface of platelets.
- In the carboxylase reaction, vitamin K gets oxidized to the nonfunctional epoxide form. **Warfarin**, a synthetic analog of vitamin K used clinically to reduce clotting, inhibits the enzyme **VKOR** that regenerates the functional, reduced vitamin K.
- The extrinsic pathway is initiated by exposure of **FIII (TF)**, an **accessory protein** in vascular subendothelium. Circulating FVII binds to and is activated by TF forming **TF**–**FVIIa**, a complex that in turn activates FX by proteolysis. FXa allows **thrombin** (FIIa) production by the common pathway. Thrombin then activates components of the intrinsic pathway. The extrinsic pathway is rapidly inhibited by **TFPI.**
- The intrinsic pathway is initiated by **thrombin** which activates FXI to FXIa. FXIa activates FIX to FIXa. FIXa then combines with FVIIIa, and the complex activates FX. FVIII deficiency results in **hemophilia A**, whereas FIX deficiency results in the less common **hemophilia B**.
- In the common pathway, FXa associates with **FVa** (an accessory protein), forming **prothrombinase** that cleaves **prothrombin (FII)** to **thrombin (FIIa)**. Thrombin then cleaves **fibrinogen (FI)** to **fibrin (FIa)**.
- Fibrin monomers associate, forming a **soluble** (**soft**) **fibrin clot,** and are then **cross-linked** by **FXIIIa,** forming an **insoluble (hard)** fibrin clot. The fibrin clot is cleaved **(fibrinolysis)** by the protein **plasmin,** a serine protease that is generated from **plasminogen** by **plasminogen activators** such as **TPA (or t-PA)**. Recombinant TPA is used therapeutically in ischemic stroke.
- The liver and blood vessels produce **anticoagulation** proteins that limit coagulation. **AT**, a serine protease inhibitor or **serpin**, is activated by heparan sulfate (or the anticoagulant drug heparin) and binds to and removes thrombin and FXa from the blood. **Protein C** is activated by the **thrombin**–**thrombomodulin** complex and then forms a complex with **protein S,** producing **APC**. The APC complex cleaves the accessory proteins FVa and FVIIIa. **FV Leiden** is resistant to APC and causes the most common inherited **thrombophilic** condition in the United States.
- **Platelet plug** formation is initiated when a wound to a tissue damages blood vessels and exposes collagen in the vessel subendothelium to the vessel lumen. Platelets (thrombocytes) adhere to the exposed collagen through an interaction between GPIb on their surface and VWF that is bound to collagen in the subendothelium. Deficiency of VWF results in VWD, the most common inherited coagulopathy.
- Once adhered, platelets are activated and then aggregate at the damaged site. Activation involves changes in shape and degranulation, the process by which platelets release the contents of their storage granules. Thrombin is the most potent activator of platelets.
- Activated platelets release substances that cause vasoconstriction, recruit and activate other platelets, and support the formation of a fibrin clot. Structural changes in the surface receptor GPIIb/IIIa expose binding sites for fibrinogen that links activated platelets together to make the initial loose plug of platelets (primary hemostasis).
- Fibrinogen is converted to fibrin by thrombin. Fibrin is cross-linked by FXIIIa coming both from the blood and from platelets. This strengthens the fibrin meshwork and stabilizes the platelet plug (secondary hemostasis).
- Disorders of platelets and coagulation proteins can impair the ability to clot. PT and aPTT are clinical laboratory tests used to evaluate the clotting cascade.

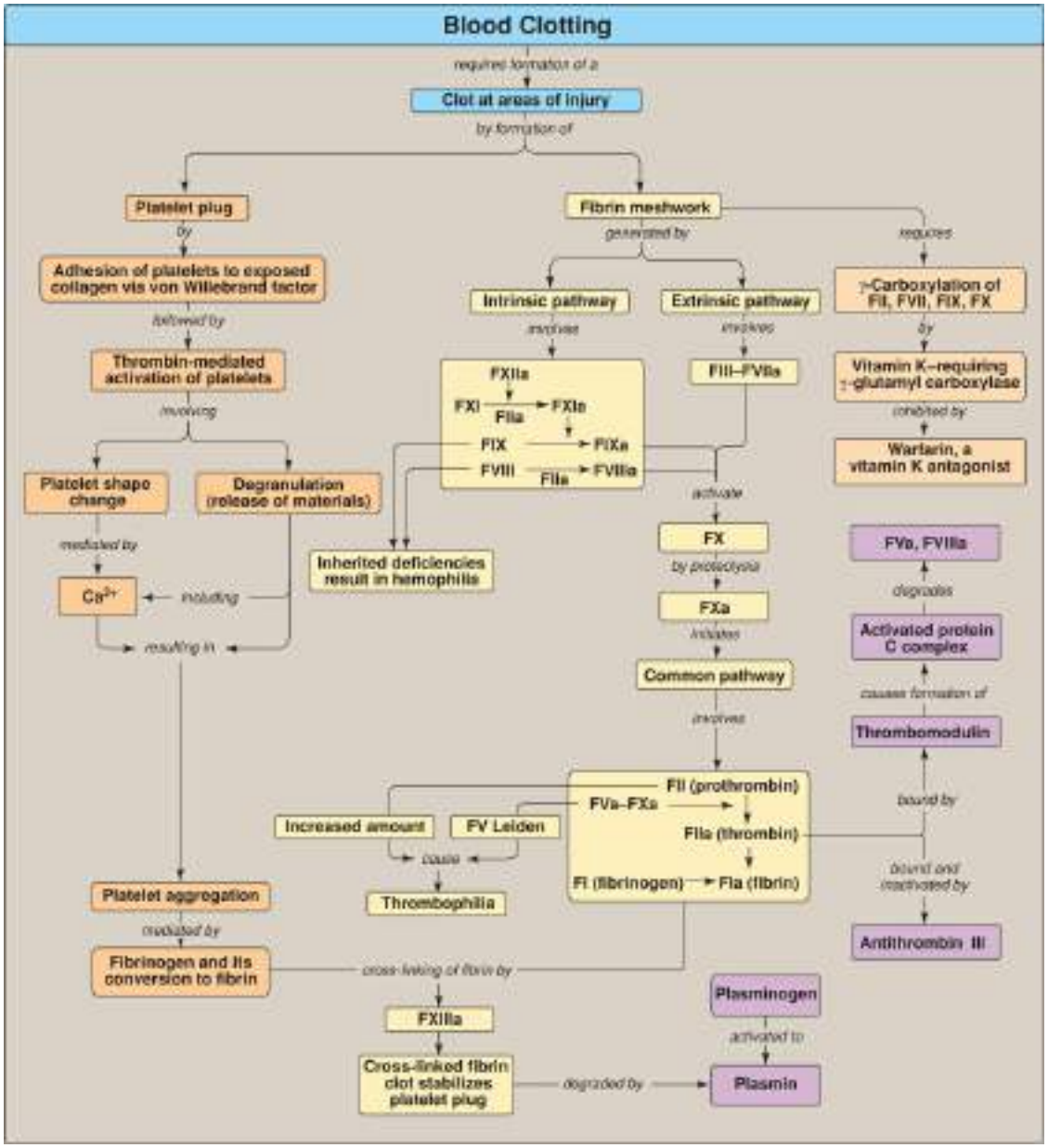


Figure 35.25
Key concept map for blood clotting. a = active; F = factor; Ca^{2+} = calcium.

Study Questions

Choose the ONE best answer.

For Questions 35.1–35.5, match the most appropriate protein factors (F) of clotting to the description.

A. FI
B. FII
C. FIII
D. FV
E. FVII
F. FVIII

G. FIX
H. FX
I. FXI
J. FXIII

35.1 This factor activates components of the intrinsic, extrinsic, and common pathways.

35.2 This factor converts the soluble clot to an insoluble clot.

35.3 This factor initiates the common pathway.

35.4 This factor is an accessory protein that potentiates the activity of factor Xa.

35.5 This factor is a γ-carboxyglutamate–containing serine protease of the extrinsic pathway.

Correct answers = B, J, H, D, E. Thrombin (FII) is formed in the common pathway and activates components in each of the three pathways of the clotting cascade. FXIII, a transglutaminase, covalently cross-links associated fibrin monomers, thereby converting a soluble clot to an insoluble one. The generation of FXa by the intrinsic and extrinsic pathways initiates the common pathway. FV increases the activity of FXa. It is one of three accessory (nonprotease) proteins. The others are FIII (tissue factor) and FVIII (complexes with FIX to activate FX). FVII is a γ-carboxyglutamate–containing serine protease that complexes with FIII in the extrinsic pathway.

35.6 In which patient would prothrombin time (PT) be unaffected and activated partial thromboplastin time (aPTT) be prolonged?

A. A patient on aspirin therapy
B. A patient with end-stage liver disease
C. A patient with hemophilia
D. A patient with thrombocytopenia

Correct answer = C. PT measures the activity of the extrinsic through the common pathways, and aPTT measures the activity of the intrinsic through the common pathways. Patients with hemophilia are deficient in either FVIII (hemophilia A) or FIX (hemophilia B), components of the common pathway. They have an intact extrinsic pathway. Therefore, the PT is unaffected, and the aPTT is prolonged. Patients on aspirin therapy and those with thrombocytopenia have alterations in platelet function and number, respectively, and not in the proteins of the clotting cascade. Therefore, both the PT and the aPTT are unaffected. Patients with end-stage liver disease have decreased ability to synthesize clotting proteins. They show prolonged PT and aPTT.

35.7 Which one of the following can be ruled out in a patient with thrombophilia?

A. A deficiency of antithrombin
B. A deficiency of FIX
C. A deficiency of protein C
D. An excess of prothrombin
E. Expression of FV Leiden

Correct answer = B. Symptomatic deficiencies in clotting factors will present with a decreased ability to clot (coagulopathy). Thrombophilia, however, is characterized by an increased tendency to clot. Choices A, C, D, and E result in thrombophilia.

35.8 Current guidelines for the treatment of patients with acute ischemic stroke (a stroke caused by a blood clot obstructing a vessel that supplies blood to the brain) include the recommendation that tissue plasminogen activator (TPA) be used shortly after the onset of symptoms. The basis of the recommendation for TPA is that it activates:

A. antithrombin.
B. the activated protein C complex.

C. the receptor for von Willebrand factor.
D. the serine protease that degrades fibrin.
E. thrombomodulin.

Correct answer = D. TPA converts plasminogen to plasmin. Plasmin (a serine protease) degrades the fibrin meshwork, removing the obstruction to blood flow. Antithrombin III in association with heparin binds thrombin and carries it to the liver, decreasing thrombin's availability in the blood. The activated protein C complex degrades the accessory proteins FV and FVIII. The platelet receptor for von Willebrand factor is not affected by TPA. Thrombomodulin binds thrombin and converts it from a protein of coagulation to one of anticoagulation by decreasing its activation of fibrinogen and increasing its activation of protein C.

35.9 The adhesion, activation, and aggregation of platelets provide the initial plug at the site of vessel injury. Which of the following statements concerning the formation of this platelet plug is correct?

A. Activated platelets undergo a shape change that decreases their surface area.
B. Formation of a platelet plug is prevented in intact vessels by the production of thromboxane A_2 by endothelial cells.
C. The activation phase requires production of cyclic adenosine monophosphate.
D. The adhesion phase is mediated by the binding of platelets to von Willebrand factor via glycoprotein Ib.
E. Thrombin activates platelets by binding to a protease- activated G protein–coupled receptor and causing activation of protein kinase A.

Correct answer = D. The adhesion phase of platelet plug formation is initiated by the binding of von Willebrand factor to a receptor (glycoprotein Ib) on the surface of platelets. Shape change from discoidal to spherical with pseudopodia increases the surface area of platelets. Thromboxane A_2 is made by platelets. It causes platelet activation and vasoconstriction. Adenosine diphosphate is released from activated platelets, and it itself activates platelets. Thrombin works primarily through receptors coupled to G_q proteins causing activation of phospholipase C.

35.10 Nephrotic syndrome is a kidney disease characterized by protein loss in the urine (≥3 g/day) that is accompanied by edema. The loss of protein results in a hypercoagulable state. Excretion of which of the following proteins would explain the thrombophilia seen in the syndrome?

A. Antithrombin
B. FV
C. FVIII
D. Prothrombin

Correct answer = A. Antithrombin III (ATIII) inhibits the action of thrombin (FIIa), a Gla-containing protein of clotting that activates the extrinsic, intrinsic, and common pathways. Excretion of ATIII in nephrotic syndrome allows the actions of FIIa to continue, resulting in a hypercoagulable state. The other choices are proteins required for clotting. Their excretion in the urine would decrease clotting.

35.11 Blocking the action of which of the following proteins would be a rational therapy for hemophilia B?

A. FIX
B. FXIII
C. Protein C
D. Tissue factor pathway inhibitor

Correct answer = D. Hemophilia B is a coagulopathy caused by decreased thrombin production by the common pathway as a result of a deficiency in FIX of the intrinsic pathway. Because the extrinsic pathway also can result in thrombin production, blocking the inhibitor of this pathway (tissue factor pathway inhibitor) should, in principle, increase thrombin production.

35.12 The parents of a newborn are unsure whether to allow the baby to be given the injection of vitamin K that is recommended shortly after birth to prevent vitamin K deficiency bleeding, which is caused by the low levels of the vitamin in newborns. The activity of which one of the following protein factors involved in clotting would be decreased in this patient if she does not receive the injection?

A. FV
B. FVII
C. FXI
D. FXIII

Correct answer = B. FVII is a γ-carboxyglutamate (Gla)-containing protein of clotting. The creation of Gla residues by γ-glutamyl carboxylase requires vitamin K as a coenzyme. FII, FIX, and FX, as well as proteins C and S that limit clotting, also contain Gla residues. The other choices do not contain Gla residues.

35.13 Thrombin, produced in the common pathway of clotting, has both procoagulant and anticoagulant activities. Which one of the following is an anticoagulant activity of thrombin?

A. Activating FXIII
B. Binding to thrombomodulin
C. Increasing nitric oxide production
D. Inhibiting FV and FVIII
E. Inhibiting platelet activation

Correct answer = B. Thrombin bound to thrombomodulin activates protein C that degrades the accessory proteins FV and FVIII, thereby inhibiting clotting. Activation of FXIII by thrombin strengthens the fibrin clot. Nitric oxide, a vasodilator made by endothelial cells, decreases clot formation. It is not affected by thrombin. Thrombin is a powerful activator of platelets.

35.14 Which of the following pattern of results would be expected for a patient with a deficiency in FXIII?

A. Both prothrombin time and activated partial thromboplastin time are decreased.
B. Both prothrombin time and activated partial thromboplastin time are increased.
C. Both prothrombin time and activated partial thromboplastin time are unchanged.
D. Only prothrombin time is affected.
E. Only activated partial thromboplastin time is affected.

Correct answer = C. FXIII is a transglutaminase that cross-links fibrin molecules in a soft clot to form a hard clot. Its deficiency does not affect the PT or aPTT tests. (Note: It is evaluated by a clot solubility test.)

35.15 Why do individuals with Scott syndrome, a rare disorder caused by mutations to scramblase in platelets, have a tendency to bleed?

Scramblase moves phosphatidylserine (PS) from the cytosolic leaflet to the extracellular leaflet in the plasma membrane of platelets. This disrupts the asymmetrical localization of membrane phospholipids created by ATP-dependent flippases (move PS from extracellular to cytosolic leaflet) and floppases (move phosphatidylcholine [PC] in the opposite direction). Having PS on the outer face of platelet membranes provides a site for protein clotting factors to interact and activate thrombin. If scramblase is inactive, PS is not available to these factors, and bleeding results.

35.16 Several days after having had their home treated for an infestation of rats, the parents of a 3-year-old female become concerned that she might be ingesting the poison-containing pellets. After calling the Poison Hotline, they take her to the emergency department. Blood studies reveal a prolonged prothrombin and activated partial thromboplastin time and a decreased concentration of thrombin, FVII, FIX, and FX. Why might administration of vitamin K be a rational approach to the treatment of this patient?

Many rodent poisons are super warfarins, drugs that have a long half-life in the body. Warfarin inhibits γ-carboxylation (production of γ-carboxyglutamate, or Gla, residues), and the clotting proteins reported as decreased are the Gla-containing proteases of the clotting cascade. (Note: Proteins C and S of anticlotting are also Gla-containing proteins.) Because warfarin functions as a vitamin K antagonist, administration of vitamin K is a rational approach to treatment.

APPENDIX
Clinical Cases

I. INTEGRATIVE CASES

Metabolic pathways, initially presented in isolation, are, in fact, linked to form an interconnected network. The following four integrative case studies illustrate how a perturbation in one process can result in perturbations in other processes of the network.

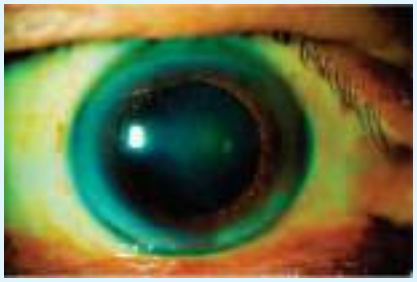
CASE 1: CHEST PAIN

Patient Presentation: A 35-year-old male with severe substernal chest pain of ~2 hours' duration is brought by ambulance to his local hospital at 5 AM. The pain is accompanied by dyspnea (shortness of breath), diaphoresis (sweating), and nausea.

Focused History: The patient reports episodes of exertional chest pain in the last few months, but they were less severe and of short duration. He smokes (2 to 3 packs per day), drinks alcohol only rarely, eats a "typical" diet, and walks with his wife most weekends. His blood pressure has been normal. Family history reveals that his father and paternal aunt died of heart disease at ages 45 and 39 years, respectively. His mother and younger (age 31 years) brother are said to be in good health.

Physical Examination (Pertinent Findings): He is pale and clammy and is in distress due to chest pain. Blood pressure and respiratory rate are elevated. Lipid deposits are noted on the periphery of his corneas (corneal arcus; see left image) and under the skin on and around his eyelids (xanthelasmas; see right image). No deposits on his tendons (xanthomas) are detected.

Corneal arcus



Xanthelasmas

Pertinent Test Results: The patient's electrocardiogram is consistent with an acute myocardial infarction

(MI). Angiography reveals areas of severe stenosis (narrowing) of several coronary arteries. Initial results from the clinical laboratory include the following:

	Patient	Reference Range
Troponin	0.5 ng/mL	Below 0.04 ng/mL
Total cholesterol	365 mg/dL (**H**)	<200
Low-density lipoprotein (LDL) cholesterol	304 mg/dL (**H**)	<130
High-density lipoprotein (HDL) cholesterol	38 mg/dL (**L**)	>45
Triglycerides (triacylglycerols)	115 mg/dL	<150

H = High; **L** = Low. [Note: the patient had not eaten for ~8 hours prior to the blood draw.]

Diagnosis: Acute myocardial infarction (MI), the irreversible necrosis (death) of heart muscle secondary to ischemia (decreased blood supply), is caused by the occlusion (blockage) of a blood vessel most commonly by a blood clot (thrombus). The patient subsequently is determined to have heterozygous familial hypercholesterolemia (FH), also known as type IIa hyperlipidemia.

Immediate Treatment: He is given O_2, a vasodilator, and pain medication, and undergoes a procedure to place stents to reestablish perfusion (restore blood flow to the heart).

Long-Term Treatment: Lipid-lowering drugs such as statins, daily aspirin, and counseling on nutrition, exercise, and smoking cessation would likely be part of the long-term treatment plan.

Prognosis: Patients with heterozygous FH have ~50% of the normal numbers of functional LDL receptors and hypercholesterolemia (two to three times normal) that puts them at high risk (>50% risk) for premature coronary heart disease (CHD). However, fewer than 5% of patients with hypercholesterolemia actually have FH.

Nutrition Nugget: Dietary recommendations for individuals with heterozygous FH include limiting saturated fats to <7% of total calories and cholesterol to <200 mg/day, substituting unsaturated fats for saturated fats, and adding soluble fiber (10 to 20 g/day) and plant sterols (2 g/day) for their hypocholesterolemic effects. Fiber increases bile acid (BA) excretion. This results in increased hepatic uptake of cholesterol-rich LDL to supply the substrate for BA synthesis. Plant sterols decrease cholesterol absorption in the intestine.

Genetics Gem: FH is caused by hundreds of different mutations in the gene for the LDL receptor (on chromosome 19) that affect receptor amount and/or function. FH is an autosomal-dominant disease in which homozygotes are more seriously affected than heterozygotes. Heterozygous FH has an incidence of ~1:500 in the general population. It is associated with increased risk of cardiovascular disease. Genetic screening of the first-degree relatives of this patient would identify affected individuals for treatment.

REVIEW QUESTIONS: Choose the ONE best answer

RQ1. Triacylglycerols are glycerol-based lipids. Which of the following is also a glycerol-based lipid?

A. Ganglioside GM_2
B. Phosphatidylcholine
C. Prostaglandin PGI_2
D. Sphingomyelin
E. Vitamin D

RQ2. Statins are of benefit to patients with hypercholesterolemia because they:

A. decrease a rate-limiting and regulated step of de novo cholesterol biosynthesis.
B. decrease expression of the gene for the LDL receptor.
C. increase the oxidation of cholesterol to $CO_2 + H_2O$.
D. interfere with the absorption of bile salts in the enterohepatic circulation.
E. reduce cholesterol by increasing steroid hormone and vitamin D synthesis.

RQ3. Statins are competitive inhibitors of HMG CoA reductase. Which of the following statements about the mechanism of action of statins is therefore correct?

A. Statins function as irreversible inhibitors.
B. Statins cause an increase in both the apparent K_m and the V_{max}.
C. Statins increase the apparent K_m and have no effect on the V_{max}.
D. Statins decrease both the apparent K_m and the apparent V_{max}.
E. Statins have no effect on the K_m and decrease the apparent V_{max}.

RQ4. Decreased tissue perfusion results in hypoxia (decreased O_2 availability). Relative to normoxia, in hypoxia the:

A. electron transport will be upregulated to provide protons for ATP synthesis.
B. ratio of NAD^+ to NADH will increase.
C. pyruvate dehydrogenase complex will be active.
D. process of substrate-level phosphorylation will be increased in the cytosol.
E. tricarboxylic acid cycle will be upregulated.

THOUGHT QUESTIONS

TQ1. Relative to an individual with familial defective LDL receptors, what would be the expected phenotype in an individual with familial defective apolipoprotein B-100? With apolipoprotein E4, the isoform that only poorly binds its receptor?

TQ2. Why was aspirin prescribed? **Hint:** What product of arachidonic acid metabolism is inhibited by aspirin?

TQ3. Heart muscle normally uses aerobic metabolism to meet its energy needs. However, in hypoxia, anaerobic glycolysis is increased. What allosteric activator of glycolysis is responsible for this effect? With hypoxia, what will be the end product of glycolysis?

TQ4. One of the reasons for encouraging smoking cessation and exercise for this patient is that these changes raise the level of HDL, and elevated HDL reduces the risk for CHD. How does a rise in HDL reduce the risk for CHD?

CASE 2: SEVERE FASTING HYPOGLYCEMIA

Patient Presentation: The patient is a 4-month-old male whose mother is concerned about the "twitching" movements he makes just before feedings. She tells the pediatrician that the movements started ~1 week ago, are most apparent in the morning, and disappear shortly after eating.

Focused History: The child was born at full term following a normal pregnancy and delivery. He appeared normal at birth. He has been at the 30th percentile for both weight and length since birth. His immunizations are up to date. He last ate a few hours ago.

Physical Examination (Pertinent Findings): The child appears sleepy and feels clammy to the touch. His respiratory rate is elevated. His temperature is normal. He has a protuberant, firm abdomen that appears to be nontender. His liver is palpable 4 cm below the right costal margin and is smooth.

Pertinent Test Results:

	Patient	Pediatric Reference Range
Glucose	50 mg/dL (**L**)	60–105
Lactate	3.4 mmol/L (**H**)	0.6–3.2
Uric acid	5.6 mg/dL (**H**)	2.4–5.4
Total cholesterol	220 mg/dL (**H**)	<170
Triglycerides (triacylglycerols)	280 mg/dL (**H**)	<90
pH	7.30 (**L**)	7.35–7.45

HCO_3^-	12 mEq/L (**L**)	19–25

H = High; **L** = Low.

The child is sent to the regional children's hospital for further evaluation. Ultrasound studies confirm hepatomegaly and enlarged, symmetrical kidneys but no evidence of tumors. A liver biopsy is performed. The hepatocytes are enlarged. Staining reveals large amounts of lipid (primarily triacylglycerol) and carbohydrate. Liver glycogen is elevated in amount and normal in structure. Enzyme assay using liver homogenate treated with detergent reveals <10% of the normal activity of glucose 6-phosphatase, an enzyme of the endoplasmic reticular (ER) membrane in the liver and the kidneys.

Diagnosis: This child has glucose 6-phosphatase deficiency (glycogen storage disease [GSD] type Ia, von Gierke disease).

Treatment (Immediate): He was given glucose intravenously, and his blood glucose level rose into the normal range. However, as the day progressed, it fell to well below normal. Administration of glucagon had no effect on blood glucose levels but increased blood lactate. His blood glucose levels were able to be maintained only by constant infusion of glucose.

Prognosis: Individuals with glucose 6-phosphatase deficiency develop hepatic adenomas starting in the second decade of life and are at increased risk for hepatic carcinoma. Kidney involvement can cause impaired tubular function resulting in acidosis, and glomerular function may also be impaired and can result in chronic kidney disease. Patients are at increased risk for developing gout, but this rarely occurs before puberty.

Nutrition Nugget: Long-term medical nutrition therapy for this child is designed to maintain his blood glucose levels in the normal range. Frequent (every 2 to 3 hours) daytime feedings that are rich in carbohydrate (provided by uncooked cornstarch that is slowly hydrolyzed) and nighttime nasogastric infusion of glucose are advised. Avoidance of fructose and galactose is recommended because they are metabolized to glycolytic intermediates and lactate, which can exacerbate the metabolic problems. Calcium and vitamin D supplements are prescribed.	**Genetics Gem:** GSD Ia is an autosomal-recessive disorder caused by >100 known mutations to the gene for glucose 6-phosphatase located on chromosome 17. It has an incidence of 1:100,000 and accounts for ~25% of all cases of GSD in the United States. It is one of the few genetic causes of hypoglycemia in newborns. GSD Ia is not routinely screened for in newborns. [Note: Deficiency of the translocase that moves glucose 6-phosphate into the ER is the cause of GSD Ib. Hypoglycemia and neutropenia are seen.]

REVIEW QUESTIONS: Choose the ONE best answer

RQ1. This patient is hypoglycemic because:

A. unphosphorylated glucose cannot be produced by glycogenolysis or gluconeogenesis.
B. glycogen phosphorylase is dephosphorylated and inactive; glycogen cannot be degraded.
C. hormone-sensitive lipase is inactive; substrates for gluconeogenesis cannot be generated.
D. his decreased insulin/glucagon ratio upregulates glucose transporters in liver and kidneys.

RQ2. The patient was prescribed calcium supplements because chronic acidosis can cause bone demineralization, resulting in osteopenia. Vitamin D (1,25-diOH-D_3) was also prescribed because vitamin D:

A. binds G_q protein–coupled membrane receptors and causes a rise in inositol trisphosphate.
B. cannot be synthesized by humans and, therefore, must be supplied in the diet.
C. is a fat-soluble vitamin that increases intestinal absorption of calcium.
D. acts as the coenzyme-prosthetic group for calbindin, a calcium transporter in the intestine.

RQ3. The hepatomegaly and renomegaly seen in this child are primarily the result of an increase in the amount of glycogen stored in these organs. What is the basis for glycogen accumulation in these organs?

A. Glycolysis is downregulated, which pushes glucose to glycogenesis.

B. Increased oxidation of fatty acids spares glucose for glycogenesis.
C. Glucose 6-phosphate is an allosteric activator of glycogen synthase b.
D. The rise in the insulin/glucagon ratio favors glycogenesis.

RQ4. Glucose 6-phosphatase is an integral protein of the ER membrane. Which of the following statements about such proteins is correct?

A. If glycosylated, the carbohydrate portion of the protein that extends into the cytosol.
B. They are synthesized on ribosomes that are free in the cytosol.
C. The membrane-spanning domain consists of hydrophilic amino acids.
D. The initial targeting signal is an amino terminal hydrophobic signal sequence.

THOUGHT QUESTIONS

TQ1. What is the likely reason for the patient's twitching movements?

TQ2. Why was the liver homogenate treated with detergent? **Hint:** Think about where the enzyme is located.

TQ3. Why is this patient's blood glucose level unaffected by glucagon? **Hint:** What is the role of glucagon in normal individuals who experience a drop in blood glucose?

TQ4. Why are urate and lactate elevated in a disorder of glycogen metabolism? **Hint:** It is the result of a decrease in inorganic phosphate (P_i), but why is P_i decreased?

TQ5. Why are triacylglycerols and cholesterol elevated? **Hint:** Glucose is the primary carbon source for their synthesis.

Why are ketone bodies not elevated?

CASE 3: HYPERGLYCEMIA AND HYPERKETONEMIA

Patient Presentation: A 40-year-old female was brought to the Emergency Department in a disoriented, confused state by her husband.

Focused History: Her husband reveals that the patient has had type 1 diabetes (T1D) for the last 24 years and this is her first medical emergency in 2 years.

Physical Examination (Pertinent Findings): The patient displayed signs of dehydration including dry mucous membranes and skin, poor skin turgor, and low blood pressure. She also had signs of acidosis such as deep, rapid breathing (Kussmaul respiration). Her breath had a faintly fruity odor. Her temperature was normal.

Pertinent Test Results: Results of blood tests performed by the clinical laboratory are shown below:

	Patient	Reference Range
Glucose	414 mg/dL (23 mmol/L) (**H**)	70–99 (3.9–5.5)
Blood urea nitrogen	8 mmol/L (**H**)	2.5–6.4
3-Hydroxybutyrate	350 mg/dL (**H**)	0–3
HCO_3^-	12 mmol/L (**L**)	22–28
Na^+	136 mmol/L	138–150
K^+	5.3 mmol/L	3.5–5.0
Cl^-	102 mmol/L	95–105
pH	7.1 (**L**)	7.35–7.45

H = High; **L** = Low.

Microscopic examination of her urine revealed white blood cells, suspicious for a urinary tract infection (UTI), which was later confirmed by urine culture.

Diagnosis: This patient is in diabetic ketoacidosis (DKA) precipitated by a UTI. [Note: Diabetes increases the risk for infections such as UTI.]

Immediate Treatment: She was administered insulin. Rehydration was initiated with normal saline given intravenously (IV). Blood glucose, ketone bodies, and electrolytes were measured periodically. Antibiotic treatment for her UTI was started.

Long-Term Treatment: Diabetes increases the risk for macrovascular complications including coronary artery disease and stroke and microvascular complications such as retinopathy, nephropathy, and neuropathy. Ongoing monitoring for these complications will be continued.

Prognosis: Diabetes is the seventh leading cause of death by disease in the United States. Individuals with diabetes have a reduced life expectancy relative to those without diabetes.

Nutrition Nugget: Monitoring total intake of carbohydrates is primary in blood glucose control. Carbohydrates should come from whole grains, vegetables, legumes, and fruits. Low-fat dairy products and nuts and fish rich in ω-3 fatty acids are encouraged. Intake of saturated and trans fats should be minimized.	**Genetics Gem:** Autoimmune destruction of pancreatic β-cells is characteristic of T1D. Of the genetic loci that confer risk for T1D, the human-leukocyte antigen (HLA) region on chromosome 6 has the strongest association. The majority of genes in the HLA region are involved in the immune response.

REVIEW QUESTIONS: Choose the ONE best answer

RQ1. Which of the following statements concerning T1D is correct?

A. Diagnosis can be made by measuring the level of glucose or glycated hemoglobin (HbA_{1c}).
B. During periods of stress, a patient's urine will likely be negative for reducing sugars.
C. T1D is associated with obesity and a sedentary lifestyle.
D. Characteristic metabolic abnormalities of T1D result from insensitivity to insulin.
E. Treatment with exogenous insulin allows normalization of blood glucose.

RQ2. Ketone bodies:

A. are made from acetyl coenzyme A (CoA) primarily produced by the oxidation of glucose.
B. are utilized by many tissues, particularly the liver, after conversion to acetyl CoA.
C. include acetoacetate, which can impart a fruity odor to the breath.
D. requires albumin for transport through the blood.
E. utilized in energy metabolism are organic acids that can add to the proton load of the body.

RQ3. Adipose lipolysis followed by β-oxidation of the fatty acid (FA) products is required for the generation of ketone bodies. Which of the following statements concerning the generation and use of FA is correct?

A. Mitochondrial β-oxidation of FA is inhibited by malonyl CoA.
B. Production of FA from adipose lipolysis is upregulated by insulin.
C. The acetyl CoA product of FA β-oxidation inhibits the use of pyruvate for gluconeogenesis.
D. The β-oxidation of FA utilizes reducing equivalents generated by gluconeogenesis.
E. The FAs produced by lipolysis are taken up by the brain and oxidized for energy.

THOUGHT QUESTIONS

TQ1. At admission, the patient was hypoinsulinemic, and she was given insulin. Why did her hypoinsulinemia result in hyperglycemia? **Hint:** What is the role of insulin in glucose metabolism?

TQ2. Why is there glucose in her urine (glucosuria)? How is the glucosuria related to her dehydrated state?

TQ3. Why is the majority of the acetyl CoA from FA β-oxidation being used for ketogenesis rather than being oxidized in the tricarboxylic acid cycle?

TQ4. Was she in positive or negative nitrogen balance when she was brought to the hospital?

TQ5. What response to the DKA is apparent in this patient? What response is likely occurring in the kidney? **Hint:** In addition to conversion to urea, how is toxic ammonia removed from the body?

TQ6. What would be true about the levels of ketone bodies and glucose during periods of physiologic stress in individuals with impaired FA oxidation?

CASE 4: HYPOGLYCEMIA, HYPERKETONEMIA, AND LIVER DYSFUNCTION

Patient Presentation: A 59-year-old male with slurred speech, ataxia (loss of skeletal muscle coordination), and abdominal pain was dropped off at the Emergency Department (ED).

Focused History: This patient is known to the Emergency Department staff from previous visits. He has a 6-year history of chronic, excessive alcohol consumption. He is not known to take illicit drugs. At this ED visit, the patient reports that he has been drinking heavily in the past day or so. He cannot recall having eaten anything in that time but admits to vomiting, without evidence of recent bleeding.

Physical Examination (Pertinent Findings): The physical examination was remarkable for the patient's emaciated appearance. (His body mass index was later determined to be 17.5, which put him in the underweight category.) His facial cheeks were erythematous (red in color) due to dilated blood vessels in the skin (telangiectasia). Eye movement was normal. Neither icterus (jaundice) nor edema (swelling due to fluid retention) were seen. The liver was slightly enlarged. Bedside tests revealed hypoglycemia and hyperketonemia (as acetoacetate). Blood was drawn and sent to the clinical laboratory.

Pertinent Test Results:

	Patient	Reference Range
Ethanol	180 mg/dL (**H**)	(>80 considered positive for DUI)
Glucose	58 mg/dL (**L**)	70–99
Lactate	23 mg/dL (**H**)	5–15
Uric acid	7.0 mg/dL	2.5–8.0
3-Hydroxybutyrate	50 mg/dL (**H**)	0–3.0
Total bilirubin	1.5 mg/dL (**H**)	0.3–1.0
Direct (conjugated) bilirubin	0.5 mg/dL (**H**)	0.1–0.3
Albumin	3.0 g/dL (**L**)	3.5–5.8
Aspartate transaminase (AST)	130 U/L (**H**)	0–35
Alanine transaminase (ALT)	75 U/L (**H**)	0–35
Prothrombin time	15.5 s (**H**)	11.0–13.2

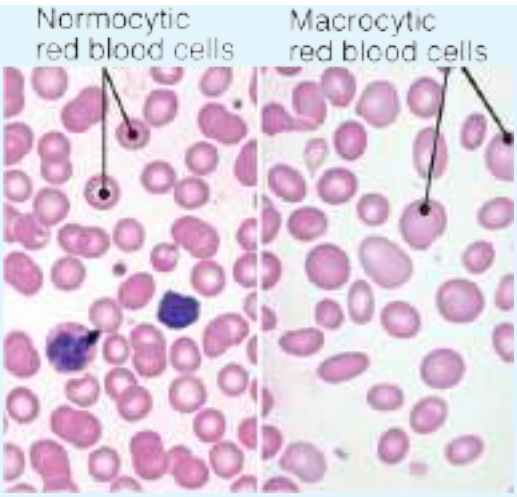
DUI = driving under the influence; **H** = High; **L** = Low.

Additional Tests: Complete blood count (CBC) and blood smear revealed a macrocytic anemia (see right image). Folate and B_{12} levels were ordered.

Diagnosis: The patient has alcohol use disorder and alcoholic ketoacidosis.

Treatment (Immediate): Thiamine and glucose were given intravenously.

Prognosis: Alcohol dependence is the third most common cause of preventable death in the United States. People with alcohol use disorder are at increased risk for vitamin deficiencies, liver cirrhosis, pancreatitis, gastrointestinal bleeding, and some cancers.



Nutrition Nugget: Those with alcohol use disorder are at risk for vitamin deficiencies as a result of decreased intake and absorption. Thiamine (vitamin B_1) deficiency is common and can have serious consequences such as Wernicke–Korsakoff syndrome with its neurologic effects. Thiamine pyrophosphate (TPP), the coenzyme form, is required for the dehydrogenase-mediated oxidation of α-keto acids (such as pyruvate) as well as the transfer of two-carbon ketol groups by transketolase in the reversible sugar interconversions in the pentose phosphate pathway.

Genetics Gem: Acetaldehyde, the product of ethanol oxidation by the hepatic, cytosolic, nicotinamide adenine dinucleotide (NAD^+)-requiring enzyme alcohol dehydrogenase (ADH), is oxidized to acetate by the mitochondrial, NAD^+-requiring aldehyde dehydrogenase (ALDH2). Individuals of East Asian heritage often have a single nucleotide polymorphism (SNP) that renders ALDH2 essentially inactive. This results in aldehyde-induced facial flushing and mild to moderate intoxication after consumption of small amounts of ethanol.

REVIEW QUESTIONS: Choose the ONE best answer

RQ1. Many of the metabolic consequences of chronic excessive alcohol consumption seen in this patient are the result of an increase in the ratio of reduced nicotinamide adenine dinucleotide (NADH) to its oxidized form (NAD^+) in both the cytoplasm and mitochondria. Which of the following statements concerning the effects of the rise in mitochondrial NADH is correct?

A. Fatty acid oxidation is increased.
B. Gluconeogenesis is increased.
C. Lipolysis is inhibited.
D. The tricarboxylic acid cycle is inhibited.
E. The reduction of malate to oxaloacetate in the malate–aspartate shuttle is increased.

RQ2. Ethanol can also be oxidized by cytochrome P450 (CYP) enzymes, and CYP2E1 is an important example. CYP2E1, which is ethanol inducible, generates reactive oxygen species (ROS) in its metabolism of ethanol. Which of the following statements concerning the CYP proteins is correct?

A. CYP proteins are heme-containing dioxygenases.
B. CYP proteins of the inner mitochondrial membrane are involved in detoxification reactions.
C. CYP proteins of the smooth endoplasmic reticular membrane are involved in the synthesis of steroid hormones, bile acids, and calcitriol.
D. ROS such as hydrogen peroxide generated by CYP2E1 can be oxidized by glutathione peroxidase.
E. The pentose phosphate pathway is an important source NADPH that provides the reducing equivalents needed for the regeneration of functional glutathione.

RQ3. Alcohol is known to modulate the levels of serotonin in the central nervous system, where the monoamine functions as a neurotransmitter. Which of the following statements about serotonin is correct? Serotonin is:

A. associated with anxiety and depression.
B. degraded via methylation by monoamine oxidase.
C. released by activated platelets.
D. synthesized from tyrosine.

RQ4. Chronic, excessive consumption of alcohol is a leading cause of acute pancreatitis, a painful inflammatory condition that results from autodigestion of the gland by premature activation of pancreatic enzymes. Which of the following statements concerning the pancreas is correct?

A. Autodigestion of the pancreas would be expected to result in a decrease in pancreatic proteins in the blood.
B. In individuals who progress from acute to chronic pancreatitis, diabetes and steatorrhea are expected findings.
C. In response to secretin, the exocrine pancreas secretes protons to lower the pH in the intestinal lumen.
D. Pancreatitis may also be seen in individuals with hypercholesterolemia.

THOUGHT QUESTIONS

TQ1. A. What effect does the rise in cytosolic NADH seen with ethanol metabolism have on glycolysis? **Hint:** What coenzyme is required in glycolysis?

B. How does this relate to the fatty liver (hepatic steatosis) commonly seen in alcohol-dependent individuals?

TQ2. Why might individuals with a history of gouty attacks be advised to reduce their consumption of ethanol?

TQ3. Why might prothrombin time be affected in alcohol-dependent individuals?

TQ4. Folate and vitamin B_{12} deficiencies cause a macrocytic anemia that may be seen in those with alcoholism. Why is it advisable to measure vitamin B_{12} levels before supplementing with folate in an individual with macrocytic anemia?

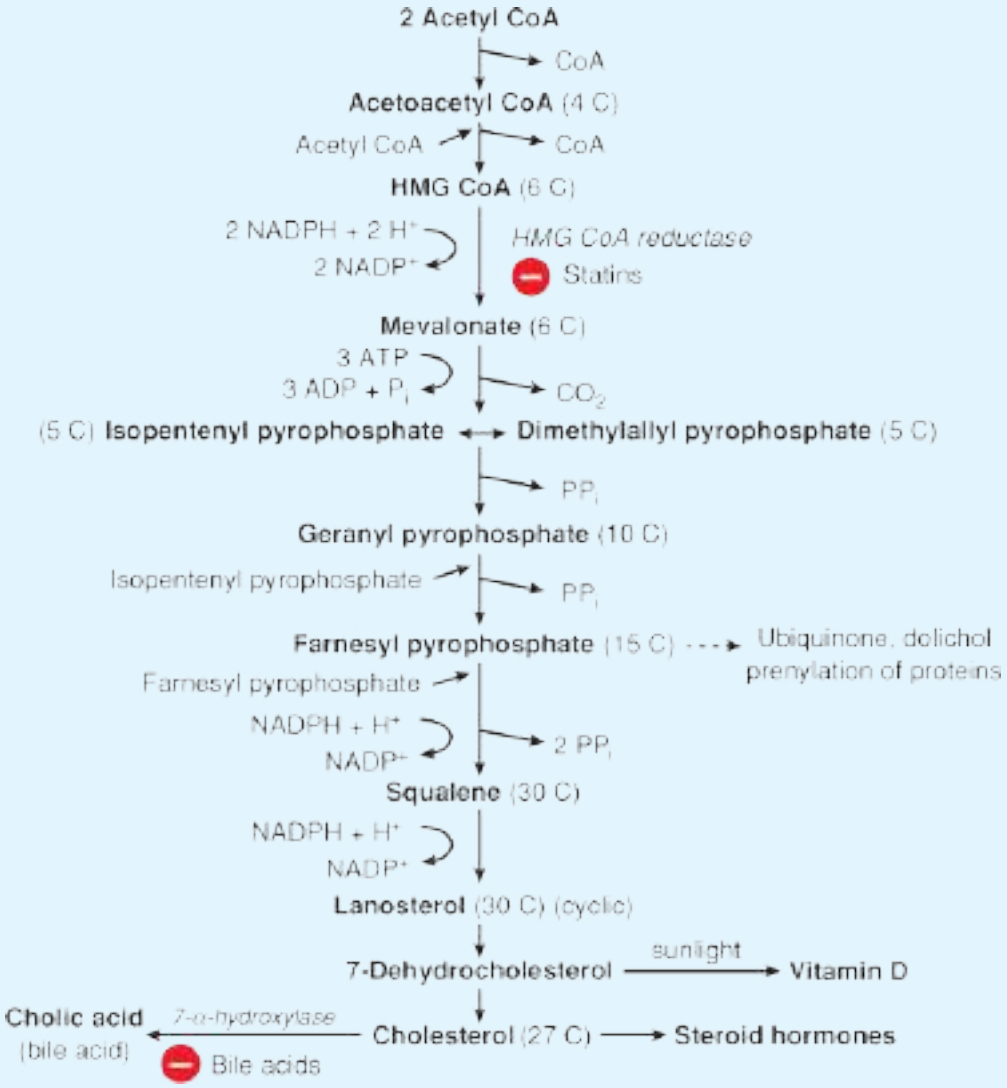
II. INTEGRATIVE CASE ANSWERS

CASE 1: Answers to Review Questions

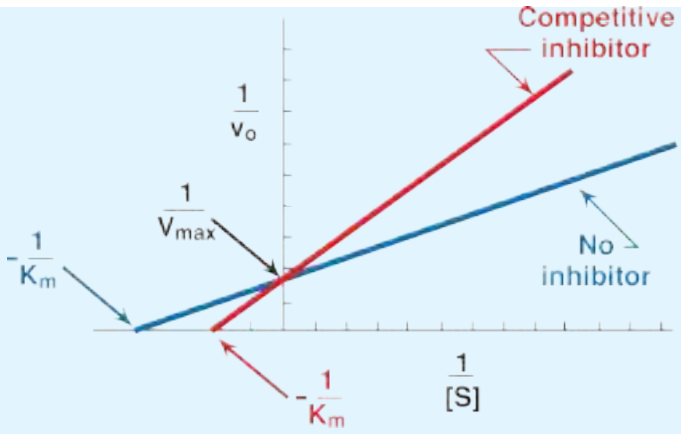
RQ1. **Answer = B.** Phosphatidylcholine is a glycerol-based phospholipid derived from diacylglycerol phosphate (phosphatidic acid) and cytidine diphosphate-choline. Gangliosides are derived from ceramides, lipids with a sphingosine backbone. Prostaglandins of the 2 series (such as PGI_2) are derived from the 20-carbon polyunsaturated fatty acid arachidonic acid. Sphingomyelin is a sphingophospholipid derived from ceramide. Vitamin D is derived from an intermediate in the biosynthetic pathway for the sterol cholesterol.

RQ2. **Answer = A.** Statins inhibit hydroxymethylglutaryl coenzyme A (HMG CoA) reductase, thereby preventing the nicotinamide adenine dinucleotide phosphate (NADPH)-dependent reduction of HMG CoA to mevalonate and decreasing cholesterol biosynthesis (see figure below). The decrease in cholesterol content caused by statins results in movement of the sterol regulatory element–binding protein-2 (SREBP-2) in complex with SREBP cleavage–activating protein (SCAP) from the endoplasmic reticular membrane to the Golgi membrane. SREBP-2 is cleaved, generating a transcription factor that moves to the nucleus and binds to the sterol regulatory element upstream of the genes for HMG CoA reductase and the low-density lipoprotein (LDL) receptor, increasing their expression. Humans are unable to degrade the steroid nucleus to CO_2 + H_2O. Bile acid (BA) sequestrants, such as cholestyramine, prevent the absorption of bile salts by the liver, thereby

increasing their excretion. The liver then takes up cholesterol via the LDL receptor and uses it to make BA, thereby reducing blood cholesterol levels. Steroid hormones are synthesized from cholesterol, and vitamin D is synthesized in skin from an intermediate (7-dehydrocholesterol) in the cholesterol biosynthetic pathway. Therefore, inhibition of cholesterol synthesis would be expected to decrease their production as well.



RQ3. **Answer = C.** Competitive inhibitors bind to the same site as the substrate (S) and prevent the S from binding. This results in an increase in the apparent K_m (Michaelis constant, or that S concentration that gives one-half of the maximal velocity [V_{max}]). However, because the inhibition can be reversed by adding additional substrate, the V_{max} is unchanged (see figure at right). It is noncompetitive inhibitors that decrease the apparent V_{max} and have no effect on K_m.



RQ4. **Answer = D.** In hypoxia, substrate-level phosphorylation in glycolysis provides ATP. Oxidative phosphorylation is inhibited by the lack of O_2. Because the rate of ATP synthesis by oxidative phosphorylation controls the rate of cellular respiration, electron transport is inhibited. The resulting rise in the ratio of the reduced form of nicotinamide adenine dinucleotide (NADH) to the oxidized form (NAD^+) inhibits the tricarboxylic acid cycle and the pyruvate dehydrogenase complex.

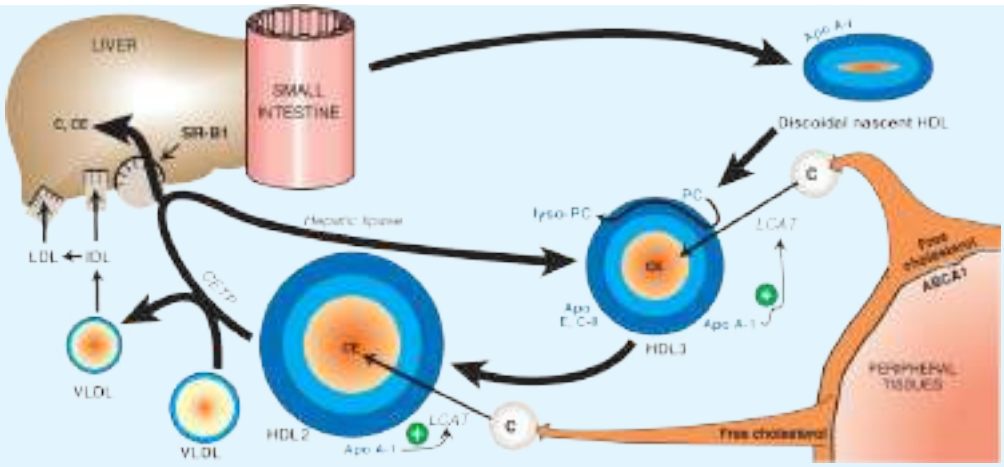
CASE 1: Answers to Thought Questions

TQ1. The phenotype would be the same. In familial defective apolipoprotein (apo) B-100, LDL receptors are normal in number and function, but the ligand for the receptor is altered such that binding to the receptor is decreased. Decreased ligand–receptor binding results in increased levels of LDL in the blood with hypercholesterolemia. [Note: The phenotype would be the same in individuals with a gain-of-function mutation to PCSK9, the protease that decreases recycling of the LDL receptor, thereby increasing its degradation.] With the apo E4 isoform, cholesterol-rich chylomicron remnants and intermediate-density lipoproteins would accumulate in blood.

TQ2. Aspirin irreversibly inhibits cyclooxygenase (COX) and, therefore, the synthesis of prostaglandins (PG), such as PGI_2 in vascular endothelial cells, and thromboxanes (TX), such as TXA_2 in activated platelets. TXA_2 promotes vasoconstriction and formation of a platelet plug, whereas PGI_2 inhibits these events. Because platelets are anucleate, they cannot overcome this inhibition by synthesizing more COX. However, endothelial cells have a nucleus. Aspirin, then, inhibits formation of blood clots by preventing production of TXA_2 for the life of the platelet.

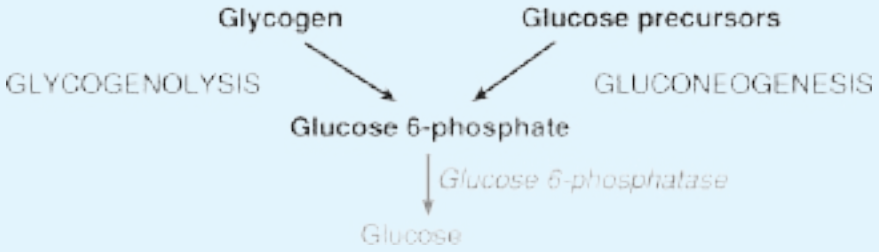
TQ3. The decrease in ATP (as the result of a decrease in O_2 and, thus, a decrease in oxidative phosphorylation) causes an increase in adenosine monophosphate (AMP). AMP allosterically activates phosphofructokinase-1, the key regulated enzyme of glycolysis. The rise in glycolysis increases the production of ATP by substrate-level phosphorylation. It also increases the ratio of the reduced to oxidized forms of NAD. Under anaerobic conditions, pyruvate produced in glycolysis is reduced to lactate by lactate dehydrogenase as NADH is oxidized to NAD^+. NAD^+ is required for continued glycolysis. Because fewer ATP molecules are produced per molecule of substrate in substrate-level phosphorylation relative to oxidative phosphorylation, there is a compensatory increase in the rate of glycolysis under anaerobic conditions.

TQ4. High-density lipoprotein (HDL) functions in reverse cholesterol transport. It takes cholesterol from nonhepatic (peripheral) tissues (e.g., the endothelial layer of arteries) and brings it to the liver (see figure on the next page). The ABCA1 transporter mediates the efflux of cholesterol to HDL. The cholesterol is esterified by extracellular lecithin–cholesterol acyltransferase (LCAT) that requires apo A-1 as a coenzyme. Some cholesteryl ester is transferred to very–low-density lipoproteins (VLDL) by cholesteryl ester transfer protein (CETP) in exchange for triacylglycerol. The remainder is taken up by a scavenger receptor (SR-B1) on the surface of hepatocytes. The liver can use the cholesterol from HDL in the synthesis of bile acids. Removal of cholesterol from endothelial cells prevents its accumulation (as cholesterol or cholesteryl ester), decreasing the risk of heart disease. [Note: In contrast, LDL carries cholesterol to peripheral tissues or back to the liver.]

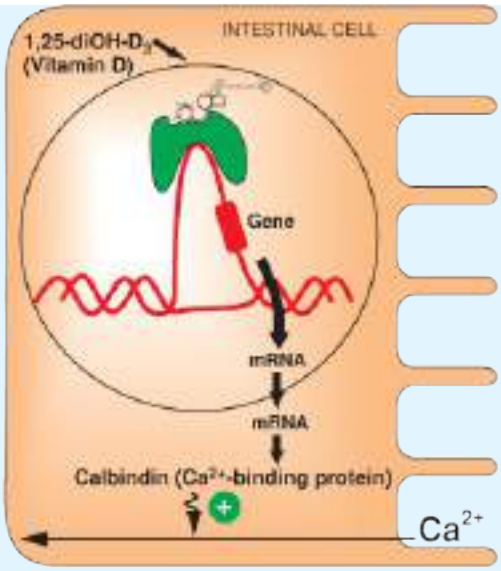


CASE 2: Answers to Review Questions

RQ1. **Answer = A.** Deficiency of glucose 6-phosphatase prevents the glucose 6-phosphate generated by glycogenolysis and gluconeogenesis from being dephosphorylated and released into the blood (see figure below). Blood glucose levels fall, and a severe, fasting hypoglycemia results. [Note: This patient's symptoms appeared only recently because, at age 4 months, his feedings are less frequent.] Hypoglycemia stimulates release of glucagon, which leads to phosphorylation and activation of glycogen phosphorylase kinase that phosphorylates and activates glycogen phosphorylase. Epinephrine is also released and leads to phosphorylation and activation of hormone-sensitive lipase. However, typical fatty acids (FAs) cannot serve as substrates for gluconeogenesis. The glucose transporters in the liver and kidneys are insulin insensitive.



RQ2. **Answer = C.** Vitamin D is a fat-soluble vitamin that functions as a steroid hormone. In complex with its intracellular nuclear receptor, it increases transcription of the gene for calbindin, a calcium (Ca^{2+}) transporter protein in the intestine (see figure at right). Vitamin D does not bind to a membrane receptor and does not produce second messengers. It can be synthesized in the skin by the action of ultraviolet light on an intermediate of cholesterol synthesis, 7-dehydrocholesterol. Of the fat-soluble vitamins (A, D, E, and K), only K functions as a coenzyme.



RQ3. **Answer = C.** Glucose 6-phosphate is a positive allosteric effector of the covalently inhibited (phosphorylated) glycogen synthase b. With the rise in glucose 6-phosphate, glycogen synthesis is activated, and glycogen stores are increased in both the liver and the kidneys. The increased availability of glucose 6-phosphate also drives glycolysis. The increase in glycolysis provides substrates for lipogenesis, thereby increasing synthesis of FA and triacylglycerols (TAG). In hypoglycemia, the insulin/glucagon ratio is low, not high.

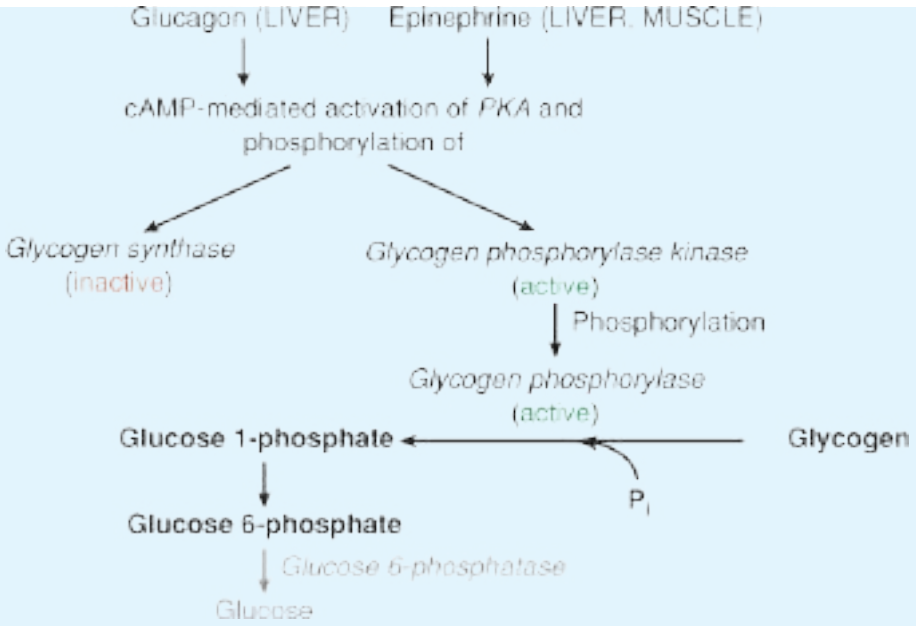
RQ4. **Answer = D.** Membrane proteins are initially targeted to the endoplasmic reticulum (ER) by an amino terminal hydrophobic signal sequence. Glycosylation is the most common posttranslational modification found in proteins. The glycosylated portion of membrane proteins is found on the extracellular face of the membrane. The membrane-spanning domain consists of ~22 hydrophobic amino acids. Proteins destined for secretion or for membranes, the ER lumen, Golgi, or lysosomes are synthesized on ribosomes associated with the ER.

CASE 2: Answers to Thought Questions

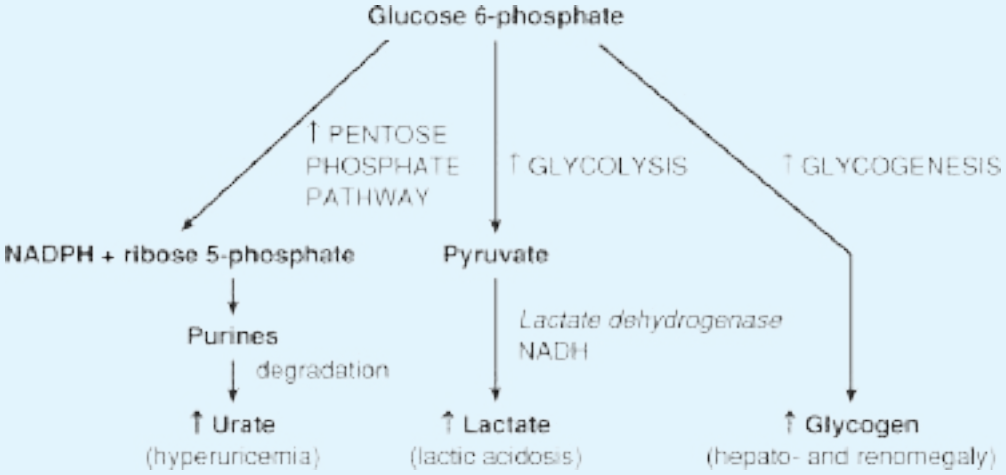
TQ1. The twitching is the result of the adrenergic response to hypoglycemia and is mediated by the rise in epinephrine. The adrenergic response includes tremor and sweating. Neuroglycopenia (impaired delivery of glucose to the brain) results in impairment of brain function that can lead to seizures, coma, and death. Neuroglycopenic symptoms develop if the hypoglycemia persists.

TQ2. Detergents are amphipathic molecules (i.e., they have both hydrophilic [polar] and hydrophobic [nonpolar] regions). Detergents solubilize membranes, thereby disrupting membrane structure. If the problem were the translocase needed to move the glucose 6-phosphate substrate into the ER, rather than the phosphatase, disruption of the ER membrane would allow the substrate access to the phosphatase.

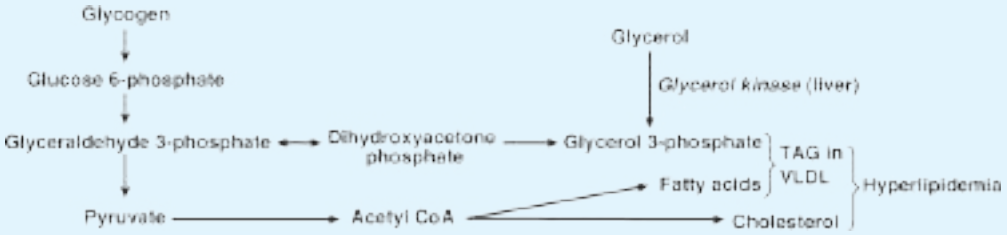
TQ3. Glucagon, a peptide hormone released from pancreatic α-cells in hypoglycemia, binds its plasma membrane G protein–coupled receptor on hepatocytes. The α_s-subunit of the associated trimeric G protein is activated (guanosine diphosphate is replaced by guanosine triphosphate), separates from the β- and γ-subunits, and activates adenylyl cyclase that generates cyclic adenosine monophosphate (cAMP) from ATP. cAMP activates protein kinase A (PKA) that phosphorylates and activates glycogen phosphorylase kinase, which phosphorylates and activates glycogen phosphorylase. The phosphorylase degrades glycogen, generating glucose 1-phosphate that is converted to glucose 6-phosphate. With glucose 6-phosphatase deficiency, the degradative process stops here (see figure below). Consequently, administration of glucagon is unable to cause a rise in blood glucose. [Note: Epinephrine would be similarly ineffective.]



TQ4. The availability of inorganic phosphate (P_i) is decreased because it is trapped as phosphorylated glycolytic intermediates as a result of the upregulation of glycolysis by the rise in glucose 6-phosphate. Urate is elevated because the trapping of P_i decreases the ability to phosphorylate adenosine diphosphate (ADP) to ATP, and the fall in ATP causes a rise in adenosine monophosphate (AMP). The AMP is degraded to urate. Additionally, the availability of glucose 6-phosphate drives the pentose phosphate pathway, resulting in a rise in ribose 5-phosphate (from ribulose 5-phosphate) and, consequently, a rise in purine synthesis. Nicotinamide adenine dinucleotide phosphate (NADPH) also rises. Purines made beyond need are degraded to urate (see figure on the next page). [Note: The decrease in P_i reduces the activity of glycogen phosphorylase, resulting in increased storage of glycogen with a normal structure.] Lactate is elevated because the decrease in phosphorylation of ADP to ATP results in a decrease in cellular respiration (respiratory control) as a result of these processes being coupled. As a consequence, reduced nicotinamide adenine dinucleotide (NADH) from glycolysis cannot be oxidized by Complex I of the electron transport chain. Instead, it is oxidized by cytosolic lactate dehydrogenase with its coenzyme NADH as pyruvate is reduced to lactate. [Note: Pyruvate is increased as a result of the increase in glycolysis.] The lactate ionizes, releasing protons (H^+) and leading to a metabolic acidosis (low pH caused here by increased production of acid). Respiratory compensation causes an increased respiratory rate.



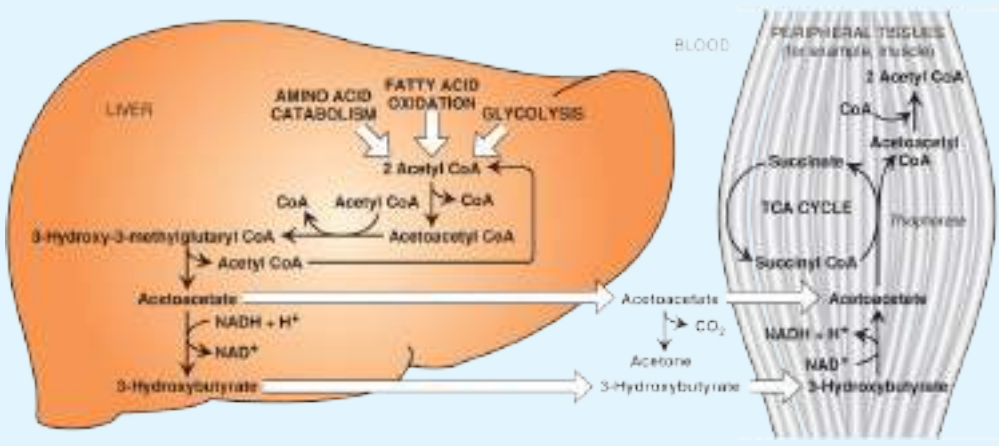
TQ5. Increased glycolysis results in increased availability of glycerol 3-phosphate for hepatic TAG synthesis. Additionally, some of the pyruvate generated in glycolysis will be oxidatively decarboxylated to acetyl coenzyme A (CoA). However, the tricarboxylic acid cycle is inhibited by the rise in NADH, and the acetyl CoA is transported to the cytosol as citrate. The rise of acetyl CoA in the cytosol results in increased fatty acid (FA) synthesis. Recall that citrate is an allosteric activator of acetyl CoA carboxylase (ACC). The malonyl product of ACC inhibits FA oxidation at the carnitine palmitoyltransferase I step. Because mitochondrial FA oxidation generates the acetyl CoA substrate for hepatic ketogenesis, ketone body levels do not rise. The FA gets esterified to the glycerol backbone, resulting in an increase in TAG that gets sent out of the liver as components of very–low-density lipoproteins (VLDL). [Note: The hypoglycemia results in release of epinephrine and the activation of TAG lipolysis with release of free FA into the blood. The FAs are oxidized, with the excess used in hepatic TAG synthesis.] The acetyl CoA is also a substrate for cholesterol synthesis. Thus, the increase in glycolysis results in the hyperlipidemia (see figure below).



CASE 3: Answers to Review Questions

RQ1. **Correct answer = A.** Diabetes is characterized by hyperglycemia. Chronic hyperglycemia can result in the nonenzymatic glycosylation (glycation) of hemoglobin (Hb), producing HbA_{1c}. Therefore, measurement of glucose or HbA_{1c} in the blood is used to diagnose diabetes. In response to physiologic stress (e.g., a urinary tract infection), secretion of counterregulatory hormones (such as the catecholamines) results in a rise in blood glucose. Glucose is a reducing sugar. It is type 2 diabetes (T2D) that is associated with obesity and a sedentary lifestyle and is caused by insensitivity to insulin (insulin resistance). T1D is caused by lack of insulin as a result of the autoimmune destruction of pancreatic β-cells. Even individuals on a program of tight glycemic control do not achieve euglycemia.

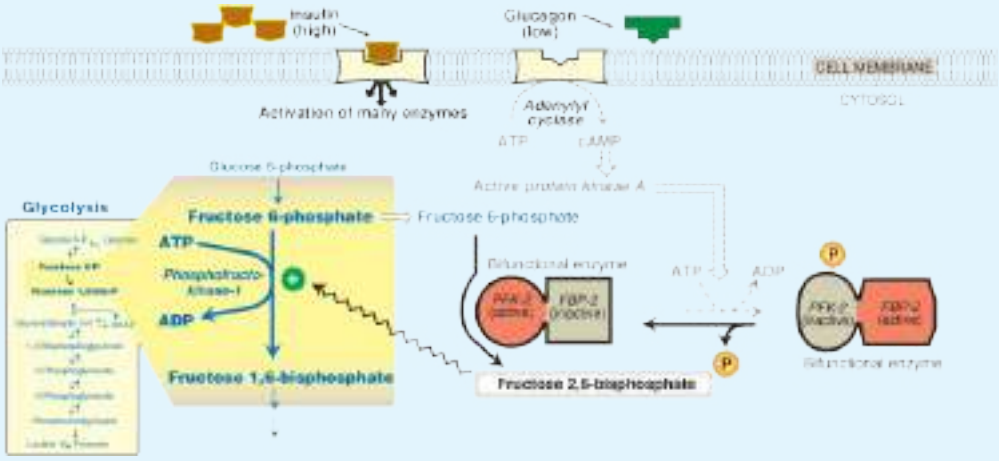
RQ2. **Correct answer = E.** The ketone bodies 3-hydroxybutyrate and acetoacetate are organic acids, and their ionization contributes to the proton load of the body. Ketone bodies are made in the mitochondria of liver cells using acetyl coenzyme A (CoA) generated primarily from the β-oxidation of fatty acids ([FAs]; see figure on the next page). Because they are water soluble, they do not require a transporter. The liver cannot use them because it lacks the enzyme thiophorase, which moves CoA from succinyl CoA to acetoacetate for conversion to two molecules of acetyl CoA. It is the acetone released in the breath that can impart a fruity odor.



RQ3. **Correct answer = A.** Malonyl CoA, an intermediate of FA synthesis, inhibits FA β-oxidation through inhibition of carnitine palmitoyltransferase I. Lipolysis occurs when the insulin/counterregulatory hormone ratio decreases. Acetyl CoA, the product of FA β-oxidation, inhibits the pyruvate dehydrogenase (PDH) complex through activation of PDH kinase and activates pyruvate carboxylase. Acetyl CoA, then, pushes pyruvate to gluconeogenesis. β-Oxidation generates reduced nicotinamide adenine dinucleotide (NADH), the reducing equivalent required for gluconeogenesis. FAs are not readily catabolized for energy by the brain.

CASE 3: Answers to Thought Questions

TQ1. Hypoinsulinemia results in hyperglycemia because insulin is required for the uptake of blood glucose by muscle and adipose tissue. Their glucose transporter (GLUT-4) is insulin dependent in that insulin is required for movement of the transporter to the cell surface from intracellular storage sites. Insulin is also required to suppress hepatic gluconeogenesis. Insulin suppresses the release of glucagon from pancreatic α-cells. The resulting rise in the insulin/glucagon ratio results in the dephosphorylation and activation of the kinase domain of bifunctional phosphofructokinase-2 (PFK-2). The fructose 2,6-bisphosphate produced by PFK-2 activates phosphofructokinase-1 of glycolysis (see figure below). It also inhibits fructose 1,6-bisphosphatase (FBP-2), thereby inhibiting gluconeogenesis. With hypoinsulinemia, the failure to take up glucose from the blood while simultaneously sending it out into the blood results in hyperglycemia.



TQ2. The blood glucose level has exceeded the capacity of the kidney to reabsorb glucose (via a sodium-dependent glucose transporter [SGLT]). The high concentration of glucose in the urine osmotically draws water from the body. This causes increased urination (polyuria) with loss of water that results in dehydration.

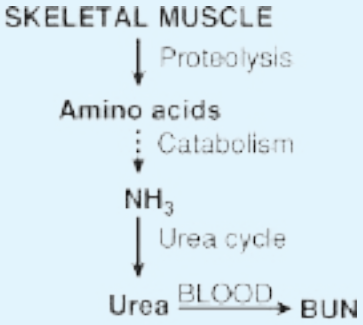
TQ3. The NADH generated in FA β-oxidation inhibits the tricarboxylic acid (TCA) cycle at the three NADH-producing dehydrogenase steps. This shifts acetyl CoA away from oxidation in the TCA cycle and toward use as a substrate in hepatic ketogenesis.

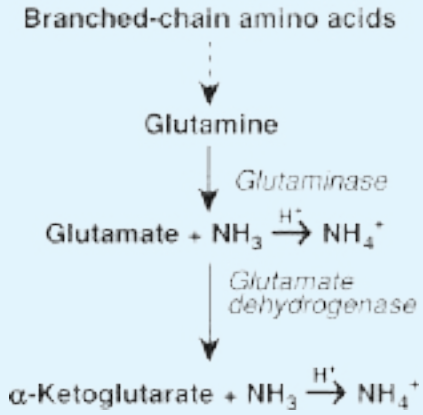
$$H^+ + HCO_3^- \leftrightarrow H_2CO_3 \text{ (carbonic acid)} \leftrightarrow CO_2 + H_2O.$$

TQ4. She was in negative nitrogen balance: More nitrogen was going out than coming in. This is reflected in the elevated blood urea nitrogen (BUN) level seen in the patient (see figure at top right). [Note: The BUN value also reflects dehydration.] Muscle proteolysis and amino acid catabolism are occurring as a result of the fall in insulin. (Recall that skeletal muscle does not express the glucagon receptor.) Amino acid catabolism produces ammonia (NH_3), which is converted to urea by the hepatic urea cycle and sent into the blood. [Note: Urea in the urine is reported as urinary urea nitrogen.]

TQ5. The Kussmaul respiration seen in this patient is a respiratory response to the metabolic acidosis. Hyperventilation blows off CO_2 and water, reducing the concentration of protons (H^+) and

bicarbonate (HCO_3^-) as reflected in the following equation:





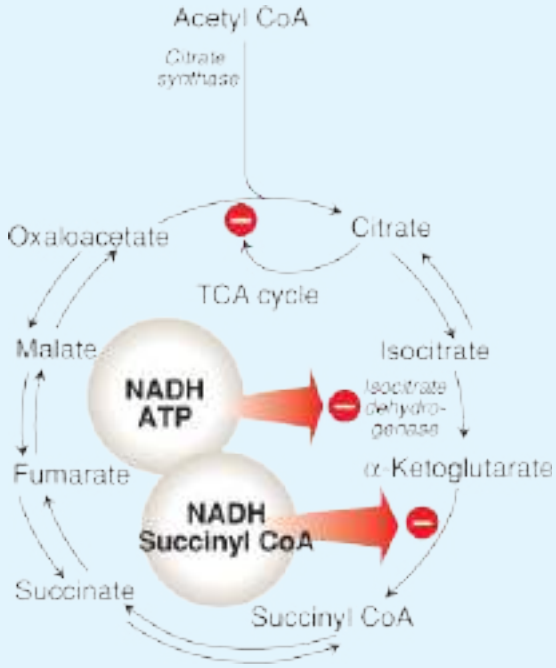
The renal response includes, in part, the excretion of H^+ as ammonium (NH_4^+). Degradation of branched-chain amino acids in skeletal muscle results in the release of large amounts of glutamine (Gln) into the blood. The kidneys take up and catabolize the Gln, generating NH_3 in the process. The NH_3 is converted to NH_4^+ by secreted H^+ and is excreted (see figure at middle right). [Note: When ketone bodies are plentiful, enterocytes shift to using them as a fuel instead of Gln. This increases the amount of Gln going to the kidney.]

TQ6. Because FA β-oxidation supplies the acetyl CoA substrate for ketogenesis, impaired β-oxidation decreases the ability to make ketone bodies. Ketone bodies are an alternate to the use of glucose, and, thus, dependence on glucose increases. Because FA β-oxidation supplies the NADH and the nucleoside triphosphates needed for gluconeogenesis, glucose production decreases. The result is a hypoketotic hypoglycemia. Recall that this was seen with medium-chain acyl CoA dehydrogenase (MCAD) deficiency.

CASE 4: Answers to Review Questions

RQ1. **Answer = D.** The rise in reduced nicotinamide adenine dinucleotide (NADH) in the mitochondria decreases the tricarboxylic acid (TCA) cycle, fatty acid (FA) oxidation, and gluconeogenesis. NADH inhibits the isocitrate dehydrogenase reaction, the key regulated step of the TCA cycle, and the α-ketoglutarate dehydrogenase reaction (see figure at bottom right). It also favors the reduction of oxaloacetate (OAA) to malate (not malate to OAA), decreasing the availability of OAA for condensation with acetyl coenzyme A (CoA) in the TCA cycle and for gluconeogenesis. FA oxidation requires the oxidized form of nicotinamide adenine dinucleotide (NAD^+) for the 3-hydroxyacyl CoA dehydrogenase step and, thus, is inhibited by the rise in NADH. The decrease in FA oxidation decreases the production of ATP and acetyl CoA (the allosteric activator of pyruvate carboxylase) needed for gluconeogenesis. Lipolysis is activated in fasting as a consequence of the

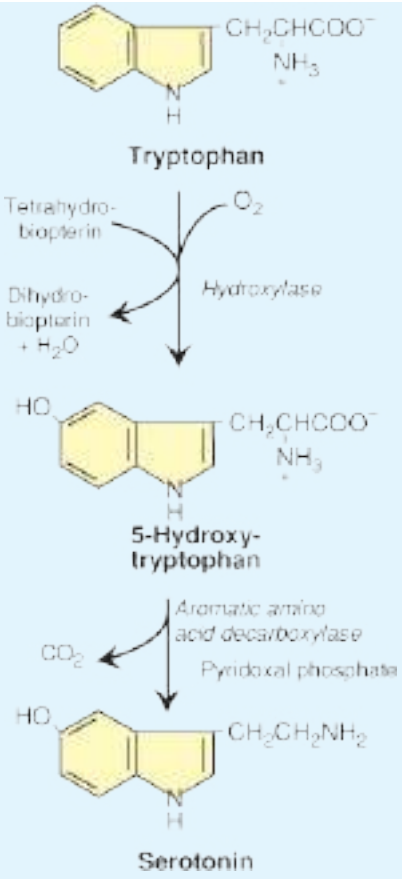
fall in insulin and the rise in catecholamines that result in activation of hormone-sensitive lipase.



RQ2. **Answer = E.** The irreversible, oxidative portion of the pentose phosphate pathway provides the nicotinamide adenine dinucleotide phosphate (NADPH) that supplies the reducing equivalents needed for activity of cytochrome P450 (CYP) proteins and for the regeneration of functional (reduced) glutathione. It is also an important source of NADPH for reductive biosynthetic processes in the cytosol, such as FA and cholesterol synthesis. [Note: Malic enzyme is another source.] CYP proteins are monooxygenases (mixed-function oxidases). They incorporate one O atom from O_2 into the substrate as the other is reduced to water. It is the CYP proteins of the smooth endoplasmic reticular membrane that are involved in detoxification reactions. Those of the inner mitochondrial membrane are involved in the synthesis of steroid hormones, bile acids, and vitamin D. Reactive oxygen species are reduced by glutathione peroxidase as glutathione is oxidized.

RQ3. **Answer = C.** Serotonin is released by activated platelets and causes vasoconstriction and platelet aggregation. [Note: Platelets do not synthesize serotonin, but they take up that which was made in the intestine and secreted into the blood.] Serotonin is associated with a feeling of well-being. It is degraded to 5-hydroxyindoleacetic acid by monoamine oxidase that catalyzes oxidative deamination. It is catechol-O-methyltransferase that catalyzes the methylation step in the degradation of the catecholamines. Serotonin is synthesized from tryptophan in a two-step process that utilizes tetrahydrobiopterin (BH_4)-requiring tryptophan hydroxylase and a pyridoxal phosphate (PLP)-requiring decarboxylase (see figure at right).

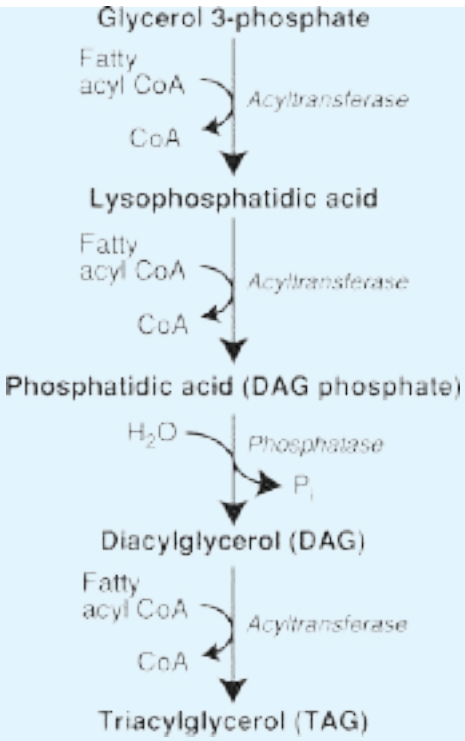
RQ4. **Answer = B.** The exocrine pancreas secretes enzymes required for the digestion of dietary carbohydrate, protein, and fat. The endocrine pancreas secretes the peptide hormones insulin and glucagon. Damage that affects the functions of the pancreas would lead to diabetes (decreased insulin) and steatorrhea (fatty stool), with the latter the consequence of maldigestion of dietary fat. As was seen with the rise of troponins in a myocardial infarction and transaminases in liver damage, loss of cellular integrity (as would be seen in autodigestion of the pancreas) results in proteins that normally are intracellular being found in higher-than-normal concentrations in the blood. Secretin causes the pancreas to release bicarbonate to raise the pH of the chyme coming to the intestine from the stomach. Pancreatic enzymes work best at neutral or slightly alkaline pH. Pancreatitis is seen in individuals with hypertriglyceridemia as a result of a deficiency in lipoprotein lipase or its coenzyme, apolipoprotein C-II.



CASE 4: Answers to Thought Questions

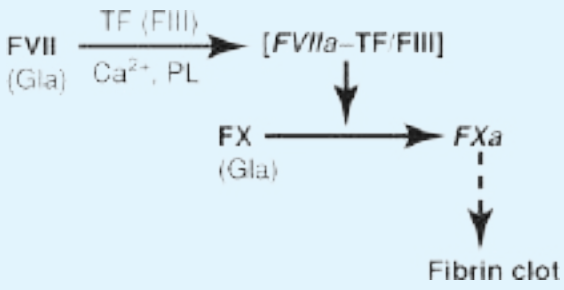
TQ1. A. The rise in cytosolic NADH seen with ethanol metabolism inhibits glycolysis. The glyceraldehyde 3-phosphate dehydrogenase step requires NAD^+, which gets reduced as glyceraldehyde 3-phosphate gets oxidized. With the rise in NADH, glyceraldehyde 3-phosphate accumulates.

B. Glyceraldehyde 3-phosphate from glycolysis is converted to glycerol 3-phosphate, the initial acceptor of FA in triacylglycerol (TAG) synthesis (see figure at right). FAs are available because of increased synthesis (from acetyl CoA, which is increased as a result of both increased production from the acetate product of acetaldehyde oxidation and decreased use in the TCA cycle), increased availability from lipolysis in adipose tissue, and decreased degradation. The TAG produced in the liver accumulate (due, in part, to decreased production of very–low-density lipoproteins) and cause fatty liver (steatosis). Hepatic steatosis is an early (and reversible) stage in alcohol-related liver disease. Subsequent stages are alcohol-related hepatitis (sometimes reversible) and cirrhosis (irreversible).



TQ2. The rise in NADH favors the reduction of pyruvate to lactate by lactate dehydrogenase. Lactate decreases the renal excretion of uric acid, thereby causing hyperuricemia, a necessary step in an acute gouty attack. [Note: The shift from pyruvate to lactate decreases the availability of pyruvate, a substrate for gluconeogenesis. This contributes to the hypoglycemia seen in AK.]

TQ3. Prothrombin time (PT) measures the time it takes for plasma to clot after the addition of tissue factor, thereby allowing evaluation of the extrinsic (and common) pathways of coagulation. In the extrinsic pathway, Tissue Factor forms a complex with Factor VII and the complex is activated in a calcium (Ca^{2+})- and phospholipid (PL)-dependent process (see figure at bottom right). Factor VII, like most of the proteins of clotting, is made by the liver. Alcohol-induced liver damage can decrease its synthesis. Additionally, Factor VII has a short half-life, and, as a γ-carboxyglutamate (Gla)-containing protein, its synthesis requires vitamin K. Poor nutrition can result in decreased availability of vitamin K and, therefore, decreased ability to clot. [Note: Severe liver disease results in prolonged PT and activated partial thromboplastin time, or aPPt.]



TQ4. Administration of folate can mask a deficiency in vitamin B_{12} by reversing the hematologic manifestation (macrocytic anemia) of the deficiency. However, folate has no effect on the neurologic damage caused by B_{12} deficiency. Over time, then, the neurologic effects can become severe and irreversible. Thus, folate can mask a deficiency of B_{12} and prevent treatment until the neuropathy is apparent.

III. FOCUSED CASES

CASE 1: MICROCYTIC ANEMIA

Patient Presentation: A 24-year-old male is being evaluated as a follow-up to a pre-employment medical evaluation.

Focused History: He has no significant medical issues. His family history is unremarkable.

Pertinent Findings: The physical examination was normal. Routine analysis of his blood included the following results:

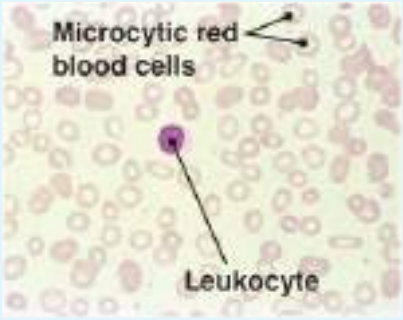
	Patient	Reference Range
Red blood cells	$4.8 \times 10^6/mm^3$	4.3–5.9
Hemoglobin	9.6 g/dL (**L**)	13.5–7.5 (men)
Mean corpuscular volume	70 μm^3 (**L**)	80–100
Serum iron	150 µg/dL	50–170

Based on the data, hemoglobin (Hb) electrophoresis was performed. The results are as follows:

	Patient	Reference Range
HbA	90% (**L**)	96–98
HbA_2	6% (**H**)	<3
HbF	4% (**H**)	<2

H = High; **L** = Low. [Note: HbA includes HbA_{1c}.]

Diagnosis: This patient has β-thalassemia trait (β-thalassemia minor) that is causing a microcytic anemia (see image at right).



Treatment: None is required at this time. Patients are advised that iron supplements will not prevent their anemia.

Prognosis: β-Thalassemia trait does not cause mortality or significant morbidity. Patients should be informed of the genetic nature of their autosomal-recessive condition for family planning considerations because homozygous β-thalassemia (Cooley anemia) is a serious disorder.

CASE-RELATED QUESTIONS: Choose the ONE best answer

Q1. Mutations to the gene for β-globin that result in decreased production of the protein are the cause of β-thalassemia. The mutations primarily affect gene transcription or posttranscriptional processing of the messenger RNA (mRNA) product. Which of the following statements concerning mRNA is correct?

A. Eukaryotic mRNA is polycistronic.

B. mRNA synthesis involves trans-acting factors binding to cis-acting elements.
C. mRNA synthesis is terminated at the DNA base sequence thymine adenine guanine (TAG).
D. Polyadenylation of the 5′-end of eukaryotic mRNA requires a methyl donor.
E. Splicing of eukaryotic mRNA involves removal of exons and joining of introns.

Q2. HbA, a tetramer of 2α- and 2β-globin chains, delivers O_2 from the lungs to the tissues and protons and CO_2 from the tissues to the lungs. Increased concentration of which of the following will result in decreased O_2 delivery by HbA?

A. 2,3-Bisphosphoglycerate
B. Carbon dioxide
C. Carbon monoxide
D. Protons

Q3. What is the basis for the increase in HbA_2 and HbF (fetal Hb) in the β-thalassemias?

Q4. Why is the allele-specific oligonucleotide (ASO) hybridization technique useful in the diagnosis of all cases of sickle cell anemia but not all cases of β-thalassemia?

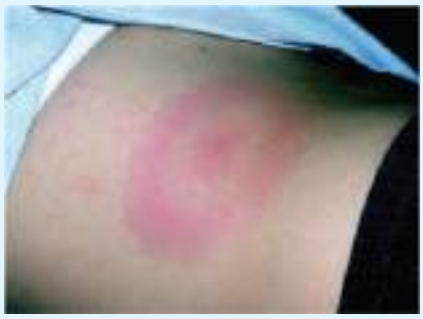
CASE 2: SKIN RASH

Patient Presentation: A 34-year-old female presents with a red, nonitchy rash on her left thigh along with flu-like symptoms.

Focused History: She reports that the rash first appeared a little over 2 weeks ago. It started out small but has gotten larger. She also thinks she is getting the flu because her muscles and joints ache (myalgia and arthralgia, respectively), and she has had a headache for the last few days. She reports that she and her husband took a camping trip last month.

Pertinent Findings: The physical examination is remarkable for the presence of a red, circular, flat lesion ~11 cm in size that resembles a bullseye (erythema migrans) (see image at right). She also has a low-grade fever.

Diagnosis: The patient has Lyme disease caused by the bacterium *Borrelia burgdorferi*, which is transmitted by the bite of a tick in the genus *Ixodes*. Infected ticks are endemic in several regions of the United States.



Treatment: She is prescribed doxycycline, an antibiotic in the tetracycline family. Monitoring of the patient will continue until all symptoms have completely resolved. Blood is drawn for clinical laboratory tests.

Prognosis: Patients treated with the appropriate antibiotic in the early stages of Lyme disease typically recover quickly and completely.

CASE-RELATED QUESTIONS: Choose the ONE best answer

Q1. Antibiotics in the tetracycline class inhibit protein synthesis (translation) of prokaryotic mRNA at the initiation step. Which of the following statements about translation is correct?

A. In eukaryotic translation, the initiating amino acid is formylated methionine.

B. Only the charged initiating transfer RNA goes directly to the ribosomal A site.
C. Peptidyltransferase is a ribozyme that forms the peptide bond between two amino acids.
D. Prokaryotic translation can be inhibited by the phosphorylation of initiation factor 2.
E. Termination of translation is independent of guanosine triphosphate hydrolysis.
F. The Shine-Dalgarno sequence facilitates the binding of the large ribosomal subunit to mRNA.

Q2. The Centers for Disease Control and Prevention recommends a two-tier testing procedure for Lyme disease that involves a screening enzyme-linked immunosorbent assay (ELISA) followed by a confirmatory western blot analysis on any sample with a positive or equivocal ELISA result. Which of the following statements about these testing procedures is correct?

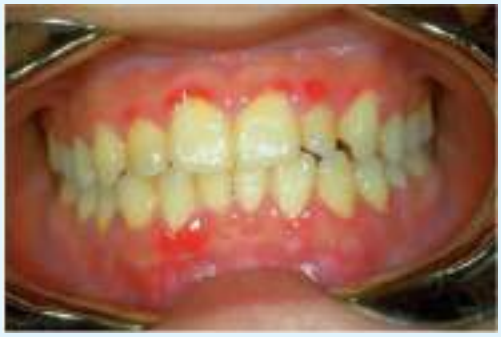
A. Both techniques are used to detect specific mRNA.
B. Both techniques involve the use of antibodies to detect proteins.
C. ELISA requires the use of electrophoresis.
D. Western blots require use of the polymerase chain reaction.

Q3. Why are eukaryotic cells unaffected by antibiotics in the tetracycline class?

CASE 3: BLOOD ON THE TOOTHBRUSH

Patient Presentation: An 84-year-old male presents for evaluation of bruising and bleeding gums.

Focused History: The patient has lived alone since the death of his wife 11 months ago. He has been isolated and finds it hard to get out of the house. His appetite has changed, and he is content with cereal, coffee, and packaged snacks. Chewing is difficult.



Pertinent Findings: The physical examination was remarkable for the presence of swollen dark-colored gums (see image at right). Several of his teeth were loose, including one that anchors his dental bridge. Several black and blue marks (ecchymoses) were noted on the legs, and an unhealed sore was present on the right wrist. Inspection of his scalp revealed tiny red spots (petechiae) around some of the hair follicles. Blood was drawn for testing.

The results of blood tests are as follows:

	Patient	Reference Range
Red blood cells	$4.0 \times 10^6/mm^3$ (**L**)	4.3–5.9
Hemoglobin	10 g/dL (**L**)	13.5–17.5 (men)
Mean corpuscular volume	$78\ \mu m^3$ (**L**)	80–100
Serum iron	40 µg/dL (**L**)	50–170
Serum ferritin	23 µg/L (**L**)	40–160 µg/L
Total iron-binding capacity	375 µg/dL (**H**)	300–360 µg/dL
Platelets	$250 \times 10^9/L$	$150–350 \times 10^9$

The test for blood in his stool (occult blood test) was negative.

Results of follow-up tests (obtained several days after the appointment) included the following:

	Patient	Reference Range
Vitamin C (plasma)	0.16 mg/dL (**L**)	0.2–2

H = High; **L** = Low.

Diagnosis: He has vitamin C deficiency with a microcytic, hypochromic anemia secondary to iron deficiency.

Treatment: He was prescribed vitamin C (as oral ascorbic acid) and iron (as oral ferrous sulfate) supplements. He will also be referred to social services.

Prognosis: The prognosis for recovery is good.

CASE-RELATED QUESTIONS: Choose the ONE best answer

Q1. Which of the following statements about vitamin C is correct? Vitamin C is:

A. a competitive inhibitor of iron absorption in the intestine.
B. a fat-soluble vitamin with a 3-month supply typically stored in adipose tissue.
C. a coenzyme required for the hydroxylation of prolyl and lysyl residues in collagen.
D. required for the cross-linking of collagen.

Q2. In contrast to the microcytic anemia characteristic of iron deficiency (common in older adults), a macrocytic anemia is seen with deficiencies of vitamin B_{12} and/or folic acid. These vitamin deficiencies are also common in older adults. Which of the following statements concerning these vitamins is correct?

A. An inability to absorb B_{12} results in pernicious anemia.
B. Both vitamins cause changes in gene expression.
C. Folic acid plays a key role in energy metabolism in most cells.
D. Treatment with methotrexate can result in toxic levels of the coenzyme form of folic acid.
E. Vitamin B_{12} is the coenzyme for amino acid deaminations, decarboxylations, and transaminations.

Q3. How do hemolytic anemias differ from nutritional anemias?

CASE 4: RAPID HEART RATE, HEADACHE, AND SWEATING

Patient Presentation: A 45-year-old female presents with concerns about sudden (paroxysmal), intense, brief episodes of headache, sweating (diaphoresis), and a racing heart (palpitations).

Focused History: She reports that the attacks started ~3 weeks ago. They last from 2 to 10 minutes, during which time she feels quite anxious. During the attacks, it feels as though her heart is skipping beats (arrhythmia). At first, she thought the attacks were related to recent stress at work and maybe even menopause. The last time it happened, she was in a pharmacy and had her blood pressure taken. She was told it was 165/110 mm Hg. The patient notes that she has lost weight (~8 lb) in this period even though her appetite has been good.

Pertinent Findings: The physical examination was remarkable for her thin, pale appearance. Blood pressure was elevated (150/100 mm Hg), as was the heart rate (110 to 120 beats/min). Based on her history, blood levels of normetanephrine and metanephrine were ordered. They were found to be elevated.

Diagnosis: She has a pheochromocytoma, a rare catecholamine-secreting tumor of the adrenal medulla.

Treatment: Imaging studies of the abdomen locate the tumor in her right adrenal gland and laparoscopic surgical removal of the tumor was performed. The tumor was found to be nonmalignant. Following surgery, her blood pressure returned to normal. Follow-up measurement of plasma metanephrines was performed 2 weeks later and was in the normal range.

Prognosis: The 5-year survival rate for nonmalignant pheochromocytomas is >95%.

CASE-RELATED QUESTIONS: Choose the ONE best answer

Q1. Pheochromocytomas secrete norepinephrine (NE) and epinephrine. Which of the following statements concerning the synthesis and degradation of these two biogenic amines is correct?

A. The substrate for their synthesis is tryptophan, which is hydroxylated to 3,4-dihydroxyphenylalanine (DOPA) by tetrahydrobiopterin-requiring tryptophan hydroxylase.
B. The conversion of DOPA to dopamine utilizes a pyridoxal phosphate–requiring carboxylase.
C. The conversion of norepinephrine to epinephrine requires vitamin C.
D. Degradation involves methylation by catechol-O-methyltransferase and produces normetanephrine from norepinephrine and metanephrine from epinephrine.
E. Normetanephrine and metanephrine are oxidatively deaminated to homovanillic acid by monoamine oxidase.

Q2. Which of the following statements concerning the actions of epinephrine and/or NE are correct?

A. NE functions as a neurotransmitter and a hormone.
B. They are initiated by autophosphorylation of select tyrosine residues in their receptors.
C. They are mediated by binding to adrenergic receptors, a class of nuclear receptors.
D. They result in the activation of glycogen and triacylglycerol synthesis.

Q3. NE bound to certain receptors causes vasoconstriction and an increase in blood pressure. Why might NE be used clinically in the treatment of septic shock?

CASE 5: SUN SENSITIVITY

Patient Presentation: A 6-year-old male is evaluated for freckle-like areas of hyperpigmentation on his face, neck, forearms, and lower legs.

Focused History: His father reports that the child has always been quite sensitive to the sun. His skin turns red (erythema) and his eyes hurt (photophobia) if he is exposed to the sun for any period of time.

Pertinent Findings: The physical examination was remarkable for the presence of thickened, scaly areas (actinic keratosis) and hyperpigmented areas on skin exposed to ultraviolet (UV) radiation from the sun. Small dilated blood vessels (telangiectasia) were also seen. Tissue from several sites on his arms and legs was biopsied, and two were later determined to be squamous cell carcinomas.

Diagnosis: He has xeroderma pigmentosum, a rare defect in nucleotide excision repair of DNA.

Treatment: Protection from sunlight through use of sunscreens such as protective clothing that reflect UV radiation and chemicals that absorb it is essential. Frequent skin and eye examinations are recommended.

Prognosis: Most patients with xeroderma pigmentosum die at an early age from skin cancers. However, survival beyond middle age is possible.

CASE-RELATED QUESTIONS: Choose the ONE best answer

Q1. DNA repair:

A. is performed only by eukaryotes.
B. of double-strand breaks is error free.
C. of mismatched bases involves repair of the parental strand.
D. of UV radiation–induced pyrimidine dimers involves removal of a short oligonucleotide containing the dimer.
E. of uracil produced by the deamination of cytosine requires the actions of endo- and exonucleases to remove the uracil base.

Q2. Regarding DNA synthesis or replication:

A. in both eukaryotes and prokaryotes requires an RNA primer.
B. in eukaryotes requires condensation of chromatin.
C. in prokaryotes is accomplished by a single DNA polymerase.
D. is initiated at random sites in the genome.
E. produces a polymer of deoxyribonucleoside monophosphates linked by 5′→3′-phosphodiester bonds.

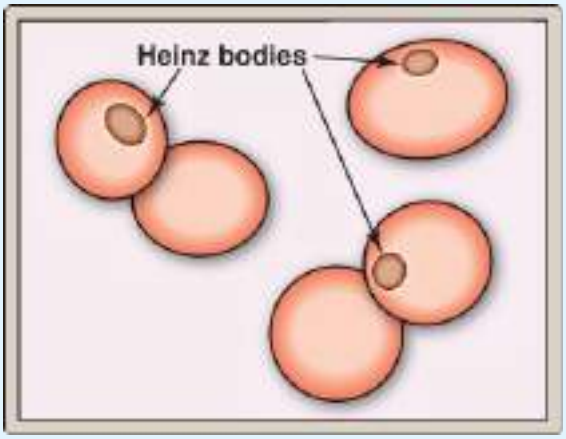
Q3. What is the difference between DNA proofreading and repair?

CASE 6: DARK URINE AND YELLOW SCLERAE

Patient Presentation: A 63-year-old male patient presents with fatigue and scleral icterus.

Focused History: He began treatment ~4 days ago with a sulfonamide antibiotic and a urinary analgesic for a urinary tract infection. He had been told that his urine would change color (become reddish) with the analgesic, but he reports that it has gotten darker (more brownish) over the last 2 days. Last night, his wife noticed that his eyes had a yellow tint. He says he feels as though he has no energy.

Pertinent Findings: The physical examination was remarkable for the patient's pale appearance, mild scleral icterus (jaundice), mild splenomegaly, and increased heart rate (tachycardia). His urine tested positive for hemoglobin (hemoglobinuria). A peripheral blood smear reveals a lower-than-normal number of red blood cells (RBC), with some containing precipitated hemoglobin (Heinz bodies; see image at right), and a higher-than-normal number of reticulocytes (immature RBC). Results of the complete blood count (CBC) and blood chemistry tests are pending.



Diagnosis: This patient has glucose 6-phosphate dehydrogenase (G6PD) deficiency, an X-linked disorder that causes hemolysis (RBC lysis).

Treatment: G6PD deficiency can result in a hemolytic anemia in affected individuals exposed to oxidative agents, including infection, certain drugs and fava beans. He will be switched to a different antibiotic and advised to avoid certain agents and to always report his condition to medical providers. He likely was not previously exposed to a strong oxidant stressor and was unaware he had this genetic defect.

Prognosis: In the absence of exposure to oxidative agents, G6PD deficiency does not cause mortality or significant morbidity.

CASE-RELATED QUESTIONS: Choose the ONE best answer

Q1. Which of the following statements concerning G6PD and the pentose phosphate pathway is correct?

A. Deficiency of G6PD occurs only in RBC.
B. Deficiency of G6PD results in an inability to keep glutathione in its reduced form.
C. The pentose phosphate pathway begins with one reversible reductive reaction followed by a series of phosphorylated sugar interconversions.

D. The NADPH produced in the pentose phosphate pathway is utilized in processes such as fatty acid oxidation.

Q2. The results of his CBC were consistent with a hemolytic anemia. Blood chemistry tests revealed an elevation in the bilirubin level. Which of the following statements concerning bilirubin is correct?

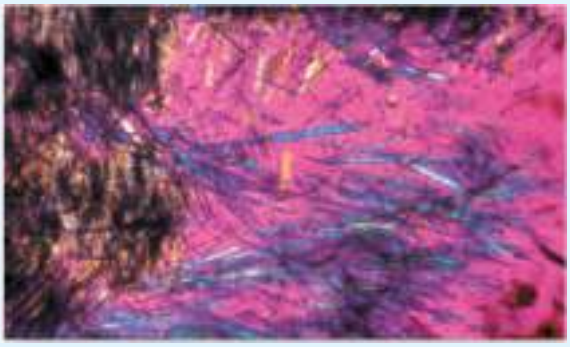
A. Hyperbilirubinemia results in deposition of bilirubin in the skin and sclerae resulting in jaundice.
B. The solubility of bilirubin is increased by adding to two molecules of ascorbic acid in the liver.
C. The conjugated form of bilirubin increases in the blood with a hemolytic anemia.
D. Phototherapy can increase the solubility of the excess bilirubin generated in the porphyrias.

Q3. Why is urinary urobilinogen increased relative to normal in hemolytic jaundice and absent in obstructive jaundice?

CASE 7: JOINT PAIN

Patient Presentation: A 22-year-old male presents for follow-up 10 days after having been treated in the Emergency Department (ED) for severe inflammation at the base of his thumb.

Focused History: This was his first occurrence of severe joint pain. In the ED, he was given an anti-inflammatory medication. Fluid aspirated from the carpometacarpal joint of the thumb was negative for organisms but positive for needle-shaped monosodium urate (MSU) crystals (see image at right). The inflammatory symptoms have since resolved. He reports he is in good health otherwise, with no significant past medical history. His body mass index (BMI) is 31. No tophi (deposits of MSU crystals under the skin) were detected in the physical examination.



Pertinent Findings: Results on a 24-hour urine specimen and blood tests requested in advance of this visit revealed normal kidney function and uric acid section. His blood urate was 8.5 mg/dL (reference = 2.5–8.0). The unusually young age of presentation is suggestive of an enzymopathy of purine metabolism, and additional blood tests are ordered.

Diagnosis: The patient has gout (MSU crystal deposition disease), a type of inflammatory arthritis.

Treatment: He was given prescriptions for pain medication and for allopurinol and colchicine. The treatment goals are to reduce his blood urate levels to <6.0 mg/dL and to prevent additional attacks. He was advised to lose weight because being overweight or obese is a risk factor for gout. His BMI of 31 puts him in the obese category. He was also given written information on the association between diet and gout.

Prognosis: Gout increases the risk of developing renal stones. It is also associated with hypertension, diabetes, and heart disease.

CASE-RELATED QUESTIONS: Choose the ONE best answer

Q1. Allopurinol is converted in the body to oxypurinol, which functions as a noncompetitive inhibitor of an enzyme in purine metabolism. Which of the following statements concerning purine metabolism and its regulation is correct?

A. As a noncompetitive inhibitor, oxypurinol increases the apparent K_m of the target enzyme.

B. Colchicine inhibits xanthine oxidase, an enzyme of purine degradation.
C. Glutamate provides two of the nitrogen atoms of the purine ring.
D. In purine nucleotide synthesis, the ring system is first constructed and then attached to ribose 5-phosphate.
E. Oxypurinol inhibits the amidotransferase that initiates degradation of the purine ring system.
F. Partial or complete enzymic deficiencies in the salvage of purine bases are characterized by hyperuricemia.

Q2. Which of the following statements is true of the pyrimidines?

A. Carbamoyl phosphate synthetase I is the regulated enzymic activity in pyrimidine ring synthesis.
B. Methotrexate decreases synthesis of the pyrimidine nucleotide thymidine monophosphate.
C. Orotic aciduria is a pathology of pyrimidine degradation.
D. Pyrimidine nucleotide synthesis is independent of 5-phosphoribosyl-1-pyrophosphate (PRPP).

Q3. The patient is subsequently shown to have a form of PRPP synthetase that shows increased enzymic activity. Why does this result in hyperuricemia?

CASE 8: NO BOWEL MOVEMENT

Patient Presentation: A 2-day-old female has not yet had a bowel movement.

Focused History: The infant was born at full term following a normal pregnancy and delivery. She appeared normal at birth. She is the first child of parents who are both in good health, with unremarkable family histories.

Pertinent Findings: The child has a distended abdomen. She recently vomited small amounts of bilious (green-colored) material.

Diagnosis: Meconium ileus (obstruction of the ileum by meconium, the first stool produced by newborns) was confirmed by abdominal X-rays. About 98% of full-term newborns with meconium ileus have cystic fibrosis (CF). Diagnosis of cystic fibrosis was subsequently confirmed with a chloride sweat test and genetic analysis.

Treatment: The ileus was successfully treated without surgery. The family was referred to the CF center at the regional children's hospital.

Prognosis: CF is the most common life-limiting autosomal-recessive disease in Caucasians and is seen in approximately 1/3,300 live births in the United States.

CASE-RELATED QUESTIONS: Choose the ONE best answer

Q1. Which of the following statements concerning CF is correct?

A. Clinical manifestations of CF are the consequence of chloride retention with increased water reabsorption that causes mucus on the epithelial surface to be excessively thick and sticky.
B. Excessive pancreatic secretion of insulin in CF commonly results in hypoglycemia.
C. Some mutations result in premature degradation of the CFTR protein through tagging with ubiquinone followed by proteasome-mediated proteolysis.
D. The most common mutation, ΔF508, results in the loss of a codon for phenylalanine (F) and is classified as a frameshift mutation.

Q2. The CFTR protein is an intrinsic plasma membrane glycoprotein. Targeting of proteins destined to function as components of membranes:

A. includes transport to and through the Golgi.
B. involves an amino-terminal signal sequence that is retained in the functional protein.
C. occurs after the protein has been completely synthesized (i.e., posttranslationally).
D. requires the presence of mannose 6-phosphate residues on the protein.

Q3. Why might steatorrhea be seen with CF?

CASE 9: ELEVATED AMMONIA

Patient Presentation: A 40-hour-old male with signs of cerebral edema.

Focused History: The child was born at full term after a normal pregnancy and delivery. He appeared normal at birth. At the age of 36 hours, he became irritable, lethargic, and hypothermic. He fed only poorly and vomited. He also displayed tachypneic (rapid) breathing and neurologic posturing. At age 38 hours, he had a seizure.

Pertinent Findings: Respiratory alkalosis (increased pH, decreased CO_2 [hypocapnia]), increased ammonia, and decreased blood urea nitrogen were found. An amino acid screen revealed that argininosuccinate was increased >60-fold over baseline, and citrulline was increased 4-fold. Glutamine was elevated, and arginine (Arg) was decreased relative to normal.

Diagnosis: The patient has a urea cycle enzyme defect with neonatal onset.

Treatment: Hemodialysis was performed to remove ammonia. Sodium phenylacetate and sodium benzoate were administered to aid in excretion of waste nitrogen, as was Arg. Long-term treatment will include lifelong limitation of dietary protein; supplementation with essential amino acids; and administration of Arg, sodium phenylacetate, and sodium phenylbutyrate.

Prognosis: Survival into adulthood is possible. The degree of neurologic impairment is related to the degree and extent of the hyperammonemia.

CASE-RELATED QUESTIONS: Choose the ONE best answer

Q1. Based on the findings, which enzyme of the urea cycle is most likely to be deficient in this patient?

A. Arginase
B. Argininosuccinate lyase
C. Argininosuccinate synthetase
D. Carbamoyl phosphate synthetase I
E. Ornithine transcarbamoylase

Q2. Why is Arg supplementation helpful in this case?

Q3. In individuals with partial (milder) deficiency of urea cycle enzymes, the level of which one of the following would be expected to be decreased during periods of physiologic stress?

A. Alanine
B. Ammonia
C. Glutamine
D. Insulin
E. pH

CASE 10: CALF PAIN

Patient Presentation: A 19-year-old female is being evaluated for pain and swelling in her right calf.

Focused History: Ten days ago, the patient had her spleen removed following a bicycle accident in which she fractured her tibial eminence, necessitating immobilization of the right knee. She has had a good recovery from the surgery. She is no longer taking pain medication but has continued her oral contraceptives (OCP).

Pertinent Findings: Her right calf is reddish in color (erythematous) and warm to the touch. It is visibly swollen. The left calf is normal in appearance and is without pain. An ultrasound is ordered.

Diagnosis: She has a deep venous thrombosis (DVT). OCP are a risk factor for DVT, as are surgery and immobilization.

Treatment (Immediate): Heparin is administered for anticoagulation.

Prognosis: In the 10 years following a DVT, about one-third of individuals have a recurrence.

CASE-RELATED QUESTIONS: Choose the ONE best answer

Q1. Which one of the following would increase the risk of thrombosis?

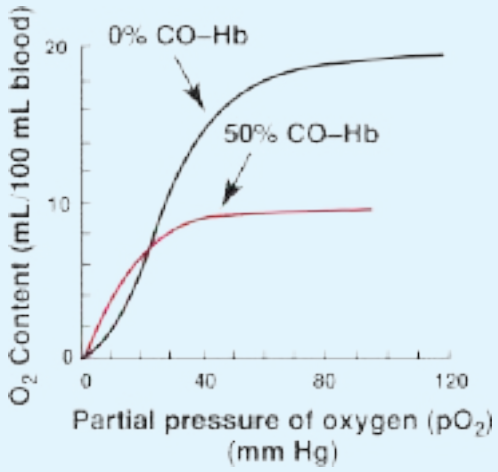
A. Excess production of antithrombin
B. Excess production of protein S
C. Expression of FV Leiden
D. Hypoprothrombinemia
E. von Willebrand disease

Q2. Compare and contrast the actions of heparin and warfarin.

IV. FOCUSED CASES: ANSWERS TO CASE-BASED QUESTIONS

CASE 1: Anemia with β-Thalassemia Minor

Q1. **Answer = B.** Transcription (synthesis of single-stranded RNA from the template strand of double-stranded DNA) requires the binding of proteins (trans-acting factors) to sequences on the DNA (cis-acting elements). Eukaryotic messenger RNA (mRNA) is monocistronic because it contains information from just one gene (cistron). The base sequence TAG (thymine adenine guanine) in the coding strand of DNA is U(uracil)AG in the mRNA. UAG is a signal that terminates translation (protein synthesis), not transcription. It is formation of the 5′-cap of eukaryotic mRNA that requires methylation (using S-adenosylmethionine), not 3′-end polyadenylation. Splicing is the spliceosome-mediated process by which introns are removed from eukaryotic mRNA and exons joined.

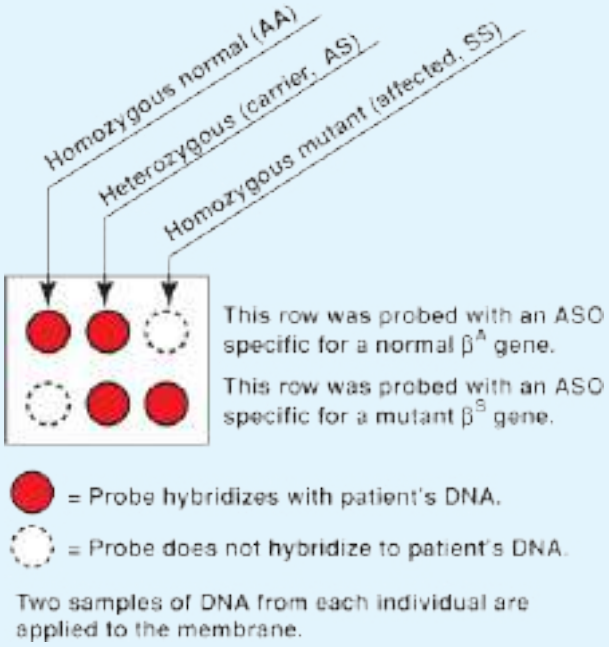


Q2. **Answer = C.** Carbon monoxide (CO) increases the affinity of hemoglobin (Hb)A for O_2, thereby decreasing the ability of HbA to offload O_2 in the tissues. CO stabilizes the R (relaxed) or oxygenated form and shifts the O_2 dissociation curve to the left, decreasing O_2 delivery (see figure at top right). The other choices decrease the affinity for O_2, stabilize the T (tense) or deoxygenated form, and cause a right shift in the curve.

Q3. HbA_2 and fetal Hb (HbF) do not contain β-globin. As β-globin production decreases, synthesis of HbA_2 ($\alpha_2\delta_2$) and HbF ($\alpha_2\gamma_2$) increases.

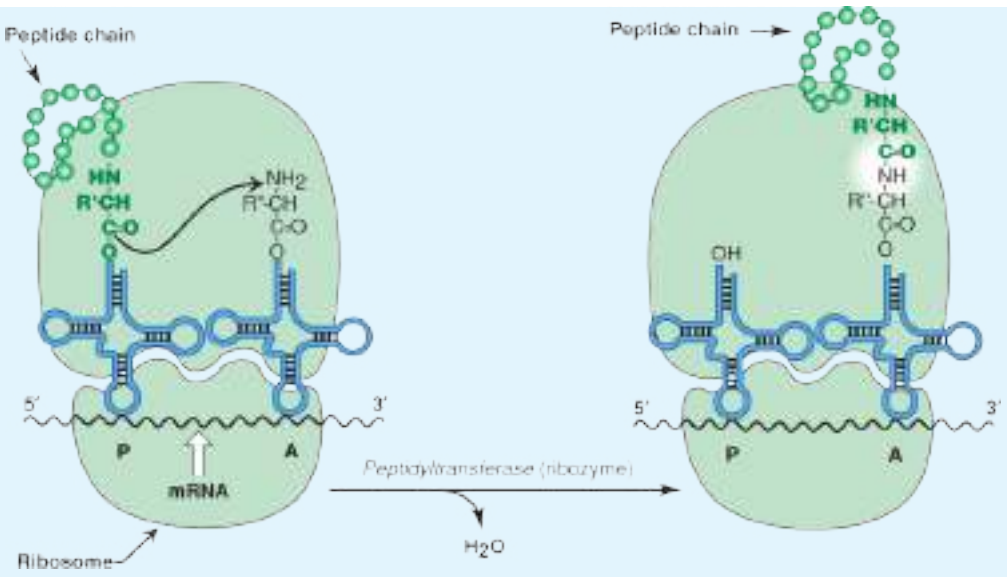
Q4. Sickle cell anemia is caused by a single point mutation (A→T) in the gene for β-globin that results in the replacement of glutamate by valine at the sixth amino acid position in the protein. Mutational analysis using allele-specific oligonucleotide (ASO) probes for that mutation (β^S) and for the normal sequence (β^A) is used in diagnosis (see figure at lower right). β-Thalassemia, in contrast, is caused

by hundreds of different mutations. Mutational analysis using ASO probes can assess common mutations, including point mutations, in at-risk populations (e.g., those of Mediterranean ancestry). β-Thalassemia is also know as Mediterranean anemia. However, less common mutations are often not included in the panel and can be detected only by DNA sequencing.



CASE 2: Skin Rash with Lyme Disease

Q1. **Answer = C.** Peptide-bond formation between the amino acid in the A site of the ribosome and the amino acid last added to the growing peptide in the P site is catalyzed by an RNA of the large ribosomal subunit. Any RNA with catalytic activity is referred to as a ribozyme (see figure on the next page). Formylated methionine is used to initiate prokaryotic translation. The charged initiating transfer RNA ($tRNA_i$) is the only tRNA that goes directly to the P site, leaving the A site available for the tRNA carrying the next amino acid of the protein being made. Eukaryotic translation is inhibited by the phosphorylation of initiation factor 2 (eIF-2). The Shine-Dalgarno sequence is found in prokaryotic messenger RNA (mRNA) and facilitates the interaction of the mRNA with the small ribosomal subunit. In eukaryotes, the cap-binding proteins perform that task.

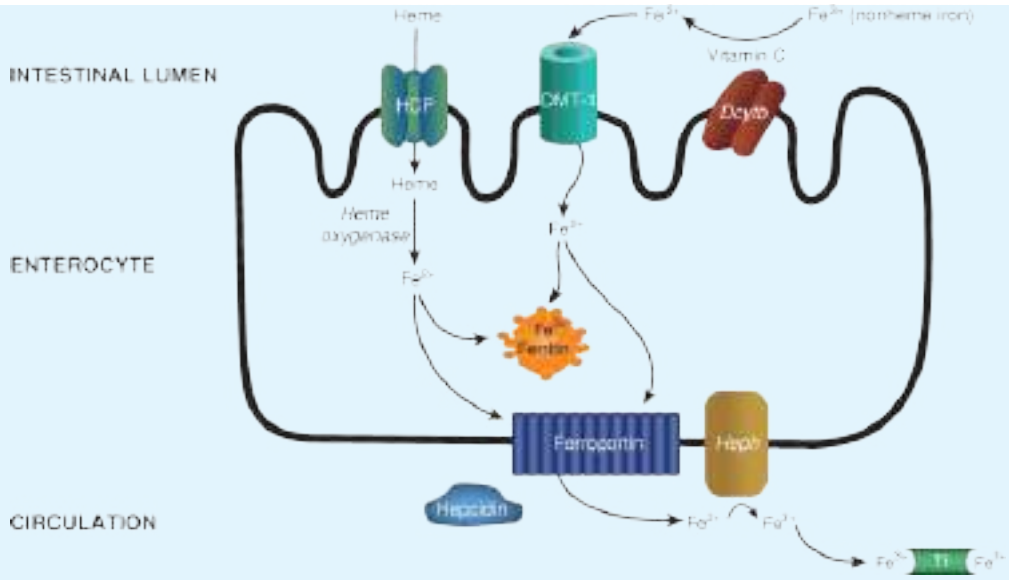


Q2. **Answer = B.** The enzyme-linked immunosorbent assay (ELISA) and western blot are used to analyze proteins. Each makes use of antibodies to detect and quantify the protein of interest. It is western blots that utilize electrophoresis. The polymerase chain reaction (PCR) is used to amplify DNA.

Q3. Antibiotics in the tetracycline family inhibit protein synthesis by binding to and blocking the A site of the small (30S) ribosomal subunit in prokaryotes. Tetracycline specifically interacts with the 16S ribosomal RNA (rRNA) component of the 30S subunit, inhibiting translation initiation. Eukaryotes do not contain 16S rRNA. Their small (40S) subunit contains 18S rRNA, which does not bind tetracycline.

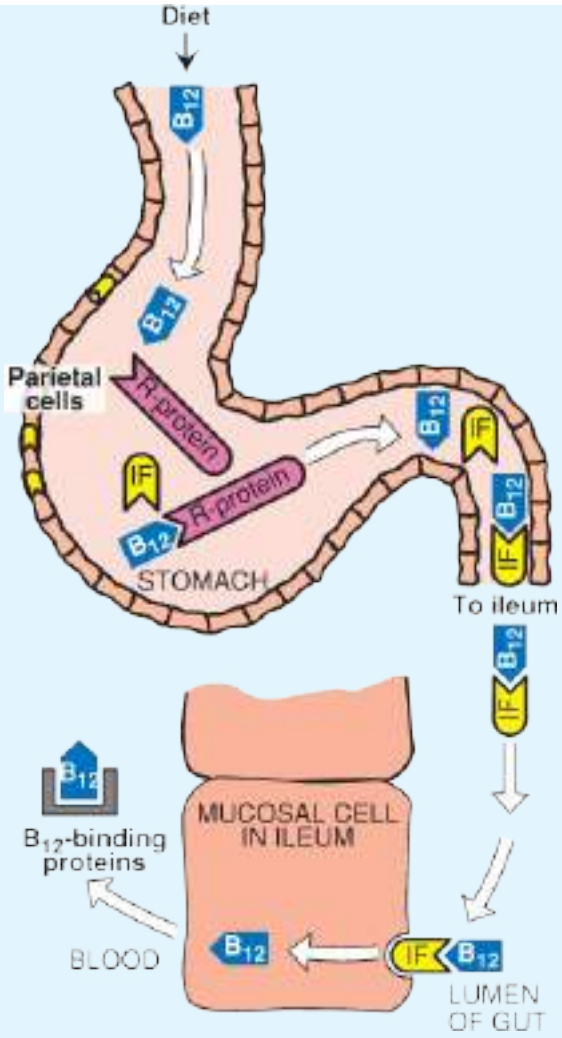
CASE 3: Blood on the Toothbrush with Vitamin C Deficiency

Q1. **Answer = C.** Vitamin C (ascorbic acid) functions as a coenzyme in the hydroxylation of proline and lysine in the synthesis of collagen, a fibrous protein of the extracellular matrix. Vitamin C is also the coenzyme for duodenal cytochrome b (Dcytb) that reduces dietary iron from the ferric (Fe^{3+}) to the ferrous (Fe^{2+}) form that is required for absorption via the divalent metal transporter (DMT) of enterocytes (see figure below). With a deficiency of vitamin C, uptake of dietary iron is impaired and results in a microcytic, hypochromic anemia. As a water-soluble vitamin, vitamin C is not stored. Cross-linking of collagen by lysyl oxidase requires copper, not vitamin C.



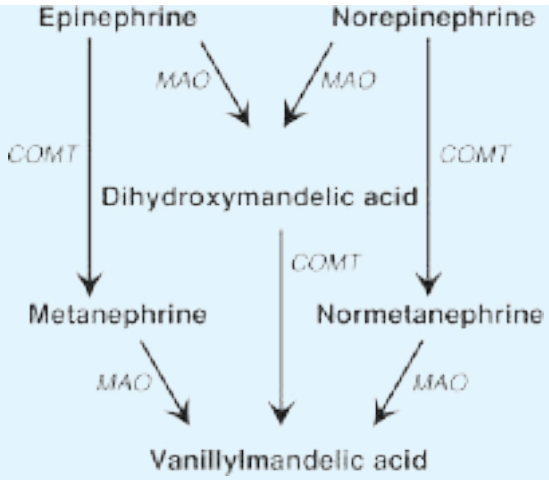
Q2. **Answer = A.** An inability to absorb vitamin B_{12} leads to pernicious anemia and is most commonly caused by decreased production of intrinsic factor (IF) by the parietal cells of the stomach (see figure at right). Vitamins D and A, in complex with their receptors, bind to DNA and alter gene expression. Thiamine (vitamin B_1) is a coenzyme in the oxidative decarboxylation of pyruvate and α-ketoglutarate and, therefore, is important in energy metabolism in most cells. Methotrexate inhibits dihydrofolate reductase, the enzyme that reduces dihydrofolate to tetrahydrofolate (THF), the functional coenzyme form of folate. This results in decreased availability of THF. It is pyridoxine (vitamin B_6) as pyridoxal phosphate that is the coenzyme for most reactions involving amino acids. [Note: Tetrahydrobiopterin is required by aromatic amino acid hydroxylases and nitric oxide synthases.]

Q3. Nutritional anemias are characterized by either increased red blood cell (RBC) size (folate and B_{12} deficiencies) or decreased RBC size (iron and vitamin C deficiencies). In hemolytic anemias, such as is seen in glucose 6-phosphate dehydrogenase and pyruvate kinase deficiencies and in sickle cell anemia, RBC size typically is normal, but RBC number is decreased.



CASE 4: Rapid Heart Rate, Headache, and Sweating with a Pheochromocytoma

Q1. **Answer = D.** Degradation of both epinephrine and norepinephrine (NE) involves methylation by catechol-O-methyltransferase (COMT) that produces normetanephrine from NE and metanephrine from epinephrine (see figure at right). Both of these products are deaminated to vanillylmandelic acid by monoamine oxidase (MAO). The substrate for the synthesis of the catecholamines is tyrosine, which gets hydroxylated to 3,4-dihydroxyphenylalanine (DOPA) by tetrahydrobiopterin-requiring tyrosine hydroxylase. DOPA is converted to dopamine by a pyridoxal phosphate–requiring decarboxylase. [Note: Most carboxylases require biotin.] NE is converted to epinephrine by methylation, and S-adenosylmethionine provides the methyl group.

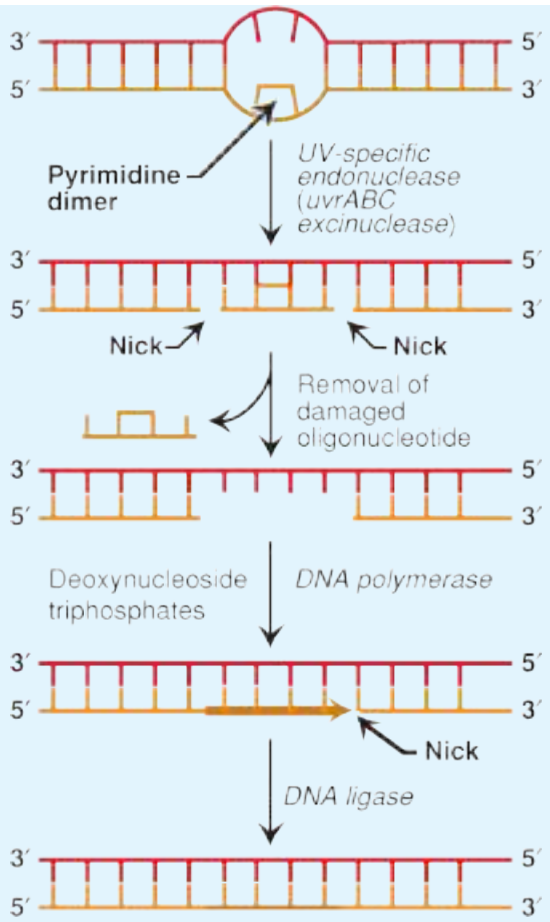


Q2. **Answer = A.** NE released from the sympathetic nervous system functions as a neurotransmitter that acts on postsynaptic neurons and causes, for example, increased heart rate. It is also released from the adrenal medulla and, along with epinephrine, functions as a counterregulatory hormone that results in mobilization of stored fuels (e.g., glucose and triacylglycerols). These actions are mediated by the binding of NE to adrenergic receptors, which are G protein–coupled receptors of the plasma membrane, and not to nuclear receptors like those of steroid hormones or membrane tyrosine kinase receptors like that of insulin.

Q3. Septic shock is vasodilatory hypotension (low blood pressure caused by blood vessel dilation) resulting from the production of large amounts of nitric oxide by inducible nitric oxide synthase in response to infection. NE bound to receptors on smooth muscle cells causes vasoconstriction and, thus, raises blood pressure.

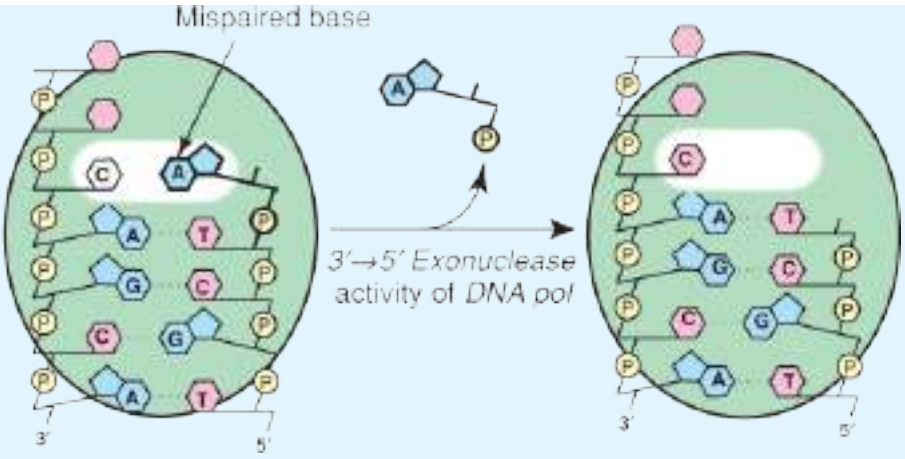
CASE 5: Sun Sensitivity with Xeroderma Pigmentosum

Q1. **Answer = D.** Pyrimidine dimers are the characteristic DNA lesions caused by ultraviolet (UV) radiation. Their repair involves the excision of an oligonucleotide containing the dimer and replacement of that oligonucleotide, a process known as nucleotide excision repair (NER). (See figure at right for a representation of the process in prokaryotes.) DNA repair systems are found in prokaryotes and eukaryotes. Nothing is error free, but the homologous recombination (HR) method of double-strand break repair is much less prone to error than is the nonhomologous end joining (NHEJ) method because any DNA that was lost is replaced. Mismatch-base repair (MMR) involves identification and repair of the newly synthesized (daughter) strand. In prokaryotes, the extent of strand methylation is used to discriminate between the strands. Base excision repair (BER), the mechanism by which uracil is removed from DNA, utilizes a glycosylase to remove the base, creating an apyrimidinic or apurinic (AP) site. The sugar-phosphate is then removed by the actions of an endo- and exonuclease.



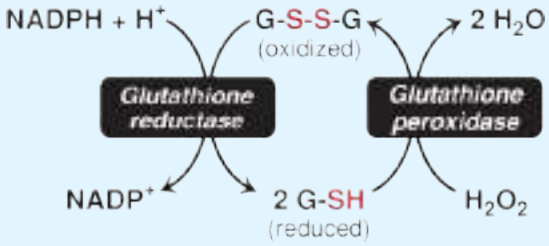
Q2. **Answer = A.** All replication requires an RNA primer because DNA polymerases (pol) cannot initiate DNA synthesis. The chromatin of eukaryotes gets decondensed (relaxed) for replication. Relaxation can be accomplished, for example, by acetylation via histone acetyltransferases. Prokaryotes have more than one DNA pol. For example, pol III extends the RNA primer with DNA, and pol I removes the primer and replaces it with DNA. Replication is initiated at specific locations (one in prokaryotes, many in eukaryotes) that are recognized by proteins (e.g., DnaA in prokaryotes). Deoxynucleoside monophosphates (dNMP) are joined by a phosphodiester bond that links the 3′-hydroxyl group of the last dNMP (deoxyribonucleoside monophosphate) added with the 5′-phosphate group of the incoming nucleotide, thereby forming a 3′→5′-phosphodiester bond as pyrophosphate is released.

Q3. Proofreading occurs during replication in the S (synthesis of DNA) phase of the cell cycle and involves the 3′→5′ exonuclease activity possessed by some DNA pol (see figure below). Because repair can occur independently of replication, it can be performed outside of the S phase.

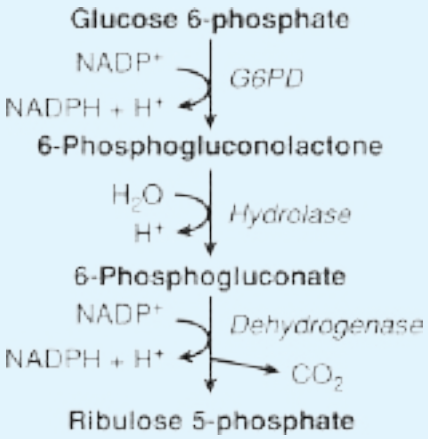


CASE 6: Dark Urine and Yellow Sclerae with Glucose 6-Phosphate Dehydrogenase Deficiency

Q1. **Answer = B.** Glutathione in its reduced form (G-SH) is an important antioxidant. The selenium-containing enzyme glutathione peroxidase reduces hydrogen peroxide (H_2O_2, a reactive oxygen species) to water as glutathionine is oxidized (G-S-S-G). Reduced nicotinamide adenine dinucleotide phosphate (NADPH)-requiring glutathionine reductase regenerates G-SH from G-S-S-G (see Figure A). The NADPH is supplied by the oxidative reactions of the pentose phosphate pathway (see Figure B), which is regulated by the availability of NADPH at the glucose 6-phosphate dehydrogenase (G6PD)-catalyzed step (the first step). Deficiency of G6PD occurs in all cells, but the effects are seen in red blood cells where the pentose phosphate pathway is the only source of NADPH. The pathway involves two irreversible oxidative reactions, each of which generates NADPH. The NADPH is used in reductive processes such as fatty acid synthesis (not oxidation) as well as steroid hormone and cholesterol synthesis.



A.

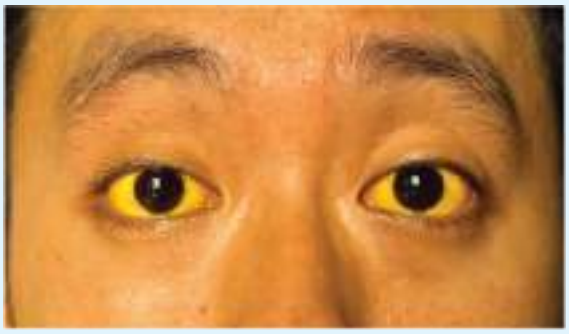


B.

Q2. **Answer = A.** Jaundice (icterus) refers to the yellow color of the skin, nail beds, and sclerae that results from bilirubin deposition when the bilirubin level in the blood is elevated (hyperbilirubinemia;

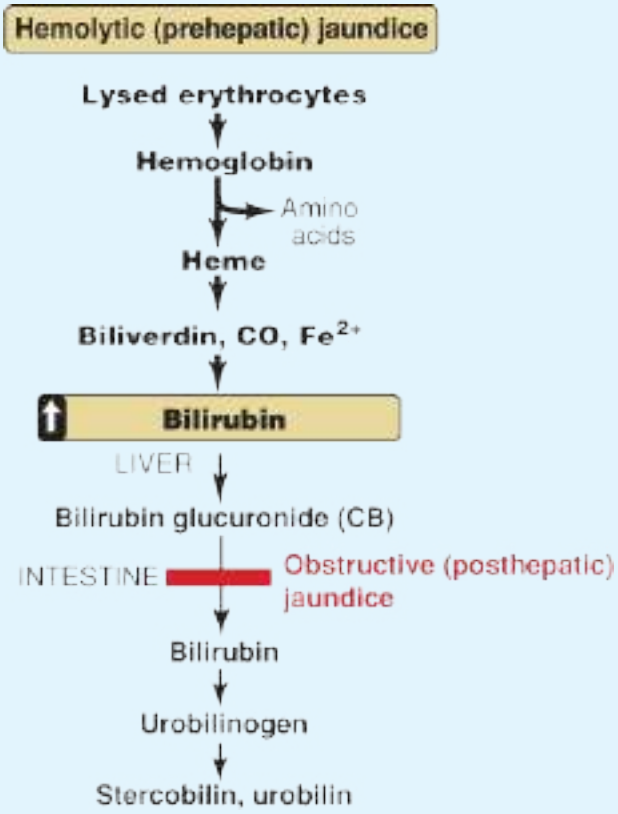
see Image C). Bilirubin has low solubility in aqueous solutions, and its solubility is increased by conjugation with uridine diphosphate–glucuronic acid in the liver, forming bilirubin diglucuronide or conjugated bilirubin (CB). In hemolytic conditions, such as G6PD deficiency, both CB and unconjugated bilirubin (UCB) are increased, but it is UCB that is found in the blood. CB is sent into the intestine. Phototherapy is used to treat unconjugated hyperbilirubinemia because it converts bilirubin to isomeric forms that are more water soluble. Bilirubin is the product of heme degradation in cells of the mononuclear phagocyte system, particularly in the liver and the spleen. The porphyrias are pathologies of heme synthesis and, therefore, are not characterized by hyperbilirubinemia.

C.



Q3. With hemolysis, more bilirubin is produced and conjugated. Conjugated bilirubin is sent to the intestine where it is converted to urobilinogen, some of which is reabsorbed, enters the portal blood, and travels to the kidney. Because the source of urinary urobilinogen is intestinal urobilinogen, urinary urobilinogen will be low in obstructive jaundice because intestinal urobilinogen will be low as a result of the obstruction of the common bile duct (see Figure D).

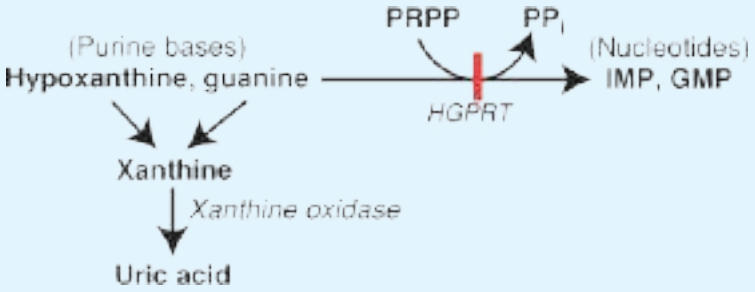
D.



CASE 7: Joint Pain with Gout

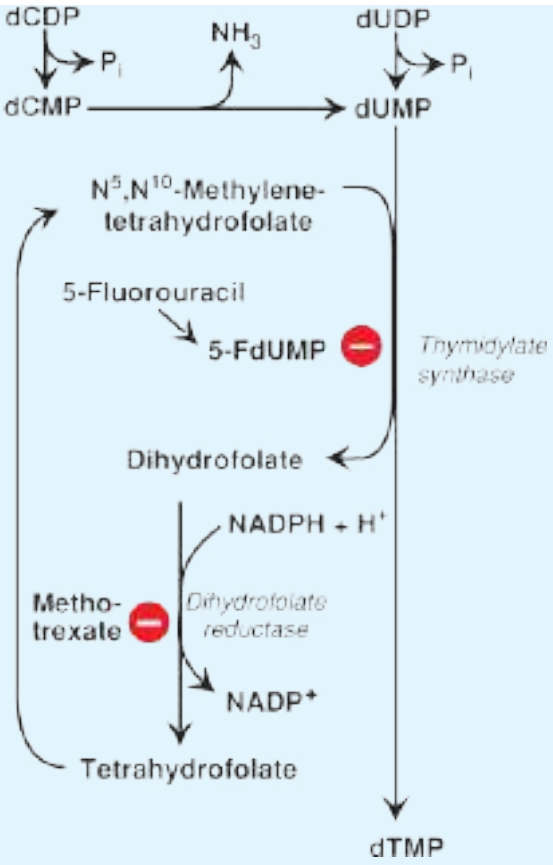
Q1. **Answer = F.** Salvage of the purine bases hypoxanthine and guanine to the purine nucleotides inosine monophosphate (IMP) and guanosine monophosphate (GMP) by hypoxanthine-guanine

phosphoribosyltransferase (HGPRT) requires 5-phosphoribosyl-1-pyrophosphate (PRPP) as the source of the ribose 1-phosphate. Salvage decreases the amount of substrate available for degradation to uric acid. Therefore, a deficiency in salvage results in hyperuricemia (see figure at right). Noncompetitive inhibitors such as oxypurinol have no effect on the Michaelis constant (K_m) but decrease the apparent maximal velocity (V_{max}). Colchicine is an anti-inflammatory drug. It has no effect on the enzymes of purine synthesis or degradation. Glutamine (not glutamate) is a nitrogen source for purine ring synthesis. In purine nucleotide synthesis, the purine ring system is constructed on the ribose 5-phosphate provided by PRPP. Allopurinol and its metabolite, oxypurinol, inhibit xanthine oxidase of purine degradation. The amidotransferase is the regulated enzyme of purine synthesis. Its activity is decreased by purine nucleotides and increased by PRPP.



Q2. **Answer = B.** Methotrexate inhibits dihydrofolate reductase, decreasing the availability of N^5,N^{10}-methylene tetrahydrofolate needed for synthesis of deoxythymidine monophosphate (dTMP) from deoxyuridine monophosphate (dUMP) by thymidylate synthase (see figure at right). Carbamoyl phosphate synthetase (CPS) II is the regulated enzymic activity of pyrimidine biosynthesis in humans. CPS I is an enzyme of the urea cycle. Orotic aciduria is a rare pathology of pyrimidine synthesis caused by a deficiency in one or both enzymic activities of bifunctional uridine monophosphate synthase. Pyrimidine nucleotide synthesis, like purine synthesis and salvage, requires PRPP.

Q3. Increased activity of PRPP synthetase results in increased synthesis of PRPP. This results in an increase in purine nucleotide synthesis beyond need. The excess purine nucleotides get degraded to uric acid, thereby causing hyperuricemia.

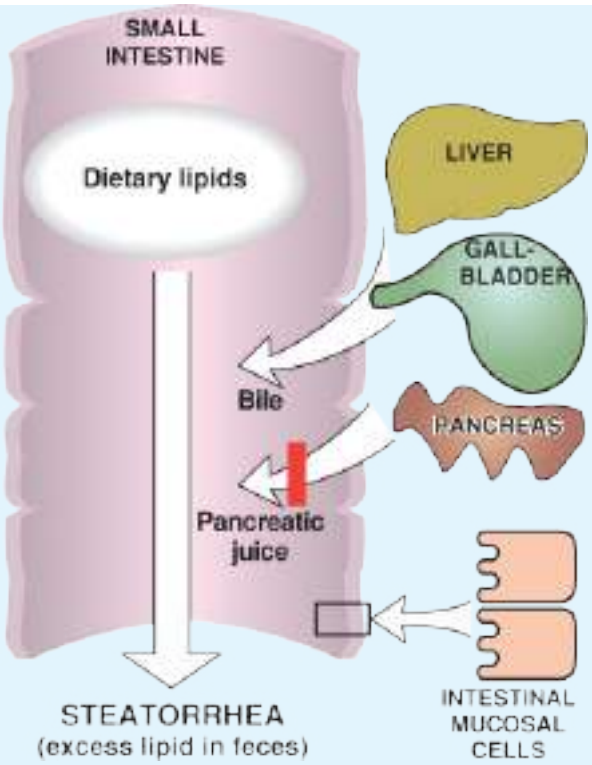


CASE 8: No Bowel Movement with Cystic Fibrosis

Q1. **Answer = A.** The clinical manifestations of cystic fibrosis (CF) are the consequence of chloride retention with increased water absorption that causes mucus on an epithelial surface to be excessively thick and sticky. The result is pulmonary and gastrointestinal problems such as respiratory infection and impaired exocrine and endocrine pancreatic functions (pancreatic insufficiency). Impaired endocrine pancreatic function can result in diabetes with associated hyperglycemia. Some mutations do result in increased degradation of the CF transmembrane conductance regulator (CFTR) protein, but degradation is initiated by tagging the protein with ubiquitin. Frameshift mutations alter the reading frame through the addition or deletion of nucleotides by a number not divisible by three. Because the ΔF509 mutation is caused by the loss of three nucleotides that code for phenylalanine (F) at position 509 in the CFTR protein, it is not a frameshift mutation.

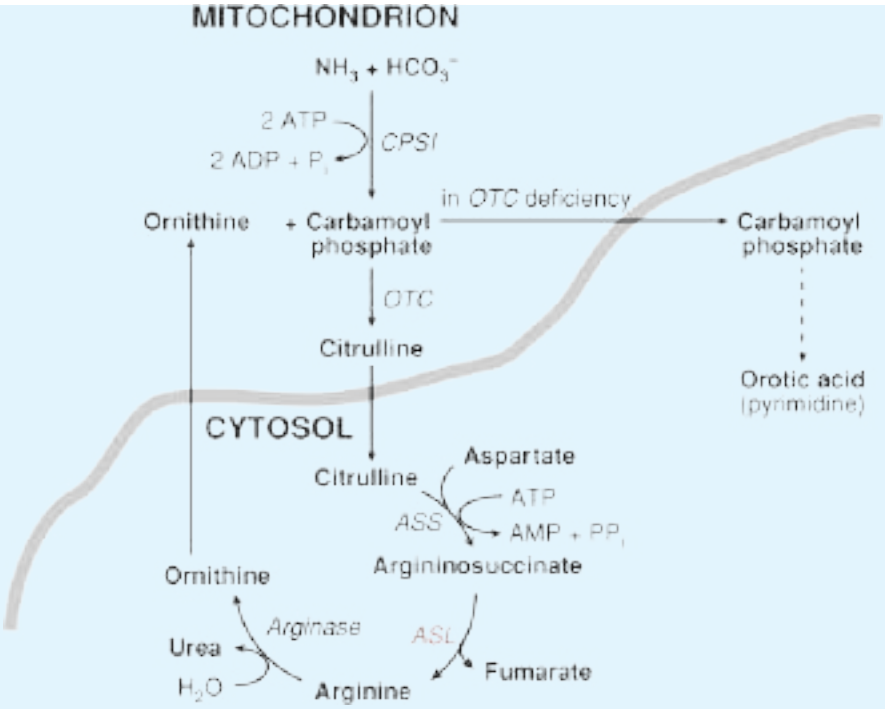
Q2. **Answer = A.** Targeting of proteins destined to function as components of the plasma membrane is an example of cotranslational targeting. It involves the initiation of translation on cytosolic ribosomes; recognition of the amino (N)-terminal signal sequence in the protein by the signal recognition particle; movement of the protein-synthesizing complex to the outer face of the membrane of the endoplasmic reticulum (ER); and continuation of protein synthesis, such that the protein is threaded into the lumen of the ER and packaged into vesicles that travel to and through the Golgi and eventually fuse with the plasma membrane. The N-terminal signal sequence is removed by a peptidase in the lumen of the ER. Mannose 6-phosphate is the signal that cotranslationally targets proteins to the matrix of the lysosome where they function as acid hydrolases.

Q3. The pancreatic insufficiency seen in some patients with CF results in a decreased ability to digest food, and digestion is required for absorption. Dietary fats move through the intestine and are excreted in the stool (see figure at right), which is foul-smelling and bulky and may float. Patients are at risk for malnutrition and deficiencies in fat-soluble vitamins. Oral supplementation of pancreatic enzymes is the treatment.



CASE 9: Hyperammonemia with a Urea Cycle Defect

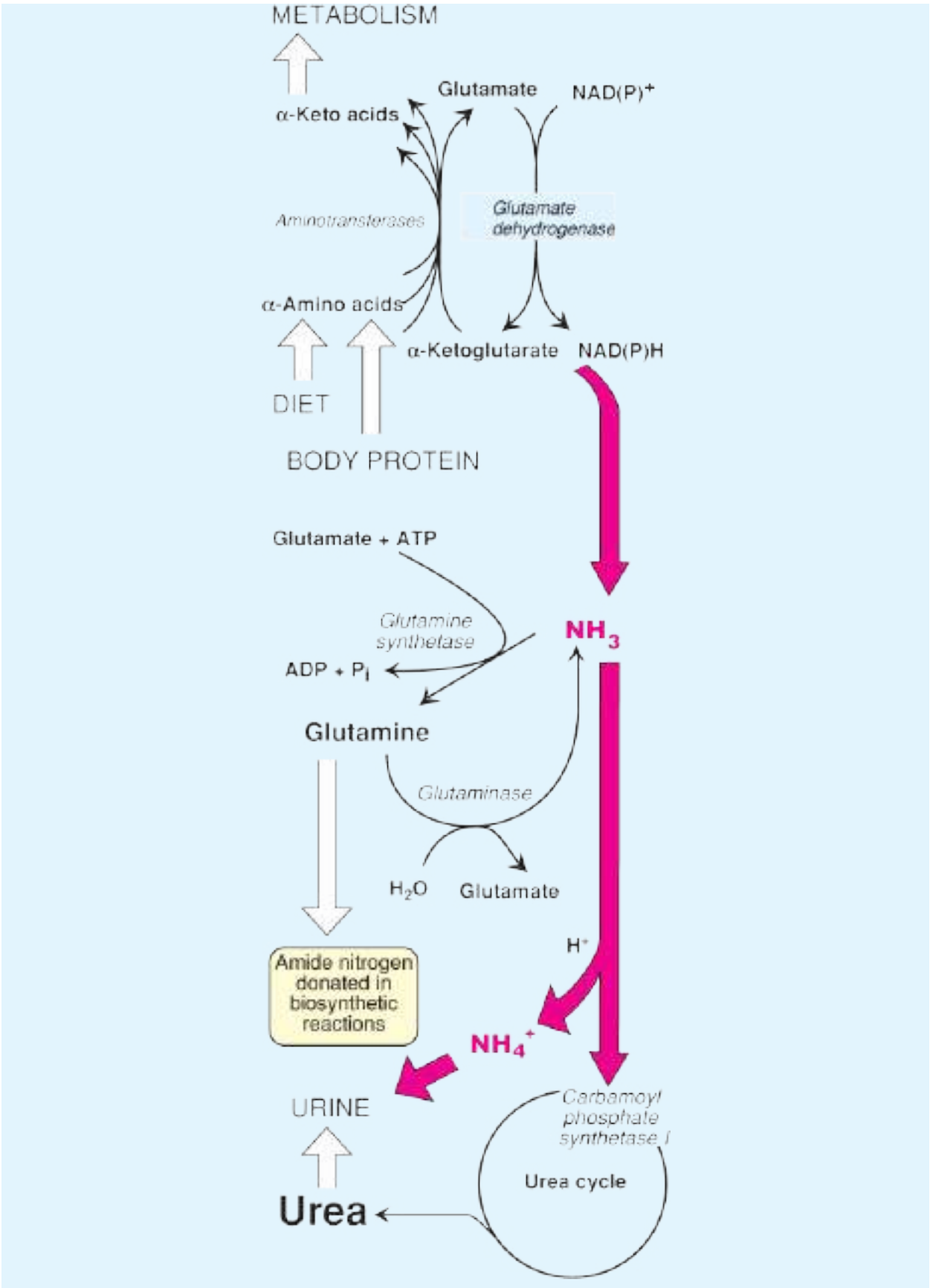
Q1. **Answer = B.** Argininosuccinate lyase (ASL) cleaves argininosuccinate to arginine (Arg) and fumarate. The increase in argininosuccinate and citrulline and the decrease in Arg indicate a deficiency in ASL (see figure below). With arginase deficiency, Arg would be increased, not decreased. Additionally, with arginase deficiency, the hyperammonemia would be less severe because two nitrogens are excreted. Deficiency of argininosuccinate synthetase (ASS) would also cause an increase in citrulline, but argininosuccinate would be low to absent. Deficiency of carbamoyl phosphate synthetase (CPS) I is characterized by low levels of Arg and citrulline. Deficiency of ornithine transcarbamoylase (OTC), the only X-linked enzyme of the urea cycle, would result in low levels of Arg and citrulline and elevated levels of urinary orotic acid. [Note: The orotic acid is elevated because the carbamoyl phosphate (CP) substrate of OTC is being used in the cytosol as a substrate for pyrimidine synthesis.]

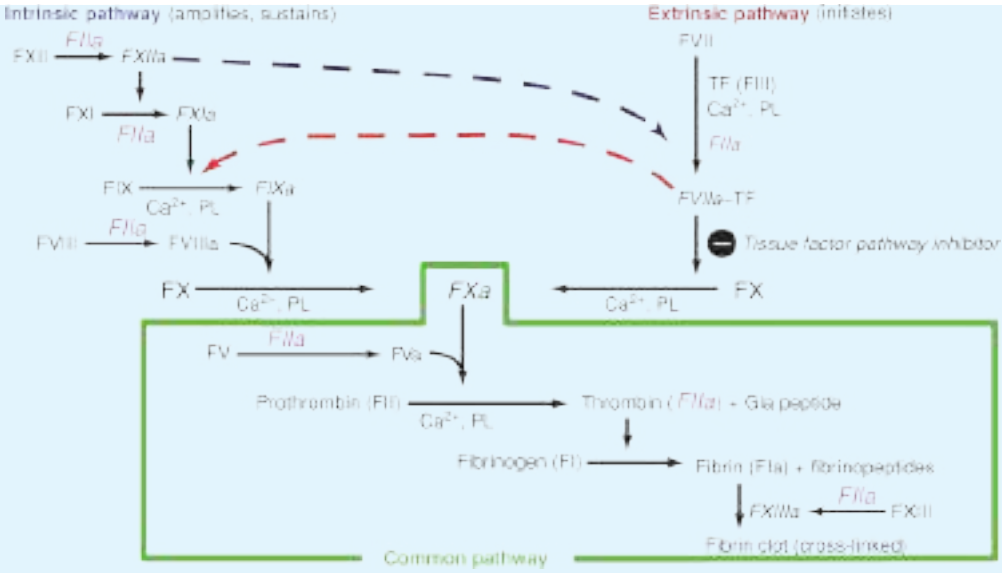


Q2. Arg supplementation is helpful because the Arg will be hydrolyzed to urea + ornithine by arginase. The ornithine will be combined with CP to form citrulline (see figure above). With ASL (and ASS) deficiency, citrulline accumulates and is excreted, thereby carrying waste nitrogen out of the body.

Q3. **Answer = D.** In individuals with milder (partial) deficiencies in the enzymes of the urea cycle, hyperammonemia may be triggered by physiologic stress (e.g., an illness or prolonged fasting) that decreases the insulin/counterregulatory hormone ratio. [Note: The degree of the hyperammonemia is usually less severe than that seen in the neonatal onset forms.] The shift in the ratio results, in part, in skeletal muscle proteolysis, and the amino acids that are released get degraded. Degradation involves transamination by pyridoxal phosphate–requiring aminotransferases that generate the α-keto acid derivative of the amino acid + glutamate. The glutamate undergoes oxidative deamination to α-ketoglutarate and ammonia (NH_3) by glutamate dehydrogenase (GDH; see figure at right). [Note: GDH is unusual in that it uses both nicotinamide adenine dinucleotide [NAD] and nicotinamide adenine dinucleotide phosphate [NADP] as coenzymes.]

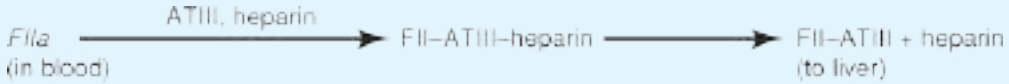
The NH_3, which is toxic, can be transported to the liver as glutamine (Gln) and alanine (Ala). The Gln is generated by the amination of glutamate by ATP-requiring glutamine synthetase. In the liver, the enzyme glutaminase removes the NH_3, which can be converted to urea by the urea cycle or excreted as ammonium (NH_4^+) (see figure at right). Gln, then, is a nontoxic vehicle of NH_3 transport in the blood. Ala is generated in skeletal muscle from the catabolism of the branched-chain amino acids (BCAA). In the liver, Ala is transaminated by alanine transaminase (ALT) to pyruvate (used in gluconeogenesis) and glutamate. Thus, Ala carries nitrogen to the liver for conversion to urea (see figure below). Therefore, defects in the urea cycle would result in an elevation in NH_3, Gln, and Ala. The elevated NH_3 drives respiration, and the hyperventilation causes a rise in pH (respiratory alkalosis). [Note: Hyperammonemia is toxic to the nervous system. Although the exact mechanisms are not completely understood, it is known that the metabolism of large amounts of NH_3 to Gln [in the astrocytes of the brain] results in osmotic effects that cause the brain to swell. Additionally, the rise in Gln decreases the availability of glutamate, an excitatory neurotransmitter.]





CASE 10: Swollen, Painful Calf with Deep Venous Thrombosis

Q1. **Answer = C.** Factor V Leiden is a mutant form of Factor V that is resistant to proteolysis by the activated protein C complex. Decreased ability to degrade Factor V allows continued production of activated thrombin and leads to an increased risk of clot formation or thrombophilia. Antithrombin (ATIII) and protein S are proteins of anticoagulation. Increased, not decreased, production of prothrombin would result in thrombophilia. Deficiency of von Willebrand factor causes a coagulopathy or a deficiency in clotting through effects as a carrier of Factor VIII and also on platelet aggregation.



Q2. Heparin, a glycosaminoglycan, functions as an anticoagulant. It activates antithrombin (sometimes called ATIII), enabling antithrombin to inhibit thrombin and Factor Xa. Antithrombin functions by cleaving thrombin and Factor Xa, rendering them inactive. Warfarin, a synthetic analog of vitamin K, inhibits vitamin K, the coenzyme that is required for the γ-carboxylation of glutamate residues to γ-carboxyglutamate (Gla) residues in Factors II, VII, IX and X (see figures below).

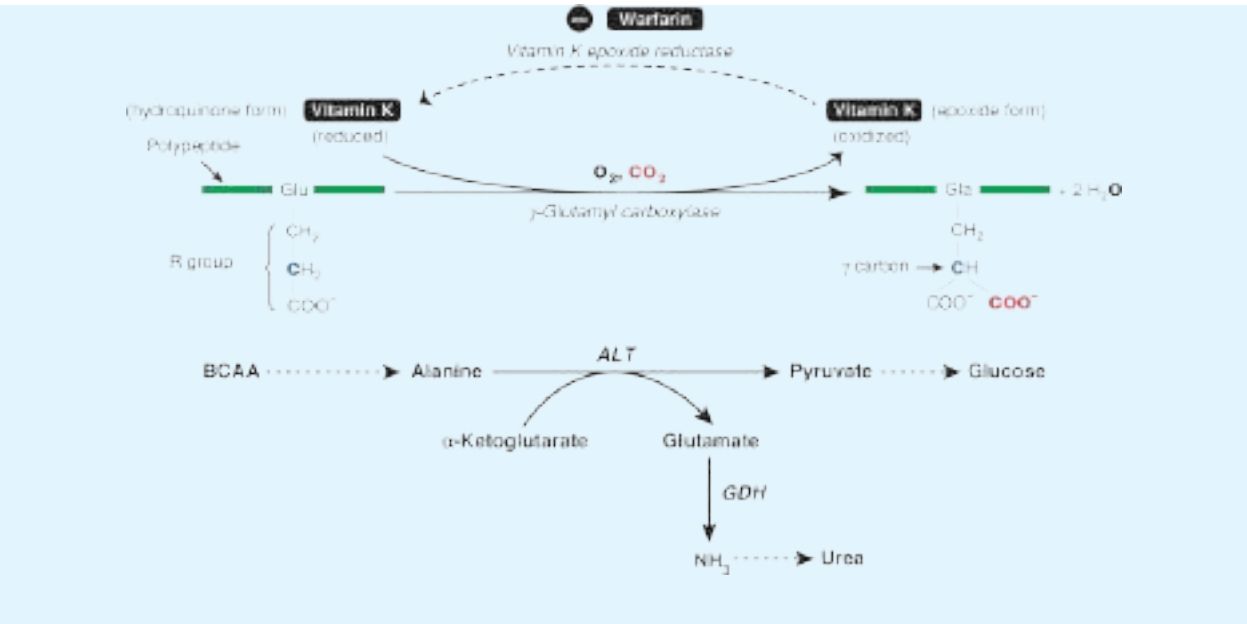


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Figure Sources

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Figure 3.20. Photo From Fizkes/Shutterstock.com.

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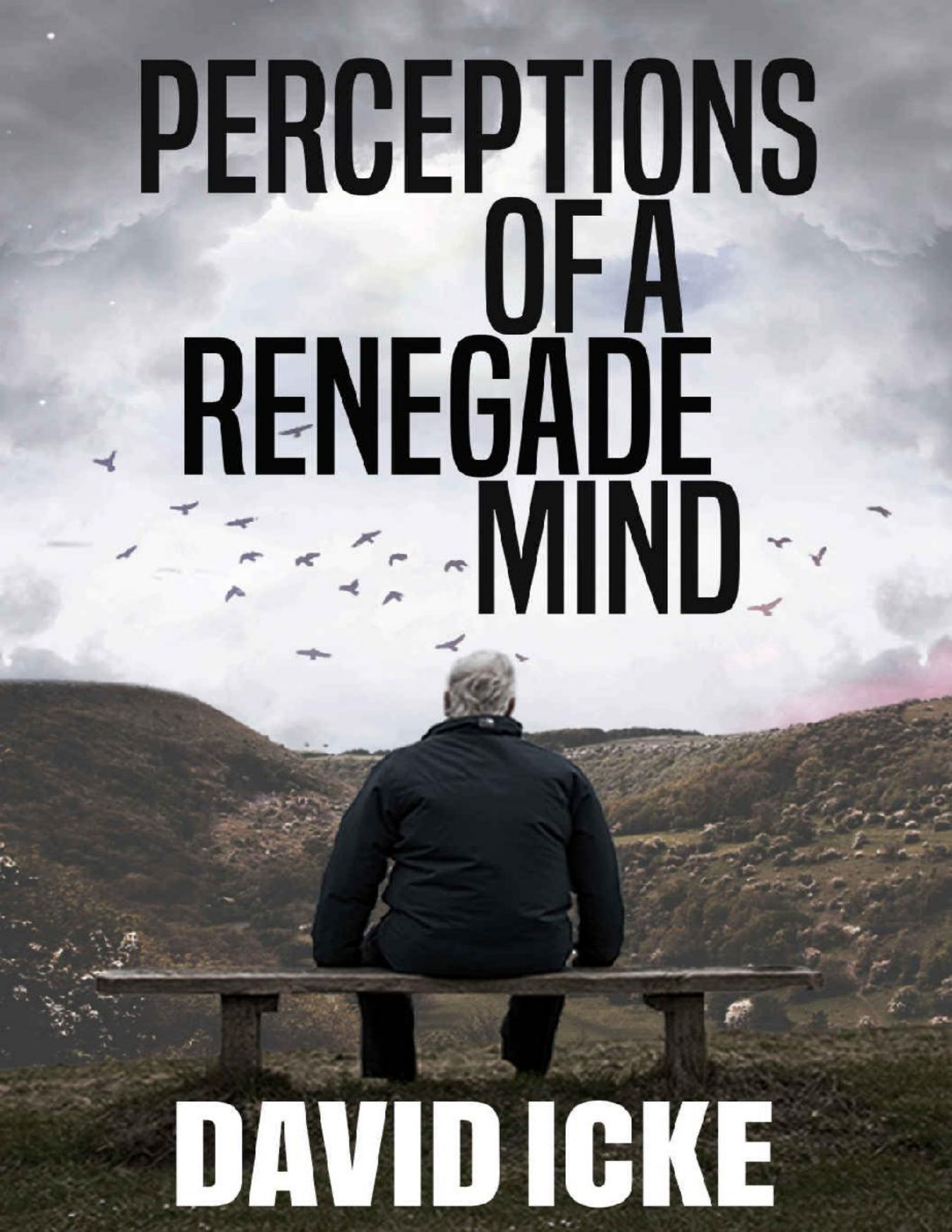
PERCEPTIONS OF A RENEGADE MIND



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PERCEPTIONS OF A RENEGADE MIND

DAVID ICKE

Dedication:

To *Freeeeeedom!*

Renegade:
Adjective
‘Having rejected tradition: Unconventional.’
Merriam-Webster Dictionary

Acquiescence to tyranny is the death of the spirit

You may be 38 years old, as I happen to be. And one day, some great opportunity stands before you and calls you to stand up for some great principle, some great issue, some great cause. And you refuse to do it because you are afraid … You refuse to do it because you want to live longer … You're afraid that you will lose your job, or you are afraid that you will be criticised or that you will lose your popularity, or you're afraid that somebody will stab you, or shoot at you or bomb your house; so you refuse to take the stand.

Well, you may go on and live until you are 90, but you're just as dead at 38 as you would be at 90. And the cessation of breathing in your life is but the belated announcement of an earlier death of the spirit.

Martin Luther King

How the few control the many and always have – the many do whatever they're told

'Forward, the Light Brigade!'
Was there a man dismayed?
Not though the soldier knew
Someone had blundered.
Theirs not to make reply,
Theirs not to reason why,
Theirs but to do and die.
Into the valley of Death
Rode the six hundred.

Cannon to right of them,
Cannon to left of them,
Cannon in front of them
Volleyed and thundered;
Stormed at with shot and shell,
Boldly they rode and well,
Into the jaws of Death,
Into the mouth of hell
Rode the six hundred

Alfred Lord Tennyson (1809-1892)

The mist is lifting slowly
I can see the way ahead
And I've left behind the empty streets
That once inspired my life
And the strength of the emotion
Is like thunder in the air
'Cos the promise that we made each other
Haunts me to the end

The secret of your beauty
And the mystery of your soul
I've been searching for in everyone I meet
And the times I've been mistaken
It's impossible to say
And the grass is growing
Underneath our feet

The words that I remember
From my childhood still are true
That there's none so blind
As those who will not see
And to those who lack the courage
And say it's dangerous to try
Well they just don't know
That love eternal will not be denied

I know you're out there somewhere
Somewhere, somewhere
I know you're out there somewhere
Somewhere you can hear my voice

I know I'll find you somehow
Somehow, somehow
I know I'll find you somehow
And somehow I'll return again to you

The Moody Blues

Are you a gutless wonder - or a Renegade Mind?

Monuments put from pen to paper,
Turns me into a gutless wonder,
And if you tolerate this,
Then your children will be next.
Gravity keeps my head down,
Or is it maybe shame ...

Manic Street Preachers

Rise like lions after slumber
In unvanquishable number.
Shake your chains to earth like dew
Which in sleep have fallen on you.
Ye are many – they are few.

Percy Shelley

Contents

CHAPTER ONE

I'm thinking' – Oh, but *are* you?

Think for yourself and let others enjoy the privilege of doing so too
Voltaire

French-born philosopher, mathematician and scientist René Descartes became famous for his statement in Latin in the 17th century which translates into English as: 'I think, therefore I am.'

On the face of it that is true. Thought reflects perception and perception leads to both behaviour and self-identity. In that sense 'we' are what we think. But who or what is doing the thinking and is thinking the only route to perception? Clearly, as we shall see, 'we' are not always the source of 'our' perception, indeed with regard to humanity as a whole this is rarely the case; and thinking is far from the only means of perception. Thought is the village idiot compared with other expressions of consciousness that we all have the potential to access and tap into. This has to be true when we *are* those other expressions of consciousness which are infinite in nature. We have forgotten this, or, more to the point, been manipulated to forget.

These are not just the esoteric musings of the navel. The whole foundation of human control and oppression is control of perception. Once perception is hijacked then so is behaviour which is dictated by perception. Collective perception becomes collective behaviour and collective behaviour is what we call human society. Perception is all and those behind human control know that which is why perception is the target 24/7 of the psychopathic manipulators that I call the Global Cult. They know that if they dictate perception they will dictate behaviour and collectively dictate

the nature of human society. They are further aware that perception is formed from information received and if they control the circulation of information they will to a vast extent direct human behaviour. Censorship of information and opinion has become globally Nazi-like in recent years and never more blatantly than since the illusory 'virus pandemic' was triggered out of China in 2019 and across the world in 2020. Why have billions submitted to house arrest and accepted fascistic societies in a way they would have never believed possible? Those controlling the information spewing from government, mainstream media and Silicon Valley (all controlled by the same Global Cult networks) told them they were in danger from a 'deadly virus' and only by submitting to house arrest and conceding their most basic of freedoms could they and their families be protected. This monumental and provable lie became the *perception* of the billions and therefore the *behaviour* of the billions. In those few words you have the whole structure and modus operandi of human control. Fear is a perception – **F**alse **E**motion **A**ppearing **R**eal – and fear is the currency of control. In short … get them by the balls (or give them the impression that you have) and their hearts and minds will follow. Nothing grips the dangly bits and freezes the rear-end more comprehensively than fear.

World number 1

There are two 'worlds' in what appears to be one 'world' and the prime difference between them is knowledge. First we have the mass of human society in which the population is maintained in coldly-calculated ignorance through control of information and the 'education' (indoctrination) system. That's all you really need to control to enslave billions in a perceptual delusion in which what are perceived to be *their* thoughts and opinions are ever-repeated mantras that the system has been downloading all their lives through 'education', media, science, medicine, politics and academia in which the personnel and advocates are themselves overwhelmingly the perceptual products of the same repetition. Teachers and academics in general are processed by the same programming machine as everyone else, but unlike the great majority they never leave the 'education' program. It gripped them as students and continues to grip them as programmers of subsequent generations of students. The programmed become the programmers – the programmed programmers. The same can largely be

said for scientists, doctors and politicians and not least because as the American writer Upton Sinclair said: 'It is difficult to get a man to understand something when his salary depends upon his not understanding it.' If your career and income depend on thinking the way the system demands then you will – bar a few free-minded exceptions – concede your mind to the Perceptual Mainframe that I call the Postage Stamp Consensus. This is a tiny band of perceived knowledge and possibility 'taught' (downloaded) in the schools and universities, pounded out by the mainstream media and on which all government policy is founded. Try thinking, and especially speaking and acting, outside of the 'box' of consensus and see what that does for your career in the Mainstream Everything which bullies, harasses, intimidates and ridicules the population into compliance. Here we have the simple structure which enslaves most of humanity in a perceptual prison cell for an entire lifetime and I'll go deeper into this process shortly. Most of what humanity is taught as fact is nothing more than programmed belief. American science fiction author Frank Herbert was right when he said: 'Belief can be manipulated. Only knowledge is dangerous.' In the 'Covid' age belief is promoted and knowledge is censored. It was always so, but never to the extreme of today.

World number 2

A 'number 2' is slang for 'doing a poo' and how appropriate that is when this other 'world' is doing just that on humanity every minute of every day. World number 2 is a global network of secret societies and semi-secret groups dictating the direction of society via governments, corporations and authorities of every kind. I have spent more than 30 years uncovering and exposing this network that I call the Global Cult and knowing its agenda is what has made my books so accurate in predicting current and past events. Secret societies are secret for a reason. They want to keep their hoarded knowledge to themselves and their chosen initiates and to hide it from the population which they seek through ignorance to control and subdue. The whole foundation of the division between World 1 and World 2 is *knowledge*. What number 1 knows number 2 must not. Knowledge they have worked so hard to keep secret includes (a) the agenda to enslave humanity in a centrally-controlled global dictatorship, and (b) the nature of reality and life itself. The latter (b) must be suppressed to allow the former

(a) to prevail as I shall be explaining. The way the Cult manipulates and interacts with the population can be likened to a spider's web. The 'spider' sits at the centre in the shadows and imposes its will through the web with each strand represented in World number 2 by a secret society, satanic or semi-secret group, and in World number 1 – the world of the seen – by governments, agencies of government, law enforcement, corporations, the banking system, media conglomerates and Silicon Valley (Fig 1 overleaf). The spider and the web connect and coordinate all these organisations to pursue the same global outcome while the population sees them as individual entities working randomly and independently. At the level of the web governments *are* the banking system *are* the corporations *are* the media *are* Silicon Valley *are* the World Health Organization working from their inner cores as one unit. Apparently unconnected countries, corporations, institutions, organisations and people are on the *same team* pursuing the same global outcome. Strands in the web immediately around the spider are the most secretive and exclusive secret societies and their membership is emphatically restricted to the Cult inner-circle emerging through the generations from particular bloodlines for reasons I will come to. At the core of the core you would get them in a single room. That's how many people are dictating the direction of human society and its transformation through the 'Covid' hoax and other means. As the web expands out from the spider we meet the secret societies that many people will be aware of – the Freemasons, Knights Templar, Knights of Malta, Opus Dei, the inner sanctum of the Jesuit Order, and such like. Note how many are connected to the Church of Rome and there is a reason for that. The Roman Church was established as a revamp, a rebranding, of the relocated 'Church' of Babylon and the Cult imposing global tyranny today can be tracked back to Babylon and Sumer in what is now Iraq.

Figure 1: The global web through which the few control the many. (Image Neil Hague.)

Inner levels of the web operate in the unseen away from the public eye and then we have what I call the cusp organisations located at the point where the hidden meets the seen. They include a series of satellite organisations answering to a secret society founded in London in the late 19th century called the Round Table and among them are the Royal Institute of International Affairs (UK, founded in 1920); Council on Foreign Relations (US, 1921); Bilderberg Group (worldwide, 1954); Trilateral Commission (US/worldwide, 1972); and the Club of Rome (worldwide, 1968) which was created to exploit environmental concerns to justify the centralisation of global power to 'save the planet'. The Club of Rome instigated with others the human-caused climate change hoax which has led to all the 'green new deals' demanding that very centralisation of control. Cusp organisations, which include endless 'think tanks' all over the world, are designed to coordinate a single global policy between political and business leaders, intelligence personnel, media organisations and anyone who can influence the direction of policy in their own sphere of operation. Major players and regular attenders will know what is happening – or some of it – while others come and go and are kept overwhelmingly in the dark about the big picture. I refer to these cusp groupings as semi-secret in that they can be publicly identified, but what goes on at the inner-core is kept very much 'in house' even from most of their members and participants through a fiercely-imposed system of compartmentalisation. Only let them know what they need to know to serve your interests and no more. The

structure of secret societies serves as a perfect example of this principle. Most Freemasons never get higher than the bottom three levels of 'degree' (degree of knowledge) when there are 33 official degrees of the Scottish Rite. Initiates only qualify for the next higher 'compartment' or degree if those at that level choose to allow them. Knowledge can be carefully assigned only to those considered 'safe'. I went to my local Freemason's lodge a few years ago when they were having an 'open day' to show how cuddly they were and when I chatted to some of them I was astonished at how little the rank and file knew even about the most ubiquitous symbols they use. The mushroom technique – keep them in the dark and feed them bullshit – applies to most people in the web as well as the population as a whole. Sub-divisions of the web mirror in theme and structure transnational corporations which have a headquarters somewhere in the world dictating to all their subsidiaries in different countries. Subsidiaries operate in their methodology and branding to the same centrally-dictated plan and policy in pursuit of particular ends. The Cult web functions in the same way. Each country has its own web as a subsidiary of the global one. They consist of networks of secret societies, semi-secret groups and bloodline families and their job is to impose the will of the spider and the global web in their particular country. Subsidiary networks control and manipulate the national political system, finance, corporations, media, medicine, etc. to ensure that they follow the globally-dictated Cult agenda. These networks were the means through which the 'Covid' hoax could be played out with almost every country responding in the same way.

The 'Yessir' pyramid

Compartmentalisation is the key to understanding how a tiny few can dictate the lives of billions when combined with a top-down sequence of imposition and acquiescence. The inner core of the Cult sits at the peak of the pyramidal hierarchy of human society (Fig 2 overleaf). It imposes its will – its agenda for the world – on the level immediately below which acquiesces to that imposition. This level then imposes the Cult will on the level below them which acquiesces and imposes on the next level. Very quickly we meet levels in the hierarchy that have no idea there even is a Cult, but the sequence of imposition and acquiescence continues down the pyramid in just the same way. 'I don't know why we are doing this but the

order came from “on-high” and so we better just do it.’ Alfred Lord Tennyson said of the cannon fodder levels in his poem *The Charge of the Light Brigade*: ‘Theirs not to reason why; theirs but to do and die.’ The next line says that ‘into the valley of death rode the six hundred’ and they died because they obeyed without question what their perceived ‘superiors’ told them to do. In the same way the population capitulated to ‘Covid’. The whole hierarchical pyramid functions like this to allow the very few to direct the enormous many. Eventually imposition-acquiescence-imposition-acquiescence comes down to the mass of the population at the foot of the pyramid. If they acquiesce to those levels of the hierarchy imposing on them (governments/law enforcement/doctors/media) a circuit is completed between the population and the handful of super-psychopaths in the Cult inner core at the top of the pyramid. Without a circuit-breaking refusal to obey, the sequence of imposition and acquiescence allows a staggeringly few people to impose their will upon the entirety of humankind. We are looking at the very sequence that has subjugated billions since the start of 2020. Our freedom has not been taken from us. Humanity has given it away. Fascists do not impose fascism because there are not enough of them. Fascism is imposed by the population acquiescing to fascism. Put another way allowing their perceptions to be programmed to the extent that leads to the population giving their freedom away by giving their perceptions – their mind – away. If this circuit is not broken by humanity ceasing to cooperate with their own enslavement then nothing can change. For that to happen people have to critically think and see through the lies and window dressing and then summon the backbone to act upon what they see. The Cult spends its days working to stop either happening and its methodology is systematic and highly detailed, but it can be overcome and that is what this book is all about.

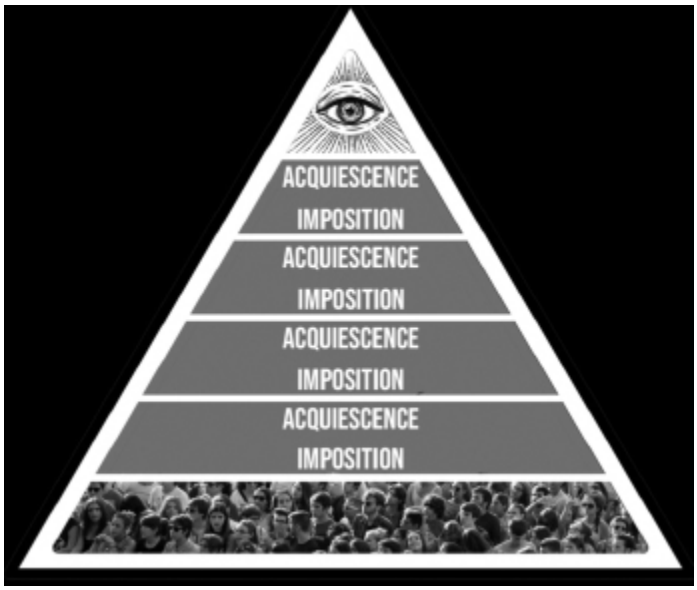

Figure 2: The simple sequence of imposition and compliance that allows a handful of people at the peak of the pyramid to dictate the lives of billions.

The Life Program

Okay, back to world number 1 or the world of the 'masses'. Observe the process of what we call 'life' and it is a perceptual download from cradle to grave. The Cult has created a global structure in which perception can be programmed and the program continually topped-up with what appears to be constant confirmation that the program is indeed true reality. The important word here is 'appears'. This is the structure, the fly-trap, the Postage Stamp Consensus or Perceptual Mainframe, which represents that incredibly narrow band of perceived possibility delivered by the 'education' system, mainstream media, science and medicine. From the earliest age the download begins with parents who have themselves succumbed to the very programming their children are about to go through. Most parents don't do this out of malevolence and mostly it is quite the opposite. They do what they believe is best for their children and that is what the program has told them is best. Within three or four years comes the major transition from parental programming to full-blown state (Cult) programming in school, college and university where perceptually-programmed teachers and academics pass on their programming to the next generations. Teachers who resist are soon marginalised and their careers ended while children who resist are called a problem child for whom Ritalin may need to be

prescribed. A few years after entering the ‘world’ children are under the control of authority figures representing the state telling them when they have to be there, when they can leave and when they can speak, eat, even go to the toilet. This is calculated preparation for a lifetime of obeying authority in all its forms. Reflex-action fear of authority is instilled by authority from the start. Children soon learn the carrot and stick consequences of obeying or defying authority which is underpinned daily for the rest of their life. Fortunately I daydreamed through this crap and never obeyed authority simply because it told me to. This approach to my alleged ‘betters’ continues to this day. There can be consequences of pursuing open-minded freedom in a world of closed-minded conformity. I spent a lot of time in school corridors after being ejected from the classroom for not taking some of it seriously and now I spend a lot of time being ejected from Facebook, YouTube and Twitter. But I can tell you that being true to yourself and not compromising your self-respect is far more exhilarating than bowing to authority for authority’s sake. You don’t have to be a sheep to the shepherd (authority) and the sheep dog (fear of not obeying authority).

The perceptual download continues throughout the formative years in school, college and university while script-reading ‘teachers’, ‘academics’ ‘scientists’, ‘doctors’ and ‘journalists’ insist that ongoing generations must be as programmed as they are. Accept the program or you will not pass your ‘exams’ which confirm your ‘degree’ of programming. It is tragic to think that many parents pressure their offspring to work hard at school to download the program and qualify for the next stage at college and university. The late, great, American comedian George Carlin said: ‘Here’s a bumper sticker I’d like to see: We are proud parents of a child who has resisted his teachers’ attempts to break his spirit and bend him to the will of his corporate masters.’ Well, the best of luck finding many of those, George. Then comes the moment to leave the formal programming years in academia and enter the ‘adult’ world of work. There you meet others in your chosen or prescribed arena who went through the same Postage Stamp Consensus program before you did. There is therefore overwhelming agreement between almost everyone on the basic foundations of Postage Stamp reality and the rejection, even contempt, of the few who have a mind of their own and are prepared to use it. This has two major effects. Firstly, the consensus confirms to the programmed that their download is really

how things are. I mean, everyone knows that, right? Secondly, the arrogance and ignorance of Postage Stamp adherents ensure that anyone questioning the program will have unpleasant consequences for seeking their own truth and not picking their perceptions from the shelf marked: 'Things you must believe without question and if you don't you're a dangerous lunatic conspiracy theorist and a harebrained nutter'.

Every government, agency and corporation is founded on the same Postage Stamp prison cell and you can see why so many people believe the same thing while calling it their own 'opinion'. Fusion of governments and corporations in pursuit of the same agenda was the definition of fascism described by Italian dictator Benito Mussolini. The pressure to conform to perceptual norms downloaded for a lifetime is incessant and infiltrates society right down to family groups that become censors and condemners of their own 'black sheep' for not, ironically, being sheep. We have seen an explosion of that in the 'Covid' era. Cult-owned global media unleashes its propaganda all day every day in support of the Postage Stamp and targets with abuse and ridicule anyone in the public eye who won't bend their mind to the will of the tyranny. Any response to this is denied (certainly in my case). They don't want to give a platform to expose official lies. Cult-owned-and-created Internet giants like Facebook, Google, YouTube and Twitter delete you for having an unapproved opinion. Facebook boasts that its AI censors delete 97-percent of 'hate speech' before anyone even reports it. Much of that 'hate speech' will simply be an opinion that Facebook and its masters don't want people to see. Such perceptual oppression is widely known as fascism. Even Facebook executive Benny Thomas, a 'CEO Global Planning Lead', said in comments secretly recorded by investigative journalism operation Project Veritas that Facebook is 'too powerful' and should be broken up:

> I mean, no king in history has been the ruler of two billion people, but Mark Zuckerberg is … And he's 36. That's too much for a 36-year-old ... You should not have power over two billion people. I just think that's wrong.

Thomas said Facebook-owned platforms like Instagram, Oculus, and WhatsApp needed to be separate companies. 'It's too much power when they're all one together'. That's the way the Cult likes it, however. We have

an executive of a Cult organisation in Benny Thomas that doesn't know there is a Cult such is the compartmentalisation. Thomas said that Facebook and Google 'are no longer companies, they're countries'. Actually they are more powerful than countries on the basis that if you control information you control perception and control human society.

I love my oppressor

Another expression of this psychological trickery is for those who realise they are being pressured into compliance to eventually convince themselves to believe the official narratives to protect their self-respect from accepting the truth that they have succumbed to meek and subservient compliance. Such people become some of the most vehement defenders of the system. You can see them everywhere screaming abuse at those who prefer to think for themselves and by doing so reminding the compliers of their own capitulation to conformity. 'You are talking dangerous nonsense you Covidiot!!' Are you trying to convince me or yourself? It is a potent form of Stockholm syndrome which is defined as: 'A psychological condition that occurs when a victim of abuse identifies and attaches, or bonds, positively with their abuser.' An example is hostages bonding and even 'falling in love' with their kidnappers. The syndrome has been observed in domestic violence, abused children, concentration camp inmates, prisoners of war and many and various Satanic cults. These are some traits of Stockholm syndrome listed at goodtherapy.org:

- Positive regard towards perpetrators of abuse or captor [see 'Covid'].
- Failure to cooperate with police and other government authorities when it comes to holding perpetrators of abuse or kidnapping accountable [or in the case of 'Covid' cooperating with the police to enforce and defend their captors' demands].
- Little or no effort to escape [see 'Covid'].
- Belief in the goodness of the perpetrators or kidnappers [see 'Covid'].
- Appeasement of captors. This is a manipulative strategy for maintaining one's safety. As victims get rewarded – perhaps with less

abuse or even with life itself – their appeasing behaviours are reinforced [see 'Covid'].

• Learned helplessness. This can be akin to 'if you can't beat 'em, join 'em'. As the victims fail to escape the abuse or captivity, they may start giving up and soon realize it's just easier for everyone if they acquiesce all their power to their captors [see 'Covid'].

• Feelings of pity toward the abusers, believing they are actually victims themselves. Because of this, victims may go on a crusade or mission to 'save' [protect] their abuser [see the venom unleashed on those challenging the official 'Covid' narrative].

• Unwillingness to learn to detach from their perpetrators and heal. In essence, victims may tend to be less loyal to themselves than to their abuser [*definitely* see 'Covid'].

Ponder on those traits and compare them with the behaviour of great swathes of the global population who have defended governments and authorities which have spent every minute destroying their lives and livelihoods and those of their children and grandchildren since early 2020 with fascistic lockdowns, house arrest and employment deletion to 'protect' them from a 'deadly virus' that their abusers' perceptually created to bring about this very outcome. We are looking at mass Stockholm syndrome. All those that agree to concede their freedom will believe those perceptions are originating in their own independent 'mind' when in fact by conceding their reality to Stockholm syndrome they have by definition conceded any independence of mind. Listen to the 'opinions' of the acquiescing masses in this 'Covid' era and what gushes forth is the repetition of the official version of everything delivered unprocessed, unfiltered and unquestioned. The whole programming dynamic works this way. I must be free because I'm told that I am and so I think that I am.

You can see what I mean with the chapter theme of 'I'm thinking – Oh, but *are* you?' The great majority are not thinking, let alone for themselves. They are repeating what authority has told them to believe which allows them to be controlled. Weaving through this mentality is the fear that the 'conspiracy theorists' are right and this again explains the often hysterical abuse that ensues when you dare to contest the official narrative of anything. Denial is the mechanism of hiding from yourself what you don't

want to be true. Telling people what they want to hear is easy, but it's an infinitely greater challenge to tell them what they would rather not be happening. One is akin to pushing against an open door while the other is met with vehement resistance no matter what the scale of evidence. I don't want it to be true so I'll convince myself that it's not. Examples are everywhere from the denial that a partner is cheating despite all the signs to the reflex-action rejection of any idea that world events in which country after country act in exactly the same way are centrally coordinated. To accept the latter is to accept that a force of unspeakable evil is working to destroy your life and the lives of your children with nothing too horrific to achieve that end. Who the heck wants that to be true? But if we don't face reality the end is duly achieved and the consequences are far worse and ongoing than breaking through the walls of denial today with the courage to make a stand against tyranny.

Connect the dots – but how?

A crucial aspect of perceptual programming is to portray a world in which everything is random and almost nothing is connected to anything else. Randomness cannot be coordinated by its very nature and once you perceive events as random the idea they could be connected is waved away as the rantings of the tinfoil-hat brigade. You can't plan and coordinate random you idiot! No, you can't, but you can hide the coldly-calculated and long-planned behind the *illusion* of randomness. A foundation manifestation of the Renegade Mind is to scan reality for patterns that connect the apparently random and turn pixels and dots into pictures. This is the way I work and have done so for more than 30 years. You look for similarities in people, modus operandi and desired outcomes and slowly, then ever quicker, the picture forms. For instance: There would seem to be no connection between the 'Covid pandemic' hoax and the human-caused global-warming hoax and yet they are masks (appropriately) on the same face seeking the same outcome. Those pushing the global warming myth through the Club of Rome and other Cult agencies are driving the lies about 'Covid' – Bill Gates is an obvious one, but they are endless. Why would the same people be involved in both when they are clearly not connected? Oh, but they *are*. Common themes with personnel are matched by common goals. The 'solutions' to both 'problems' are centralisation of global power

to impose the will of the few on the many to 'save' humanity from 'Covid' and save the planet from an 'existential threat' (we need 'zero Covid' and 'zero carbon emissions'). These, in turn, connect with the 'dot' of globalisation which was coined to describe the centralisation of global power in every area of life through incessant political and corporate expansion, trading blocks and superstates like the European Union. If you are the few and you want to control the many you have to centralise power and decision-making. The more you centralise power the more power the few at the centre will have over the many; and the more that power is centralised the more power those at the centre have to centralise even quicker. The momentum of centralisation gets faster and faster which is exactly the process we have witnessed. In this way the hoaxed 'pandemic' and the fakery of human-caused global warming serve the interests of globalisation and the seizure of global power in the hands of the Cult inner-circle which is behind 'Covid', 'climate change' *and* globalisation. At this point random 'dots' become a clear and obvious picture or pattern.

Klaus Schwab, the classic Bond villain who founded the Cult's Gates-funded World Economic Forum, published a book in 2020, *The Great Reset*, in which he used the 'problem' of 'Covid' to justify a total transformation of human society to 'save' humanity from 'climate change'. Schwab said: 'The pandemic represents a rare but narrow window of opportunity to reflect, reimagine, and reset our world.' What he didn't mention is that the Cult he serves is behind both hoaxes as I show in my book *The Answer*. He and the Cult don't have to reimagine the world. They know precisely what they want and that's why they destroyed human society with 'Covid' to 'build back better' in their grand design. Their job is not to imagine, but to get humanity to imagine and agree with their plans while believing it's all random. It must be pure coincidence that 'The Great Reset' has long been the Cult's code name for the global imposition of fascism and replaced previous code-names of the 'New World Order' used by Cult frontmen like Father George Bush and the 'New Order of the Ages' which emerged from Freemasonry and much older secret societies. New Order of the Ages appears on the reverse of the Great Seal of the United States as 'Novus ordo seclorum' underneath the Cult symbol used since way back of the pyramid and all seeing-eye (Fig 3). The pyramid is the hierarchy of human control headed by the illuminated eye that symbolises the force behind the Cult which I will expose in later chapters. The term

‘Annuit Coeptis’ translates as ‘He favours our undertaking’. We are told the ‘He’ is the Christian god, but ‘He’ is not as I will be explaining.

Figure 3: The all-seeing eye of the Cult ‘god’ on the Freemason-designed Great Seal of the United States and also on the dollar bill.

Having you on

Two major Cult techniques of perceptual manipulation that relate to all this are what I have called since the 1990s Problem-Reaction-Solution (PRS) and the Totalitarian Tiptoe (TT). They can be uncovered by the inquiring mind with a simple question: Who benefits? The answer usually identifies the perpetrators of a given action or happening through the concept of ‘he who most benefits from a crime is the one most likely to have committed it’. The Latin ‘Cue bono?’ – Who benefits? – is widely attributed to the Roman orator and statesman Marcus Tullius Cicero. No wonder it goes back so far when the concept has been relevant to human behaviour since history was recorded. Problem-Reaction-Solution is the technique used to manipulate us every day by covertly creating a problem (or the illusion of one) and offering the solution to the problem (or the illusion of one). In the first phase you create the problem and blame someone or something else for why it has happened. This may relate to a financial collapse, terrorist attack, war, global warming or pandemic, anything in fact that will allow you to impose the ‘solution’ to change society in the way you desire at that time. The ‘problem’ doesn’t have to be real. PRS is manipulation of perception and all you need is the population to believe the problem is real. Human-

caused global warming and the 'Covid pandemic' only have to be *perceived* to be real for the population to accept the 'solutions' of authority. I refer to this technique as NO-Problem-Reaction-Solution. Billions did not meekly accept house arrest from early 2020 because there was a real deadly 'Covid pandemic' but because they perceived – believed – that to be the case. The antidote to Problem-Reaction-Solution is to ask who benefits from the proposed solution. Invariably it will be anyone who wants to justify more control through deletion of freedom and centralisation of power and decision-making.

The two world wars were Problem-Reaction-Solutions that transformed and realigned global society. Both were manipulated into being by the Cult as I have detailed in books since the mid-1990s. They dramatically centralised global power, especially World War Two, which led to the United Nations and other global bodies thanks to the overt and covert manipulations of the Rockefeller family and other Cult bloodlines like the Rothschilds. The UN is a stalking horse for full-blown world government that I will come to shortly. The land on which the UN building stands in New York was donated by the Rockefellers and the same Cult family was behind Big Pharma scalpel and drug 'medicine' and the creation of the World Health Organization as part of the UN. They have been stalwarts of the eugenics movement and funded Hitler's race-purity expert' Ernst Rudin. The human-caused global warming hoax has been orchestrated by the Club of Rome through the UN which is manufacturing both the 'problem' through its Intergovernmental Panel on Climate Change and imposing the 'solution' through its Agenda 21 and Agenda 2030 which demand the total centralisation of global power to 'save the world' from a climate hoax the United Nations is itself perpetrating. What a small world the Cult can be seen to be particularly among the inner circles. The bedfellow of Problem-Reaction-Solution is the Totalitarian Tiptoe which became the Totalitarian Sprint in 2020. The technique is fashioned to hide the carefully-coordinated behind the cover of apparently random events. You start the sequence at 'A' and you know you are heading for 'Z'. You don't want people to know that and each step on the journey is presented as a random happening while all the steps strung together lead in the same direction. The speed may have quickened dramatically in recent times, but you can still see the incremental approach of the Tiptoe in the case of 'Covid' as each new imposition takes us deeper into fascism. Tell people they have to do this or that to get back to

'normal', then this and this and this. With each new demand adding to the ones that went before the population's freedom is deleted until it disappears. The spider wraps its web around the flies more comprehensively with each new diktat. I'll highlight this in more detail when I get to the 'Covid' hoax and how it has been pulled off. Another prime example of the Totalitarian Tiptoe is how the Cult-created European Union went from a 'free-trade zone' to a centralised bureaucratic dictatorship through the Tiptoe of incremental centralisation of power until nations became mere administrative units for Cult-owned dark suits in Brussels.

The antidote to ignorance is knowledge which the Cult seeks vehemently to deny us, but despite the systematic censorship to that end the Renegade Mind can overcome this by vociferously seeking out the facts no matter the impediments put in the way. There is also a method of thinking and perceiving – *knowing* – that doesn't even need names, dates, place-type facts to identify the patterns that reveal the story. I'll get to that in the final chapter. All you need to know about the manipulation of human society and to what end is still out there – *at the time of writing* – in the form of books, videos and websites for those that really want to breach the walls of programmed perception. To access this knowledge requires the abandonment of the mainstream media as a source of information in the awareness that this is owned and controlled by the Cult and therefore promotes mass perceptions that suit the Cult. Mainstream media lies all day, every day. That is its function and very reason for being. Where it does tell the truth, here and there, is only because the truth and the Cult agenda very occasionally coincide. If you look for fact and insight to the BBC, CNN and virtually all the rest of them you are asking to be conned and perceptually programmed.

Know the outcome and you'll see the journey

Events seem random when you have no idea where the world is being taken. Once you do the random becomes the carefully planned. Know the outcome and you'll see the journey is a phrase I have been using for a long time to give context to daily happenings that appear unconnected. Does a problem, or illusion of a problem, trigger a proposed 'solution' that further drives society in the direction of the outcome? Invariably the answer will be yes and the random – *abracadabra* – becomes the clearly coordinated. So

what is this outcome that unlocks the door to a massively expanded understanding of daily events? I will summarise its major aspects – the fine detail is in my other books – and those new to this information will see that the world they thought they were living in is a very different place. The foundation of the Cult agenda is the incessant centralisation of power and all such centralisation is ultimately in pursuit of Cult control on a global level. I have described for a long time the planned world structure of top-down dictatorship as the Hunger Games Society. The term obviously comes from the movie series which portrayed a world in which a few living in military-protected hi-tech luxury were the overlords of a population condemned to abject poverty in isolated 'sectors' that were not allowed to interact. 'Covid' lockdowns and travel bans anyone? The 'Hunger Games' pyramid of structural control has the inner circle of the Cult at the top with pretty much the entire population at the bottom under their control through dependency for survival on the Cult. The whole structure is planned to be protected and enforced by a military-police state (Fig 4).

Here you have the reason for the global lockdowns of the fake pandemic to coldly destroy independent incomes and livelihoods and make everyone dependent on the 'state' (the Cult that controls the 'states'). I have warned in my books for many years about the plan to introduce a 'guaranteed income' – a barely survivable pittance – designed to impose dependency when employment was destroyed by AI technology and now even more comprehensively at great speed by the 'Covid' scam. Once the pandemic was played and lockdown consequences began to delete independent income the authorities began to talk right on cue about the need for a guaranteed income and a 'Great Reset'. Guaranteed income will be presented as benevolent governments seeking to help a desperate people – desperate as a direct result of actions of the same governments. The truth is that such payments are a trap. You will only get them if you do exactly what the authorities demand including mass vaccination (genetic manipulation). We have seen this theme already in Australia where those dependent on government benefits have them reduced if parents don't agree to have their children vaccinated according to an insane health-destroying government-dictated schedule. Calculated economic collapse applies to governments as well as people. The Cult wants rid of countries through the creation of a world state with countries broken up into regions ruled by a world government and super states like the European Union. Countries must be

bankrupted, too, to this end and it's being achieved by the trillions in 'rescue packages' and furlough payments, trillions in lost taxation, and money-no-object spending on 'Covid' including constant all-medium advertising (programming) which has made the media dependent on government for much of its income. The day of reckoning is coming – as planned – for government spending and given that it has been made possible by printing money and not by production/taxation there is inflation on the way that has the potential to wipe out monetary value. In that case there will be no need for the Cult to steal your money. It just won't be worth anything (see the German Weimar Republic before the Nazis took over). Many have been okay with lockdowns while getting a percentage of their income from so-called furlough payments without having to work. Those payments are dependent, however, on people having at least a theoretical job with a business considered non-essential and ordered to close. As these business go under because they are closed by lockdown after lockdown the furlough stops and it will for everyone eventually. Then what? The 'then what?' is precisely the idea.

Figure 4: The Hunger Games Society structure I have long warned was planned and now the 'Covid' hoax has made it possible. This is the real reason for lockdowns.

Hired hands

Between the Hunger Games Cult elite and the dependent population is planned to be a vicious military-police state (a fusion of the two into one force). This has been in the making for a long time with police looking ever more like the military and carrying weapons to match. The pandemic scam has seen this process accelerate so fast as lockdown house arrest is brutally enforced by carefully recruited fascist minds and gormless system-servers. The police and military are planned to merge into a centrally-directed world army in a global structure headed by a world government which wouldn't be elected even by the election fixes now in place. The world army is not planned even to be human and instead wars would be fought, primarily against the population, using robot technology controlled by artificial intelligence. I have been warning about this for decades and now militaries around the world are being transformed by this very AI technology. The global regime that I describe is a particular form of fascism known as a technocracy in which decisions are not made by clueless and co-opted politicians but by unelected technocrats – scientists, engineers, technologists and bureaucrats. Cult-owned-and-controlled Silicon Valley giants are examples of technocracy and they already have far more power to direct world events than governments. They are with their censorship *selecting* governments. I know that some are calling the 'Great Reset' a Marxist communist takeover, but fascism and Marxism are different labels for the same tyranny. Tell those who lived in fascist Germany and Stalinist Russia that there was a difference in the way their freedom was deleted and their lives controlled. I could call it a fascist technocracy or a Marxist technocracy and they would be equally accurate. The Hunger Games society with its world government structure would oversee a world army, world central bank and single world cashless currency imposing its will on a microchipped population (Fig 5). Scan its different elements and see how the illusory pandemic is forcing society in this very direction at great speed. Leaders of 23 countries and the World Health Organization (WHO) backed the idea in March, 2021, of a global treaty for 'international cooperation' in 'health emergencies' and nations should 'come together as a global community for peaceful cooperation that extends beyond this crisis'. Cut the Orwellian bullshit and this means another step towards global government. The plan includes a cashless digital money system that I first warned about in 1993. Right at the start of 'Covid' the deeply corrupt

Tedros Adhanom Ghebreyesus, the crooked and merely gofer ‘head’ of the World Health Organization, said it was possible to catch the ‘virus’ by touching cash and it was better to use cashless means. The claim was ridiculous nonsense and like the whole ‘Covid’ mind-trick it was nothing to do with ‘health’ and everything to do with pushing every aspect of the Cult agenda. As a result of the Tedros lie the use of cash has plummeted. The Cult script involves a single world digital currency that would eventually be technologically embedded in the body. China is a massive global centre for the Cult and if you watch what is happening there you will know what is planned for everywhere. The Chinese government is developing a digital currency which would allow fines to be deducted immediately via AI for anyone caught on camera breaking its fantastic list of laws and the money is going to be programmable with an expiry date to ensure that no one can accrue wealth except the Cult and its operatives.

Figure 5: The structure of global control the Cult has been working towards for so long and this has been enormously advanced by the ‘Covid’ illusion.

Serfdom is so smart

The Cult plan is far wider, extreme, and more comprehensive than even most conspiracy researchers appreciate and I will come to the true depths of deceit and control in the chapters ‘Who controls the Cult?’ and ‘Escaping Wetiko’. Even the world that we know is crazy enough. We are being deluged with ever more sophisticated and controlling technology under the heading of ‘smart’. We have smart televisions, smart meters, smart cards,

smart cars, smart driving, smart roads, smart pills, smart patches, smart watches, smart skin, smart borders, smart pavements, smart streets, smart cities, smart communities, smart environments, smart growth, smart planet ... smart *everything* around us. Smart technologies and methods of operation are designed to interlock to create a global Smart Grid connecting the entirety of human society including human minds to create a centrally-dictated 'hive' mind. 'Smart cities' is code for densely-occupied megacities of total surveillance and control through AI. Ever more destructive frequency communication systems like 5G have been rolled out without any official testing for health and psychological effects (colossal). 5G/6G/7G systems are needed to run the Smart Grid and each one becomes more destructive of body and mind. Deleting independent income is crucial to forcing people into these AI-policed prisons by ending private property ownership (except for the Cult elite). The Cult's Great Reset now openly foresees a global society in which no one will own any possessions and everything will be rented while the Cult would own literally everything under the guise of government and corporations. The aim has been to use the lockdowns to destroy sources of income on a mass scale and when the people are destitute and in unrepayable amounts of debt (problem) Cult assets come forward with the pledge to write-off debt in return for handing over all property and possessions (solution). Everything – literally everything including people – would be connected to the Internet via AI. I was warning years ago about the coming Internet of Things (IoT) in which all devices and technology from your car to your fridge would be plugged into the Internet and controlled by AI. Now we are already there with much more to come. The next stage is the Internet of Everything (IoE) which is planned to include the connection of AI to the human brain and body to replace the human mind with a centrally-controlled AI mind. Instead of perceptions being manipulated through control of information and censorship those perceptions would come direct from the Cult through AI. What do you think? You think whatever AI decides that you think. In human terms there would be no individual 'think' any longer. Too incredible? The ravings of a lunatic? Not at all. Cult-owned crazies in Silicon Valley have been telling us the plan for years without explaining the real motivation and calculated implications. These include Google executive and 'futurist' Ray Kurzweil who highlights the year 2030 for when this would be underway. He said:

Our thinking ... will be a hybrid of biological and non-biological thinking ... humans will be able to extend their limitations and 'think in the cloud' ... We're going to put gateways to the cloud in our brains ... We're going to gradually merge and enhance ourselves ... In my view, that's the nature of being human – we transcend our limitations.

As the technology becomes vastly superior to what we are then the small proportion that is still human gets smaller and smaller and smaller until it's just utterly negligible.

The sales-pitch of Kurzweil and Cult-owned Silicon Valley is that this would make us 'super-human' when the real aim is to make us post-human and no longer 'human' in the sense that we have come to know. The entire global population would be connected to AI and become the centrally-controlled 'hive-mind' of externally-delivered perceptions. The Smart Grid being installed to impose the Cult's will on the world is being constructed to allow particular locations – even one location – to control the whole global system. From these prime control centres, which absolutely include China and Israel, anything connected to the Internet would be switched on or off and manipulated at will. Energy systems could be cut, communication via the Internet taken down, computer-controlled driverless autonomous vehicles driven off the road, medical devices switched off, the potential is limitless given how much AI and Internet connections now run human society. We have seen nothing yet if we allow this to continue. Autonomous vehicle makers are working with law enforcement to produce cars designed to automatically pull over if they detect a police or emergency vehicle flashing from up to 100 feet away. At a police stop the car would be unlocked and the window rolled down automatically. Vehicles would only take you where the computer (the state) allowed. The end of petrol vehicles and speed limiters on all new cars in the UK and EU from 2022 are steps leading to electric computerised transport over which ultimately you have no control. The picture is far bigger even than the Cult global network or web and that will become clear when I get to the nature of the 'spider'. There is a connection between all these happenings and the instigation of DNA-manipulating 'vaccines' (which aren't 'vaccines') justified by the 'Covid' hoax. That connection is the unfolding plan to transform the human body from a biological to a synthetic biological state and this is why synthetic biology is such a fast-emerging discipline of mainstream science. 'Covid vaccines' are infusing self-replicating synthetic genetic material into the cells to cumulatively take us on the Totalitarian Tiptoe from Human 1.0

to the synthetic biological Human 2.0 which will be physically and perceptually attached to the Smart Grid to one hundred percent control every thought, perception and deed. Humanity needs to wake up and *fast*.

This is the barest explanation of where the 'outcome' is planned to go but it's enough to see the journey happening all around us. Those new to this information will already see 'Covid' in a whole new context. I will add much more detail as we go along, but for the minutiae evidence see my mega-works, *The Answer, The Trigger* and *Everything You Need to Know But Have Never Been Told*.

Now – how does a Renegade Mind see the 'world'?

CHAPTER TWO

Renegade Perception

It is one thing to be clever and another to be wise
George R.R. Martin

A simple definition of the difference between a programmed mind and a Renegade Mind would be that one sees only dots while the other connects them to see the picture. Reading reality with accuracy requires the observer to (a) know the planned outcome and (b) realise that everything, but *everything*, is connected.

The entirety of infinite reality is connected – that's its very nature – and with human society an expression of infinite reality the same must apply. Simple cause and effect is a connection. The effect is triggered by the cause and the effect then becomes the cause of another effect. Nothing happens in isolation because it *can't*. Life in whatever reality is simple choice and consequence. We make choices and these lead to consequences. If we don't like the consequences we can make different choices and get different consequences which lead to other choices and consequences. The choice and the consequence are not only connected they are indivisible. You can't have one without the other as an old song goes. A few cannot control the world unless those being controlled allow that to happen – cause and effect, choice and consequence. Control – who has it and who doesn't – is a two-way process, a symbiotic relationship, involving the controller and controlled. 'They took my freedom away!!' Well, yes, but you also gave it to them. Humanity is subjected to mass control because humanity has acquiesced to that control. This is all cause and effect and literally a case of

give and take. In the same way world events of every kind are connected and the Cult works incessantly to sell the illusion of the random and coincidental to maintain the essential (to them) perception of dots that hide the picture. Renegade Minds know this and constantly scan the world for patterns of connection. This is absolutely pivotal in understanding the happenings in the world and without that perspective clarity is impossible. First you know the planned outcome and then you identify the steps on the journey – the day-by-day apparently random which, when connected in relation to the outcome, no longer appear as individual events, but as the proverbial *chain* of events leading in the same direction. I'll give you some examples:

Political puppet show

We are told to believe that politics is 'adversarial' in that different parties with different beliefs engage in an endless tussle for power. There may have been some truth in that up to a point – and only a point – but today divisions between 'different' parties are rhetorical not ideological. Even the rhetorical is fusing into one-speak as the parties eject any remaining free thinkers while others succumb to the ever-gathering intimidation of anyone with the 'wrong' opinion. The Cult is not a new phenomenon and can be traced back thousands of years as my books have documented. Its intergenerational initiates have been manipulating events with increasing effect the more that global power has been centralised. In ancient times the Cult secured control through the system of monarchy in which 'special' bloodlines (of which more later) demanded the right to rule as kings and queens simply by birthright and by vanquishing others who claimed the same birthright. There came a time, however, when people had matured enough to see the unfairness of such tyranny and demanded a say in who governed them. Note the word – *governed* them. Not served them – *governed* them, hence government defined as 'the political direction and control exercised over the actions of the members, citizens, or inhabitants of communities, societies, and states; direction of the affairs of a state, community, etc.' Governments exercise control over rather than serve just like the monarchies before them. Bizarrely there are still countries like the United Kingdom which are ruled by a monarch *and* a government that officially answers to the monarch. The UK head of state and that of Commonwealth

countries such as Canada, Australia and New Zealand is ‘selected’ by who in a *single family* had unprotected sex with whom and in what order. Pinch me it can’t be true. Ouch! Shit, it is. The demise of monarchies in most countries offered a potential vacuum in which some form of free and fair society could arise and the Cult had that base covered. Monarchies had served its interests but they couldn’t continue in the face of such widespread opposition and, anyway, replacing a ‘royal’ dictatorship that people could see with a dictatorship ‘of the people’ hiding behind the concept of ‘democracy’ presented far greater manipulative possibilities and ways of hiding coordinated tyranny behind the illusion of ‘freedom’.

Democracy is quite wrongly defined as government selected by the population. This is not the case at all. It is government selected by *some* of the population (and then only in theory). This ‘some’ doesn’t even have to be the majority as we have seen so often in first-past-the-post elections in which the so-called majority party wins fewer votes than the ‘losing’ parties combined. Democracy can give total power to a party in government from a minority of the votes cast. It’s a sleight of hand to sell tyranny as freedom. Seventy-four million Trump-supporting Americans didn’t vote for the ‘Democratic’ Party of Joe Biden in the distinctly dodgy election in 2020 and yet far from acknowledging the wishes and feelings of that great percentage of American society the Cult-owned Biden government set out from day one to destroy them and their right to a voice and opinion. Empty shell Biden and his Cult handlers said they were doing this to ‘protect democracy’. Such is the level of lunacy and sickness to which politics has descended. Connect the dots and relate them to the desired outcome – a world government run by self-appointed technocrats and no longer even elected politicians. While operating through its political agents in government the Cult is at the same time encouraging public distain for politicians by putting idiots and incompetents in theoretical power on the road to deleting them. The idea is to instil a public reaction that says of the technocrats: ‘Well, they couldn’t do any worse than the pathetic politicians.’ It’s all about controlling perception and Renegade Minds can see through that while programmed minds cannot when they are ignorant of both the planned outcome and the manipulation techniques employed to secure that end. This knowledge can be learned, however, and fast if people choose to get informed.

Politics may at first sight appear very difficult to control from a central point. I mean look at the 'different' parties and how would you be able to oversee them all and their constituent parts? In truth, it's very straightforward because of their structure. We are back to the pyramid of imposition and acquiescence. Organisations are structured in the same way as the system as a whole. Political parties are not open forums of free expression. They are hierarchies. I was a national spokesman for the British Green Party which claimed to be a different kind of politics in which influence and power was devolved; but I can tell you from direct experience – and it's far worse now – that Green parties are run as hierarchies like all the others however much they may try to hide that fact or kid themselves that it's not true. A very few at the top of all political parties are directing policy and personnel. They decide if you are elevated in the party or serve as a government minister and to do that you have to be a yes man or woman. Look at all the maverick political thinkers who never ascended the greasy pole. If you want to progress within the party or reach 'high-office' you need to fall into line and conform. Exceptions to this are rare indeed. Should you want to run for parliament or Congress you have to persuade the local or state level of the party to select you and for that you need to play the game as dictated by the hierarchy. If you secure election and wish to progress within the greater structure you need to go on conforming to what is acceptable to those running the hierarchy from the peak of the pyramid. Political parties are perceptual gulags and the very fact that there are party 'Whips' appointed to 'whip' politicians into voting the way the hierarchy demands exposes the ridiculous idea that politicians are elected to serve the people they are supposed to represent. Cult operatives and manipulation has long seized control of major parties that have any chance of forming a government and at least most of those that haven't. A new party forms and the Cult goes to work to infiltrate and direct. This has reached such a level today that you see video compilations of 'leaders' of all parties whether Democrats, Republicans, Conservative, Labour and Green parroting the same Cult mantra of 'Build Back Better' and the 'Great Reset' which are straight off the Cult song-sheet to describe the transformation of global society in response to the Cult-instigated hoaxes of the 'Covid pandemic' and human-caused 'climate change'. To see Caroline Lucas, the Green Party MP that I knew when I was in the party in the

1980s, speaking in support of plans proposed by Cult operative Klaus Schwab representing the billionaire global elite is a real head-shaker.

Many parties – one master

The party system is another mind-trick and was instigated to change the nature of the dictatorship by swapping 'royalty' for dark suits that people believed – though now ever less so – represented their interests. Understanding this trick is to realise that a single force (the Cult) controls all parties either directly in terms of the major ones or through manipulation of perception and ideology with others. You don't need to manipulate Green parties to demand your transformation of society in the name of 'climate change' when they are obsessed with the lie that this is essential to 'save the planet'. You just give them a platform and away they go serving your interests while believing they are being environmentally virtuous. America's political structure is a perfect blueprint for how the two or multi-party system is really a one-party state. The Republican Party is controlled from one step back in the shadows by a group made up of billionaires and their gofers known as neoconservatives or Neocons. I have exposed them in fine detail in my books and they were the driving force behind the policies of the imbecilic presidency of Boy George Bush which included 9/11 (see *The Trigger* for a comprehensive demolition of the official story), the subsequent 'war on terror' (war *of* terror) and the invasions of Afghanistan and Iraq. The latter was a No-Problem-Reaction-Solution based on claims by Cult operatives, including Bush and British Prime Minister Tony Blair, about Saddam Hussein's 'weapons of mass destruction' which did not exist as war criminals Bush and Blair well knew.

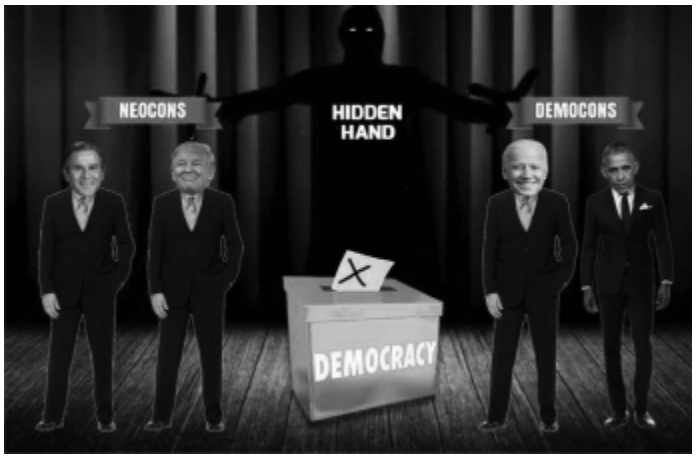

Figure 6: Different front people, different parties – same control system.

The Democratic Party has its own 'Neocon' group controlling from the background which I call the 'Democons' and here's the penny-drop – the Neocons and Democons answer to the same masters one step further back into the shadows (Fig 6). At that level of the Cult the Republican and Democrat parties are controlled by the same people and no matter which is in power the Cult is in power. This is how it works in almost every country and certainly in Britain with Conservative, Labour, Liberal Democrat and Green parties now all on the same page whatever the rhetoric may be in their feeble attempts to appear different. Neocons operated at the time of Bush through a think tank called The Project for the New American Century which in September, 2000, published a document entitled *Rebuilding America's Defenses: Strategies, Forces, and Resources For a New Century* demanding that America fight 'multiple, simultaneous major theatre wars' as a 'core mission' to force regime-change in countries including Iraq, Libya and Syria. Neocons arranged for Bush ('Republican') and Blair ('Labour Party') to front-up the invasion of Iraq and when they departed the Democons orchestrated the targeting of Libya and Syria through Barack Obama ('Democrat') and British Prime Minister David Cameron ('Conservative Party'). We have 'different' parties and 'different' people, but the same unfolding script. The more the Cult has seized the reigns of parties and personnel the more their policies have transparently pursued the same agenda to the point where the fascist 'Covid' impositions of the Conservative junta of Jackboot Johnson in Britain were opposed by the Labour Party because they were not fascist enough. The Labour Party is likened to the US Democrats while the Conservative Party is akin to a

British version of the Republicans and on both sides of the Atlantic they all speak the same language and support the direction demanded by the Cult although some more enthusiastically than others. It's a similar story in country after country because it's all centrally controlled. Oh, but what about Trump? I'll come to him shortly. Political 'choice' in the 'party' system goes like this: You vote for Party A and they get into government. You don't like what they do so next time you vote for Party B and they get into government. You don't like what they do when it's pretty much the same as Party A and why wouldn't that be with both controlled by the same force? Given that only two, sometimes three, parties have any chance of forming a government to get rid of Party B that you don't like you have to vote again for Party A which … you don't like. This, ladies and gentlemen, is what they call 'democracy' which we are told – wrongly – is a term interchangeable with 'freedom'.

The cult of cults

At this point I need to introduce a major expression of the Global Cult known as Sabbatian-Frankism. Sabbatian is also spelt as Sabbatean. I will summarise here. I have published major exposés and detailed background in other works. Sabbatian-Frankism combines the names of two frauds posing as 'Jewish' men, Sabbatai Zevi (1626-1676), a rabbi, black magician and occultist who proclaimed he was the Jewish messiah; and Jacob Frank (1726-1791), the Polish 'Jew', black magician and occultist who said he was the reincarnation of 'messiah' Zevi and biblical patriarch Jacob. They worked across two centuries to establish the Sabbatian-Frankist cult that plays a major, indeed central, role in the manipulation of human society by the Global Cult which has its origins much further back in history than Sabbatai Zevi. I should emphasise two points here in response to the shrill voices that will scream 'anti-Semitism': (1) Sabbatian-Frankists are NOT Jewish and only pose as such to hide their cult behind a Jewish façade; and (2) my information about this cult has come from Jewish sources who have long realised that their society and community has been infiltrated and taken over by interloper Sabbatian-Frankists. Infiltration has been the foundation technique of Sabbatian-Frankism from its official origin in the 17th century. Zevi's Sabbatian sect attracted a massive following described as the biggest messianic movement in Jewish history, spreading as far as

Africa and Asia, and he promised a return for the Jews to the 'Promised Land' of Israel. Sabbatianism was not Judaism but an inversion of everything that mainstream Judaism stood for. So much so that this sinister cult would have a feast day when Judaism had a fast day and whatever was forbidden in Judaism the Sabbatians were encouraged and even commanded to do. This included incest and what would be today called Satanism. Members were forbidden to marry outside the sect and there was a system of keeping their children ignorant of what they were part of until they were old enough to be trusted not to unknowingly reveal anything to outsiders. The same system is employed to this day by the Global Cult in general which Sabbatian-Frankism has enormously influenced and now largely controls.

Zevi and his Sabbatians suffered a setback with the intervention by the Sultan of the Islamic Ottoman Empire in the Middle East and what is now the Republic of Turkey where Zevi was located. The Sultan gave him the choice of proving his 'divinity', converting to Islam or facing torture and death. Funnily enough Zevi chose to convert or at least appear to. Some of his supporters were disillusioned and drifted away, but many did not with 300 families also converting – only in theory – to Islam. They continued behind this Islamic smokescreen to follow the goals, rules and rituals of Sabbatianism and became known as 'crypto-Jews' or the 'Dönmeh' which means 'to turn'. This is rather ironic because they didn't 'turn' and instead hid behind a fake Islamic persona. The process of appearing to be one thing while being very much another would become the calling card of Sabbatianism especially after Zevi's death and the arrival of the Satanist Jacob Frank in the 18th century when the cult became Sabbatian-Frankism and plumbed still new depths of depravity and infiltration which included – still includes – human sacrifice and sex with children. Wherever Sabbatians go paedophilia and Satanism follow and is it really a surprise that Hollywood is so infested with child abuse and Satanism when it was established by Sabbatian-Frankists and is still controlled by them? Hollywood has been one of the prime vehicles for global perceptual programming and manipulation. How many believe the version of 'history' portrayed in movies when it is a travesty and inversion (again) of the truth? Rabbi Marvin Antelman describes Frankism in his book, *To Eliminate the Opiate*, as 'a movement of complete evil' while Jewish professor Gershom Scholem said of Frank in *The Messianic Idea in Judaism*: 'In all his actions

[he was] a truly corrupt and degenerate individual ... one of the most frightening phenomena in the whole of Jewish history.' Frank was excommunicated by traditional rabbis, as was Zevi, but Frank was undeterred and enjoyed vital support from the House of Rothschild, the infamous banking dynasty whose inner-core are Sabbatian-Frankists and not Jews. Infiltration of the Roman Church and Vatican was instigated by Frank with many Dönmeh 'turning' again to convert to Roman Catholicism with a view to hijacking the reins of power. This was the ever-repeating modus operandi and continues to be so. Pose as an advocate of the religion, culture or country that you want to control and then manipulate your people into the positions of authority and influence largely as advisers, administrators and Svengalis for those that appear to be in power. They did this with Judaism, Christianity (Christian Zionism is part of this), Islam and other religions and nations until Sabbatian-Frankism spanned the world as it does today.

Sabbatian Saudis and the terror network

One expression of the Sabbatian-Frankist Dönmeh within Islam is the ruling family of Saudi Arabia, the House of Saud, through which came the vile distortion of Islam known as Wahhabism. This is the violent creed followed by terrorist groups like Al-Qaeda and ISIS or Islamic State. Wahhabism is the hand-chopping, head-chopping 'religion' of Saudi Arabia which is used to keep the people in a constant state of fear so the interloper House of Saud can continue to rule. Al-Qaeda and Islamic State were lavishly funded by the House of Saud while being created and directed by the Sabbatian-Frankist network in the United States that operates through the Pentagon, CIA and the government in general of whichever 'party'. The front man for the establishment of Wahhabism in the middle of the 18th century was a Sabbatian-Frankist 'crypto-Jew' posing as Islamic called Muhammad ibn Abd al-Wahhab. His daughter would marry the son of Muhammad bin Saud who established the first Saudi state before his death in 1765 with support from the British Empire. Bin Saud's successors would establish modern Saudi Arabia in league with the British and Americans in 1932 which allowed them to seize control of Islam's major shrines in Mecca and Medina. They have dictated the direction of Sunni Islam ever since while Iran is the major centre of the Shiite version and here we have

the source of at least the public conflict between them. The Sabbatian network has used its Wahhabi extremists to carry out Problem-Reaction-Solution terrorist attacks in the name of 'Al-Qaeda' and 'Islamic State' to justify a devastating 'war on terror', ever-increasing surveillance of the population and to terrify people into compliance. Another insight of the Renegade Mind is the streetwise understanding that just because a country, location or people are attacked doesn't mean that those apparently representing that country, location or people are not behind the attackers. Often they are *orchestrating* the attacks because of the societal changes that can be then justified in the name of 'saving the population from terrorists'.

I show in great detail in *The Trigger* how Sabbatian-Frankists were the real perpetrators of 9/11 and not '19 Arab hijackers' who were blamed for what happened. Observe what was justified in the name of 9/11 alone in terms of Middle East invasions, mass surveillance and control that fulfilled the demands of the Project for the New American Century document published by the Sabbatian Neocons. What appear to be enemies are on the deep inside players on the same Sabbatian team. Israel and Arab 'royal' dictatorships are all ruled by Sabbatians and the recent peace agreements between Israel and Saudi Arabia, the United Arab Emirates (UAE) and others are only making formal what has always been the case behind the scenes. Palestinians who have been subjected to grotesque tyranny since Israel was bombed and terrorised into existence in 1948 have never stood a chance. Sabbatian-Frankists have controlled Israel (so the constant theme of violence and war which Sabbatians love) and they have controlled the Arab countries that Palestinians have looked to for real support that never comes. 'Royal families' of the Arab world in Saudi Arabia, Bahrain, UAE, etc., are all Sabbatians with allegiance to the aims of the cult and not what is best for their Arabic populations. They have stolen the oil and financial resources from their people by false claims to be 'royal dynasties' with a genetic right to rule and by employing vicious militaries to impose their will.

Satanic 'illumination'

The Satanist Jacob Frank formed an alliance in 1773 with two other Sabbatians, Mayer Amschel Rothschild (1744-1812), founder of the Rothschild banking dynasty, and Jesuit-educated fraudulent Jew, Adam Weishaupt, and this led to the formation of the Bavarian Illuminati, firstly

under another name, in 1776. The Illuminati would be the manipulating force behind the French Revolution (1789-1799) and was also involved in the American Revolution (1775-1783) before and after the Illuminati's official creation. Weishaupt would later become (in public) a Protestant Christian in archetypal Sabbatian style. I read that his name can be decoded as Adam-Weis-haupt or 'the first man to lead those who know'. He wasn't a leader in the sense that he was a subordinate, but he did lead those below him in a crusade of transforming human society that still continues today. The theme was confirmed as early as 1785 when a horseman courier called Lanz was reported to be struck by lighting and extensive Illuminati documents were found in his saddlebags. They made the link to Weishaupt and detailed the plan for world takeover. Current events with 'Covid' fascism have been in the making for a very long time. Jacob Frank was jailed for 13 years by the Catholic Inquisition after his arrest in 1760 and on his release he headed for Frankfurt, Germany, home city and headquarters of the House of Rothschild where the alliance was struck with Mayer Amschel Rothschild and Weishaupt. Rothschild arranged for Frank to be given the title of Baron and he became a wealthy nobleman with a big following of Jews in Germany, the Austro-Hungarian Empire and other European countries. Most of them would have believed he was on their side.

The name 'Illuminati' came from the Zohar which is a body of works in the Jewish mystical 'bible' called the Kabbalah. 'Zohar' is the foundation of Sabbatian-Frankist belief and in Hebrew 'Zohar' means 'splendour', 'radiance', 'illuminated', and so we have 'Illuminati'. They claim to be the 'Illuminated Ones' from their knowledge systematically hidden from the human population and passed on through generations of carefully-chosen initiates in the global secret society network or Cult. Hidden knowledge includes an awareness of the Cult agenda for the world and the nature of our collective reality that I will explore later. Cult 'illumination' is symbolised by the torch held by the Statue of Liberty which was gifted to New York by French Freemasons in Paris who knew exactly what it represents. 'Liberty' symbolises the goddess worshipped in Babylon as Queen Semiramis or Ishtar. The significance of this will become clear. Notice again the ubiquitous theme of inversion with the Statue of 'Liberty' really symbolising mass control (Fig 7). A mirror-image statute stands on an island in the River Seine in Paris from where New York Liberty

originated (Fig 8). A large replica of the Liberty flame stands on top of the Pont de l'Alma tunnel in Paris where Princess Diana died in a Cult ritual described in *The Biggest Secret*. Lucifer 'the light bringer' is related to all this (and much more as we'll see) and 'Lucifer' is a central figure in Sabbatian-Frankism and its associated Satanism. Sabbatians reject the Jewish Torah, or Pentateuch, the 'five books of Moses' in the Old Testament known as Genesis, Exodus, Leviticus, Numbers, and Deuteronomy which are claimed by Judaism and Christianity to have been dictated by 'God' to Moses on Mount Sinai. Sabbatians say these do not apply to them and they seek to replace them with the Zohar to absorb Judaism and its followers into their inversion which is an expression of a much greater global inversion. They want to delete all religions and force humanity to worship a one-world religion – Sabbatian Satanism that also includes worship of the Earth goddess. Satanic themes are being more and more introduced into mainstream society and while Christianity is currently the foremost target for destruction the others are planned to follow.

Figure 7: The Cult goddess of Babylon disguised as the Statue of Liberty holding the flame of Lucifer the 'light bringer'.

Figure 8: Liberty's mirror image in Paris where the New York version originated.

Marx brothers

Rabbi Marvin Antelman connects the Illuminati to the Jacobins in *To Eliminate the Opiate* and Jacobins were the force behind the French Revolution. He links both to the Bund der Gerechten, or League of the Just, which was the network that inflicted communism/Marxism on the world. Antelman wrote:

> The original inner circle of the Bund der Gerechten consisted of born Catholics, Protestants and Jews [Sabbatian-Frankist infiltrators], and those representatives of respective subdivisions formulated schemes for the ultimate destruction of their faiths. The heretical Catholics laid plans which they felt would take a century or more for the ultimate destruction of the church; the apostate Jews for the ultimate destruction of the Jewish religion.

Sabbatian-created communism connects into this anti-religion agenda in that communism does not allow for the free practice of religion. The Sabbatian 'Bund' became the International Communist Party and Communist League and in 1848 'Marxism' was born with the Communist Manifesto of Sabbatian assets Karl Marx and Friedrich Engels. It is absolutely no coincidence that Marxism, just a different name for fascist and other centrally-controlled tyrannies, is being imposed worldwide as a result of the 'Covid' hoax and nor that Marxist/fascist China was the place where the hoax originated. The reason for this will become very clear in the chapter 'Covid: The calculated catastrophe'. The so-called 'Woke' mentality has hijacked traditional beliefs of the political left and replaced

them with far-right make-believe ‘social justice’ better known as Marxism. Woke will, however, be swallowed by its own perceived ‘revolution’ which is really the work of billionaires and billionaire corporations feigning being ‘Woke’. Marxism is being touted by Wokers as a replacement for ‘capitalism’ when we don’t have ‘capitalism’. We have cartelism in which the market is stitched up by the very Cult billionaires and corporations bankrolling Woke. Billionaires love Marxism which keeps the people in servitude while they control from the top. Terminally naïve Wokers think they are ‘changing the world’ when it’s the Cult that is doing the changing and when they have played their vital part and become surplus to requirements they, too, will be targeted. The Illuminati-Jacobins were behind the period known as ‘The Terror’ in the French Revolution in 1793 and 1794 when Jacobin Maximillian de Robespierre and his Orwellian ‘Committee of Public Safety’ killed 17,000 ‘enemies of the Revolution’ who had once been ‘friends of the Revolution’. Karl Marx (1818-1883), whose Sabbatian creed of Marxism has cost the lives of at least 100 million people, is a hero once again to Wokers who have been systematically kept ignorant of real history by their ‘education’ programming. As a result they now promote a Sabbatian ‘Marxist’ abomination destined at some point to consume them. Rabbi Antelman, who spent decades researching the Sabbatian plot, said of the League of the Just and Karl Marx:

Contrary to popular opinion Karl Marx did not originate the Communist Manifesto. He was paid for his services by the League of the Just, which was known in its country of origin, Germany, as the Bund der Geaechteten.

Antelman said the text attributed to Marx was the work of other people and Marx ‘was only repeating what others already said’. Marx was ‘a hired hack – lackey of the wealthy Illuminists’. Marx famously said that religion was the ‘opium of the people’ (part of the Sabbatian plan to demonise religion) and Antelman called his books, *To Eliminate the Opiate*. Marx was born Jewish, but his family converted to Christianity (Sabbatian modus operandi) and he attacked Jews, not least in his book, *A World Without Jews*. In doing so he supported the Sabbatian plan to destroy traditional Jewishness and Judaism which we are clearly seeing today with the vindictive targeting of orthodox Jews by the Sabbatian government of Israel over ‘Covid’ laws. I

don't follow any religion and it has done much damage to the world over centuries and acted as a perceptual straightjacket. Renegade Minds, however, are always asking *why* something is being done. It doesn't matter if they agree or disagree with what is happening – *why* is it happening is the question. The 'why?' can be answered with regard to religion in that religions create interacting communities of believers when the Cult wants to dismantle all discourse, unity and interaction (see 'Covid' lockdowns) and the ultimate goal is to delete all religions for a one-world religion of Cult Satanism worshipping their 'god' of which more later. We see the same 'why?' with gun control in America. I don't have guns and don't want them, but why is the Cult seeking to disarm the population at the same time that law enforcement agencies are armed to their molars and why has every tyrant in history sought to disarm people before launching the final takeover? They include Hitler, Stalin, Pol Pot and Mao who followed confiscation with violent seizing of power. You know it's a Cult agenda by the people who immediately race to the microphones to exploit dead people in multiple shootings. Ultra-Zionist Cult lackey Senator Chuck Schumer was straight on the case after ten people were killed in Boulder, Colorado in March, 2121. Simple rule … if Schumer wants it the Cult wants it and the same with his ultra-Zionist mate the wild-eyed Senator Adam Schiff. At the same time they were calling for the disarmament of Americans, many of whom live a long way from a police response, Schumer, Schiff and the rest of these pampered clowns were sitting on Capitol Hill behind a razor-wired security fence protected by thousands of armed troops in addition to their own armed bodyguards. Mom and pop in an isolated home? They're just potential mass shooters.

Zion Mainframe

Sabbatian-Frankists and most importantly the Rothschilds were behind the creation of 'Zionism', a political movement that demanded a Jewish homeland in Israel as promised by Sabbatai Zevi. The very symbol of Israel comes from the German meaning of the name Rothschild. Dynasty founder Mayer Amschel Rothschild changed the family name from Bauer to Rothschild, or 'Red-Shield' in German, in deference to the six-pointed 'Star of David' hexagram displayed on the family's home in Frankfurt. The symbol later appeared on the flag of Israel after the Rothschilds were

centrally involved in its creation. Hexagrams are not a uniquely Jewish symbol and are widely used in occult ('hidden') networks often as a symbol for Saturn (see my other books for why). Neither are Zionism and Jewishness interchangeable. Zionism is a political movement and philosophy and not a 'race' or a people. Many Jews oppose Zionism and many non-Jews, including US President Joe Biden, call themselves Zionists as does Israel-centric Donald Trump. America's support for the Israel government is pretty much a gimme with ultra-Zionist billionaires and corporations providing fantastic and dominant funding for both political parties. Former Congresswoman Cynthia McKinney has told how she was approached immediately she ran for office to 'sign the pledge' to Israel and confirm that she would always vote in that country's best interests. All American politicians are approached in this way. Anyone who refuses will get no support or funding from the enormous and all-powerful Zionist lobby that includes organisations like mega-lobby group AIPAC, the American Israel Public Affairs Committee. Trump's biggest funder was ultra-Zionist casino and media billionaire Sheldon Adelson while major funders of the Democratic Party include ultra-Zionist George Soros and ultra-Zionist financial and media mogul, Haim Saban. Some may reel back at the suggestion that Soros is an Israel-firster (Sabbatian-controlled Israel-firster), but Renegade Minds watch the actions not the words and everywhere Soros donates his billions the Sabbatian agenda benefits. In the spirit of Sabbatian inversion Soros pledged $1 billion for a new university network to promote 'liberal values and tackle intolerance'. He made the announcement during his annual speech at the Cult-owned World Economic Forum in Davos, Switzerland, in January, 2020, after his 'harsh criticism' of 'authoritarian rulers' around the world. You can only laugh at such brazen mendacity. How *he* doesn't laugh is the mystery. Translated from the Orwellian 'liberal values and tackle intolerance' means teaching non-white people to hate white people and for white people to loathe themselves for being born white. The reason for that will become clear.

The 'Anti-Semitism' fraud

Zionists support the Jewish homeland in the land of Palestine which has been the Sabbatian-Rothschild goal for so long, but not for the benefit of Jews. Sabbatians and their global Anti-Semitism Industry have skewed

public and political opinion to equate opposing the violent extremes of Zionism to be a blanket attack and condemnation of all Jewish people. Sabbatians and their global Anti-Semitism Industry have skewed public and political opinion to equate opposing the violent extremes of Zionism to be a blanket attack and condemnation of all Jewish people. This is nothing more than a Sabbatian protection racket to stop legitimate investigation and exposure of their agendas and activities. The official definition of 'anti-Semitism' has more recently been expanded to include criticism of Zionism – a *political movement* – and this was done to further stop exposure of Sabbatian infiltrators who created Zionism as we know it today in the 19th century. Renegade Minds will talk about these subjects when they know the shit that will come their way. People must decide if they want to know the truth or just cower in the corner in fear of what others will say. Sabbatians have been trying to label me as 'anti-Semitic' since the 1990s as I have uncovered more and more about their background and agendas. Useless, gutless, fraudulent 'journalists' then just repeat the smears without question and on the day I was writing this section a pair of unquestioning repeaters called Ben Quinn and Archie Bland (how appropriate) outright called me an 'anti-Semite' in the establishment propaganda sheet, the London *Guardian*, with no supporting evidence. The Sabbatian Anti-Semitism Industry said so and who are they to question that? They wouldn't dare. Ironically 'Semitic' refers to a group of languages in the Middle East that are almost entirely Arabic. 'Anti-Semitism' becomes 'anti-Arab' which if the consequences of this misunderstanding were not so grave would be hilarious. Don't bother telling Quinn and Bland. I don't want to confuse them, bless 'em. One reason I am dubbed 'anti-Semitic' is that I wrote in the 1990s that Jewish operatives (Sabbatians) were heavily involved in the Russian Revolution when Sabbatians overthrew the Romanov dynasty. This apparently made me 'anti-Semitic'. Oh, really? Here is a section from *The Trigger*:

British journalist Robert Wilton confirmed these themes in his 1920 book *The Last Days of the Romanovs* when he studied official documents from the Russian government to identify the members of the Bolshevik ruling elite between 1917 and 1919. The Central Committee included 41 Jews among 62 members; the Council of the People's Commissars had 17 Jews out of 22 members; and 458 of the 556 most important Bolshevik positions between 1918 and 1919 were occupied by Jewish people. Only 17 were Russian. Then there were the 23 Jews among the 36 members of the vicious Cheka Soviet secret police established in 1917 who would soon appear all across the country.

Professor Robert Service of Oxford University, an expert on 20th century Russian history, found evidence that ['Jewish'] Leon Trotsky had sought to make sure that Jews were enrolled in the Red Army and were disproportionately represented in the Soviet civil bureaucracy that included the Cheka which performed mass arrests, imprisonment and executions of 'enemies of the people'. A US State Department Decimal File (861.00/5339) dated November 13th, 1918, names [Rothschild banking agent in America] Jacob Schiff and a list of ultra-Zionists as funders of the Russian Revolution leading to claims of a 'Jewish plot', but the key point missed by all is they were not 'Jews' – they were Sabbatian-Frankists.

Britain's Winston Churchill made the same error by mistake or otherwise. He wrote in a 1920 edition of the *Illustrated Sunday Herald* that those behind the Russian revolution were part of a 'worldwide conspiracy for the overthrow of civilisation and for the reconstitution of society on the basis of arrested development, of envious malevolence, and impossible equality' (see 'Woke' today because that has been created by the same network). Churchill said there was no need to exaggerate the part played in the creation of Bolshevism and in the actual bringing about of the Russian Revolution 'by these international and for the most part atheistical Jews' ['atheistical Jews' = Sabbatians]. Churchill said it is certainly a very great one and probably outweighs all others: 'With the notable exception of Lenin, the majority of the leading figures are Jews.' He went on to describe, knowingly or not, the Sabbatian modus operandi of placing puppet leaders nominally in power while they control from the background:

Moreover, the principal inspiration and driving power comes from the Jewish leaders. Thus Tchitcherin, a pure Russian, is eclipsed by his nominal subordinate, Litvinoff, and the influence of Russians like Bukharin or Lunacharski cannot be compared with the power of Trotsky, or of Zinovieff, the Dictator of the Red Citadel (Petrograd), or of Krassin or Radek – all Jews. In the Soviet institutions the predominance of Jews is even more astonishing. And the prominent, if not indeed the principal, part in the system of terrorism applied by the Extraordinary Commissions for Combatting Counter-Revolution has been taken by Jews, and in some notable cases by Jewesses.

What I said about seriously disproportionate involvement in the Russian Revolution by Jewish 'revolutionaries' (Sabbatians) is provable fact, but truth is no defence against the Sabbatian Anti-Semitism Industry, its repeater parrots like Quinn and Bland, and the now breathtaking network of so-called 'Woke' 'anti-hate' groups with interlocking leaderships and funding which have the role of discrediting and silencing anyone who gets too close to exposing the Sabbatians. We have seen 'truth is no defence'

confirmed in legal judgements with the Saskatchewan Human Rights Commission in Canada decreeing this: ‘Truthful statements can be presented in a manner that would meet the definition of hate speech, and not all truthful statements must be free from restriction.’ Most ‘anti-hate’ activists, who are themselves consumed by hatred, are too stupid and ignorant of the world to know how they are being used. They are far too far up their own virtue-signalling arses and it’s far too dark for them to see anything.

The ‘revolution’ game

The background and methods of the ‘Russian’ Revolution are straight from the Sabbatian playbook seen in the French Revolution and endless others around the world that appear to start as a revolution of the people against tyrannical rule and end up with a regime change to more tyrannical rule overtly or covertly. Wars, terror attacks and regime overthrows follow the Sabbatian cult through history with its agents creating them as Problem-Reaction-Solutions to remove opposition on the road to world domination. Sabbatian dots connect the Rothschilds with the Illuminati, Jacobins of the French Revolution, the ‘Bund’ or League of the Just, the International Communist Party, Communist League and the Communist Manifesto of Karl Marx and Friedrich Engels that would lead to the Rothschild-funded Russian Revolution. The sequence comes under the heading of ‘creative destruction’ when you advance to your global goal by continually destroying the status quo to install a new status quo which you then also destroy. The two world wars come to mind. With each new status quo you move closer to your planned outcome. Wars and mass murder are to Sabbatians a collective blood sacrifice ritual. They are obsessed with death for many reasons and one is that death is an inversion of life. Satanists and Sabbatians are obsessed with death and often target churches and churchyards for their rituals. Inversion-obsessed Sabbatians explain the use of inverted symbolism including the *inverted* pentagram and *inverted* cross. The inversion of the cross has been related to targeting Christianity, but the cross was a religious symbol long before Christianity and its inversion is a statement about the Sabbatian mentality and goals more than any single religion.

Sabbatians operating in Germany were behind the rise of the occult-obsessed Nazis and the subsequent Jewish exodus from Germany and Europe to Palestine and the United States after World War Two. The Rothschild dynasty was at the forefront of this both as political manipulators and by funding the operation. Why would Sabbatians help to orchestrate the horrors inflicted on Jews by the Nazis and by Stalin after they organised the Russian Revolution? Sabbatians hate Jews and their religion, that's why. They pose as Jews and secure positions of control within Jewish society and play the 'anti-Semitism' card to protect themselves from exposure through a global network of organisations answering to the Sabbatian-created-and-controlled globe-spanning intelligence network that involves a stunning web of military-intelligence operatives and operations for a tiny country of just nine million. Among them are Jewish assets who are not Sabbatians but have been convinced by them that what they are doing is for the good of Israel and the Jewish community to protect them from what they have been programmed since childhood to believe is a Jew-hating hostile world. The Jewish community is just a highly convenient cover to hide the true nature of Sabbatians. Anyone getting close to exposing their game is accused by Sabbatian place-people and gofers of 'anti-Semitism' and claiming that all Jews are part of a plot to take over the world. I am not saying that. I am saying that Sabbatians – the *real* Jew-haters – have infiltrated the Jewish community to use them both as a cover and an 'anti-Semitic' defence against exposure. Thus we have the Anti-Semitism Industry targeted researchers in this way and most Jewish people think this is justified and genuine. They don't know that their 'Jewish' leaders and institutions of state, intelligence and military are not controlled by Jews at all, but cultists and stooges of Sabbatian-Frankism. I once added my name to a pro-Jewish freedom petition online and the next time I looked my name was gone and text had been added to the petition blurb to attack me as an 'anti-Semite' such is the scale of perceptual programming.

Moving on America

I tell the story in *The Trigger* and a chapter called 'Atlantic Crossing' how particularly after Israel was established the Sabbatians moved in on the United States and eventually grasped control of government administration,

the political system via both Democrats and Republicans, the intelligence community like the CIA and National Security Agency (NSA), the Pentagon and mass media. Through this seriously compartmentalised network Sabbatians and their operatives in Mossad, Israeli Defense Forces (IDF) and US agencies pulled off 9/11 and blamed it on 19 'Al-Qaeda hijackers' dominated by men from, or connected to, Sabbatian-ruled Saudi Arabia. The '19' were not even on the planes let alone flew those big passenger jets into buildings while being largely incompetent at piloting one-engine light aircraft. 'Hijacker' Hani Hanjour who is said to have flown American Airlines Flight 77 into the Pentagon with a turn and manoeuvre most professional pilots said they would have struggled to do was banned from renting a small plane by instructors at the Freeway Airport in Bowie, Maryland, just *six weeks* earlier on the grounds that he was an incompetent pilot. The Jewish population of the world is just 0.2 percent with even that almost entirely concentrated in Israel (75 percent Jewish) and the United States (around two percent). This two percent and globally 0.2 percent refers to *Jewish* people and not Sabbatian interlopers who are a fraction of that fraction. What a sobering thought when you think of the fantastic influence on world affairs of tiny Israel and that the Project for the New America Century (PNAC) which laid out the blueprint in September, 2000, for America's war on terror and regime change wars in Iraq, Libya and Syria was founded and dominated by Sabbatians known as 'Neocons'. The document conceded that this plan would not be supported politically or publicly without a major attack on American soil and a Problem-Reaction-Solution excuse to send troops to war across the Middle East. Sabbatian Neocons said:

> ... [The] process of transformation ... [war and regime change] ... is likely to be a long one, absent some catastrophic and catalysing event – like a new Pearl Harbor.

Four months later many of those who produced that document came to power with their inane puppet George Bush from the long-time Sabbatian Bush family. They included Sabbatian Dick Cheney who was officially vice-president, but really de-facto president for the entirety of the 'Bush' government. Nine months after the 'Bush' inauguration came what Bush called at the time 'the Pearl Harbor of the 21st century' and with typical

Sabbatian timing and symbolism 2001 was the 60th anniversary of the attack in 1941 by the Japanese Air Force on Pearl Harbor, Hawaii, which allowed President Franklin Delano Roosevelt to take the United States into a Sabbatian-instigated Second World War that he said in his election campaign that he never would. The evidence is overwhelming that Roosevelt and his military and intelligence networks knew the attack was coming and did nothing to stop it, but they did make sure that America's most essential naval ships were not in Hawaii at the time. Three thousand Americans died in the Pearl Harbor attacks as they did on September 11th. By the 9/11 year of 2001 Sabbatians had widely infiltrated the US government, military and intelligence operations and used their compartmentalised assets to pull off the 'Al-Qaeda' attacks. If you read *The Trigger* it will blow your mind to see the utterly staggering concentration of 'Jewish' operatives (Sabbatian infiltrators) in essential positions of political, security, legal, law enforcement, financial and business power before, during, and after the attacks to make them happen, carry them out, and then cover their tracks – and I do mean *staggering* when you think of that 0.2 percent of the world population and two percent of Americans which are Jewish while Sabbatian infiltrators are a fraction of that. A central foundation of the 9/11 conspiracy was the hijacking of government, military, Air Force and intelligence computer systems in real time through 'back-door' access made possible by Israeli (Sabbatian) 'cyber security' software. Sabbatian-controlled Israel is on the way to rivalling Silicon Valley for domination of cyberspace and is becoming the dominant force in cyber-security which gives them access to entire computer systems and their passcodes across the world. Then add to this that Zionists head (officially) Silicon Valley giants like Google (Larry Page and Sergey Brin), Google-owned YouTube (Susan Wojcicki), Facebook (Mark Zuckerberg and Sheryl Sandberg), and Apple (Chairman Arthur D. Levinson), and that ultra-Zionist hedge fund billionaire Paul Singer has a $1 billion stake in Twitter which is only nominally headed by 'CEO' pothead Jack Dorsey. As cable news host Tucker Carlson said of Dorsey: 'There used to be debate in the medical community whether dropping a ton of acid had permanent effects and I think that debate has now ended.' Carlson made the comment after Dorsey told a hearing on Capitol Hill (if you cut through his bullshit) that he believed in free speech so long as he got to decide what you can hear and see. These 'big names' of Silicon Valley are only front men and

women for the Global Cult, not least the Sabbatians, who are the true controllers of these corporations. Does anyone still wonder why these same people and companies have been ferociously censoring and banning people (like me) for exposing any aspect of the Cult agenda and especially the truth about the 'Covid' hoax which Sabbatians have orchestrated?

The Jeffrey Epstein paedophile ring was a Sabbatian operation. He was officially 'Jewish' but he was a Sabbatian and women abused by the ring have told me about the high number of 'Jewish' people involved. The Epstein horror has Sabbatian written all over it and matches perfectly their modus operandi and obsession with sex and ritual. Epstein was running a Sabbatian blackmail ring in which famous people with political and other influence were provided with young girls for sex while everything was being filmed and recorded on hidden cameras and microphones at his New York house, Caribbean island and other properties. Epstein survivors have described this surveillance system to me and some have gone public. Once the famous politician or other figure knew he or she was on video they tended to do whatever they were told. Here we go again …when you've got them by the balls their hearts and minds will follow. Sabbatians use this blackmail technique on a wide scale across the world to entrap politicians and others they need to act as demanded. Epstein's private plane, the infamous 'Lolita Express', had many well-known passengers including Bill Clinton while Bill Gates has flown on an Epstein plane and met with him four years after Epstein had been jailed for paedophilia. They subsequently met many times at Epstein's home in New York according to a witness who was there. Epstein's infamous side-kick was Ghislaine Maxwell, daughter of Mossad agent and ultra-Zionist mega-crooked British businessman, Bob Maxwell, who at one time owned the *Daily Mirror* newspaper. Maxwell was murdered at sea on his boat in 1991 by Sabbatian-controlled Mossad when he became a liability with his business empire collapsing as a former Mossad operative has confirmed (see *The Trigger*).

Money, money, money, funny money …

Before I come to the Sabbatian connection with the last three US presidents I will lay out the crucial importance to Sabbatians of controlling banking and finance. Sabbatian Mayer Amschel Rothschild set out to dominate this arena in his family's quest for total global control. What is freedom? It is, in

effect, choice. The more choices you have the freer you are and the fewer your choices the more you are enslaved. In the global structure created over centuries by Sabbatians the biggest decider and restrictor of choice is … money. Across the world if you ask people what they would like to do with their lives and why they are not doing that they will reply 'I don't have the money'. This is the idea. A global elite of multi-billionaires are described as 'greedy' and that is true on one level; but control of money – who has it and who doesn't – is not primarily about greed. It's about control. Sabbatians have seized ever more control of finance and sucked the wealth of the world out of the hands of the population. We talk now, after all, about the 'One-percent' and even then the wealthiest are a lot fewer even than that. This has been made possible by a money scam so outrageous and so vast it could rightly be called the scam of scams founded on creating 'money' out of nothing and 'loaning' that with interest to the population. Money out of nothing is called 'credit'. Sabbatians have asserted control over governments and banking ever more completely through the centuries and secured financial laws that allow banks to lend hugely more than they have on deposit in a confidence trick known as fractional reserve lending. Imagine if you could lend money that doesn't exist and charge the recipient interest for doing so. You would end up in jail. Bankers by contrast end up in mansions, private jets, Malibu and Monaco.

Banks are only required to keep a fraction of their deposits and wealth in their vaults and they are allowed to lend 'money' they don't have called 'credit. Go into a bank for a loan and if you succeed the banker will not move any real wealth into your account. They will type into your account the amount of the agreed 'loan' – say £100,000. This is not wealth that really exists; it is non-existent, fresh-air, created-out-of-nothing 'credit' which has never, does not, and will never exist except in theory. Credit is backed by nothing except wind and only has buying power because people think that it has buying power and accept it in return for property, goods and services. I have described this situation as like those cartoon characters you see chasing each other and when they run over the edge of a cliff they keep running forward on fresh air until one of them looks down, realises what's happened, and they all crash into the ravine. The whole foundation of the Sabbatian financial system is to stop people looking down except for periodic moments when they want to crash the system (as in 2008 and 2020 ongoing) and reap the rewards from all the property, businesses and wealth

their borrowers had signed over as ‘collateral’ in return for a ‘loan’ of fresh air. Most people think that money is somehow created by governments when it comes into existence from the start as a debt through banks ‘lending’ illusory money called credit. Yes, the very currency of exchange is a *debt* from day one issued as an interest-bearing loan. Why don’t governments create money interest-free and lend it to their people interest-free? Governments are controlled by Sabbatians and the financial system is controlled by Sabbatians for whom interest-free money would be a nightmare come true. Sabbatians underpin their financial domination through their global network of central banks, including the privately-owned US Federal Reserve and Britain’s Bank of England, and this is orchestrated by a privately-owned central bank coordination body called the Bank for International Settlements in Basle, Switzerland, created by the usual suspects including the Rockefellers and Rothschilds. Central bank chiefs don’t answer to governments or the people. They answer to the Bank for International Settlements or, in other words, the Global Cult which is dominated today by Sabbatians.

Built-in disaster

There are so many constituent scams within the overall banking scam. When you take out a loan of thin-air credit only the amount of that loan is theoretically brought into circulation to add to the amount in circulation; but you are paying back the principle plus interest. The additional interest is not created and this means that with every ‘loan’ there is a shortfall in the money in circulation between what is borrowed and what has to be paid back. There is never even close to enough money in circulation to repay all outstanding public and private debt including interest. Coldly weaved in the very fabric of the system is the certainty that some will lose their homes, businesses and possessions to the banking ‘lender’. This is less obvious in times of ‘boom’ when the amount of money in circulation (and the debt) is expanding through more people wanting and getting loans. When a downturn comes and the money supply contracts it becomes painfully obvious that there is not enough money to service all debt and interest. This is less obvious in times of ‘boom’ when the amount of money in circulation (and the debt) is expanding through more people wanting and getting loans. When a downturn comes and the money supply contracts and it becomes

painfully obvious – as in 2008 and currently – that there is not enough money to service all debt and interest. Sabbatian banksters have been leading the human population through a calculated series of booms (more debt incurred) and busts (when the debt can't be repaid and the banks get the debtor's tangible wealth in exchange for non-existent 'credit'). With each 'bust' Sabbatian bankers have absorbed more of the world's tangible wealth and we end up with the One-percent. Governments are in bankruptcy levels of debt to the same system and are therefore owned by a system they do not control. The Federal Reserve, 'America's central bank', is privately-owned and American presidents only nominally appoint its chairman or woman to maintain the illusion that it's an arm of government. It's not. The 'Fed' is a cartel of private banks which handed billions to its associates and friends after the crash of 2008 and has been Sabbatian-controlled since it was manipulated into being in 1913 through the covert trickery of Rothschild banking agents Jacob Schiff and Paul Warburg, and the Sabbatian Rockefeller family. Somehow from a Jewish population of two-percent and globally 0.2 percent (Sabbatian interlopers remember are far smaller) ultra-Zionists headed the Federal Reserve for 31 years between 1987 and 2018 in the form of Alan Greenspan, Bernard Bernanke and Janet Yellen (now Biden's Treasury Secretary) with Yellen's deputy chairman a Israeli-American duel citizen and ultra-Zionist Stanley Fischer, a former governor of the Bank of Israel. Ultra-Zionist Fed chiefs spanned the presidencies of Ronald Reagan ('Republican'), Father George Bush ('Republican'), Bill Clinton ('Democrat'), Boy George Bush ('Republican') and Barack Obama ('Democrat'). We should really add the pre-Greenspan chairman, Paul Adolph Volcker, 'appointed' by Jimmy Carter ('Democrat') who ran the Fed between 1979 and 1987 during the Carter and Reagan administrations before Greenspan took over. Volcker was a long-time associate and business partner of the Rothschilds. No matter what the 'party' officially in power the United States economy was directed by the same force. Here are members of the Obama, Trump and Biden administrations and see if you can make out a common theme.

Barack Obama ('Democrat')

Ultra-Zionists Robert Rubin, Larry Summers, and Timothy Geithner ran the US Treasury in the Clinton administration and two of them reappeared with

Obama. Ultra-Zionist Fed chairman Alan Greenspan had manipulated the crash of 2008 through deregulation and jumped ship just before the disaster to make way for ultra-Zionist Bernard Bernanke to hand out trillions to Sabbatian 'too big to fail' banks and businesses, including the ubiquitous ultra-Zionist Goldman Sachs which has an ongoing staff revolving door operation between itself and major financial positions in government worldwide. Obama inherited the fallout of the crash when he took office in January, 2009, and fortunately he had the support of his ultra-Zionist White House Chief of Staff Rahm Emmanuel, son of a terrorist who helped to bomb Israel into being in 1948, and his ultra-Zionist senior adviser David Axelrod, chief strategist in Obama's two successful presidential campaigns. Emmanuel, later mayor of Chicago and former senior fundraiser and strategist for Bill Clinton, is an example of the Sabbatian policy after Israel was established of migrating insider families to America so their children would be born American citizens. 'Obama' chose this financial team throughout his administration to respond to the Sabbatian-instigated crisis:

Timothy Geithner (ultra-Zionist) Treasury Secretary; Jacob J. Lew, Treasury Secretary; Larry Summers (ultra-Zionist), director of the White House National Economic Council; Paul Adolph Volcker (Rothschild business partner), chairman of the Economic Recovery Advisory Board; Peter Orszag (ultra-Zionist), director of the Office of Management and Budget overseeing all government spending; Penny Pritzker (ultra-Zionist), Commerce Secretary; Jared Bernstein (ultra-Zionist), chief economist and economic policy adviser to Vice President Joe Biden; Mary Schapiro (ultra-Zionist), chair of the Securities and Exchange Commission (SEC); Gary Gensler (ultra-Zionist), chairman of the Commodity Futures Trading Commission (CFTC); Sheila Bair (ultra-Zionist), chair of the Federal Deposit Insurance Corporation (FDIC); Karen Mills (ultra-Zionist), head of the Small Business Administration (SBA); Kenneth Feinberg (ultra-Zionist), Special Master for Executive [bail-out] Compensation. Feinberg would be appointed to oversee compensation (with strings) to 9/11 victims and families in a campaign to stop them having their day in court to question the official story. At the same time ultra-Zionist Bernard Bernanke was chairman of the Federal Reserve and these are only some of the ultra-Zionists with allegiance to Sabbatian-controlled Israel in the Obama government. Obama's biggest corporate donor was ultra-Zionist Goldman Sachs which had employed many in his administration.

Donald Trump (‘Republican’)

Trump claimed to be an outsider (he wasn’t) who had come to ‘drain the swamp’. He embarked on this goal by immediately appointing ultra-Zionist Steve Mnuchin, a Goldman Sachs employee for 17 years, as his Treasury Secretary. Others included Gary Cohn (ultra-Zionist), chief operating officer of Goldman Sachs, his first Director of the National Economic Council and chief economic adviser, who was later replaced by Larry Kudlow (ultra-Zionist). Trump’s senior adviser throughout his four years in the White House was his sinister son-in-law Jared Kushner, a life-long friend of Israel Prime Minister Benjamin Netanyahu. Kushner is the son of a convicted crook who was pardoned by Trump in his last days in office. Other ultra-Zionists in the Trump administration included: Stephen Miller, Senior Policy Adviser; Avrahm Berkowitz, Deputy Adviser to Trump and his Senior Adviser Jared Kushner; Ivanka Trump, Adviser to the President, who converted to Judaism when she married Jared Kushner; David Friedman, Trump lawyer and Ambassador to Israel; Jason Greenblatt, Trump Organization executive vice president and chief legal officer, who was made Special Representative for International Negotiations and the Israeli-Palestinian Conflict; Rod Rosenstein, Deputy Attorney General; Elliot Abrams, Special Representative for Venezuela, then Iran; John Eisenberg, National Security Council Legal Adviser and Deputy Council to the President for National Security Affairs; Anne Neuberger, Deputy National Manager, National Security Agency; Ezra Cohen-Watnick, Acting Under Secretary of Defense for Intelligence; Elan Carr, Special Envoy to monitor and combat anti-Semitism; Len Khodorkovsky, Deputy Special Envoy to monitor and combat anti-Semitism; Reed Cordish, Assistant to the President, Intragovernmental and Technology Initiatives. Trump Vice President Mike Pence and Secretary of State Mike Pompeo, both Christian Zionists, were also vehement supporters of Israel and its goals and ambitions.

Donald ‘free-speech believer’ Trump pardoned a number of financial and violent criminals while ignoring calls to pardon Julian Assange and Edward Snowden whose crimes are revealing highly relevant information about government manipulation and corruption and the widespread illegal surveillance of the American people by US ‘security’ agencies. It’s so good to know that Trump is on the side of freedom and justice and not mega-criminals with allegiance to Sabbatian-controlled Israel. These included a

pardon for Israeli spy Jonathan Pollard who was jailed for life in 1987 under the Espionage Act. Aviem Sella, the Mossad agent who recruited Pollard, was also pardoned by Trump while Assange sat in jail and Snowden remained in exile in Russia. Sella had 'fled' (was helped to escape) to Israel in 1987 and was never extradited despite being charged under the Espionage Act. A Trump White House statement said that Sella's clemency had been 'supported by Benjamin Netanyahu, Ron Dermer, Israel's US Ambassador, David Friedman, US Ambassador to Israel and Miriam Adelson, wife of leading Trump donor Sheldon Adelson who died shortly before. Other friends of Jared Kushner were pardoned along with Sholom Weiss who was believed to be serving the longest-ever white-collar prison sentence of more than 800 years in 2000. The sentence was commuted of Ponzi-schemer Eliyahu Weinstein who defrauded Jews and others out of $200 million. I did mention that Assange and Snowden were ignored, right? Trump gave Sabbatians almost everything they asked for in military and political support, moving the US Embassy from Tel Aviv to Jerusalem with its critical symbolic and literal implications for Palestinian statehood, and the 'deal of the Century' designed by Jared Kushner and David Friedman which gave the Sabbatian Israeli government the green light to substantially expand its already widespread program of building illegal Jewish-only settlements in the occupied land of the West Bank. This made a two-state 'solution' impossible by seizing all the land of a potential Palestinian homeland and that had been the plan since 1948 and then 1967 when the Arab-controlled Gaza Strip, West Bank, Sinai Peninsula and Syrian Golan Heights were occupied by Israel. All the talks about talks and road maps and delays have been buying time until the West Bank was physically occupied by Israeli real estate. Trump would have to be a monumentally ill-informed idiot not to see that this was the plan he was helping to complete. The Trump administration was in so many ways the Kushner administration which means the Netanyahu administration which means the Sabbatian administration. I understand why many opposing Cult fascism in all its forms gravitated to Trump, but he was a crucial part of the Sabbatian plan and I will deal with this in the next chapter.

Joe Biden ('Democrat')

A barely cognitive Joe Biden took over the presidency in January, 2021, along with his fellow empty shell, Vice-President Kamala Harris, as the latest Sabbatian gofers to enter the White House. Names on the door may have changed and the 'party' – the force behind them remained the same as Zionists were appointed to a stream of pivotal areas relating to Sabbatian plans and policy. They included: Janet Yellen, Treasury Secretary, former head of the Federal Reserve, and still another ultra-Zionist running the US Treasury after Mnuchin (Trump), Lew and Geithner (Obama), and Summers and Rubin (Clinton); Anthony Blinken, Secretary of State; Wendy Sherman, Deputy Secretary of State (so that's 'Biden's' Sabbatian foreign policy sorted); Jeff Zients, White House coronavirus coordinator; Rochelle Walensky, head of the Centers for Disease Control; Rachel Levine, transgender deputy health secretary (that's 'Covid' hoax policy under control); Merrick Garland, Attorney General; Alejandro Mayorkas, Secretary of Homeland Security; Cass Sunstein, Homeland Security with responsibility for new immigration laws; Avril Haines, Director of National Intelligence; Anne Neuberger, National Security Agency cybersecurity director (note, cybersecurity); David Cohen, CIA Deputy Director; Ronald Klain, Biden's Chief of Staff (see Rahm Emanuel); Eric Lander, a 'leading geneticist', Office of Science and Technology Policy director (see Smart Grid, synthetic biology agenda); Jessica Rosenworcel, acting head of the Federal Communications Commission (FCC) which controls Smart Grid technology policy and electromagnetic communication systems including 5G. How can it be that so many pivotal positions are held by two-percent of the American population and 0.2 percent of the world population administration after administration no matter who is the president and what is the party? It's a coincidence? Of course it's not and this is why Sabbatians have built their colossal global web of interlocking 'anti-hate' hate groups to condemn anyone who asks these glaring questions as an 'anti-Semite'. The way that Jewish people horrifically abused in Sabbatian-backed Nazi Germany are exploited to this end is stomach-turning and disgusting beyond words.

Political fusion

Sabbatian manipulation has reversed the roles of Republicans and Democrats and the same has happened in Britain with the Conservative and

Labour Parties. Republicans and Conservatives were always labelled the 'right' and Democrats and Labour the 'left', but look at the policy positions now and the Democrat-Labour 'left' has moved further to the 'right' than Republicans and Conservatives under the banner of 'Woke', the Cult-created far-right tyranny. Where once the Democrat-Labour 'left' defended free speech and human rights they now seek to delete them and as I said earlier despite the 'Covid' fascism of the Jackboot Johnson Conservative government in the UK the Labour Party of leader Keir Starmer demanded even more extreme measures. The Labour Party has been very publicly absorbed by Sabbatians after a political and media onslaught against the previous leader, the weak and inept Jeremy Corbyn, over made-up allegations of 'anti-Semitism' both by him and his party. The plan was clear with this 'anti-Semite' propaganda and what was required in response was a swift and decisive 'fuck off' from Corbyn and a statement to expose the Anti-Semitism Industry (Sabbatian) attempt to silence Labour criticism of the Israeli government (Sabbatians) and purge the party of all dissent against the extremes of ultra-Zionism (Sabbatians). Instead Corbyn and his party fell to their knees and appeased the abusers which, by definition, is impossible. Appeasing one demand leads only to a new demand to be appeased until takeover is complete. Like I say – 'fuck off' would have been a much more effective policy and I have used it myself with great effect over the years when Sabbatians are on my case which is most of the time. I consider that fact a great compliment, by the way. The outcome of the Labour Party capitulation is that we now have a Sabbatian-controlled Conservative Party 'opposed' by a Sabbatian-controlled Labour Party in a one-party Sabbatian state that hurtles towards the extremes of tyranny (the Sabbatian cult agenda). In America the situation is the same. Labour's Keir Starmer spends his days on his knees with his tongue out pointing to Tel Aviv, or I guess now Jerusalem, while Boris Johnson has an 'anti-Semitism czar' in the form of former Labour MP John Mann who keeps Starmer company on his prayer mat.

Sabbatian influence can be seen in Jewish members of the Labour Party who have been ejected for criticism of Israel including those from families that suffered in Nazi Germany. Sabbatians despise real Jewish people and target them even more harshly because it is so much more difficult to dub them 'anti-Semitic' although in their desperation they do try.

CHAPTER THREE

The Pushbacker sting

Until you realize how easy it is for your mind to be manipulated, you remain the puppet of someone else's game
Evita Ochel

I will use the presidencies of Trump and Biden to show how the manipulation of the one-party state plays out behind the illusion of political choice across the world. No two presidencies could – on the face of it – be more different and apparently at odds in terms of direction and policy.

A Renegade Mind sees beyond the obvious and focuses on outcomes and consequences and not image, words and waffle. The Cult embarked on a campaign to divide America between those who blindly support its agenda (the mentality known as 'Woke') and those who are pushing back on where the Cult and its Sabbatians want to go. This presents infinite possibilities for dividing and ruling the population by setting them at war with each other and allows a perceptual ring fence of demonisation to encircle the Pushbackers in a modern version of the Little Big Horn in 1876 when American cavalry led by Lieutenant Colonel George Custer were drawn into a trap, surrounded and killed by Native American tribes defending their land of thousands of years from being seized by the government. In this modern version the roles are reversed and it's those defending themselves from the Sabbatian government who are surrounded and the government that's seeking to destroy them. This trap was set years ago and to explain how we must return to 2016 and the emergence of Donald Trump as a candidate to be President of the United States. He set out to overcome the

best part of 20 other candidates in the Republican Party before and during the primaries and was not considered by many in those early stages to have a prayer of living in the White House. The Republican Party was said to have great reservations about Trump and yet somehow he won the nomination. When you know how American politics works – politics in general – there is no way that Trump could have become the party's candidate unless the Sabbatian-controlled 'Neocons' that run the Republican Party wanted that to happen. We saw the proof in emails and documents made public by WikiLeaks that the Democratic Party hierarchy, or Democons, systematically undermined the campaign of Bernie Sanders to make sure that Sabbatian gofer Hillary Clinton won the nomination to be their presidential candidate. If the Democons could do that then the Neocons in the Republican Party could have derailed Trump in the same way. But they didn't and at that stage I began to conclude that Trump could well be the one chosen to be president. If that was the case the 'why' was pretty clear to see – the goal of dividing America between Cult agenda-supporting Wokers and Pushbackers who gravitated to Trump because he was telling them what they wanted to hear. His constituency of support had been increasingly ignored and voiceless for decades and profoundly through the eight years of Sabbatian puppet Barack Obama. Now here was someone speaking their language of pulling back from the incessant globalisation of political and economic power, the exporting of American jobs to China and elsewhere by 'American' (Sabbatian) corporations, the deletion of free speech, and the mass immigration policies that had further devastated job opportunities for the urban working class of all races and the once American heartlands of the Midwest.

Beware the forked tongue

Those people collectively sighed with relief that at last a political leader was apparently on their side, but another trait of the Renegade Mind is that you look even harder at people telling you what you want to hear than those who are telling you otherwise. Obviously as I said earlier people wish what they want to hear to be true and genuine and they are much more likely to believe that than someone saying what they don't want to here and don't want to be true. Sales people are taught to be skilled in eliciting by calculated questioning what their customers want to hear and repeating that

back to them as their own opinion to get their targets to like and trust them. Assets of the Cult are also sales people in the sense of selling perception. To read Cult manipulation you have to play the long and expanded game and not fall for the Vaudeville show of party politics. Both American parties are vehicles for the Cult and they exploit them in different ways depending on what the agenda requires at that moment. Trump and the Republicans were used to be the focus of dividing America and isolating Pushbackers to open the way for a Biden presidency to become the most extreme in American history by advancing the full-blown Woke (Cult) agenda with the aim of destroying and silencing Pushbackers now labelled Nazi Trump supporters and white supremacists.

Sabbatians wanted Trump in office for the reasons described by ultra-Zionist Saul Alinsky (1909-1972) who was promoting the Woke philosophy through 'community organising' long before anyone had heard of it. In those days it still went by its traditional name of Marxism. The reason for the manipulated Trump phenomenon was laid out in Alinsky's 1971 book, *Rules for Radicals*, which was his blueprint for overthrowing democratic and other regimes and replacing them with Sabbatian Marxism. Not surprisingly his to-do list was evident in the Sabbatian French and Russian 'Revolutions' and that in China which will become very relevant in the next chapter about the 'Covid' hoax. Among Alinsky's followers have been the deeply corrupt Barack Obama, House Speaker Nancy Pelosi and Hillary Clinton who described him as a 'hero'. All three are Sabbatian stooges with Pelosi personifying the arrogant corrupt idiocy that so widely fronts up for the Cult inner core. Predictably as a Sabbatian advocate of the 'light-bringer' Alinsky features Lucifer on the dedication page of his book as the original radical who gained his own kingdom ('Earth' as we shall see). One of Alinsky's golden radical rules was to pick an individual and focus all attention, hatred and blame on them and not to target faceless bureaucracies and corporations. *Rules for Radicals* is really a Sabbatian handbook with its contents repeatedly employed all over the world for centuries and why wouldn't Sabbatians bring to power their designer-villain to be used as the individual on which all attention, hatred and blame was bestowed? This is what they did and the only question for me is how much Trump knew that and how much he was manipulated. A bit of both, I suspect. This was Alinsky's Trump technique from a man who died in 1972. The technique has spanned history:

Pick the target, freeze it, personalize it, polarize it. Don't try to attack abstract corporations or bureaucracies. Identify a responsible individual. Ignore attempts to shift or spread the blame.

From the moment Trump came to illusory power everything was about him. It wasn't about Republican policy or opinion, but all about Trump. Everything he did was presented in negative, derogatory and abusive terms by the Sabbatian-dominated media led by Cult operations such as CNN, MSNBC, *The New York Times* and the Jeff Bezos-owned *Washington Post* – 'Pick the target, freeze it, personalize it, polarize it.' Trump was turned into a demon to be vilified by those who hated him and a demi-god loved by those who worshipped him. This, in turn, had his supporters, too, presented as equally demonic in preparation for the punchline later down the line when Biden was about to take office. It was here's a Trump, there's a Trump, everywhere a Trump, Trump. Virtually every news story or happening was filtered through the lens of 'The Donald'. You loved him or hated him and which one you chose was said to define you as Satan's spawn or a paragon of virtue. Even supporting some Trump policies or statements and not others was enough for an assault on your character. No shades of grey were or are allowed. Everything is black and white (literally and figuratively). A Californian I knew had her head utterly scrambled by her hatred for Trump while telling people they should love each other. She was so totally consumed by Trump Derangement Syndrome as it became to be known that this glaring contradiction would never have occurred to her. By definition anyone who criticised Trump or praised his opponents was a hero and this lady described Joe Biden as 'a kind, honest gentleman' when he's a provable liar, mega-crook and vicious piece of work to boot. Sabbatians had indeed divided America using Trump as the fall-guy and all along the clock was ticking on the consequences for his supporters.

In hock to his masters

Trump gave Sabbatians via Israel almost everything they wanted in his four years. Ask and you shall receive was the dynamic between himself and Benjamin Netanyahu orchestrated by Trump's ultra-Zionist son-in-law Jared Kushner, his ultra-Zionist Ambassador to Israel, David Friedman, and ultra-Zionist 'Israel adviser', Jason Greenblatt. The last two were central to the running and protecting from collapse of his business empire, the Trump

Organisation, and colossal business failures made him forever beholding to Sabbatian networks that bailed him out. By the start of the 1990s Trump owed $4 billion to banks that he couldn't pay and almost $1billion of that was down to him personally and not his companies. This mega-disaster was the result of building two new casinos in Atlantic City and buying the enormous Taj Mahal operation which led to crippling debt payments. He had borrowed fantastic sums from 72 banks with major Sabbatian connections and although the scale of debt should have had him living in a tent alongside the highway they never foreclosed. A plan was devised to lift Trump from the mire by BT Securities Corporation and Rothschild Inc. and the case was handled by Wilber Ross who had worked for the Rothschilds for 27 years. Ross would be named US Commerce Secretary after Trump's election. Another crucial figure in saving Trump was ultra-Zionist 'investor' Carl Icahn who bought the Taj Mahal casino. Icahn was made special economic adviser on financial regulation in the Trump administration. He didn't stay long but still managed to find time to make a tidy sum of a reported $31.3 million when he sold his holdings affected by the price of steel three days before Trump imposed a 235 percent tariff on steel imports. What amazing bits of luck these people have. Trump and Sabbatian operatives have long had a close association and his mentor and legal adviser from the early 1970s until 1986 was the dark and genetically corrupt ultra-Zionist Roy Cohn who was chief counsel to Senator Joseph McCarthy's 'communist' witch-hunt in the 1950s. *Esquire* magazine published an article about Cohn with the headline 'Don't mess with Roy Cohn'. He was described as the most feared lawyer in New York and 'a ruthless master of dirty tricks ... [with] ... more than one Mafia Don on speed dial'. Cohn's influence, contacts, support and protection made Trump a front man for Sabbatians in New York with their connections to one of Cohn's many criminal employers, the 'Russian' Sabbatian Mafia. Israel-centric media mogul Rupert Murdoch was introduced to Trump by Cohn and they started a long friendship. Cohn died in 1986 weeks after being disbarred for unethical conduct by the Appellate Division of the New York State Supreme Court. The wheels of justice do indeed run slow given the length of Cohn's crooked career.

QAnon-sense

We are asked to believe that Donald Trump with his fundamental connections to Sabbatian networks and operatives has been leading the fight to stop the Sabbatian agenda for the fascistic control of America and the world. Sure he has. A man entrapped during his years in the White House by Sabbatian operatives and whose biggest financial donor was casino billionaire Sheldon Adelson who was Sabbatian to his DNA?? Oh, do come on. Trump has been used to divide America and isolate Pushbackers on the Cult agenda under the heading of 'Trump supporters', 'insurrectionists' and 'white supremacists'. The US Intelligence/Mossad Psyop or psychological operation known as QAnon emerged during the Trump years as a central pillar in the Sabbatian campaign to lead Pushbackers into the trap set by those that wished to destroy them. I knew from the start that QAnon was a scam because I had seen the same scenario many times before over 30 years under different names and I had written about one in particular in the books. 'Not again' was my reaction when QAnon came to the fore. The same script is pulled out every few years and a new name added to the letterhead. The story always takes the same form: 'Insiders' or 'the good guys' in the government-intelligence-military 'Deep State' apparatus were going to instigate mass arrests of the 'bad guys' which would include the Rockefellers, Rothschilds, Barack Obama, Hillary Clinton, George Soros, etc., etc. Dates are given for when the 'good guys' are going to move in, but the dates pass without incident and new dates are given which pass without incident. The central message to Pushbackers in each case is that they don't have to do anything because there is 'a plan' and it is all going to be sorted by the 'good guys' on the inside. 'Trust the plan' was a QAnon mantra when the only plan was to misdirect Pushbackers into putting their trust in a Psyop they believed to be real. Beware, beware, those who tell you what you want to hear and always check it out. Right up to Biden's inauguration QAnon was still claiming that 'the Storm' was coming and Trump would stay on as president when Biden and his cronies were arrested and jailed. It was never going to happen and of course it didn't, but what did happen as a result provided that punchline to the Sabbatian Trump/QAnon Psyop.

On January 6th, 2021, a very big crowd of Trump supporters gathered in the National Mall in Washington DC down from the Capitol Building to protest at what they believed to be widespread corruption and vote fraud that stopped Trump being re-elected for a second term as president in November, 2020. I say as someone that does not support Trump or Biden

that the evidence is clear that major vote-fixing went on to favour Biden, a man with cognitive problems so advanced he can often hardly string a sentence together without reading the words written for him on the Teleprompter. Glaring ballot discrepancies included serious questions about electronic voting machines that make vote rigging a comparative cinch and hundreds of thousands of paper votes that suddenly appeared during already advanced vote counts and virtually all of them for Biden. Early Trump leads in crucial swing states suddenly began to close and disappear. The pandemic hoax was used as the excuse to issue almost limitless numbers of mail-in ballots with no checks to establish that the recipients were still alive or lived at that address. They were sent to streams of people who had not even asked for them. Private organisations were employed to gather these ballots and who knows what they did with them before they turned up at the counts. The American election system has been manipulated over decades to become a sick joke with more holes than a Swiss cheese for the express purpose of dictating the results. Then there was the criminal manipulation of information by Sabbatian tech giants like Facebook, Twitter and Google-owned YouTube which deleted pro-Trump, anti-Biden accounts and posts while everything in support of Biden was left alone. Sabbatians wanted Biden to win because after the dividing of America it was time for full-on Woke and every aspect of the Cult agenda to be unleashed.

Hunter gatherer

Extreme Silicon Valley bias included blocking information by the *New York Post* exposing a Biden scandal that should have ended his bid for president in the final weeks of the campaign. Hunter Biden, his monumentally corrupt son, is reported to have sent a laptop to be repaired at a local store and failed to return for it. Time passed until the laptop became the property of the store for non-payment of the bill. When the owner saw what was on the hard drive he gave a copy to the FBI who did nothing even though it confirmed widespread corruption in which the Joe Biden family were using his political position, especially when he was vice president to Obama, to make multiple millions in countries around the world and most notably Ukraine and China. Hunter Biden's one-time business partner Tony Bobulinski went public when the story broke in the *New York Post* to confirm the corruption he saw and that Joe Biden not only knew what was

going on he also profited from the spoils. Millions were handed over by a Chinese company with close connections – like all major businesses in China – to the Chinese communist party of President Xi Jinping. Joe Biden even boasted at a meeting of the Cult's World Economic Forum that as vice president he had ordered the government of Ukraine to fire a prosecutor. What he didn't mention was that the same man just happened to be investigating an energy company which was part of Hunter Biden's corrupt portfolio. The company was paying him big bucks for no other reason than the influence his father had. Overnight Biden's presidential campaign should have been over given that he had lied publicly about not knowing what his son was doing. Instead almost the entire Sabbatian-owned mainstream media and Sabbatian-owned Silicon Valley suppressed circulation of the story. This alone went a mighty way to rigging the election of 2020. Cult assets like Mark Zuckerberg at Facebook also spent hundreds of millions to be used in support of Biden and vote 'administration'.

The Cult had used Trump as the focus to divide America and was now desperate to bring in moronic, pliable, corrupt Biden to complete the double-whammy. No way were they going to let little things like the will of the people thwart their plan. Silicon Valley widely censored claims that the election was rigged because it *was* rigged. For the same reason anyone claiming it was rigged was denounced as a 'white supremacist' including the pathetically few Republican politicians willing to say so. Right across the media where the claim was mentioned it was described as a 'false claim' even though these excuses for 'journalists' would have done no research into the subject whatsoever. Trump won seven million more votes than any sitting president had ever achieved while somehow a cognitively-challenged soon to be 78-year-old who was hidden away from the public for most of the campaign managed to win more votes than any presidential candidate in history. It makes no sense. You only had to see election rallies for both candidates to witness the enthusiasm for Trump and the apathy for Biden. Tens of thousands would attend Trump events while Biden was speaking in empty car parks with often only television crews attending and framing their shots to hide the fact that no one was there. It was pathetic to see footage come to light of Biden standing at a podium making speeches only to TV crews and party fixers while reading the words written for him on massive Teleprompter screens. So, yes, those protestors on January 6th

had a point about election rigging, but some were about to walk into a trap laid for them in Washington by the Cult Deep State and its QAnon Psyop. This was the Capitol Hill riot ludicrously dubbed an ‘insurrection’.

The spider and the fly

Renegade Minds know there are not two ‘sides’ in politics, only one side, the Cult, working through all ‘sides’. It’s a stage show, a puppet show, to direct the perceptions of the population into focusing on diversions like parties and candidates while missing the puppeteers with their hands holding all the strings. The Capitol Hill ‘insurrection’ brings us back to the Little Big Horn. Having created two distinct opposing groupings – Woke and Pushbackers – the trap was about to be sprung. Pushbackers were to be encircled and isolated by associating them all in the public mind with Trump and then labelling Trump as some sort of Confederate leader. I knew immediately that the Capitol riot was a set-up because of two things. One was how easy the rioters got into the building with virtually no credible resistance and secondly I could see – as with the ‘Covid’ hoax in the West at the start of 2020 – how the Cult could exploit the situation to move its agenda forward with great speed. My experience of Cult techniques and activities over more than 30 years has showed me that while they do exploit situations they haven’t themselves created this never happens with events of fundamental agenda significance. Every time major events giving cultists the excuse to rapidly advance their plan you find they are manipulated into being for the specific reason of providing that excuse – Problem-Reaction-Solution. Only a tiny minority of the huge crowd of Washington protestors sought to gain entry to the Capitol by smashing windows and breaching doors. That didn’t matter. The whole crowd and all Pushbackers, even if they did not support Trump, were going to be lumped together as dangerous insurrectionists and conspiracy theorists. The latter term came into widespread use through a CIA memo in the 1960s aimed at discrediting those questioning the nonsensical official story of the Kennedy assassination and it subsequently became widely employed by the media. It’s still being used by inept ‘journalists’ with no idea of its origin to discredit anyone questioning anything that authority claims to be true. When you are perpetrating a conspiracy you need to discredit the very word itself even though the dictionary definition of conspiracy is merely ‘the

activity of secretly planning with other people to do something bad or illegal‘ and ‘a general agreement to keep silent about a subject for the purpose of keeping it secret’. On that basis there are conspiracies almost wherever you look. For obvious reasons the Cult and its lapdog media have to claim there are no conspiracies even though the word appears in state laws as with conspiracy to defraud, to murder, and to corrupt public morals.

Agent provocateurs are widely used by the Cult Deep State to manipulate genuine people into acting in ways that suit the desired outcome. By genuine in this case I mean protestors genuinely supporting Trump and claims that the election was stolen. In among them, however, were agents of the state wearing the garb of Trump supporters and QAnon to pump-prime the Capital riot which some genuine Trump supporters naively fell for. I described the situation as ‘Come into my parlour said the spider to the fly’. Leaflets appeared through the Woke paramilitary arm Antifa, the anti-fascist fascists, calling on supporters to turn up in Washington looking like Trump supporters even though they hated him. Some of those arrested for breaching the Capitol Building were sourced to Antifa and its stable mate Black Lives Matter. Both organisations are funded by Cult billionaires and corporations. One man charged for the riot was according to his lawyer a former FBI agent who had held top secret security clearance for 40 years. Attorney Thomas Plofchan said of his client, 66-year-old Thomas Edward Caldwell:

> He has held a Top Secret Security Clearance since 1979 and has undergone multiple Special Background Investigations in support of his clearances. After retiring from the Navy, he worked as a section chief for the Federal Bureau of Investigation from 2009-2010 as a GS-12 [mid-level employee].
>
> He also formed and operated a consulting firm performing work, often classified, for U.S government customers including the US. Drug Enforcement Agency, Department of Housing and Urban Development, the US Coast Guard, and the US Army Personnel Command.

A judge later released Caldwell pending trial in the absence of evidence about a conspiracy or that he tried to force his way into the building. *The New York Post* reported a ‘law enforcement source‘ as saying that ‘at least two known Antifa members were spotted’ on camera among Trump supporters during the riot while one of the rioters arrested was John Earle Sullivan, a seriously extreme Black Lives Matter Trump-hater from Utah

who was previously arrested and charged in July, 2020, over a BLM-Antifa riot in which drivers were threatened and one was shot. Sullivan is the founder of Utah-based Insurgence USA which is an affiliate of the Cult-created-and-funded Black Lives Matter movement. Footage appeared and was then deleted by Twitter of Trump supporters calling out Antifa infiltrators and a group was filmed changing into pro-Trump clothing before the riot. Security at the building was *pathetic* – as planned. Colonel Leroy Fletcher Prouty, a man with long experience in covert operations working with the US security apparatus, once described the tell-tale sign to identify who is involved in an assassination. He said:

> No one has to direct an assassination – it happens. The active role is played secretly by permitting it to happen. This is the greatest single clue. Who has the power to call off or reduce the usual security precautions?

This principle applies to many other situations and certainly to the Capitol riot of January 6th, 2021.

The sting

With such a big and potentially angry crowd known to be gathering near the Capitol the security apparatus would have had a major police detail to defend the building with National Guard troops on standby given the strength of feeling among people arriving from all over America encouraged by the QAnon Psyop and statements by Donald Trump. Instead Capitol Police 'security' was flimsy, weak, and easily breached. The same number of officers was deployed as on a regular day and that is a blatant red flag. They were not staffed or equipped for a possible riot that had been an obvious possibility in the circumstances. No protective and effective fencing worth the name was put in place and there were no contingency plans. The whole thing was basically a case of standing aside and waving people in. Once inside police mostly backed off apart from one Capitol police officer who ridiculously shot dead unarmed Air Force veteran protestor Ashli Babbitt without a warning as she climbed through a broken window. The 'investigation' refused to name or charge the officer after what must surely be considered a murder in the circumstances. They just

lifted a carpet and swept. The story was endlessly repeated about five people dying in the 'armed insurrection' when there was no report of rioters using weapons. Apart from Babbitt the other four died from a heart attack, strokes and apparently a drug overdose. Capitol police officer Brian Sicknick was reported to have died after being bludgeoned with a fire extinguisher when he was alive after the riot was over and died later of what the Washington Medical Examiner's Office said was a stroke. Sicknick had no external injuries. The lies were delivered like rapid fire. There was a narrative to build with incessant repetition of the lie until the lie became the accepted 'everybody knows that' truth. The 'Big Lie' technique of Nazi Propaganda Minister Joseph Goebbels is constantly used by the Cult which was behind the Nazis and is today behind the 'Covid' and 'climate change' hoaxes. Goebbels said:

If you tell a lie big enough and keep repeating it, people will eventually come to believe it. The lie can be maintained only for such time as the State can shield the people from the political, economic and/or military consequences of the lie. It thus becomes vitally important for the State to use all of its powers to repress dissent, for the truth is the mortal enemy of the lie, and thus by extension, the truth is the greatest enemy of the State.

Most protestors had a free run of the Capitol Building. This allowed pictures to be taken of rioters in iconic parts of the building including the Senate chamber which could be used as propaganda images against all Pushbackers. One Congresswoman described the scene as 'the worst kind of non-security anybody could ever imagine'. Well, the first part was true, but someone obviously did imagine it and made sure it happened. Some photographs most widely circulated featured people wearing QAnon symbols and now the Psyop would be used to dub all QAnon followers with the ubiquitous fit-all label of 'white supremacist' and 'insurrectionists'. When a Muslim extremist called Noah Green drove his car at two police officers at the Capitol Building killing one in April, 2021, there was no such political and media hysteria. They were just disappointed he wasn't white.

The witch-hunt

Government prosecutor Michael Sherwin, an aggressive, dark-eyed, professional Rottweiler led the 'investigation' and to call it over the top

would be to understate reality a thousand fold. Hundreds were tracked down and arrested for the crime of having the wrong political views and people were jailed who had done nothing more than walk in the building, committed no violence or damage to property, took a few pictures and left. They were labelled a 'threat to the Republic' while Biden sat in the White House signing executive orders written for him that were dismantling 'the Republic'. Even when judges ruled that a mother and son should not be in jail the government kept them there. Some of those arrested have been badly beaten by prison guards in Washington and lawyers for one man said he suffered a fractured skull and was made blind in one eye. Meanwhile a woman is shot dead for no reason by a Capitol Police officer and we are not allowed to know who he is never mind what has happened to him although that will be *nothing*. The Cult's QAnon/Trump sting to identify and isolate Pushbackers and then target them on the road to crushing and deleting them was a resounding success. You would have thought the Russians had invaded the building at gunpoint and lined up senators for a firing squad to see the political and media reaction. Congresswoman Alexandria Ocasio-Cortez is a child in a woman's body, a terrible-twos, me, me, me, Woker narcissist of such proportions that words have no meaning. She said she thought she was going to die when 'insurrectionists' banged on her office door. It turned out she wasn't even in the Capitol Building when the riot was happening and the 'banging' was a Capitol Police officer. She referred to herself as a 'survivor' which is an insult to all those true survivors of violent and sexual abuse while she lives her pampered and privileged life talking drivel for a living. Her Woke colleague and fellow mega-narcissist Rashida Tlaib broke down describing the devastating effect on her, too, of *not being* in the building when the rioters were there. Ocasio-Cortez and Tlaib are members of a fully-Woke group of Congresswomen known as 'The Squad' along with Ilhan Omar and Ayanna Pressley. The Squad from what I can see can be identified by its vehement anti-white racism, anti-white men agenda, and, as always in these cases, the absence of brain cells on active duty.

The usual suspects were on the riot case immediately in the form of Democrat ultra-Zionist senators and operatives Chuck Schumer and Adam Schiff demanding that Trump be impeached for 'his part in the insurrection'. The same pair of prats had led the failed impeachment of Trump over the invented 'Russia collusion' nonsense which claimed Russia

had helped Trump win the 2016 election. I didn't realise that Tel Aviv had been relocated just outside Moscow. I must find an up-to-date map. The Russia hoax was a Sabbatian operation to keep Trump occupied and impotent and to stop any rapport with Russia which the Cult wants to retain as a perceptual enemy to be pulled out at will. Puppet Biden began attacking Russia when he came to office as the Cult seeks more upheaval, division and war across the world. A two-year stage show 'Russia collusion inquiry' headed by the not-very-bright former 9/11 FBI chief Robert Mueller, with support from 19 lawyers, 40 FBI agents plus intelligence analysts, forensic accountants and other staff, devoured tens of millions of dollars and found no evidence of Russia collusion which a ten-year-old could have told them on day one. Now the same moronic Schumer and Schiff wanted a second impeachment of Trump over the Capitol 'insurrection' (riot) which the arrested development of Schumer called another 'Pearl Harbor' while others compared it with 9/11 in which 3,000 died and, in the case of CNN, with the Rwandan genocide in the 1990s in which an estimated 500,000 to 600,000 were murdered, between 250, 000 and 500,000 women were raped, and populations of whole towns were hacked to death with machetes. To make those comparisons purely for Cult political reasons is beyond insulting to those that suffered and lost their lives and confirms yet again the callous inhumanity that we are dealing with. Schumer is a monumental idiot and so is Schiff, but they serve the Cult agenda and do whatever they're told so they get looked after. Talking of idiots – another inane man who spanned the Russia and Capitol impeachment attempts was Senator Eric Swalwell who had the nerve to accuse Trump of collusion with the Russians while sleeping with a Chinese spy called Christine Fang or 'Fang Fang' which is straight out of a Bond film no doubt starring Klaus Schwab as the bloke living on a secret island and controlling laser weapons positioned in space and pointing at world capitals. Fang Fang plays the part of Bond's infiltrator girlfriend which I'm sure she would enjoy rather more than sharing a bed with the brainless Swalwell, lying back and thinking of China. The FBI eventually warned Swalwell about Fang Fang which gave her time to escape back to the Chinese dictatorship. How very thoughtful of them. The second Trump impeachment also failed and hardly surprising when an impeachment is supposed to remove a sitting president and by the time it happened Trump

was no longer president. These people are running your country America, well, officially anyway. Terrifying isn't it?

Outcomes tell the story - always

The outcome of all this – and it's the *outcome* on which Renegade Minds focus, not the words – was that a vicious, hysterical and obviously pre-planned assault was launched on Pushbackers to censor, silence and discredit them and even targeted their right to earn a living. They have since been condemned as 'domestic terrorists' that need to be treated like Al-Qaeda and Islamic State. 'Domestic terrorists' is a label the Cult has been trying to make stick since the period of the Oklahoma bombing in 1995 which was blamed on 'far-right domestic terrorists'. If you read *The Trigger* you will see that the bombing was clearly a Problem-Reaction-Solution carried out by the Deep State during a Bill Clinton administration so corrupt that no dictionary definition of the term would even nearly suffice. Nearly 30, 000 troops were deployed from all over America to the empty streets of Washington for Biden's inauguration. Ten thousand of them stayed on with the pretext of protecting the capital from insurrectionists when it was more psychological programming to normalise the use of the military in domestic law enforcement in support of the Cult plan for a police-military state. Biden's fascist administration began a purge of 'wrong-thinkers' in the military which means anyone that is not on board with Woke. The Capitol Building was surrounded by a fence with razor wire and the Land of the Free was further symbolically and literally dismantled. The circle was completed with the installation of Biden and the exploitation of the QAnon Psyop.

America had never been so divided since the civil war of the 19th century, Pushbackers were isolated and dubbed terrorists and now, as was always going to happen, the Cult immediately set about deleting what little was left of freedom and transforming American society through a swish of the hand of the most controlled 'president' in American history leading (officially at least) the most extreme regime since the country was declared an independent state on July 4th, 1776. Biden issued undebated, dictatorial executive orders almost by the hour in his opening days in office across the whole spectrum of the Cult wish-list including diluting controls on the border with Mexico allowing thousands of migrants to illegally enter the

United States to transform the demographics of America and import an election-changing number of perceived Democrat voters. Then there were Biden deportation amnesties for the already illegally resident (estimated to be as high as 20 or even 30 million). A bill before Congress awarded American citizenship to anyone who could prove they had worked in agriculture for just 180 days in the previous two years as 'Big Ag' secured its slave labour long-term. There were the plans to add new states to the union such as Puerto Rico and making Washington DC a state. They are all parts of a plan to ensure that the Cult-owned Woke Democrats would be permanently in power.

Border – what border?

I have exposed in detail in other books how mass immigration into the United States and Europe is the work of Cult networks fuelled by the tens of billions spent to this and other ends by George Soros and his global Open Society (open borders) Foundations. The impact can be seen in America alone where the population has increased by *100 million* in little more than 30 years mostly through immigration. I wrote in *The Answer* that the plan was to have so many people crossing the southern border that the numbers become unstoppable and we are now there under Cult-owned Biden. El Salvador in Central America puts the scale of what is happening into context. A third of the population now lives in the United States, much of it illegally, and many more are on the way. The methodology is to crush Central and South American countries economically and spread violence through machete-wielding psychopathic gangs like MS-13 based in El Salvador and now operating in many American cities. Biden-imposed lax security at the southern border means that it is all but open. He said before his 'election' that he wanted to see a surge towards the border if he became president and that was the green light for people to do just that after election day to create the human disaster that followed for both America and the migrants. When that surge came the imbecilic Alexandria Ocasio-Cortez said it wasn't a 'surge' because they are 'children, not insurgents' and the term 'surge' (used by Biden) was a claim of 'white supremacists'. This disingenuous lady may one day enter the realm of the most basic intelligence, but it won't be any time soon.

Sabbatians and the Cult are in the process of destroying America by importing violent people and gangs in among the genuine to terrorise American cities and by overwhelming services that cannot cope with the sheer volume of new arrivals. Something similar is happening in Europe as Western society in general is targeted for demographic and cultural transformation and upheaval. The plan demands violence and crime to create an environment of intimidation, fear and division and Soros has been funding the election of district attorneys across America who then stop prosecuting many crimes, reduce sentences for violent crimes and free as many violent criminals as they can. Sabbatians are creating the chaos from which order – their order – can respond in a classic Problem-Reaction-Solution. A Freemasonic moto says 'Ordo Ab Chao' (Order out of Chaos) and this is why the Cult is constantly creating chaos to impose a new 'order'. Here you have the reason the Cult is constantly creating chaos. The 'Covid' hoax can be seen with those entering the United States by plane being forced to take a 'Covid' test while migrants flooding through southern border processing facilities do not. Nothing is put in the way of mass migration and if that means ignoring the government's own 'Covid' rules then so be it. They know it's all bullshit anyway. Any pushback on this is denounced as 'racist' by Wokers and Sabbatian fronts like the ultra-Zionist Anti-Defamation League headed by the appalling Jonathan Greenblatt which at the same time argues that Israel should not give citizenship and voting rights to more Palestinian Arabs or the 'Jewish population' (in truth the Sabbatian network) will lose control of the country.

Society-changing numbers

Biden's masters have declared that countries like El Salvador are so dangerous that their people must be allowed into the United States for humanitarian reasons when there are fewer murders in large parts of many Central American countries than in US cities like Baltimore. That is not to say Central America cannot be a dangerous place and Cult-controlled American governments have been making it so since way back, along with the dismantling of economies, in a long-term plan to drive people north into the United States. Parts of Central America are very dangerous, but in other areas the story is being greatly exaggerated to justify relaxing immigration criteria. Migrants are being offered free healthcare and education in the

United States as another incentive to head for the border and there is no requirement to be financially independent before you can enter to prevent the resources of America being drained. You can't blame migrants for seeking what they believe will be a better life, but they are being played by the Cult for dark and nefarious ends. The numbers since Biden took office are huge. In February, 2021, more than 100,000 people were known to have tried to enter the US illegally through the southern border (it was 34,000 in the same month in 2020) and in March it was 170,000 – a 418 percent increase on March, 2020. These numbers are only known people, not the ones who get in unseen. The true figure for migrants illegally crossing the border in a single month was estimated by one congressman at 250,000 and that number will only rise under Biden's current policy. Gangs of murdering drug-running thugs that control the Mexican side of the border demand money – thousands of dollars – to let migrants cross the Rio Grande into America. At the same time gun battles are breaking out on the border several times a week between rival Mexican drug gangs (which now operate globally) who are equipped with sophisticated military-grade weapons, grenades and armoured vehicles. While the Capitol Building was being 'protected' from a non-existent 'threat' by thousands of troops, and others were still deployed at the time in the Cult Neocon war in Afghanistan, the southern border of America was left to its fate. This is not incompetence, it is cold calculation.

By March, 2021, there were 17,000 unaccompanied children held at border facilities and many of them are ensnared by people traffickers for paedophile rings and raped on their journey north to America. This is not conjecture – this is fact. Many of those designated children are in reality teenage boys or older. Meanwhile Wokers posture their self-purity for encouraging poor and tragic people to come to America and face this nightmare both on the journey and at the border with the disgusting figure of House Speaker Nancy Pelosi giving disingenuous speeches about caring for migrants. The woman's evil. Wokers condemned Trump for having children in cages at the border (so did Obama, *Shhhh*), but now they are sleeping on the floor without access to a shower with one border facility 729 percent over capacity. The Biden insanity even proposed flying migrants from the southern border to the northern border with Canada for 'processing'. The whole shambles is being overseen by ultra-Zionist Secretary of Homeland Security, the moronic liar Alejandro Mayorkas, who

banned news cameras at border facilities to stop Americans seeing what was happening. Mayorkas said there was not a ban on news crews; it was just that they were not allowed to film. Alongside him at Homeland Security is another ultra-Zionist Cass Sunstein appointed by Biden to oversee new immigration laws. Sunstein despises conspiracy researchers to the point where he suggests they should be banned or *taxed* for having such views. The man is not bonkers or anything. He's perfectly well-adjusted, but adjusted to what is the question. Criticise what is happening and you are a 'white supremacist' when earlier non-white immigrants also oppose the numbers which effect their lives and opportunities. Black people in poor areas are particularly damaged by uncontrolled immigration and the increased competition for work opportunities with those who will work for less. They are also losing voting power as Hispanics become more dominant in former black areas. It's a downward spiral for them while the billionaires behind the policy drone on about how much they care about black people and 'racism'. None of this is about compassion for migrants or black people – that's just wind and air. Migrants are instead being mercilessly exploited to transform America while the countries they leave are losing their future and the same is true in Europe. Mass immigration may now be the work of Woke Democrats, but it can be traced back to the 1986 Immigration Reform and Control Act (it wasn't) signed into law by Republican hero President Ronald Reagan which gave amnesty to millions living in the United States illegally and other incentives for people to head for the southern border. Here we have the one-party state at work again.

Save me syndrome

Almost every aspect of what I have been exposing as the Cult agenda was on display in even the first days of 'Biden' with silencing of Pushbackers at the forefront of everything. A Renegade Mind will view the Trump years and QAnon in a very different light to their supporters and advocates as the dots are connected. The QAnon/Trump Psyop has given the Cult all it was looking for. We may not know how much, or little, that Trump realised he was being used, but that's a side issue. This pincer movement produced the desired outcome of dividing America and having Pushbackers isolated. To turn this around we have to look at new routes to empowerment which do not include handing our power to other people and groups through what I

will call the ‘Save Me Syndrome’ – ‘I want someone else to do it so that I don’t have to’. We have seen this at work throughout human history and the QAnon/Trump Psyop is only the latest incarnation alongside all the others. Religion is an obvious expression of this when people look to a ‘god’ or priest to save them or tell them how to be saved and then there are ‘save me’ politicians like Trump. Politics is a diversion and not a ‘saviour’. It is a means to block positive change, not make it possible.

Save Me Syndrome always comes with the same repeating theme of handing your power to whom or what you believe will save you while your real ‘saviour’ stares back from the mirror every morning. Renegade Minds are constantly vigilant in this regard and always asking the question ‘What can I do?’ rather than ‘What can someone else do for me?’ Gandhi was right when he said: ‘You must be the change you want to see in the world.’ We are indeed the people we have been waiting for. We are presented with a constant raft of reasons to concede that power to others and forget where the real power is. Humanity has the numbers and the Cult does not. It has to use diversion and division to target the unstoppable power that comes from unity. Religions, governments, politicians, corporations, media, QAnon, are all different manifestations of this power-diversion and dilution. Refusing to give your power to governments and instead handing it to Trump and QAnon is not to take a new direction, but merely to recycle the old one with new names on the posters. I will explore this phenomenon as we proceed and how to break the cycles and recycles that got us here through the mists of repeating perception and so repeating history.

For now we shall turn to the most potent example in the entire human story of the consequences that follow when you give your power away. I am talking, of course, of the ‘Covid’ hoax.

CHAPTER FOUR

‘Covid’: Calculated catastrophe

Facts are threatening to those invested in fraud
DaShanne Stokes

We can easily unravel the real reason for the ‘Covid pandemic’ hoax by employing the Renegade Mind methodology that I have outlined this far. We’ll start by comparing the long-planned Cult outcome with the ‘Covid pandemic’ outcome. Know the outcome and you’ll see the journey.

I have highlighted the plan for the Hunger Games Society which has been in my books for so many years with the very few controlling the very many through ongoing dependency. To create this dependency it is essential to destroy independent livelihoods, businesses and employment to make the population reliant on the state (the Cult) for even the basics of life through a guaranteed pittance income. While independence of income remained these Cult ambitions would be thwarted. With this knowledge it was easy to see where the ‘pandemic’ hoax was going once talk of ‘lockdowns’ began and the closing of all but perceived ‘essential’ businesses to ‘save’ us from an alleged ‘deadly virus’. Cult corporations like Amazon and Walmart were naturally considered ‘essential’ while mom and pop shops and stores had their doors closed by fascist decree. As a result with every new lockdown and new regulation more small and medium, even large businesses not owned by the Cult, went to the wall while Cult giants and their frontmen and women grew financially fatter by the second. Mom and pop were denied an income and the right to earn a living and the wealth of people like Jeff Bezos (Amazon), Mark Zuckerberg (Facebook) and Sergei Brin and

Larry Page (Google/Alphabet) have reached record levels. The Cult was increasing its own power through further dramatic concentrations of wealth while the competition was being destroyed and brought into a state of dependency. Lockdowns have been instigated to secure that very end and were never anything to do with health. My brother Paul spent 45 years building up a bus repair business, but lockdowns meant buses were running at a fraction of normal levels for months on end. Similar stories can told in their hundreds of millions worldwide. Efforts of a lifetime coldly destroyed by Cult multi-billionaires and their lackeys in government and law enforcement who continued to earn their living from the taxation of the people while denying the right of the same people to earn theirs. How different it would have been if those making and enforcing these decisions had to face the same financial hardships of those they affected, but they never do.

Gates of Hell

Behind it all in the full knowledge of what he is doing and why is the psychopathic figure of Cult operative Bill Gates. His puppet Tedros at the World Health Organization declared ‘Covid’ a pandemic in March, 2020. The WHO had changed the definition of a ‘pandemic’ in 2009 just a month before declaring the ‘swine flu pandemic’ which would not have been so under the previous definition. The same applies to ‘Covid’. The definition had included… ‘an infection by an infectious agent, occurring simultaneously in different countries, with a significant mortality rate relative to the proportion of the population infected’. The new definition removed the need for ‘significant mortality’. The ‘pandemic’ has been fraudulent even down to the definition, but Gates demanded economy-destroying lockdowns, school closures, social distancing, mandatory masks, a ‘vaccination’ for every man, woman and child on the planet and severe consequences and restrictions for those that refused. Who gave him this power? The Cult did which he serves like a little boy in short trousers doing what his daddy tells him. He and his psychopathic missus even smiled when they said that much worse was to come (what they knew was planned to come). Gates responded in the matter-of-fact way of all psychopaths to a question about the effect on the world economy of what he was doing:

Well, it won't go to zero but it will shrink. Global GDP is probably going to take the biggest hit ever [Gates was smiling as he said this] … in my lifetime this will be the greatest economic hit. But you don't have a choice. People act as if you have a choice. People don't feel like going to the stadium when they might get infected … People are deeply affected by seeing these stats, by knowing they could be part of the transmission chain, old people, their parents and grandparents, could be affected by this, and so you don't get to say ignore what is going on here.

There will be the ability to open up, particularly in rich countries, if things are done well over the next few months, but for the world at large normalcy only returns when we have largely vaccinated the entire population.

The man has no compassion or empathy. How could he when he's a psychopath like all Cult players? My own view is that even beyond that he is very seriously mentally ill. Look in his eyes and you can see this along with his crazy flailing arms. You don't do what he has done to the world population since the start of 2020 unless you are mentally ill and at the most extreme end of psychopathic. You especially don't do it when to you know, as we shall see, that cases and deaths from 'Covid' are fakery and a product of monumental figure massaging. 'These stats' that Gates referred to are based on a 'test' that's not testing for the 'virus' as he has known all along. He made his fortune with big Cult support as an infamously ruthless software salesman and now buys global control of 'health' (death) policy without the population he affects having any say. It's a breathtaking outrage. Gates talked about people being deeply affected by fear of 'Covid' when that was because of *him* and his global network lying to them minute-by-minute supported by a lying media that he seriously influences and funds to the tune of hundreds of millions. He's handed big sums to media operations including the BBC, NBC, Al Jazeera, Univision, *PBS NewsHour, ProPublica, National Journal, The Guardian, The Financial Times, The Atlantic, Texas Tribune, USA Today* publisher Gannett, *Washington Monthly, Le Monde,* Center for Investigative Reporting, Pulitzer Center on Crisis Reporting, National Press Foundation, International Center for Journalists, Solutions Journalism Network, the Poynter Institute for Media Studies, and many more. Gates is everywhere in the 'Covid' hoax and the man must go to prison – or a mental facility – for the rest of his life and his money distributed to those he has taken such enormous psychopathic pleasure in crushing.

The Muscle

The Hunger Games global structure demands a police-military state – a fusion of the two into one force – which viciously imposes the will of the Cult on the population and protects the Cult from public rebellion. In that regard, too, the 'Covid' hoax just keeps on giving. Often unlawful, ridiculous and contradictory 'Covid' rules and regulations have been policed across the world by moronic automatons and psychopaths made faceless by face-nappy masks and acting like the Nazi SS and fascist blackshirts and brownshirts of Hitler and Mussolini. The smallest departure from the rules decreed by the psychos in government and their clueless gofers were jumped upon by the face-nappy fascists. Brutality against public protestors soon became commonplace even on girls, women and old people as the brave men with the batons – the Face-Nappies as I call them – broke up peaceful protests and handed out fines like confetti to people who couldn't earn a living let alone pay hundreds of pounds for what was once an accepted human right. Robot Face-Nappies of Nottingham police in the English East Midlands fined one group £11,000 for attending a child's birthday party. For decades I charted the transformation of law enforcement as genuine, decent officers were replaced with psychopaths and the brain dead who would happily and brutally do whatever their masters told them. Now they were let loose on the public and I would emphasise the point that none of this just happened. The step-by-step change in the dynamic between police and public was orchestrated from the shadows by those who knew where this was all going and the same with the perceptual reframing of those in all levels of authority and official administration through 'training courses' by organisations such as Common Purpose which was created in the late 1980s and given a massive boost in Blair era Britain until it became a global phenomenon. Supposed public 'servants' began to view the population as the enemy and the same was true of the police. This was the start of the explosion of behaviour manipulation organisations and networks preparing for the all-war on the human psyche unleashed with the dawn of 2020. I will go into more detail about this later in the book because it is a core part of what is happening.

Police desecrated beauty spots to deter people gathering and arrested women for walking in the countryside alone 'too far' from their homes. We had arrogant, clueless sergeants in the Isle of Wight police where I live posting on Facebook what they insisted the population must do or else. A

schoolmaster sergeant called Radford looked young enough for me to ask if his mother knew he was out, but he was posting what he *expected* people to do while a Sergeant Wilkinson boasted about fining lads for meeting in a McDonald's car park where they went to get a lockdown takeaway. Wilkinson added that he had even cancelled their order. What a pair of prats these people are and yet they have increasingly become the norm among Jackboot Johnson's Yellowshirts once known as the British police. This was the theme all over the world with police savagery common during lockdown protests in the United States, the Netherlands, and the fascist state of Victoria in Australia under its tyrannical and again moronic premier Daniel Andrews. Amazing how tyrannical and moronic tend to work as a team and the same combination could be seen across America as arrogant, narcissistic Woke governors and mayors such as Gavin Newsom (California), Andrew Cuomo (New York), Gretchen Whitmer (Michigan), Lori Lightfoot (Chicago) and Eric Garcetti (Los Angeles) did their Nazi and Stalin impressions with the full support of the compliant brutality of their enforcers in uniform as they arrested small business owners defying fascist shutdown orders and took them to jail in ankle shackles and handcuffs. This happened to bistro owner Marlena Pavlos-Hackney in Gretchen Whitmer's fascist state of Michigan when police arrived to enforce an order by a state-owned judge for 'putting the community at risk' at a time when other states like Texas were dropping restrictions and migrants were pouring across the southern border without any 'Covid' questions at all. I'm sure there are many officers appalled by what they are ordered to do, but not nearly enough of them. If they were truly appalled they would not do it. As the months passed every opportunity was taken to have the military involved to make their presence on the streets ever more familiar and 'normal' for the longer-term goal of police-military fusion.

Another crucial element to the Hunger Games enforcement network has been encouraging the public to report neighbours and others for 'breaking the lockdown rules'. The group faced with £11,000 in fines at the child's birthday party would have been dobbed-in by a neighbour with a brain the size of a pea. The technique was most famously employed by the Stasi secret police in communist East Germany who had public informants placed throughout the population. A police chief in the UK says his force doesn't need to carry out 'Covid' patrols when they are flooded with so many calls from the public reporting other people for visiting the beach.

Dorset police chief James Vaughan said people were so enthusiastic about snitching on their fellow humans they were now operating as an auxiliary arm of the police: 'We are still getting around 400 reports a week from the public, so we will respond to reports …We won't need to be doing hotspot patrols because people are very quick to pick the phone up and tell us.' Vaughan didn't say that this is a pillar of all tyrannies of whatever complexion and the means to hugely extend the reach of enforcement while spreading distrust among the people and making them wary of doing anything that might get them reported. Those narcissistic Isle of Wight sergeants Radford and Wilkinson never fail to add a link to their Facebook posts where the public can inform on their fellow slaves. Neither would be self-aware enough to realise they were imitating the Stasi which they might well never have heard of. Government psychologists that I will expose later laid out a policy to turn communities against each other in the same way.

A coincidence? Yep, and I can knit fog

I knew from the start of the alleged pandemic that this was a Cult operation. It presented limitless potential to rapidly advance the Cult agenda and exploit manipulated fear to demand that every man, woman and child on the planet was 'vaccinated' in a process never used on humans before which infuses self-replicating *synthetic* material into human cells. Remember the plan to transform the human body from a biological to a synthetic biological state. I'll deal with the 'vaccine' (that's not actually a vaccine) when I focus on the genetic agenda. Enough to say here that mass global 'vaccination' justified by this 'new virus' set alarms ringing after 30 years of tracking these people and their methods. The 'Covid' hoax officially beginning in China was also a big red flag for reasons I will be explaining. The agenda potential was so enormous that I could dismiss any idea that the 'virus' appeared naturally. Major happenings with major agenda implications never occur without Cult involvement in making them happen. My questions were twofold in early 2020 as the media began its campaign to induce global fear and hysteria: Was this alleged infectious agent released on purpose by the Cult or did it even exist at all? I then did what I always do in these situations. I sat, observed and waited to see where the evidence and information would take me. By March and early April synchronicity was strongly – and ever more so since then – pointing me in

the direction of *there is no 'virus'*. I went public on that with derision even from swathes of the alternative media that voiced a scenario that the Chinese government released the 'virus' in league with Deep State elements in the United States from a top-level bio-lab in Wuhan where the 'virus' is said to have first appeared. I looked at that possibility, but I didn't buy it for several reasons. Deaths from the 'virus' did not in any way match what they would have been with a 'deadly bioweapon' and it is much more effective if you sell the *illusion* of an infectious agent rather than having a real one unless you can control through injection who has it and who doesn't. Otherwise you lose control of events. A made-up 'virus' gives you a blank sheet of paper on which you can make it do whatever you like and have any symptoms or mutant 'variants' you choose to add while a real infectious agent would limit you to what it actually does. A phantom disease allows you to have endless ludicrous 'studies' on the 'Covid' dollar to widen the perceived impact by inventing ever more 'at risk' groups including one study which said those who walk slowly may be almost four times more likely to die from the 'virus'. People are in psychiatric wards for less.

A real 'deadly bioweapon' can take out people in the hierarchy that are not part of the Cult, but essential to its operation. Obviously they don't want that. Releasing a real disease means you immediately lose control of it. Releasing an illusory one means you don't. Again it's vital that people are extra careful when dealing with what they want to hear. A bioweapon unleashed from a Chinese laboratory in collusion with the American Deep State may fit a conspiracy narrative, but is it true? Would it not be far more effective to use the excuse of a 'virus' to justify the real bioweapon – the 'vaccine'? That way your disease agent does not have to be transmitted and arrives directly through a syringe. I saw a French virologist Luc Montagnier quoted in the alternative media as saying he had discovered that the alleged 'new' severe acute respiratory syndrome coronavirus , or SARS-CoV-2, was made artificially and included elements of the human immunodeficiency 'virus' (HIV) and a parasite that causes malaria. SARS-CoV-2 is alleged to trigger an alleged illness called Covid-19. I remembered Montagnier's name from my research years before into claims that an HIV 'retrovirus' causes AIDs – claims that were demolished by Berkeley virologist Peter Duesberg who showed that no one had ever proved that HIV causes acquired immunodeficiency syndrome or AIDS. Claims that become accepted as fact, publicly and medically, with no proof whatsoever

are an ever-recurring story that profoundly applies to ‘Covid’. Nevertheless, despite the lack of proof, Montagnier’s team at the Pasteur Institute in Paris had a long dispute with American researcher Robert Gallo over which of them discovered and isolated the HIV ‘virus’ and with *no evidence* found it to cause AIDS. You will see later that there is also no evidence that any ‘virus’ causes any disease or that there is even such a thing as a ‘virus’ in the way it is said to exist. The claim to have ‘isolated’ the HIV ‘virus’ will be presented in its real context as we come to the shocking story – and it is a story – of SARS-CoV-2 and so will Montagnier’s assertion that he identified the full SARS-CoV-2 genome.

Hoax in the making

We can pick up the ‘Covid’ story in 2010 and the publication by the Rockefeller Foundation of a document called ‘Scenarios for the Future of Technology and International Development’. The inner circle of the Rockefeller family has been serving the Cult since John D. Rockefeller (1839-1937) made his fortune with Standard Oil. It is less well known that the same Rockefeller – the Bill Gates of his day – was responsible for establishing what is now referred to as ‘Big Pharma’, the global network of pharmaceutical companies that make outrageous profits dispensing scalpel and drug ‘medicine’ and are obsessed with pumping vaccines in ever-increasing number into as many human arms and backsides as possible. John D. Rockefeller was the driving force behind the creation of the ‘education’ system in the United States and elsewhere specifically designed to program the perceptions of generations thereafter. The Rockefeller family donated exceptionally valuable land in New York for the United Nations building and were central in establishing the World Health Organization in 1948 as an agency of the UN which was created from the start as a Trojan horse and stalking horse for world government. Now enter Bill Gates. His family and the Rockefellers have long been extremely close and I have seen genealogy which claims that if you go back far enough the two families fuse into the same bloodline. Gates has said that the Bill and Melinda Gates Foundation was inspired by the Rockefeller Foundation and why not when both are serving the same Cult? Major tax-exempt foundations are overwhelmingly criminal enterprises in which Cult assets fund the Cult agenda in the guise of ‘philanthropy’ while avoiding tax in the

process. Cult operatives can become mega-rich in their role of front men and women for the psychopaths at the inner core and they, too, have to be psychopaths to knowingly serve such evil. Part of the deal is that a big percentage of the wealth gleaned from representing the Cult has to be spent advancing the ambitions of the Cult and hence you have the Rockefeller Foundation, Bill and Melinda Gates Foundation (and *so* many more) and people like George Soros with his global Open Society Foundations spending their billions in pursuit of global Cult control. Gates is a global public face of the Cult with his interventions in world affairs including Big Tech influence; a central role in the 'Covid' and 'vaccine' scam; promotion of the climate change shakedown; manipulation of education; geoengineering of the skies; and his food-control agenda as the biggest owner of farmland in America, his GMO promotion and through other means. As one writer said: 'Gates monopolizes or wields disproportionate influence over the tech industry, global health and vaccines, agriculture and food policy (including biopiracy and fake food), weather modification and other climate technologies, surveillance, education and media.' The almost limitless wealth secured through Microsoft and other not-allowed-to-fail ventures (including vaccines) has been ploughed into a long, long list of Cult projects designed to enslave the entire human race. Gates and the Rockefellers have been working as one unit with the Rockefeller-established World Health Organization leading global 'Covid' policy controlled by Gates through his mouth-piece Tedros. Gates became the WHO's biggest funder when Trump announced that the American government would cease its donations, but Biden immediately said he would restore the money when he took office in January, 2021. The Gates Foundation (the Cult) owns through limitless funding the world health system and the major players across the globe in the 'Covid' hoax.

Okay, with that background we return to that Rockefeller Foundation document of 2010 headed 'Scenarios for the Future of Technology and International Development' and its 'imaginary' epidemic of a virulent and deadly influenza strain which infected 20 percent of the global population and killed eight million in seven months. The Rockefeller scenario was that the epidemic destroyed economies, closed shops, offices and other businesses and led to governments imposing fierce rules and restrictions that included mandatory wearing of face masks and body-temperature checks to enter communal spaces like railway stations and supermarkets.

The document predicted that even after the height of the Rockefeller-envisaged epidemic the authoritarian rule would continue to deal with further pandemics, transnational terrorism, environmental crises and rising poverty. Now you may think that the Rockefellers are our modern-day seers or alternatively, and rather more likely, that they well knew what was planned a few years further on. Fascism had to be imposed, you see, to 'protect citizens from risk and exposure'. The Rockefeller scenario document said:

During the pandemic, national leaders around the world flexed their authority and imposed airtight rules and restrictions, from the mandatory wearing of face masks to body-temperature checks at the entries to communal spaces like train stations and supermarkets. Even after the pandemic faded, this more authoritarian control and oversight of citizens and their activities stuck and even intensified. In order to protect themselves from the spread of increasingly global problems – from pandemics and transnational terrorism to environmental crises and rising poverty – leaders around the world took a firmer grip on power.

At first, the notion of a more controlled world gained wide acceptance and approval. Citizens willingly gave up some of their sovereignty – and their privacy – to more paternalistic states in exchange for greater safety and stability. Citizens were more tolerant, and even eager, for top-down direction and oversight, and national leaders had more latitude to impose order in the ways they saw fit.

In developed countries, this heightened oversight took many forms: biometric IDs for all citizens, for example, and tighter regulation of key industries whose stability was deemed vital to national interests. In many developed countries, enforced cooperation with a suite of new regulations and agreements slowly but steadily restored both order and, importantly, economic growth.

There we have the prophetic Rockefellers in 2010 and three years later came their paper for the Global Health Summit in Beijing, China, when government representatives, the private sector, international organisations and groups met to discuss the next 100 years of 'global health'. The Rockefeller Foundation-funded paper was called 'Dreaming the Future of Health for the Next 100 Years and more prophecy ensued as it described a dystopian future: 'The abundance of data, digitally tracking and linking people may mean the 'death of privacy' and may replace physical interaction with transient, virtual connection, generating isolation and raising questions of how values are shaped in virtual networks.' Next in the 'Covid' hoax preparation sequence came a 'table top' simulation in 2018 for another 'imaginary' pandemic of a disease called Clade X which was said to kill 900 million people. The exercise was organised by the Gates-

funded Johns Hopkins University's Center for Health Security in the United States and this is the very same university that has been compiling the disgustingly and systematically erroneous global figures for 'Covid' cases and deaths. Similar Johns Hopkins health crisis scenarios have included the Dark Winter exercise in 2001 and Atlantic Storm in 2005.

Nostradamus 201

For sheer predictive genius look no further prophecy-watchers than the Bill Gates-funded Event 201 held only six weeks before the 'coronavirus pandemic' is supposed to have broken out in China and Event 201 was based on a scenario of a global 'coronavirus pandemic'. Melinda Gates, the great man's missus, told the BBC that he had 'prepared for years' for a coronavirus pandemic which told us what we already knew. Nostradamugates had predicted in a TED talk in 2015 that a pandemic was coming that would kill a lot of people and demolish the world economy. My god, the man is a machine – possibly even literally. Now here he was only weeks before the real thing funding just such a simulated scenario and involving his friends and associates at Johns Hopkins, the World Economic Forum Cult-front of Klaus Schwab, the United Nations, Johnson & Johnson, major banks, and officials from China and the Centers for Disease Control in the United States. What synchronicity – Johns Hopkins would go on to compile the fraudulent 'Covid' figures, the World Economic Forum and Schwab would push the 'Great Reset' in response to 'Covid', the Centers for Disease Control would be at the forefront of 'Covid' policy in the United States, Johnson & Johnson would produce a 'Covid vaccine', and everything would officially start just weeks later in China. Spooky, eh? They were even accurate in creating a simulation of a 'virus' pandemic because the 'real thing' would also be a simulation. Event 201 was not an exercise preparing for something that might happen; it was a rehearsal for what those in control knew was *going* to happen and very shortly. Hours of this simulation were posted on the Internet and the various themes and responses mirrored what would soon be imposed to transform human society. News stories were inserted and what they said would be commonplace a few weeks later with still more prophecy perfection. Much discussion focused on the need to deal with misinformation and the 'anti-

vax movement' which is exactly what happened when the 'virus' arrived – was said to have arrived – in the West.

Cult-owned social media banned criticism and exposure of the official 'virus' narrative and when I said there *was* no 'virus' in early April, 2020, I was banned by one platform after another including YouTube, Facebook and later Twitter. The mainstream broadcast media in Britain was in effect banned from interviewing me by the Tony-Blair-created government broadcasting censor Ofcom headed by career government bureaucrat Melanie Dawes who was appointed just as the 'virus' hoax was about to play out in January, 2020. At the same time the Ickonic media platform was using Vimeo, another ultra-Zionist-owned operation, while our own player was being created and they deleted in an instant hundreds of videos, documentaries, series and shows to confirm their unbelievable vindictiveness. We had copies, of course, and they had to be restored one by one when our player was ready. These people have no class. Sabbatian Facebook promised free advertisements for the Gates-controlled World Health Organization narrative while deleting 'false claims and conspiracy theories' to stop 'misinformation' about the alleged coronavirus. All these responses could be seen just a short while earlier in the scenarios of Event 201. Extreme censorship was absolutely crucial for the Cult because the official story was so ridiculous and unsupportable by the evidence that it could never survive open debate and the free-flow of information and opinion. If you can't win a debate then don't have one is the Cult's approach throughout history. Facebook's little boy front man – front boy – Mark Zuckerberg equated 'credible and accurate information' with official sources and exposing their lies with 'misinformation'.

Silencing those that can see

The censorship dynamic of Event 201 is now the norm with an army of narrative-supporting 'fact-checker' organisations whose entire reason for being is to tell the public that official narratives are true and those exposing them are lying. One of the most appalling of these 'fact-checkers' is called NewsGuard founded by ultra-Zionist Americans Gordon Crovitz and Steven Brill. Crovitz is a former publisher of *The Wall Street Journal*, former Executive Vice President of Dow Jones, a member of the Council on Foreign Relations (CFR), and on the board of the American Association of

Rhodes Scholars. The CFR and Rhodes Scholarships, named after Rothschild agent Cecil Rhodes who plundered the gold and diamonds of South Africa for his masters and the Cult, have featured widely in my books. NewsGuard don't seem to like me for some reason – I really can't think why – and they have done all they can to have me censored and discredited which is, to quote an old British politician, like being savaged by a dead sheep. They are, however, like all in the censorship network, very well connected and funded by organisations themselves funded by, or connected to, Bill Gates. As you would expect with anything associated with Gates NewsGuard has an offshoot called HealthGuard which 'fights online health care hoaxes'. How very kind. Somehow the NewsGuard European Managing Director Anna-Sophie Harling, a remarkably young-looking woman with no broadcasting experience and little hands-on work in journalism, has somehow secured a position on the 'Content Board' of UK government broadcast censor Ofcom. An executive of an organisation seeking to discredit dissidents of the official narratives is making decisions for the government broadcast 'regulator' about content?? Another appalling 'fact-checker' is Full Fact funded by George Soros and global censors Google and Facebook.

It's amazing how many activists in the 'fact-checking', 'anti-hate', arena turn up in government-related positions – people like UK Labour Party activist Imran Ahmed who heads the Center for Countering Digital Hate founded by people like Morgan McSweeney, now chief of staff to the Labour Party's hapless and useless 'leader' Keir Starmer. Digital Hate – which is what it really is – uses the American spelling of Center to betray its connection to a transatlantic network of similar organisations which in 2020 shapeshifted from attacking people for 'hate' to attacking them for questioning the 'Covid' hoax and the dangers of the 'Covid vaccine'. It's just a coincidence, you understand. This is one of Imran Ahmed's hysterical statements: 'I would go beyond calling anti-vaxxers conspiracy theorists to say they are an extremist group that pose a national security risk.' No one could ever accuse this prat of understatement and he's including in that those parents who are now against vaccines after their children were damaged for life or killed by them. He's such a nice man. Ahmed does the rounds of the Woke media getting soft-ball questions from spineless 'journalists' who never ask what right he has to campaign to destroy the freedom of speech of others while he demands it for himself. There also

seems to be an overrepresentation in Ofcom of people connected to the narrative-worshipping BBC. This incredible global network of narrative-support was super-vital when the ‘Covid’ hoax was played in the light of the mega-whopper lies that have to be defended from the spotlight cast by the most basic intelligence.

Setting the scene

The Cult plays the long game and proceeds step-by-step ensuring that everything is in place before major cards are played and they don’t come any bigger than the ‘Covid’ hoax. The psychopaths can’t handle events where the outcome isn’t certain and as little as possible – preferably nothing – is left to chance. Politicians, government and medical officials who would follow direction were brought to illusory power in advance by the Cult web whether on the national stage or others like state governors and mayors of America. For decades the dynamic between officialdom, law enforcement and the public was changed from one of service to one of control and dictatorship. Behaviour manipulation networks established within government were waiting to impose the coming ‘Covid’ rules and regulations specifically designed to subdue and rewire the psyche of the people in the guise of protecting health. These included in the UK the Behavioural Insights Team part-owned by the British government Cabinet Office; the Scientific Pandemic Insights Group on Behaviours (SPI-B); and a whole web of intelligence and military groups seeking to direct the conversation on social media and control the narrative. Among them are the cyberwarfare (on the people) 77th Brigade of the British military which is also coordinated through the Cabinet Office as civilian and military leadership continues to combine in what they call the Fusion Doctrine. The 77th Brigade is a British equivalent of the infamous Israeli (Sabbatian) military cyberwarfare and Internet manipulation operation Unit 8200 which I expose at length in *The Trigger*. Also carefully in place were the medical and science advisers to government – many on the payroll past or present of Bill Gates – and a whole alternative structure of unelected government stood by to take control when elected parliaments were effectively closed down once the ‘Covid’ card was slammed on the table. The structure I have described here and so much more was installed in every major country through the Cult networks. The top-down control hierarchy looks like this:

The Cult – Cult-owned Gates – the World Health Organization and Tedros – Gates-funded or controlled chief medical officers and science 'advisers' (dictators) in each country – political 'leaders'– law enforcement – The People. Through this simple global communication and enforcement structure the policy of the Cult could be imposed on virtually the entire human population so long as they acquiesced to the fascism. With everything in place it was time for the button to be pressed in late 2019/early 2020.

These were the prime goals the Cult had to secure for its will to prevail:

1) Locking down economies, closing all but designated 'essential' businesses (Cult-owned corporations were 'essential'), and putting the population under house arrest was an imperative to destroy independent income and employment and ensure dependency on the Cult-controlled state in the Hunger Games Society. Lockdowns had to be established as the global blueprint from the start to respond to the 'virus' and followed by pretty much the entire world.

2) The global population had to be terrified into believing in a deadly 'virus' that didn't actually exist so they would unquestioningly obey authority in the belief that authority must know how best to protect them and their families. Software salesman Gates would suddenly morph into the world's health expert and be promoted as such by the Cult-owned media.

3) A method of testing that wasn't testing for the 'virus', but was only claimed to be, had to be in place to provide the illusion of 'cases' and subsequent 'deaths' that had a very different cause to the 'Covid-19' that would be scribbled on the death certificate.

4) Because there was no 'virus' and the great majority testing positive with a test not testing for the 'virus' would have no symptoms of anything the lie had to be sold that people without symptoms (without the 'virus') could still pass it on to others. This was crucial to justify for the first time quarantining – house arresting – healthy people. Without this the economy-destroying lockdown of *everybody* could not have been credibly sold.

5) The 'saviour' had to be seen as a vaccine which beyond evil drug companies were working like angels of mercy to develop as quickly as possible, with all corners cut, to save the day. The public must absolutely not know that the 'vaccine' had nothing to do with a 'virus' or that the contents were ready and waiting with a very different motive long before the 'Covid' card was even lifted from the pack.

I said in March, 2020, that the 'vaccine' would have been created way ahead of the 'Covid' hoax which justified its use and the following December an article in the New York *Intelligencer* magazine said the Moderna 'vaccine' had been 'designed' by January, 2020. This was 'before China had even acknowledged that the disease could be transmitted from human to human, more than a week before the first confirmed coronavirus

case in the United States'. The article said that by the time the first American death was announced a month later 'the vaccine had already been manufactured and shipped to the National Institutes of Health for the beginning of its Phase I clinical trial'. The 'vaccine' was actually 'designed' long before that although even with this timescale you would expect the article to ask how on earth it could have been done that quickly. Instead it asked why the 'vaccine' had not been rolled out then and not months later. Journalism in the mainstream is truly dead. I am going to detail in the next chapter why the 'virus' has never existed and how a hoax on that scale was possible, but first the foundation on which the Big Lie of 'Covid' was built.

The test that doesn't test

Fraudulent 'testing' is the bottom line of the whole 'Covid' hoax and was the means by which a 'virus' that did not exist *appeared* to exist. They could only achieve this magic trick by using a test not testing for the 'virus'. To use a test that *was* testing for the 'virus' would mean that every test would come back negative given there was no 'virus'. They chose to exploit something called the RT-PCR test invented by American biochemist Kary Mullis in the 1980s who said publicly that his PCR test … *cannot detect infectious disease*. Yes, the 'test' used worldwide to detect infectious 'Covid' to produce all the illusory 'cases' and 'deaths' compiled by Johns Hopkins and others *cannot detect infectious disease*. This fact came from the mouth of the man who invented PCR and was awarded the Nobel Prize in Chemistry in 1993 for doing so. Sadly, and incredibly conveniently for the Cult, Mullis died in August, 2019, at the age of 74 just before his test would be fraudulently used to unleash fascism on the world. He was said to have died from pneumonia which was an irony in itself. A few months later he would have had 'Covid-19' on his death certificate. I say the timing of his death was convenient because had he lived Mullis, a brilliant, honest and decent man, would have been vociferously speaking out against the use of his test to detect 'Covid' when it was never designed, or able, to do that. I know that to be true given that Mullis made the same point when his test was used to 'detect' – not detect – HIV. He had been seriously critical of the Gallo/Montagnier claim to have isolated the HIV 'virus' and shown it to cause AIDS for which Mullis said there was no evidence. AIDS is actually

not a disease but a series of diseases from which people die all the time. When they die from those *same diseases* after a positive 'test' for HIV then AIDS goes on their death certificate. I think I've heard that before somewhere. Countries instigated a policy with 'Covid' that anyone who tested positive with a test not testing for the 'virus' and died of any other cause within 28 days and even longer 'Covid-19' had to go on the death certificate. Cases have come from the test that can't test for infectious disease and the deaths are those who have died of *anything* after testing positive with a test not testing for the 'virus'. I'll have much more later about the death certificate scandal.

Mullis was deeply dismissive of the now US 'Covid' star Anthony Fauci who he said was a liar who didn't know anything about anything – 'and I would say that to his face – nothing.' He said of Fauci: 'The man thinks he can take a blood sample, put it in an electron microscope and if it's got a virus in there you'll know it – he doesn't understand electron microscopy and he doesn't understand medicine and shouldn't be in a position like he's in.' That position, terrifyingly, has made him the decider of 'Covid' fascism policy on behalf of the Cult in his role as director since 1984 of the National Institute of Allergy and Infectious Diseases (NIAID) while his record of being wrong is laughable; but being wrong, so long as it's the *right kind* of wrong, is why the Cult loves him. He'll say anything the Cult tells him to say. Fauci was made Chief Medical Adviser to the President immediately Biden took office. Biden was installed in the White House by Cult manipulation and one of his first decisions was to elevate Fauci to a position of even more control. This is a coincidence? Yes, and I identify as a flamenco dancer called Lola. How does such an incompetent criminal like Fauci remain in that pivotal position in American health since *the 1980s*? When you serve the Cult it looks after you until you are surplus to requirements. Kary Mullis said prophetically of Fauci and his like: 'Those guys have an agenda and it's not an agenda we would like them to have … they make their own rules, they change them when they want to, and Tony Fauci does not mind going on television in front of the people who pay his salary and lie directly into the camera.' Fauci has done that almost daily since the 'Covid' hoax began. Lying is in Fauci's DNA. To make the situation crystal clear about the PCR test this is a direct quote from its inventor Kary Mullis:

It [the PCR test] doesn't tell you that you're sick and doesn't tell you that the thing you ended up with was really going to hurt you ...'

Ask yourself why governments and medical systems the world over have been using this very test to decide who is 'infected' with the SARS-CoV-2 'virus' and the alleged disease it allegedly causes, 'Covid-19'. The answer to that question will tell you what has been going on. By the way, here's a little show-stopper – the 'new' SARS-CoV-2 'virus' was 'identified' as such right from the start using … *the PCR test not testing for the 'virus'*. If you are new to this and find that shocking then stick around. I have hardly started yet. Even worse, other 'tests', like the 'Lateral Flow Device' (LFD), are considered so useless that they have to be *confirmed* by the PCR test! Leaked emails written by Ben Dyson, adviser to UK 'Health' Secretary Matt Hancock, said they were 'dangerously unreliable'. Dyson, executive director of strategy at the Department of Health, wrote: 'As of today, someone who gets a positive LFD result in (say) London has at best a 25 per cent chance of it being a true positive, but if it is a self-reported test potentially as low as 10 per cent (on an optimistic assumption about specificity) or as low as 2 per cent (on a more pessimistic assumption).' These are the 'tests' that schoolchildren and the public are being urged to have twice a week or more and have to isolate if they get a positive. Each fake positive goes in the statistics as a 'case' no matter how ludicrously inaccurate and the 'cases' drive lockdown, masks and the pressure to 'vaccinate'. The government said in response to the email leak that the 'tests' were accurate which confirmed yet again what shocking bloody liars they are. The real false positive rate is *100 percent* as we'll see. In another 'you couldn't make it up' the UK government agreed to pay £2.8 billion to California's Innova Medical Group to supply the irrelevant lateral flow tests. The company's primary test-making centre is in China. Innova Medical Group, established in March, 2020, is owned by Pasaca Capital Inc, chaired by Chinese-American millionaire Charles Huang who was born in Wuhan.

How it works – and how it doesn't

The RT-PCR test, known by its full title of Polymerase chain reaction, is used across the world to make millions, even billions, of copies of a

DNA/RNA genetic information sample. The process is called ‘amplification’ and means that a tiny sample of genetic material is amplified to bring out the detailed content. I stress that it is not testing for an infectious disease. It is simply amplifying a sample of genetic material. In the words of Kary Mullis: ‘PCR is … just a process that’s used to make a whole lot of something out of something.’ To emphasise the point companies that make the PCR tests circulated around the world to ‘test’ for ‘Covid’ warn on the box that it can’t be used to detect ‘Covid’ or infectious disease and is for research purposes only. It’s okay, rest for a minute and you’ll be fine. This is the test that produces the ‘cases’ and ‘deaths’ that have been used to destroy human society. All those global and national medical and scientific ‘experts’ demanding this destruction to ‘save us’ *KNOW* that the test is not testing for the ‘virus’ and the cases and deaths they claim to be real are an almost unimaginable fraud. Every one of them and so many others including politicians and psychopaths like Gates and Tedros must be brought before Nuremburg-type trials and jailed for the rest of their lives. The more the genetic sample is amplified by PCR the more elements of that material become sensitive to the test and by that I don’t mean sensitive for a ‘virus’ but for elements of the genetic material which is *naturally* in the body or relates to remnants of old conditions of various kinds lying dormant and causing no disease. Once the amplification of the PCR reaches a certain level *everyone* will test positive. So much of the material has been made sensitive to the test that everyone will have some part of it in their body. Even lying criminals like Fauci have said that once PCR amplifications pass 35 cycles everything will be a false positive that cannot be trusted for the reasons I have described. I say, like many proper doctors and scientists, that 100 percent of the ‘positives’ are false, but let’s just go with Fauci for a moment.

He says that any amplification over 35 cycles will produce false positives and yet the US Centers for Disease Control (CDC) and Food and Drug Administration (FDA) have recommended up to *40 cycles* and the National Health Service (NHS) in Britain admitted in an internal document for staff that it was using *45 cycles* of amplification. A long list of other countries has been doing the same and at least one ‘testing’ laboratory has been using *50 cycles*. Have you ever heard a doctor, medical ‘expert’ or the media ask what level of amplification has been used to claim a ‘positive’. The ‘test’ comes back ‘positive’ and so you have the ‘virus’, end of story. Now we

can see how the government in Tanzania could send off samples from a goat and a pawpaw fruit under human names and both came back positive for ‘Covid-19’. Tanzania president John Magufuli mocked the ‘Covid’ hysteria, the PCR test and masks and refused to import the DNA-manipulating ‘vaccine’. The Cult hated him and an article sponsored by the Bill Gates Foundation appeared in the London *Guardian* in February, 2021, headed ‘It’s time for Africa to rein in Tanzania’s anti-vaxxer president’. Well, ‘reined in’ he shortly was. Magufuli appeared in good health, but then, in March, 2021, he was dead at 61 from ‘heart failure’. He was replaced by Samia Hassan Suhulu who is connected to Klaus Schwab’s World Economic Forum and she immediately reversed Magufuli’s ‘Covid’ policy. A sample of cola tested positive for ‘Covid’ with the PCR test in Germany while American actress and singer-songwriter Erykah Badu tested positive in one nostril and negative in the other. Footballer Ronaldo called the PCR test ‘bullshit’ after testing positive three times and being forced to quarantine and miss matches when there was nothing wrong with him. The mantra from Tedros at the World Health Organization and national governments (same thing) has been test, test, test. They know that the more tests they can generate the more fake ‘cases’ they have which go on to become ‘deaths’ in ways I am coming to. The UK government has its Operation Moonshot planned to test multiple millions every day in workplaces and schools with free tests for everyone to use twice a week at home in line with the Cult plan from the start to make testing part of life. A government advertisement for an ‘Interim Head of Asymptomatic Testing Communication’ said the job included responsibility for delivering a ‘communications strategy’ (propaganda) ‘to support the expansion of asymptomatic testing that *‘normalises testing as part of everyday life*’. More tests means more fake ‘cases’, ‘deaths’ and fascism. I have heard of, and from, many people who booked a test, couldn’t turn up, and yet got a positive result through the post for a test they’d never even had. The whole thing is crazy, but for the Cult there’s method in the madness. Controlling and manipulating the level of amplification of the test means the authorities can control whenever they want the number of apparent ‘cases’ and ‘deaths’. If they want to justify more fascist lockdown and destruction of livelihoods they keep the amplification high. If they want to give the illusion that lockdowns and the ‘vaccine’ are working then they lower the amplification and ‘cases’ and ‘deaths’ will appear to fall. In January, 2021,

the Cult-owned World Health Organization suddenly warned laboratories about over-amplification of the test and to lower the threshold. Suddenly headlines began appearing such as: 'Why ARE "Covid" cases plummeting?' This was just when the vaccine rollout was underway and I had predicted months before they would make cases appear to fall through amplification tampering when the 'vaccine' came. These people are so predictable.

Cow vaccines?

The question must be asked of what is on the test swabs being poked far up the nose of the population to the base of the brain? A nasal swab punctured one woman's brain and caused it to leak fluid. Most of these procedures are being done by people with little training or medical knowledge. Dr Lorraine Day, former orthopaedic trauma surgeon and Chief of Orthopaedic Surgery at San Francisco General Hospital, says the tests are really a *'vaccine'*. Cows have long been vaccinated this way. She points out that masks have to cover the nose and the mouth where it is claimed the 'virus' exists in saliva. Why then don't they take saliva from the mouth as they do with a DNA test instead of pushing a long swab up the nose towards the brain? The ethmoid bone separates the nasal cavity from the brain and within that bone is the cribriform plate. Dr Day says that when the swab is pushed up against this plate and twisted the procedure is 'depositing things back there'. She claims that among these 'things' are nanoparticles that can enter the brain. Researchers have noted that a team at the Gates-funded Johns Hopkins have designed tiny, star-shaped micro-devices that can latch onto intestinal mucosa and release drugs into the body. Mucosa is the thin skin that covers the inside surface of parts of the body such as *the nose* and mouth and produces mucus to protect them. The Johns Hopkins micro-devices are called 'theragrippers' and were 'inspired' by a parasitic worm that digs its sharp teeth into a host's intestines. Nasal swabs are also coated in the sterilisation agent ethylene oxide. The US National Cancer Institute posts this explanation on its website:

At room temperature, ethylene oxide is a flammable colorless gas with a sweet odor. It is used primarily to produce other chemicals, including antifreeze. In smaller amounts, ethylene oxide is

used as a pesticide and a sterilizing agent. The ability of ethylene oxide to damage DNA makes it an effective sterilizing agent but also accounts for its cancer-causing activity.

The Institute mentions lymphoma and leukaemia as cancers most frequently reported to be associated with occupational exposure to ethylene oxide along with stomach and breast cancers. How does anyone think this is going to work out with the constant testing regime being inflicted on adults and children at home and at school that will accumulate in the body anything that's on the swab?

Doctors know best

It is vital for people to realise that 'hero' doctors 'know' only what the Big Pharma-dominated medical authorities tell them to 'know' and if they refuse to 'know' what they are told to 'know' they are out the door. They are mostly not physicians or healers, but repeaters of the official narrative – or else. I have seen alleged professional doctors on British television make shocking statements that we are supposed to take seriously. One called 'Dr' Amir Khan, who is actually telling patients how to respond to illness, said that men could take the birth pill to 'help slow down the effects of Covid-19'. In March, 2021, another ridiculous 'Covid study' by an American doctor proposed injecting men with the female sex hormone progesterone as a 'Covid' treatment. British doctor Nighat Arif told the BBC that face coverings were now going to be part of ongoing normal. Yes, the vaccine protects you, she said (evidence?) … but the way to deal with viruses in the community was always going to come down to hand washing, face covering and keeping a physical distance. That's not what we were told before the 'vaccine' was circulating. Arif said she couldn't imagine ever again going on the underground or in a lift without a mask. I was just thanking my good luck that she was not my doctor when she said – in March, 2021 – that if 'we are *behaving* and we are doing all the right things' she thought we could 'have our nearest and dearest around us at home … around *Christmas* and N*ew Year!* Her patronising delivery was the usual school teacher talking to six-year-olds as she repeated every government talking point and probably believed them all. If we have learned anything from the 'Covid' experience surely it must be that humanity's perception of doctors needs a fundamental rethink. NHS

‘doctor’ Sara Kayat told her television audience that the ‘Covid vaccine’ would ‘100 percent prevent hospitalisation and death’. Not even Big Pharma claimed that. We have to stop taking ‘experts’ at their word without question when so many of them are clueless and only repeating the party line on which their careers depend. That is not to say there are not brilliants doctors – there are and I have spoken to many of them since all this began – but you won’t see them in the mainstream media or quoted by the psychopaths and yes-people in government.

Remember the name – Christian Drosten

German virologist Christian Drosten, Director of Charité Institute of Virology in Berlin, became a national star after the pandemic hoax began. He was feted on television and advised the German government on ‘Covid’ policy. Most importantly to the wider world Drosten led a group that produced the ‘Covid’ testing protocol for the PCR test. What a remarkable feat given the PCR cannot test for infectious disease and even more so when you think that Drosten said that his method of testing for SARS-CoV-2 was developed ‘without having virus material available’. *He developed a test for a ‘virus’ that he didn’t have and had never seen*. Let that sink in as you survey the global devastation that came from what he did. The whole catastrophe of Drosten’s ‘test’ was based on the alleged genetic sequence published by Chinese scientists on the Internet. We will see in the next chapter that this alleged ‘genetic sequence’ has never been produced by China or anyone and cannot be when there *is no* SARS-CoV-2. Drosten, however, doesn’t seem to let little details like that get in the way. He was the lead author with Victor Corman from the same Charité Hospital of the paper ‘Detection of 2019 novel coronavirus (2019-nCoV) by real-time PCR‘ published in a magazine called *Eurosurveillance*. This became known as the Corman-Drosten paper. In November, 2020, with human society devastated by the effects of the Corman-Drosten test baloney, the protocol was publicly challenged by 22 international scientists and independent researchers from Europe, the United States, and Japan. Among them were senior molecular geneticists, biochemists, immunologists, and microbiologists. They produced a document headed ‘External peer review of the RTPCR test to detect SARS-Cov-2 Reveals 10 Major Flaws At The

Molecular and Methodological Level: Consequences For False-Positive Results'. The flaws in the Corman-Drosten test included the following:

- The test is non-specific because of erroneous design
- Results are enormously variable
- The test is unable to discriminate between the whole 'virus' and viral fragments
- It doesn't have positive or negative controls
- The test lacks a standard operating procedure
- It is unsupported by proper peer view

The scientists said the PCR 'Covid' testing protocol was not founded on science and they demanded the Corman-Drosten paper be retracted by *Eurosurveillance*. They said all present and previous Covid deaths, cases, and 'infection rates' should be subject to a massive retroactive inquiry. Lockdowns and travel restrictions should be reviewed and relaxed and those diagnosed through PCR to have 'Covid-19' should not be forced to isolate. Dr Kevin Corbett, a health researcher and nurse educator with a long academic career producing a stream of peer-reviewed publications at many UK universities, made the same point about the PCR test debacle. He said of the scientists' conclusions: 'Every scientific rationale for the development of that test has been totally destroyed by this paper. It's like Hiroshima/Nagasaki to the Covid test.' He said that China hadn't given them an isolated 'virus' when Drosten developed the test. Instead they had developed the test from *a sequence in a gene bank*.' Put another way … *they made it up*! The scientists were supported in this contention by a Portuguese appeals court which ruled in November, 2020, that PCR tests are unreliable and it is unlawful to quarantine people based solely on a PCR test. The point about China not providing an isolated virus must be true when the 'virus' has never been isolated to this day and the consequences of that will become clear. Drosten and company produced this useless 'protocol' right on cue in January, 2020, just as the 'virus' was said to be moving westward and it somehow managed to successfully pass a peer-review in 24 hours. In other words there was no peer-review for a test that would be used to decide who had 'Covid' and who didn't across the world. The Cult-created, Gates-controlled World Health Organization immediately

recommended all its nearly 200 member countries to use the Drosten PCR protocol to detect ‘cases’ and ‘deaths’. The sting was underway and it continues to this day.

So who is this Christian Drosten that produced the means through which death, destruction and economic catastrophe would be justified? His education background, including his doctoral thesis, would appear to be somewhat shrouded in mystery and his track record is dire as with another essential player in the ‘Covid’ hoax, the Gates-funded Professor Neil Ferguson at the Gates-funded Imperial College in London of whom more shortly. Drosten predicted in 2003 that the alleged original SARS ‘virus’ (SARS-1’) was an epidemic that could have serious effects on economies and an effective vaccine would take at least two years to produce. Drosten’s answer to every alleged ‘outbreak’ is a vaccine which you won’t be shocked to know. What followed were just 774 official deaths worldwide and none in Germany where there were only nine cases. That is even if you believe there ever was a SARS ‘virus’ when the evidence is zilch and I will expand on this in the next chapter. Drosten claims to be co-discoverer of ‘SARS-1’ and developed a test for it in 2003. He was screaming warnings about ‘swine flu’ in 2009 and how it was a widespread infection far more severe than any dangers from a vaccine could be and people should get vaccinated. It would be helpful for Drosten’s vocal chords if he simply recorded the words ‘the virus is deadly and you need to get vaccinated’ and copies could be handed out whenever the latest made-up threat comes along. Drosten’s swine flu epidemic never happened, but Big Pharma didn’t mind with governments spending hundreds of millions on vaccines that hardly anyone bothered to use and many who did wished they hadn’t. A study in 2010 revealed that the risk of dying from swine flu, or H1N1, was no higher than that of the annual seasonal flu which is what at least most of ‘it’ really was as in the case of ‘Covid-19’. A media investigation into Drosten asked how with such a record of inaccuracy he could be *the* government adviser on these issues. The answer to that question is the same with Drosten, Ferguson and Fauci – they keep on giving the authorities the ‘conclusions’ and ‘advice’ they want to hear. Drosten certainly produced the goods for them in January, 2020, with his PCR protocol garbage and provided the foundation of what German internal medicine specialist Dr Claus Köhnlein, co-author of *Virus Mania*, called the ‘test pandemic’. The 22 scientists in the *Eurosurveillance* challenge called out conflicts of interest within the

Drosten 'protocol' group and with good reason. Olfert Landt, a regular co-author of Drosten 'studies', owns the biotech company TIB Molbiol Syntheselabor GmbH in Berlin which manufactures and sells the tests that Drosten and his mates come up with. They have done this with SARS, Enterotoxigenic E. coli (ETEC), MERS, Zika 'virus', yellow fever, and now 'Covid'. Landt told the *Berliner Zeitung* newspaper:

> The testing, design and development came from the Charité [Drosten and Corman]. We simply implemented it immediately in the form of a kit. And if we don't have the virus, which originally only existed in Wuhan, we can make a synthetic gene to simulate the genome of the virus. That's what we did very quickly.

This is more confirmation that the Drosten test was designed without access to the 'virus' and only a synthetic simulation which is what SARS-CoV-2 really is – a computer-generated synthetic fiction. It's quite an enterprise they have going here. A Drosten team decides what the test for something should be and Landt's biotech company flogs it to governments and medical systems across the world. His company must have made an absolute fortune since the 'Covid' hoax began. Dr Reiner Fuellmich, a prominent German consumer protection trial lawyer in Germany and California, is on Drosten's case and that of Tedros at the World Health Organization for crimes against humanity with a class-action lawsuit being prepared in the United States and other legal action in Germany.

Why China?

Scamming the world with a 'virus' that doesn't exist would seem impossible on the face of it, but not if you have control of the relatively few people that make policy decisions and the great majority of the global media. Remember it's not about changing 'real' reality it's about controlling *perception* of reality. You don't have to make something happen you only have make people *believe* that it's happening. Renegade Minds understand this and are therefore much harder to swindle. 'Covid-19' is not a 'real' 'virus'. It's a mind virus, like a computer virus, which has infected the minds, not the bodies, of billions. It all started, publically at least, in China and that alone is of central significance. The Cult was behind the revolution led by its asset Mao Zedong, or Chairman Mao, which established the

People's Republic of China on October 1st, 1949. It should have been called The Cult's Republic of China, but the name had to reflect the recurring illusion that vicious dictatorships are run by and for the people (see all the 'Democratic Republics' controlled by tyrants). In the same way we have the 'Biden' Democratic Republic of America officially ruled by a puppet tyrant (at least temporarily) on behalf of Cult tyrants. The creation of Mao's merciless communist/fascist dictatorship was part of a frenzy of activity by the Cult at the conclusion of World War Two which, like the First World War, it had instigated through its assets in Germany, Britain, France, the United States and elsewhere. Israel was formed in 1948; the Soviet Union expanded its 'Iron Curtain' control, influence and military power with the Warsaw Pact communist alliance in 1955; the United Nations was formed in 1945 as a Cult precursor to world government; and a long list of world bodies would be established including the World Health Organization (1948), World Trade Organization (1948 under another name until 1995), International Monetary Fund (1945) and World Bank (1944). Human society was redrawn and hugely centralised in the global Problem-Reaction-Solution that was World War Two. All these changes were significant. Israel would become the headquarters of the Sabbatians and the revolution in China would prepare the ground and control system for the events of 2019/2020.

Renegade Minds know there are no borders except for public consumption. The Cult is a seamless, borderless global entity and to understand the game we need to put aside labels like borders, nations, countries, communism, fascism and democracy. These delude the population into believing that countries are ruled within their borders by a government of whatever shade when these are mere agencies of a global power. America's illusion of democracy and China's communism/fascism are subsidiaries – vehicles – for the same agenda. We may hear about conflict and competition between America and China and on the lower levels that will be true; but at the Cult level they are branches of the same company in the way of the McDonald's example I gave earlier. I have tracked in the books over the years support by US governments of both parties for Chinese Communist Party infiltration of American society through allowing the sale of land, even military facilities, and the acquisition of American business and university influence. All this is underpinned by the infamous stealing of intellectual property and

technological know-how. Cult-owned Silicon Valley corporations waive their fraudulent 'morality' to do business with human-rights-free China; Cult-controlled Disney has become China's PR department; and China in effect owns 'American' sports such as basketball which depends for much of its income on Chinese audiences. As a result any sports player, coach or official speaking out against China's horrific human rights record is immediately condemned or fired by the China-worshipping National Basketball Association. One of the first acts of China-controlled Biden was to issue an executive order telling federal agencies to stop making references to the 'virus' by the 'geographic location of its origin'. Long-time Congressman Jerry Nadler warned that criticising China, America's biggest rival, leads to hate crimes against Asian people in the United States. So shut up you bigot. China is fast closing in on Israel as a country that must not be criticised which is apt, really, given that Sabbatians control them both. The two countries have developed close economic, military, technological and strategic ties which include involvement in China's 'Silk Road' transport and economic initiative to connect China with Europe. Israel was the first country in the Middle East to recognise the establishment of Mao's tyranny in 1950 months after it was established.

Project Wuhan – the 'Covid' Psyop

I emphasise again that the Cult plays the long game and what is happening to the world today is the result of centuries of calculated manipulation following a script to take control step-by-step of every aspect of human society. I will discuss later the common force behind all this that has spanned those centuries and thousands of years if the truth be told. Instigating the Mao revolution in China in 1949 with a 2020 'pandemic' in mind is not only how they work – the 71 years between them is really quite short by the Cult's standards of manipulation preparation. The reason for the Cult's Chinese revolution was to create a fiercely-controlled environment within which an extreme structure for human control could be incubated to eventually be unleashed across the world. We have seen this happen since the 'pandemic' emerged from China with the Chinese control-structure founded on AI technology and tyrannical enforcement sweep across the West. Until the moment when the Cult went for broke in the West and put its fascism on public display Western governments had to pay some

lip-service to freedom and democracy to not alert too many people to the tyranny-in-the-making. Freedoms were more subtly eroded and power centralised with covert government structures put in place waiting for the arrival of 2020 when that smokescreen of 'freedom' could be dispensed with. The West was not able to move towards tyranny before 2020 anything like as fast as China which was created as a tyranny and had no limits on how fast it could construct the Cult's blueprint for global control. When the time came to impose that structure on the world it was the same Cult-owned Chinese communist/fascist government that provided the excuse – the 'Covid pandemic'. It was absolutely crucial to the Cult plan for the Chinese response to the 'pandemic' – draconian lockdowns of the entire population – to become the blueprint that Western countries would follow to destroy the livelihoods and freedom of their people. This is why the Cult-owned, Gates-owned, WHO Director-General Tedros said early on:

The Chinese government is to be congratulated for the extraordinary measures it has taken to contain the outbreak. China is actually setting a new standard for outbreak response and it is not an exaggeration.

Forbes magazine said of China: '… those measures protected untold millions from getting the disease'. The Rockefeller Foundation 'epidemic scenario' document in 2010 said 'prophetically':

However, a few countries did fare better – China in particular. The Chinese government's quick imposition and enforcement of mandatory quarantine for all citizens, as well as its instant and near-hermetic sealing off of all borders, saved millions of lives, stopping the spread of the virus far earlier than in other countries and enabling a swifter post-pandemic recovery.

Once again – *spooky*.

The first official story was the 'bat theory' or rather the bat diversion. The source of the 'virus outbreak' we were told was a ''wet market' in Wuhan where bats and other animals are bought and eaten in horrifically unhygienic conditions. Then another story emerged through the alternative media that the 'virus' had been released on purpose or by accident from a BSL-4 (biosafety level 4) laboratory in Wuhan not far from the wet market. The lab was reported to create and work with lethal concoctions and

bioweapons. Biosafety level 4 is the highest in the World Health Organization system of safety and containment. Renegade Minds are aware of what I call designer manipulation. The ideal for the Cult is for people to buy its prime narrative which in the opening salvoes of the 'pandemic' was the wet market story. It knows, however, that there is now a considerable worldwide alternative media of researchers sceptical of anything governments say and they are often given a version of events in a form they can perceive as credible while misdirecting them from the real truth. In this case let them think that the conspiracy involved is a 'bioweapon virus' released from the Wuhan lab to keep them from the real conspiracy – *there is no 'virus'*. The WHO's current position on the source of the outbreak at the time of writing appears to be: 'We haven't got a clue, mate.' This is a good position to maintain mystery and bewilderment. The inner circle will know where the 'virus' came from – *nowhere*. The bottom line was to ensure the public believed there *was* a 'virus' and it didn't much matter if they thought it was natural or had been released from a lab. The belief that there was a 'deadly virus' was all that was needed to trigger global panic and fear. The population was terrified into handing their power to authority and doing what they were told. They had to or they were 'all gonna die'.

In March, 2020, information began to come my way from real doctors and scientists and my own additional research which had my intuition screaming: 'Yes, that's it! *There is no virus*.' The 'bioweapon' was not the 'virus'; it was the *'vaccine'* already being talked about that would be the bioweapon. My conclusion was further enhanced by happenings in Wuhan. The 'virus' was said to be sweeping the city and news footage circulated of people collapsing in the street (which they've never done in the West with the same 'virus'). The Chinese government was building 'new hospitals' in a matter of ten days to 'cope with demand' such was the virulent nature of the 'virus'. Yet in what seemed like no time the 'new hospitals' closed – even if they even opened – and China declared itself 'virus-free'. It was back to business as usual. This was more propaganda to promote the Chinese draconian lockdowns in the West as the way to 'beat the virus'. Trouble was that we subsequently had lockdown after lockdown, but never business as usual. As the people of the West and most of the rest of the world were caught in an ever-worsening spiral of lockdown, social distancing, masks, isolated old people, families forced apart, and livelihood destruction, it was party-time in Wuhan. Pictures emerged of thousands of

people enjoying pool parties and concerts. It made no sense until you realised there never was a ‘virus’ and the whole thing was a Cult set-up to transform human society out of one its major global strongholds – China.

How is it possible to deceive virtually the entire world population into believing there is a deadly virus when there is not even a ‘virus’ let alone a deadly one? It’s nothing like as difficult as you would think and that’s clearly true because it happened.

Postscript: See end of book Postscript for more on the ‘Wuhan lab virus release’ story which the authorities and media were pushing heavily in the summer of 2021 to divert attention from the truth that the ‘Covid virus’ is pure invention.

CHAPTER FIVE

There *is no* 'virus'

You can fool some of the people all of the time, and all of the people some of the time, but you cannot fool all of the people all of the time
Abraham Lincoln

The greatest form of mind control is repetition. The more you repeat the same mantra of alleged 'facts' the more will accept them to be true. It becomes an 'everyone knows that, mate'. If you can also censor any other version or alternative to your alleged 'facts' you are pretty much home and cooking.

By the start of 2020 the Cult owned the global mainstream media almost in its entirety to spew out its 'Covid' propaganda and ignore or discredit any other information and view. Cult-owned social media platforms in Cult-owned Silicon Valley were poised and ready to unleash a campaign of ferocious censorship to obliterate all but the official narrative. To complete the circle many demands for censorship by Silicon Valley were led by the mainstream media as 'journalists' became full-out enforcers for the Cult both as propagandists and censors. Part of this has been the influx of young people straight out of university who have become 'journalists' in significant positions. They have no experience and a headful of programmed perceptions from their years at school and university at a time when today's young are the most perceptually-targeted generations in known human history given the insidious impact of technology. They enter the media perceptually prepared and ready to repeat the narratives of the system that programmed them to repeat its narratives. The BBC has a truly

pathetic ‘specialist disinformation reporter’ called Marianna Spring who fits this bill perfectly. She is clueless about the world, how it works and what is really going on. Her role is to discredit anyone doing the job that a proper journalist would do and system-serving hacks like Spring wouldn’t dare to do or even see the need to do. They are too busy licking the arse of authority which can never be wrong and, in the case of the BBC propaganda programme, *Panorama*, contacting payments systems such as PayPal to have a donations page taken down for a film company making documentaries questioning vaccines. Even the BBC soap opera *EastEnders* included a disgracefully biased scene in which an inarticulate white working class woman was made to look foolish for questioning the ‘vaccine’ while a well-spoken black man and Asian woman promoted the government narrative. It ticked every BBC box and the fact that the black and minority community was resisting the ‘vaccine’ had nothing to do with the way the scene was written. The BBC has become a disgusting tyrannical propaganda and censorship operation that should be defunded and disbanded and a free media take its place with a brief to stop censorship instead of demanding it. A BBC ‘interview’ with Gates goes something like: ‘Mr Gates, sir, if I can call you sir, would you like to tell our audience why you are such a great man, a wonderful humanitarian philanthropist, and why you should absolutely be allowed as a software salesman to decide health policy for approaching eight billion people? Thank you, sir, please sir.’ Propaganda programming has been incessant and merciless and when all you hear is the same story from the media, repeated by those around you who have only heard the same story, is it any wonder that people on a grand scale believe absolute mendacious garbage to be true? You are about to see, too, why this level of information control is necessary when the official ‘Covid’ narrative is so nonsensical and unsupportable by the evidence.

Structure of Deceit

The pyramid structure through which the ‘Covid’ hoax has been manifested is very simple and has to be to work. As few people as possible have to be involved with full knowledge of what they are doing – and why – or the real story would get out. At the top of the pyramid are the inner core of the Cult which controls Bill Gates who, in turn, controls the World Health Organization through his pivotal funding and his puppet Director-General

mouthpiece, Tedros. Before he was appointed Tedros was chair of the Gates-founded Global Fund to 'fight against AIDS, tuberculosis and malaria', a board member of the Gates-funded 'vaccine alliance' GAVI, and on the board of another Gates-funded organisation. Gates owns him and picked him for a specific reason – Tedros is a crook and worse. 'Dr' Tedros (he's not a medical doctor, the first WHO chief not to be) was a member of the tyrannical Marxist government of Ethiopia for decades with all its human rights abuses. He has faced allegations of corruption and misappropriation of funds and was exposed three times for covering up cholera epidemics while Ethiopia's health minister. Tedros appointed the mass-murdering genocidal Zimbabwe dictator Robert Mugabe as a WHO goodwill ambassador for public health which, as with Tedros, is like appointing a psychopath to run a peace and love campaign. The move was so ridiculous that he had to drop Mugabe in the face of widespread condemnation. American economist David Steinman, a Nobel peace prize nominee, lodged a complaint with the International Criminal Court in The Hague over alleged genocide by Tedros when he was Ethiopia's foreign minister. Steinman says Tedros was a 'crucial decision maker' who directed the actions of Ethiopia's security forces from 2013 to 2015 and one of three officials in charge when those security services embarked on the 'killing' and 'torturing' of Ethiopians. You can see where Tedros is coming from and it's sobering to think that he has been the vehicle for Gates and the Cult to direct the global response to 'Covid'. Think about that. A psychopathic Cult dictates to psychopath Gates who dictates to psychopath Tedros who dictates how countries of the world must respond to a 'Covid virus' never scientifically shown to exist. At the same time psychopathic Cult-owned Silicon Valley information giants like Google, YouTube, Facebook and Twitter announced very early on that they would give the Cult/Gates/Tedros/WHO version of the narrative free advertising and censor those who challenged their intelligence-insulting, mendacious story.

The next layer in the global 'medical' structure below the Cult, Gates and Tedros are the chief medical officers and science 'advisers' in each of the WHO member countries which means virtually all of them. Medical officers and arbiters of science (they're not) then take the WHO policy and recommended responses and impose them on their country's population while the political 'leaders' say they are deciding policy (they're clearly not) by 'following the science' on the advice of the 'experts' – the same

medical officers and science ‘advisers’ (dictators). In this way with the rarest of exceptions the entire world followed the same policy of lockdown, people distancing, masks and ‘vaccines’ dictated by the psychopathic Cult, psychopathic Gates and psychopathic Tedros who we are supposed to believe give a damn about the health of the world population they are seeking to enslave. That, amazingly, is all there is to it in terms of crucial decision-making. Medical staff in each country then follow like sheep the dictates of the shepherds at the top of the national medical hierarchies – chief medical officers and science ‘advisers’ who themselves follow like sheep the shepherds of the World Health Organization and the Cult. Shepherds at the national level often have major funding and other connections to Gates and his Bill and Melinda Gates Foundation which carefully hands out money like confetti at a wedding to control the entire global medical system from the WHO down.

Follow the money

Christopher Whitty, Chief Medical Adviser to the UK Government at the centre of ‘virus’ policy, a senior adviser to the government’s Scientific Advisory Group for Emergencies (SAGE), and Executive Board member of the World Health Organization, was gifted a grant of $40 million by the Bill and Melinda Gates Foundation for malaria research in Africa. The BBC described the unelected Whitty as ‘the official who will probably have the greatest impact on our everyday lives of any individual policymaker in modern times’ and so it turned out. What Gates and Tedros have said Whitty has done like his equivalents around the world. Patrick Vallance, co-chair of SAGE and the government’s Chief Scientific Adviser, is a former executive of Big Pharma giant GlaxoSmithKline with its fundamental financial and business connections to Bill Gates. In September, 2020, it was revealed that Vallance owned a deferred bonus of shares in GlaxoSmithKline worth £600,000 while the company was ‘developing’ a ‘Covid vaccine’. Move along now – nothing to see here – what could possibly be wrong with that? Imperial College in London, a major player in ‘Covid’ policy in Britain and elsewhere with its ‘Covid-19’ Response Team, is funded by Gates and has big connections to China while the now infamous Professor Neil Ferguson, the useless ‘computer modeller’ at Imperial College is also funded by Gates. Ferguson delivered the

dramatically inaccurate excuse for the first lockdowns (much more in the next chapter). The Institute for Health Metrics and Evaluation (IHME) in the United States, another source of outrageously false 'Covid' computer models to justify lockdowns, is bankrolled by Gates who is a vehement promotor of lockdowns. America's version of Whitty and Vallance, the again now infamous Anthony Fauci, has connections to 'Covid vaccine' maker Moderna as does Bill Gates through funding from the Bill and Melinda Gates Foundation. Fauci is director of the National Institute of Allergy and Infectious Diseases (NIAID), a major recipient of Gates money, and they are very close. Deborah Birx who was appointed White House Coronavirus Response Coordinator in February, 2020, is yet another with ties to Gates. Everywhere you look at the different elements around the world behind the coordination and decision making of the 'Covid' hoax there is Bill Gates and his money. They include the World Health Organization; Centers for Disease Control (CDC) in the United States; National Institutes of Health (NIH) of Anthony Fauci; Imperial College and Neil Ferguson; the London School of Hygiene where Chris Whitty worked; Regulatory agencies like the UK Medicines & Healthcare products Regulatory Agency (MHRA) which gave emergency approval for 'Covid vaccines'; Wellcome Trust; GAVI, the Vaccine Alliance; the Coalition for Epidemic Preparedness Innovations (CEPI); Johns Hopkins University which has compiled the false 'Covid' figures; and the World Economic Forum. A Nationalfile.com article said:

> Gates has a lot of pull in the medical world, he has a multi-million dollar relationship with Dr. Fauci, and Fauci originally took the Gates line supporting vaccines and casting doubt on [the drug hydroxychloroquine]. Coronavirus response team member Dr. Deborah Birx, appointed by former president Obama to serve as United States Global AIDS Coordinator, also sits on the board of a group that has received billions from Gates' foundation, and Birx reportedly used a disputed Bill Gates-funded model for the White House's Coronavirus effort. Gates is a big proponent for a population lockdown scenario for the Coronavirus outbreak.

Another funder of Moderna is the Defense Advanced Research Projects Agency (DARPA), the technology-development arm of the Pentagon and one of the most sinister organisations on earth. DARPA had a major role with the CIA covert technology-funding operation In-Q-Tel in the development of Google and social media which is now at the centre of

global censorship. Fauci and Gates are extremely close and openly admit to talking regularly about 'Covid' policy, but then why wouldn't Gates have a seat at every national 'Covid' table after his Foundation committed $1.75 billion to the 'fight against Covid-19'. When passed through our Orwellian Translation Unit this means that he has bought and paid for the Cult-driven 'Covid' response worldwide. Research the major 'Covid' response personnel in your own country and you will find the same Gates funding and other connections again and again. Medical and science chiefs following World Health Organization 'policy' sit atop a medical hierarchy in their country of administrators, doctors and nursing staff. These 'subordinates' are told they must work and behave in accordance with the policy delivered from the 'top' of the national 'health' pyramid which is largely the policy delivered by the WHO which is the policy delivered by Gates and the Cult. The whole 'Covid' narrative has been imposed on medical staff by a climate of fear although great numbers don't even need that to comply. They do so through breathtaking levels of ignorance and include doctors who go through life simply repeating what Big Pharma and their hierarchical masters tell them to say and believe. No wonder Big Pharma 'medicine' is one of the biggest killers on Planet Earth.

The same top-down system of intimidation operates with regard to the Cult Big Pharma cartel which also dictates policy through national and global medical systems in this way. The Cult and Big Pharma agendas are the same because the former controls and owns the latter. 'Health' administrators, doctors, and nursing staff are told to support and parrot the dictated policy or they will face consequences which can include being fired. How sad it's been to see medical staff meekly repeating and imposing Cult policy without question and most of those who can see through the deceit are only willing to speak anonymously off the record. They know what will happen if their identity is known. This has left the courageous few to expose the lies about the 'virus', face masks, overwhelmed hospitals that aren't, and the dangers of the 'vaccine' that isn't a vaccine. When these medical professionals and scientists, some renowned in their field, have taken to the Internet to expose the truth their articles, comments and videos have been deleted by Cult-owned Facebook, Twitter and YouTube. What a real head-shaker to see YouTube videos with leading world scientists and highly qualified medical specialists with an added link underneath to the

notorious Cult propaganda website *Wikipedia* to find the 'facts' about the same subject.

HIV – the 'Covid' trial-run

I'll give you an example of the consequences for health and truth that come from censorship and unquestioning belief in official narratives. The story was told by PCR inventor Kary Mullis in his book *Dancing Naked in the Mind Field*. He said that in 1984 he accepted as just another scientific fact that Luc Montagnier of France's Pasteur Institute and Robert Gallo of America's National Institutes of Health had independently discovered that a 'retrovirus' dubbed HIV (human immunodeficiency virus) caused AIDS. They were, after all, Mullis writes, specialists in retroviruses. This is how the medical and science pyramids work. Something is announced or *assumed* and then becomes an everybody-knows-that purely through repetition of the assumption as if it is fact. Complete crap becomes accepted truth with no supporting evidence and only repetition of the crap. This is how a 'virus' that doesn't exist became the 'virus' that changed the world. The HIV-AIDS fairy story became a multi-billion pound industry and the media poured out propaganda terrifying the world about the deadly HIV 'virus' that caused the lethal AIDS. By then Mullis was working at a lab in Santa Monica, California, to detect retroviruses with his PCR test in blood donations received by the Red Cross. In doing so he asked a virologist where he could find a reference for HIV being the cause of AIDS. 'You don't need a reference,' the virologist said … *'Everybody knows it.'* Mullis said he wanted to quote a reference in the report he was doing and he said he felt a little funny about not knowing the source of such an important discovery when everyone else seemed to. The virologist suggested he cite a report by the Centers for Disease Control and Prevention (CDC) on morbidity and mortality. Mullis read the report, but it only said that an organism had been identified and did not say how. The report did not identify the original scientific work. Physicians, however, *assumed* (key recurring theme) that if the CDC was convinced that HIV caused AIDS then proof must exist. Mullis continues:

I did computer searches. Neither Montagnier, Gallo, nor anyone else had published papers describing experiments which led to the conclusion that HIV probably caused AIDS. I read the papers in

Science for which they had become well known as AIDS doctors, but all they had said there was that they had found evidence of a past infection by something which was probably HIV in some AIDS patients.

They found antibodies. Antibodies to viruses had always been considered evidence of past disease, not present disease. Antibodies signaled that the virus had been defeated. The patient had saved himself. There was no indication in these papers that this virus caused a disease. They didn't show that everybody with the antibodies had the disease. In fact they found some healthy people with antibodies.

Mullis asked why their work had been published if Montagnier and Gallo hadn't really found this evidence, and why had they been fighting so hard to get credit for the discovery? He says he was hesitant to write 'HIV is the probable cause of AIDS' until he found published evidence to support that. 'Tens of thousands of scientists and researchers were spending billions of dollars a year doing research based on this idea,' Mullis writes. 'The reason had to be there somewhere; otherwise these people would not have allowed their research to settle into one narrow channel of investigation.' He said he lectured about PCR at numerous meetings where people were always talking about HIV and he asked them how they knew that HIV was the cause of AIDS:

Everyone said something. Everyone had the answer at home, in the office, in some drawer. They all knew, and they would send me the papers as soon as they got back. But I never got any papers. Nobody ever sent me the news about how AIDS was caused by HIV.

Eventually Mullis was able to ask Montagnier himself about the reference proof when he lectured in San Diego at the grand opening of the University of California AIDS Research Center. Mullis says this was the last time he would ask his question without showing anger. Montagnier said he should reference the CDC report. 'I read it', Mullis said, and it didn't answer the question. 'If Montagnier didn't know the answer who the hell did?' Then one night Mullis was driving when an interview came on National Public Radio with Peter Duesberg, a prominent virologist at Berkeley and a California Scientist of the Year. Mullis says he finally understood why he could not find references that connected HIV to AIDS – *there weren't any!* No one had ever proved that HIV causes AIDS even though it had spawned a multi-billion pound global industry and the media was repeating this as

fact every day in their articles and broadcasts terrifying the shit out of people about AIDS and giving the impression that a positive test for HIV (see 'Covid') was a death sentence. Duesberg was a threat to the AIDS gravy train and the agenda that underpinned it. He was therefore abused and castigated after he told the Proceedings of the National Academy of Sciences there was no good evidence implicating the new 'virus'. Editors rejected his manuscripts and his research funds were deleted. Mullis points out that the CDC has defined AIDS as one of more than 30 diseases *if accompanied* by a positive result on a test that detects antibodies to HIV; but those same diseases are not defined as AIDS cases when antibodies are not detected:

> If an HIV-positive woman develops uterine cancer, for example, she is considered to have AIDS. If she is not HIV positive, she simply has uterine cancer. An HIV-positive man with tuberculosis has AIDS; if he tests negative he simply has tuberculosis. If he lives in Kenya or Colombia, where the test for HIV antibodies is too expensive, he is simply presumed to have the antibodies and therefore AIDS, and therefore he can be treated in the World Health Organization's clinic. It's the only medical help available in some places. And it's free, because the countries that support WHO are worried about AIDS.

Mullis accuses the CDC of continually adding new diseases (see ever more 'Covid symptoms') to the grand AIDS definition and of virtually doctoring the books to make it appear as if the disease continued to spread. He cites how in 1993 the CDC enormously broadened its AIDS definition and county health authorities were delighted because they received $2,500 per year from the Federal government for every reported AIDS case. Ladies and gentlemen, I have just described, via Kary Mullis, the 'Covid pandemic' of 2020 and beyond. Every element is the same and it's been pulled off in the same way by the same networks.

The 'Covid virus' exists? Okay – prove it. Er … still waiting

What Kary Mullis described with regard to 'HIV' has been repeated with 'Covid'. A claim is made that a new, or 'novel', infection has been found and the entire medical system of the world repeats that as fact exactly as they did with HIV and AIDS. No one in the mainstream asks rather relevant questions such as 'How do you know?' and 'Where is your proof?' The

SARS-Cov-2 ‘virus’ and the ‘Covid-19 disease’ became an overnight ‘everybody-knows-that’. The origin could be debated and mulled over, but what you could not suggest was that ‘SARS-Cov-2’ didn’t exist. That would be ridiculous. ‘Everybody knows’ the ‘virus’ exists. Well, I didn’t for one along with American proper doctors like Andrew Kaufman and Tom Cowan and long-time American proper journalist Jon Rappaport. We dared to pursue the obvious and simple question: ‘Where’s the evidence?’ The overwhelming majority in medicine, journalism and the general public did not think to ask that. After all, *everyone knew* there was a new ‘virus’. Everyone was saying so and I heard it on the BBC. Some would eventually argue that the ‘deadly virus’ was nothing like as deadly as claimed, but few would venture into the realms of its very existence. Had they done so they would have found that the evidence for that claim had gone AWOL as with HIV causes AIDS. In fact, not even that. For something to go AWOL it has to exist in the first place and scientific proof for a ‘SARS-Cov-2’ can be filed under nothing, nowhere and zilch.

Dr Andrew Kaufman is a board-certified forensic psychiatrist in New York State, a Doctor of Medicine and former Assistant Professor and Medical Director of Psychiatry at SUNY Upstate Medical University, and Medical Instructor of Hematology and Oncology at the Medical School of South Carolina. He also studied biology at the Massachusetts Institute of Technology (MIT) and trained in Psychiatry at Duke University. Kaufman is retired from allopathic medicine, but remains a consultant and educator on natural healing, I saw a video of his very early on in the ‘Covid’ hoax in which he questioned claims about the ‘virus’ in the absence of any supporting evidence and with plenty pointing the other way. I did everything I could to circulate his work which I felt was asking the pivotal questions that needed an answer. I can recommend an excellent pull-together interview he did with the website The Last Vagabond entitled *Dr Andrew Kaufman: Virus Isolation, Terrain Theory and Covid-19* and his website is andrewkaufmanmd.com. Kaufman is not only a forensic psychiatrist; he is forensic in all that he does. He always reads original scientific papers, experiments and studies instead of second-third-fourth-hand reports about the ‘virus’ in the media which are repeating the repeated repetition of the narrative. When he did so with the original Chinese ‘virus’ papers Kaufman realised that there was no evidence of a ‘SARS-Cov-2’. They had never – from the start – shown it to exist and every repeat of this

claim worldwide was based on the accepted existence of proof that was nowhere to be found – see Kary Mullis and HIV. Here we go again.

Let's postulate

Kaufman discovered that the Chinese authorities immediately concluded that the cause of an illness that broke out among about 200 initial patients in Wuhan was a 'new virus' when there were no grounds to make that conclusion. The alleged 'virus' was not isolated from other genetic material in their samples and then shown through a system known as Koch's postulates to be the causative agent of the illness. The world was told that the SARS-Cov-2 'virus' caused a disease they called 'Covid-19' which had 'flu-like' symptoms and could lead to respiratory problems and pneumonia. If it wasn't so tragic it would almost be funny. *'Flu-like' symptoms'? Pneumonia? Respiratory disease?* What in *CHINA* and particularly in *Wuhan*, one of the most polluted cities in the world with a resulting epidemic of respiratory disease?? Three hundred thousand people get pneumonia in China every year and there are nearly a billion cases worldwide of 'flu-like symptoms'. These have a whole range of causes – including pollution in Wuhan – but no other possibility was credibly considered in late 2019 when the world was told there was a new and deadly 'virus'. The global prevalence of pneumonia and 'flu-like systems' gave the Cult networks unlimited potential to re-diagnose these other causes as the mythical 'Covid-19' and that is what they did from the very start. Kaufman revealed how Chinese medical and science authorities (all subordinates to the Cult-owned communist government) took genetic material from the lungs of only a few of the first patients. The material contained their own cells, bacteria, fungi and other microorganisms living in their bodies. The only way you could prove the existence of the 'virus' and its responsibility for the alleged 'Covid-19' was to isolate the virus from all the other material – a process also known as 'purification' – and then follow the postulates sequence developed in the late 19th century by German physician and bacteriologist Robert Koch which became the 'gold standard' for connecting an alleged causation agent to a disease:

1. The microorganism (bacteria, fungus, virus, etc.) must be present in every case of the disease and all patients must have the same symptoms. It must also *not be present in healthy individuals*.

2. The microorganism must be isolated from the host with the disease. If the microorganism is a bacteria or fungus it must be grown in a pure culture. If it is a virus, it must be purified (i.e. containing no other material except the virus particles) from a clinical sample.

3. The specific disease, with all of its characteristics, must be reproduced when the infectious agent (the purified virus or a pure culture of bacteria or fungi) is inoculated into a healthy, susceptible host.

4. The microorganism must be recoverable from the experimentally infected host as in step 2.

Not one of these criteria has been met in the case of 'SARS-Cov-2' and 'Covid-19'. Not ONE. *EVER*. Robert Koch refers to bacteria and not viruses. What are called 'viral particles' are so minute (hence masks are useless by any definition) that they could only be seen after the invention of the electron microscope in the 1930s and can still only be observed through that means. American bacteriologist and virologist Thomas Milton Rivers, the so-called 'Father of Modern Virology' who was very significantly director of the Rockefeller Institute for Medical Research in the 1930s, developed a less stringent version of Koch's postulates to identify 'virus' causation known as 'Rivers criteria'. 'Covid' did not pass that process either. Some even doubt whether any 'virus' can be isolated from other particles containing genetic material in the Koch method. Freedom of Information requests in many countries asking for scientific proof that the 'Covid virus' has been purified and isolated and shown to exist have all come back with a 'we don't have that' and when this happened with a request to the UK Department of Health they added this comment:

However, outside of the scope of the [Freedom of Information Act] and on a discretionary basis, the following information has been advised to us, which may be of interest. Most infectious diseases are caused by viruses, bacteria or fungi. Some bacteria or fungi have the capacity to grow on their own in isolation, for example in colonies on a petri dish. Viruses are different in that they are what we call 'obligate pathogens' – that is, they cannot survive or reproduce without infecting a host ...

… For some diseases, it is possible to establish causation between a microorganism and a disease by isolating the pathogen from a patient, growing it in pure culture and reintroducing it to a healthy organism. These are known as 'Koch's postulates' and were developed in 1882. However, as our understanding of disease and different disease-causing agents has advanced, these are no longer the method for determining causation [Andrew Kaufman asks why in that case are there two published articles falsely claiming to satisfy Koch's postulates].

It has long been known that viral diseases cannot be identified in this way as viruses cannot be grown in 'pure culture'. When a patient is tested for a viral illness, this is normally done by looking for the presence of antigens, or viral genetic code in a host with molecular biology techniques [Kaufman

asks how you could know the origin of these chemicals without having a pure culture for comparison].

For the record ‘antigens’ are defined so:

Invading microorganisms have antigens on their surface that the human body can recognise as being foreign – meaning not belonging to it. When the body recognises a foreign antigen, lymphocytes (white blood cells) produce antibodies, which are complementary in shape to the antigen.

Notwithstanding that this is open to question in relation to ‘SARS-Cov-2’ the presence of ‘antibodies’ can have many causes and they are found in people that are perfectly well. Kary Mullis said: ‘Antibodies … had always been considered evidence of past disease, not present disease.’

‘Covid’ really is a *computer* ‘virus’

Where the UK Department of Health statement says ‘viruses’ are now ‘diagnosed’ through a ‘viral genetic code in a host with molecular biology techniques’, they mean … *the PCR test* which its inventor said cannot test for infectious disease. They have no credible method of connecting a ‘virus’ to a disease and we will see that there is no scientific proof that any ‘virus’ causes any disease or there is any such thing as a ‘virus’ in the way that it is described. Tenacious Canadian researcher Christine Massey and her team made some 40 Freedom of Information requests to national public health agencies in different countries asking for proof that SARS-CoV-2 has been isolated and not one of them could supply that information. Massey said of her request in Canada: ‘Freedom of Information reveals Public Health Agency of Canada has no record of ‘SARS-COV-2’ isolation performed by anyone, anywhere, ever.’ If you accept the comment from the UK Department of Health it’s because they can’t isolate a ‘virus’. Even so many ‘science’ papers claimed to have isolated the ‘Covid virus’ until they were questioned and had to admit they hadn’t. A reply from the Robert Koch Institute in Germany was typical: ‘I am not aware of a paper which purified isolated SARS-CoV-2.’ So what the hell was Christian Drosten and his gang using to design the ‘Covid’ testing protocol that has produced all the illusory Covid’ cases and ‘Covid’ deaths when the head of the Chinese version of the CDC admitted there was a problem right from the start in that the ‘virus’ had never been isolated/purified? Breathe deeply: What they are calling ‘Covid’ is actually created by a *computer program* i.e. *they made it*

up – er, that's it. They took lung fluid, with many sources of genetic material, from one single person alleged to be infected with Covid-19 by a PCR test which they *claimed*, without clear evidence, contained a 'virus'. They used several computer programs to create a model of a theoretical virus genome sequence from more than fifty-six million small sequences of RNA, each of an unknown source, assembling them like a puzzle with no known solution. The computer filled in the gaps with sequences from bits in the gene bank to make it look like a bat SARS-like coronavirus! A wave of the magic wand and poof, an in silico (computer-generated) genome, a scientific fantasy, was created. UK health researcher Dr Kevin Corbett made the same point with this analogy:

> … It's like giving you a few bones and saying that's your fish. It could be any fish. Not even a skeleton. Here's a few fragments of bones. That's your fish … It's all from gene bank and the bits of the virus sequence that weren't there they made up.
>
> They synthetically created them to fill in the blanks. That's what genetics is; it's a code. So it's ABBBCCDDD and you're missing some what you think is EEE so you put it in. It's all synthetic. You just manufacture the bits that are missing. This is the end result of the geneticization of virology. This is basically a computer virus.

Further confirmation came in an email exchange between British citizen journalist Frances Leader and the government's Medicines & Healthcare Products Regulatory Agency (the Gates-funded MHRA) which gave emergency permission for untested 'Covid vaccines' to be used. The agency admitted that the 'vaccine' is not based on an isolated 'virus', but comes from a *computer-generated model*. Frances Leader was naturally banned from Cult-owned fascist Twitter for making this exchange public. The process of creating computer-generated alleged 'viruses' is called 'in silico' or 'in silicon' – computer chips – and the term 'in silico' is believed to originate with biological experiments using only a computer in 1989. 'Vaccines' involved with 'Covid' are also produced 'in silico' or by computer not a natural process. If the original 'virus' is nothing more than a made-up computer model how can there be 'new variants' of something that never existed in the first place? They are not new 'variants'; they are new *computer models* only minutely different to the original program and designed to further terrify the population into having the 'vaccine' and submitting to fascism. You want a 'new variant'? Click, click, enter – there

you go. Tell the medical profession that you have discovered a ‘South African variant’, ‘UK variants’ or a ‘Brazilian variant’ and in the usual HIV-causes-AIDS manner they will unquestioningly repeat it with no evidence whatsoever to support these claims. They will go on television and warn about the dangers of ‘new variants’ while doing nothing more than repeating what they have been told to be true and knowing that any deviation from that would be career suicide. Big-time insiders will know it’s a hoax, but much of the medical community is clueless about the way they are being played and themselves play the public without even being aware they are doing so. What an interesting ‘coincidence’ that AstraZeneca and Oxford University were conducting ‘Covid vaccine trials’ in the three countries – the UK, South Africa and Brazil – where the first three ‘variants’ were claimed to have ‘broken out’.

Here’s your ‘virus’ – it’s a unicorn

Dr Andrew Kaufman presented a brilliant analysis describing how the ‘virus’ was imagined into fake existence when he dissected an article published by *Nature* and written by 19 authors detailing *alleged* ‘sequencing of a complete viral genome’ of the ‘new SARS-CoV-2 virus’. This computer-modelled *in silico* genome was used as a template for all subsequent genome sequencing experiments that resulted in the so-called variants which he said now number more than *6,000*. The fake genome was constructed from more than 56 million individual short strands of RNA. Those little pieces were assembled into longer pieces by finding areas of overlapping sequences. The computer programs created over two million possible combinations from which the authors simply chose the longest one. They then compared this to a ‘bat virus’ and the computer ‘alignment’ rearranged the sequence and filled in the gaps! They called this computer-generated abomination the ‘complete genome’. Dr Tom Cowan, a fellow medical author and collaborator with Kaufman, said such computer-generation constitutes scientific fraud and he makes this superb analogy:

> Here is an equivalency: A group of researchers claim to have found a unicorn because they found a piece of a hoof, a hair from a tail, and a snippet of a horn. They then add that information into a computer and program it to re-create the unicorn, and they then claim this computer re-creation is the real unicorn. Of course, they had never actually seen a unicorn so could not possibly have examined its genetic makeup to compare their samples with the actual unicorn’s hair, hooves and horn.

The researchers claim they decided which is the real genome of SARS-CoV-2 by ‘consensus’, sort of like a vote. Again, different computer programs will come up with different versions of the imaginary ‘unicorn’, so they come together as a group and decide which is the real imaginary unicorn.

This is how the ‘virus’ that has transformed the world was brought into fraudulent ‘existence’. Extraordinary, yes, but as the Nazis said the bigger the lie the more will believe it. Cowan, however, wasn’t finished and he went on to identify what he called the real blockbuster in the paper. He quotes this section from a paper written by virologists and published by the CDC and then explains what it means:

Therefore, we examined the capacity of SARS-CoV-2 to infect and replicate in several common primate and human cell lines, including human adenocarcinoma cells (A549), human liver cells (HUH 7.0), and human embryonic kidney cells (HEK-293T). In addition to Vero E6 and Vero CCL81 cells. ... Each cell line was inoculated at high multiplicity of infection and examined 24h post-infection.

No CPE was observed in any of the cell lines except in Vero cells, which grew to greater than 10 to the 7th power at 24 h post-infection. In contrast, HUH 7.0 and 293T showed only modest viral replication, and A549 cells were incompatible with SARS CoV-2 infection.

Cowan explains that when virologists attempt to prove infection they have three possible ‘hosts’ or models on which they can test. The first was humans. Exposure to humans was generally not done for ethical reasons and has never been done with SARS-CoV-2 or any coronavirus. The second possible host was animals. Cowan said that forgetting for a moment that they never actually use purified virus when exposing animals they do use solutions that they *claim* contain the virus. Exposure to animals has been done with SARS-CoV-2 in an experiment involving mice and this is what they found: *None of the wild (normal) mice got sick*. In a group of genetically-modified mice, a statistically insignificant number lost weight and had slightly bristled fur, but they experienced nothing like the illness called ‘Covid-19’. Cowan said the third method – the one they mostly rely on – is to inoculate solutions they *say* contain the virus onto a variety of tissue cultures. This process had never been shown to kill tissue *unless* the sample material was starved of nutrients and poisoned as *part of the process*. Yes, incredibly, in tissue experiments designed to show the ‘virus’ is responsible for killing the tissue they starve the tissue of nutrients and

add toxic drugs including antibiotics and they do not have control studies to see if it's the starvation and poisoning that is degrading the tissue rather than the 'virus' they allege to be in there somewhere. You want me to pinch you? Yep, I understand. Tom Cowan said this about the whole nonsensical farce as he explains what that quote from the CDC paper really means:

The shocking thing about the above quote is that using their own methods, the virologists found that solutions containing SARS-CoV-2 – even in high amounts – were NOT, I repeat NOT, infective to any of the three human tissue cultures they tested. In plain English, this means they proved, on their terms, that this 'new coronavirus' is not infectious to human beings. It is ONLY infective to monkey kidney cells, and only then when you add two potent drugs (gentamicin and amphotericin), known to be toxic to kidneys, to the mix.

My friends, read this again and again. These virologists, published by the CDC, performed a clear proof, on their terms, showing that the SARS-CoV-2 virus is harmless to human beings. That is the only possible conclusion, but, unfortunately, this result is not even mentioned in their conclusion. They simply say they can provide virus stocks cultured only on monkey Vero cells, thanks for coming.

Cowan concluded: 'If people really understood how this "science" was done, I would hope they would storm the gates and demand honesty, transparency and truth.' Dr Michael Yeadon, former Vice President and Chief Scientific Adviser at drug giant Pfizer has been a vocal critic of the 'Covid vaccine' and its potential for multiple harm. He said in an interview in April, 2021, that 'not one [vaccine] has the virus. He was asked why vaccines normally using a 'dead' version of a disease to activate the immune system were not used for 'Covid' and instead we had the synthetic methods of the 'mRNA Covid vaccine'. Yeadon said that to do the former 'you'd have to have some of [the virus] wouldn't you?' He added: 'No-one's got any – seriously.' Yeadon said that surely they couldn't have fooled the whole world for a year without having a virus, 'but oddly enough ask around – no one's got it'. He didn't know why with all the 'great labs' around the world that the virus had not been isolated – 'Maybe they've been too busy running bad PCR tests and vaccines that people don't need.' What is today called 'science' is not 'science' at all. Science is no longer what is, but whatever people can be manipulated to *believe* that it is. Real science has been hijacked by the Cult to dispense and produce the 'expert scientists' and contentions that suit the agenda of the Cult. How big-time this has happened with the 'Covid' hoax which is entirely based on fake science

delivered by fake ‘scientists’ and fake ‘doctors’. The human-caused climate change hoax is also entirely based on fake science delivered by fake ‘scientists’ and fake ‘climate experts’. In both cases real scientists, climate experts and doctors have their views suppressed and deleted by the Cult-owned science establishment, media and Silicon Valley. This is the ‘science’ that politicians claim to be ‘following’ and a common denominator of ‘Covid’ and climate are Cult psychopaths Bill Gates and his mate Klaus Schwab at the Gates-funded World Economic Forum. But, don’t worry, it’s all just a coincidence and absolutely nothing to worry about. *Zzzzzzzz*.

What is a ‘virus’ REALLY?

Dr Tom Cowan is one of many contesting the very existence of viruses let alone that they cause disease. This is understandable when there is no scientific evidence for a disease-causing ‘virus’. German virologist Dr Stefan Lanka won a landmark case in 2017 in the German Supreme Court over his contention that there is no such thing as a measles virus. He had offered a big prize for anyone who could prove there is and Lanka won his case when someone sought to claim the money. There is currently a prize of more than 225,000 euros on offer from an Isolate Truth Fund for anyone who can prove the isolation of SARS-CoV-2 and its genetic substance. Lanka wrote in an article headed ‘The Misconception Called Virus’ that scientists think a ‘virus’ is causing tissue to become diseased and degraded when in fact it is the *processes they are using* which do that – not a ‘virus’. Lanka has done an important job in making this point clear as Cowan did in his analysis of the CDC paper. Lanka says that all claims about viruses as disease-causing pathogens are wrong and based on ‘easily recognisable, understandable and verifiable misinterpretations.’ Scientists believed they were working with ‘viruses’ in their laboratories when they were really working with ‘typical particles of specific dying tissues or cells …’ Lanka said that the tissue decaying process claimed to be caused by a ‘virus’ still happens when no alleged ‘virus’ is involved. It’s the *process* that does the damage and not a ‘virus’. The genetic sample is deprived of nutrients, removed from its energy supply through removal from the body and then doused in toxic antibiotics to remove any bacteria. He confirms again that establishment scientists do not (pinch me) conduct control experiments to

see if this is the case and if they did they would see the claims that 'viruses' are doing the damage is nonsense. He adds that during the measles 'virus' court case he commissioned an independent laboratory to perform just such a control experiment and the result was that the tissues and cells died in the exact same way as with alleged 'infected' material. This is supported by a gathering number of scientists, doctors and researchers who reject what is called 'germ theory' or the belief in the body being infected by contagious sources emitted by other people. Researchers Dawn Lester and David Parker take the same stance in their highly-detailed and sourced book *What Really Makes You Ill – Why everything you thought you knew about disease is wrong* which was recommended to me by a number of medical professionals genuinely seeking the truth. Lester and Parker say there is no provable scientific evidence to show that a 'virus' can be transmitted between people or people and animals or animals and people:

The definition also claims that viruses are the cause of many diseases, as if this has been definitively proven. But this is not the case; there is no original scientific evidence that definitively demonstrates that any virus is the cause of any disease. The burden of proof for any theory lies with those who proposed it; but none of the existing documents provides 'proof' that supports the claim that 'viruses' are pathogens.

Dr Tom Cowan employs one of his clever analogies to describe the process by which a 'virus' is named as the culprit for a disease when what is called a 'virus' is only material released by cells detoxing themselves from infiltration by chemical or radiation poisoning. The tidal wave of technologically-generated radiation in the 'smart' modern world plus all the toxic food and drink are causing this to happen more than ever. Deluded 'scientists' misread this as a gathering impact of what they wrongly label 'viruses'.

Paper can infect houses

Cowan said in an article for davidicke.com – with his tongue only mildly in his cheek – that he believed he had made a tremendous discovery that may revolutionise science. He had discovered that small bits of paper are alive, 'well alive-ish', can 'infect' houses, and then reproduce themselves inside the house. The result was that this explosion of growth in the paper inside

the house causes the house to explode, blowing it to smithereens. His evidence for this new theory is that in the past months he had carefully examined many of the houses in his neighbourhood and found almost no scraps of paper on the lawns and surrounds of the house. There was an occasional stray label, but nothing more. Then he would return to these same houses a week or so later and with a few, not all of them, particularly the old and decrepit ones, he found to his shock and surprise they were littered with stray bits of paper. He knew then that the paper had infected these houses, made copies of itself, and blew up the house. A young boy on a bicycle at one of the sites told him he had seen a demolition crew using dynamite to explode the house the previous week, but Cowan dismissed this as the idle thoughts of silly boys because 'I was on to something big'. He was on to how 'scientists' mistake genetic material in the detoxifying process for something they call a 'virus'. Cowan said of his house and paper story:

If this sounds crazy to you, it's because it should. This scenario is obviously nuts. But consider this admittedly embellished, for effect, current viral theory that all scientists, medical doctors and virologists currently believe.

He takes the example of the 'novel SARS-Cov2' virus to prove the point. First they take someone with an undefined illness called 'Covid-19' and don't even attempt to find any virus in their sputum. Never mind the scientists still describe how this 'virus', which they have not located attaches to a cell receptor, injects its genetic material, in 'Covid's' case, RNA, into the cell. The RNA once inserted exploits the cell to reproduce itself and makes 'thousands, nay millions, of copies of itself … Then it emerges victorious to claim its next victim':

If you were to look in the scientific literature for proof, actual scientific proof, that uniform SARS-CoV2 viruses have been properly isolated from the sputum of a sick person, that actual spike proteins could be seen protruding from the virus (which has not been found), you would find that such evidence doesn't exist.

If you go looking in the published scientific literature for actual pictures, proof, that these spike proteins or any viral proteins are ever attached to any receptor embedded in any cell membrane, you would also find that no such evidence exists. If you were to look for a video or documented evidence

of the intact virus injecting its genetic material into the body of the cell, reproducing itself and then emerging victorious by budding off the cell membrane, you would find that no such evidence exists.

The closest thing you would find is electron micrograph pictures of cellular particles, possibly attached to cell debris, both of which to be seen were stained by heavy metals, a process that completely distorts their architecture within the living organism. This is like finding bits of paper stuck to the blown-up bricks, thereby proving the paper emerged by taking pieces of the bricks on its way out.

The Enders baloney

Cowan describes the 'Covid' story as being just as make-believe as his paper story and he charts back this fantasy to a Nobel Prize winner called John Enders (1897-1985), an American biomedical scientist who has been dubbed 'The Father of Modern Vaccines'. Enders is claimed to have 'discovered' the process of the viral culture which 'proved' that a 'virus' caused measles. Cowan explains how Enders did this 'by using the EXACT same procedure that has been followed by every virologist to find and characterize every new virus since 1954'. Enders took throat swabs from children with measles and immersed them in 2ml of milk. Penicillin (100u/ml) and the antibiotic streptomycin (50,g/ml) were added and the whole mix was centrifuged – rotated at high speed to separate large cellular debris from small particles and molecules as with milk and cream, for example. Cowan says that if the aim is to find little particles of genetic material ('viruses') in the snot from children with measles it would seem that the last thing you would do is mix the snot with other material – milk – that also has genetic material. 'How are you ever going to know whether whatever you found came from the snot or the milk?' He points out that streptomycin is a 'nephrotoxic' or poisonous-to-the-kidney drug. You will see the relevance of that shortly. Cowan says that it gets worse, much worse, when Enders describes the culture medium upon which the virus 'grows': 'The culture medium consisted of bovine amniotic fluid (90%), beef embryo extract (5%), horse serum (5%), antibiotics and phenol red as an indicator of cell metabolism.' Cowan asks incredulously: 'Did he just say that the culture medium also contained fluids and tissues that are themselves rich sources of genetic material?' The genetic cocktail, or 'medium', is inoculated onto tissue and cells from rhesus monkey *kidney* tissue. This is where the importance of streptomycin comes in and currently-used antimicrobials and other drugs that are *poisonous to kidneys*

and used in ALL modern viral cultures (e.g. gentamicin, streptomycin, and amphotericin). Cowan asks: 'How are you ever going to know from this witch's brew where any genetic material comes from as we now have five different sources of rich genetic material in our mix?' Remember, he says, that all genetic material, whether from monkey kidney tissues, bovine serum, milk, etc., is made from the exact same components. The same central question returns: 'How are you possibly going to know that it was the virus that killed the kidney tissue and not the toxic antibiotic and starvation rations on which you are growing the tissue?' John Enders answered the question himself – *you can't*:

A second agent was obtained from an uninoculated culture of monkey kidney cells. The cytopathic changes [death of the cells] it induced in the unstained preparations could not be distinguished with confidence from the viruses isolated from measles.

The death of the cells ('cytopathic changes') happened in exactly the same manner, whether they inoculated the kidney tissue with the measles snot or not, Cowan says. 'This is evidence that the destruction of the tissue, the very proof of viral causation of illness, was not caused by anything in the snot because they saw the same destructive effect when the snot was not even used … the cytopathic, i.e., cell-killing, changes come from the process of the culture itself, not from any virus in any snot, period.' Enders quotes in his 1957 paper a virologist called Ruckle as reporting similar findings 'and in addition has isolated an agent from monkey kidney tissue that is so far indistinguishable from human measles virus'. In other words, Cowan says, these particles called 'measles viruses' are simply and clearly breakdown products of the starved and poisoned tissue. For measles 'virus' see all 'viruses' including the so-called 'Covid virus'. Enders, the 'Father of Modern Vaccines', also said:

There is a potential risk in employing cultures of primate cells for the production of vaccines composed of attenuated virus, since the presence of other agents possibly latent in primate tissues cannot be definitely excluded by any known method.

Cowan further quotes from a paper published in the journal *Viruses* in May, 2020, while the 'Covid pandemic' was well underway in the media if

not in reality. ‘EVs’ here refers to particles of genetic debris from our own tissues, such as exosomes of which more in a moment: ‘The remarkable resemblance between EVs and viruses has caused quite a few problems in the studies focused on the analysis of EVs released during viral infections.’ Later the paper adds that to date a reliable method that can actually guarantee a complete separation (of EVs from viruses) DOES NOT EXIST. This was published at a time when a fairy tale ‘virus’ was claimed in total certainty to be causing a fairy tale ‘viral disease’ called ‘Covid-19’ – a fairy tale that was already well on the way to transforming human society in the image that the Cult has worked to achieve for so long. Cowan concludes his article:

To summarize, there is no scientific evidence that pathogenic viruses exist. What we think of as ‘viruses’ are simply the normal breakdown products of dead and dying tissues and cells. When we are well, we make fewer of these particles; when we are starved, poisoned, suffocated by wearing masks, or afraid, we make more.

There is no engineered virus circulating and making people sick. People in laboratories all over the world are making genetically modified products to make people sick. These are called vaccines. There is no virome, no ‘ecosystem’ of viruses, viruses are not 8%, 50% or 100 % of our genetic material. These are all simply erroneous ideas based on the misconception called a virus.

What is ‘Covid’? Load of bollocks

The background described here by Cowan and Lanka was emphasised in the first video presentation that I saw by Dr Andrew Kaufman when he asked whether the ‘Covid virus’ was in truth a natural defence mechanism of the body called ‘exosomes’. These are released by cells when in states of toxicity – see the same themes returning over and over. They are released ever more profusely as chemical and radiation toxicity increases and think of the potential effect therefore of 5G alone as its destructive frequencies infest the human energetic information field with a gathering pace (5G went online in Wuhan in 2019 as the ‘virus’ emerged). I’ll have more about this later. Exosomes transmit a warning to the rest of the body that ‘Houston, we have a problem’. Kaufman presented images of exosomes and compared them with ‘Covid’ under an electron microscope and the similarity was remarkable. They both attach to the same cell receptors (*claimed* in the case of ‘Covid’), contain the same genetic material in the form of RNA or ribonucleic acid, and both are found in ‘viral cell cultures’ with damaged or

dying cells. James Hildreth MD, President and Chief Executive Officer of the Meharry Medical College at Johns Hopkins, said: 'The virus is fully an exosome in every sense of the word.' Kaufman's conclusion was that there is no 'virus': 'This entire pandemic is a completely manufactured crisis … there is no evidence of anyone dying from [this] illness.' Dr Tom Cowan and Sally Fallon Morell, authors of *The Contagion Myth*, published a statement with Dr Kaufman in February, 2021, explaining why the 'virus' does not exist and you can read it that in full in the Appendix.

'Virus' theory can be traced to the 'cell theory' in 1858 of German physician Rudolf Virchow (1821-1920) who contended that disease originates from a single cell infiltrated by a 'virus'. Dr Stefan Lanka said that findings and insights with respect to the structure, function and central importance of tissues in the creation of life, which were already known in 1858, comprehensively refute the cell theory. Virchow ignored them. We have seen the part later played by John Enders in the 1950s and Lanka notes that infection theories were only established as a global dogma through the policies and eugenics of the Third Reich in Nazi Germany (creation of the same Sabbatian cult behind the 'Covid' hoax). Lanka said: 'Before 1933, scientists dared to contradict this theory; after 1933, these critical scientists were silenced'. Dr Tom Cowan's view is that ill-heath is caused by too much of something, too little of something, or toxification from chemicals and radiation – not contagion. We must also highlight as a major source of the 'virus' theology a man still called the 'Father of Modern Virology' – Thomas Milton Rivers (1888-1962). There is no way given the Cult's long game policy that it was a coincidence for the 'Father of Modern Virology' to be director of the Rockefeller Institute for Medical Research from 1937 to 1956 when he is credited with making the Rockefeller Institute a leader in 'viral research'. Cult Rockefellers were the force behind the creation of Big Pharma 'medicine', established the World Health Organisation in 1948, and have long and close associations with the Gates family that now runs the WHO during the pandemic hoax through mega-rich Cult gofer and psychopath Bill Gates.

Only a Renegade Mind can see through all this bullshit by asking the questions that need to be answered, not taking 'no' or prevarication for an answer, and certainly not hiding from the truth in fear of speaking it. Renegade Minds have always changed the world for the better and they will change this one no matter how bleak it may currently appear to be.

CHAPTER SIX

Sequence of deceit

If you tell the truth, you don't have to remember anything
Mark Twain

Against the background that I have laid out this far the sequence that took us from an invented 'virus' in Cult-owned China in late 2019 to the fascist transformation of human society can be seen and understood in a whole new context.

We were told that a deadly disease had broken out in Wuhan and the world media began its campaign (coordinated by behavioural psychologists as we shall see) to terrify the population into unquestioning compliance. We were shown images of Chinese people collapsing in the street which never happened in the West with what was supposed to be the same condition. In the earliest days when alleged cases and deaths were few the fear register was hysterical in many areas of the media and this would expand into the common media narrative across the world. The real story was rather different, but we were never told that. The Chinese government, one of the Cult's biggest centres of global operation, said they had discovered a new illness with flu-like and pneumonia-type symptoms in a city with such toxic air that it is overwhelmed with flu-like symptoms, pneumonia and respiratory disease. Chinese scientists said it was a new – 'novel' – coronavirus which they called Sars-Cov-2 and that it caused a disease they labelled 'Covid-19'. There was no evidence for this and the 'virus' has never to this day been isolated, purified and its genetic code established from that. It was from the beginning a computer-generated fiction. Stories

of Chinese whistleblowers saying the number of deaths was being supressed or that the ‘new disease’ was related to the Wuhan bio-lab misdirected mainstream and alternative media into cul-de-sacs to obscure the real truth – there was no ‘virus’.

Chinese scientists took genetic material from the lung fluid of just a few people and said they had found a ‘new’ disease when this material had a wide range of content. There was no evidence for a ‘virus’ for the very reasons explained in the last two chapters. The ‘virus’ has never been shown to (a) exist and (b) cause any disease. People were diagnosed on symptoms that are so widespread in Wuhan and polluted China and with a PCR test that can’t detect infectious disease. On this farce the whole global scam was sold to the rest of the world which would also diagnose respiratory disease as ‘Covid-19’ from symptoms alone or with a PCR test not testing for a ‘virus’. Flu miraculously disappeared *worldwide* in 2020 and into 2021 as it was redesignated ‘Covid-19’. It was really the same old flu with its ‘flu-like’ symptoms attributed to ‘flu-like’ ‘Covid-19’. At the same time with very few exceptions the Chinese response of draconian lockdown and fascism was the chosen weapon to respond across the West as recommended by the Cult-owned Tedros at the Cult-owned World Health Organization run by the Cult-owned Gates. All was going according to plan. Chinese scientists – everything in China is controlled by the Cult-owned government – compared their contaminated RNA lung-fluid material with other RNA sequences and said it appeared to be just under 80 percent identical to the SARS-CoV-1 ‘virus’ claimed to be the cause of the SARS (severe acute respiratory syndrome) ‘outbreak’ in 2003. They decreed that because of this the ‘new virus’ had to be related and they called it SARS-CoV-2. There are some serious problems with this assumption and *assumption* was all it was. Most ‘factual’ science turns out to be assumptions repeated into everyone-knows-that. A match of under 80-percent is meaningless. Dr Kaufman makes the point that there’s a *96 percent* genetic correlation between humans and chimpanzees, but ‘no one would say our genetic material is part of the chimpanzee family’. Yet the Chinese authorities were claiming that a much lower percentage, less than 80 percent, proved the existence of a new ‘coronavirus’. For goodness sake human DNA is 60 percent similar to a *banana*.

You are feeling sleepy

The entire ‘Covid’ hoax is a global Psyop, a psychological operation to program the human mind into believing and fearing a complete fantasy. A crucial aspect of this was what *appeared* to happen in Italy. It was all very well streaming out daily images of an alleged catastrophe in Wuhan, but to the Western mind it was still on the other side of the world in a very different culture and setting. A reaction of ‘this could happen to me and my family’ was still nothing like as intense enough for the mind-doctors. The Cult needed a Western example to push people over that edge and it chose Italy, one of its major global locations going back to the Roman Empire. An Italian ‘Covid’ crisis was manufactured in a particular area called Lombardy which just happens to be notorious for its toxic air and therefore respiratory disease. Wuhan, China, déjà vu. An hysterical media told horror stories of Italians dying from ‘Covid’ in their droves and how Lombardy hospitals were being overrun by a tidal wave of desperately ill people needing treatment after being struck down by the ‘deadly virus’. Here was the psychological turning point the Cult had planned. Wow, if this is happening in Italy, the Western mind concluded, this indeed could happen to me and my family. Another point is that Italian authorities responded by following the Chinese blueprint so vehemently recommended by the Cult-owned World Health Organization. They imposed fascistic lockdowns on the whole country viciously policed with the help of surveillance drones sweeping through the streets seeking out anyone who escaped from mass house arrest. Livelihoods were destroyed and psychology unravelled in the way we have witnessed since in all lockdown countries. Crucial to the plan was that Italy responded in this way to set the precedent of suspending freedom and imposing fascism in a ‘Western liberal democracy’. I emphasised in an animated video explanation on davidicke.com posted in the summer of 2020 how important it was to the Cult to expand the Chinese lockdown model across the West. Without this, and the bare-faced lie that non-symptomatic people could still transmit a ‘disease’ they didn’t have, there was no way locking down the whole population, sick and not sick, could be pulled off. At just the right time and with no evidence Cult operatives and gofers claimed that people without symptoms could pass on the ‘disease’. In the name of protecting the ‘vulnerable’ like elderly people, who lockdowns would kill by the tens of thousands, we had for the first time healthy people told to isolate as well as the sick. The great majority of

people who tested positive had no symptoms because there was nothing wrong with them. It was just a trick made possible by a test not testing for the 'virus'.

Months after my animated video the Gates-funded Professor Neil Ferguson at the Gates-funded Imperial College confirmed that I was right. He didn't say it in those terms, naturally, but he did say it. Ferguson will enter the story shortly for his outrageously crazy 'computer models' that led to Britain, the United States and many other countries following the Chinese and now Italian methods of response. Put another way, following the Cult script. Ferguson said that SAGE, the UK government's scientific advisory group which has controlled 'Covid' policy from the start, wanted to follow the Chinese lockdown model (while they all continued to work and be paid), but they wondered if they could possibly, in Ferguson's words, 'get away with it in Europe'. 'Get away with it'? Who the hell do these moronic, arrogant people think they are? This appalling man Ferguson said that once Italy went into national lockdown they realised they, too, could mimic China:

It's a communist one-party state, we said. We couldn't get away with it in Europe, we thought … and then Italy did it. And we realised we could. Behind this garbage from Ferguson is a simple fact: Doing the same as China in every country was the plan from the start and Ferguson's 'models' would play a central role in achieving that. It's just a coincidence, of course, and absolutely nothing to worry your little head about.

Oops, sorry, our mistake

Once the Italian segment of the Psyop had done the job it was designed to do a very different story emerged. Italian authorities revealed that 99 percent of those who had 'died from Covid-19' in Italy had one, two, three, or more 'co-morbidities' or illnesses and health problems that could have ended their life. The US Centers for Disease Control and Prevention (CDC) published a figure of 94 percent for Americans dying of 'Covid' while having other serious medical conditions – on average two to three (some five or six) other potential causes of death. In terms of death from an unproven 'virus' I say it is 100 percent. The other one percent in Italy and six percent in the US would presumably have died from 'Covid's' flu-like symptoms with a range of other possible causes in conjunction with a test

not testing for the ‘virus’. Fox News reported that even more startling figures had emerged in one US county in which 410 of 422 deaths attributed to ‘Covid-19’ had other potentially deadly health conditions. The Italian National Health Institute said later that the average age of people dying with a ‘Covid-19’ diagnosis in Italy was about 81. Ninety percent were over 70 with ten percent over 90. In terms of other reasons to die some 80 percent had two or more chronic diseases with half having three or more including cardiovascular problems, diabetes, respiratory problems and cancer. Why is the phantom ‘Covid-19’ said to kill overwhelmingly old people and hardly affect the young? Old people continually die of many causes and especially respiratory disease which you can re-diagnose ‘Covid-19’ while young people die in tiny numbers by comparison and rarely of respiratory disease. Old people ‘die of Covid’ because they die of other things that can be redesignated ‘Covid’ and it really is that simple.

Flu has flown

The blueprint was in place. Get your illusory ‘cases’ from a test not testing for the ‘virus’ and redesignate other causes of death as ‘Covid-19’. You have an instant ‘pandemic’ from something that is nothing more than a computer-generated fiction. With near-on a billion people having ‘flu-like’ symptoms every year the potential was limitless and we can see why flu quickly and apparently miraculously disappeared *worldwide* by being diagnosed ‘Covid-19’. The painfully bloody obvious was explained away by the childlike media in headlines like this in the UK ‘*Independent*’: ‘Not a single case of flu detected by Public Health England this year as Covid restrictions suppress virus’. I kid you not. The masking, social distancing and house arrest that did not make the ‘Covid virus’ disappear somehow did so with the ‘flu virus’. Even worse the article, by a bloke called Samuel Lovett, suggested that maybe the masking, sanitising and other ‘Covid’ measures should continue to keep the flu away. With a ridiculousness that disturbs your breathing (it’s ‘Covid-19’) the said Lovett wrote: ‘With widespread social distancing and mask-wearing measures in place throughout the UK, the usual routes of transmission for influenza have been blocked.’ He had absolutely no evidence to support that statement, but look at the consequences of him acknowledging the obvious. With flu not disappearing at all and only being relabelled ‘Covid-19’ he would have to

contemplate that ‘Covid’ was a hoax on a scale that is hard to imagine. You need guts and commitment to truth to even go there and that’s clearly something Samuel Lovett does not have in abundance. He would never have got it through the editors anyway.

Tens of thousands die in the United States alone every winter from flu including many with pneumonia complications. CDC figures record *45 million* Americans diagnosed with flu in 2017-2018 of which 61,000 died and some reports claim 80,000. Where was the same hysteria then that we have seen with ‘Covid-19’? Some 250,000 Americans are admitted to hospital with pneumonia every year with about 50,000 cases proving fatal. About 65 million suffer respiratory disease every year and three million deaths makes this the third biggest cause of death worldwide. You only have to redesignate a portion of all these people ‘Covid-19’ and you have an instant global pandemic or the *appearance* of one. Why would doctors do this? They are told to do this and all but a few dare not refuse those who must be obeyed. Doctors in general are not researching their own knowledge and instead take it direct and unquestioned from the authorities that own them and their careers. The authorities say they must now diagnose these symptoms ‘Covid-19’ and not flu, or whatever, and they do it. Dark suits say put ‘Covid-19’ on death certificates no matter what the cause of death and the doctors do it. Renegade Minds don’t fall for the illusion that doctors and medical staff are all highly-intelligent, highly-principled, seekers of medical truth. *Some are*, but not the majority. They are repeaters, gofers, and yes sir, no sir, purveyors of what the system demands they purvey. The ‘Covid’ con is not merely confined to diseases of the lungs. Instructions to doctors to put ‘Covid-19’ on death certificates for anyone dying of *anything* within 28 days (or much more) of a positive test not testing for the ‘virus’ opened the floodgates. The term dying *with* ‘Covid’ and not *of* ‘Covid’ was coined to cover the truth. Whether it was a *with* or an *of* they were all added to the death numbers attributed to the ‘deadly virus’ compiled by national governments and globally by the Gates-funded Johns Hopkins operation in the United States that was so involved in those ‘pandemic’ simulations. Fraudulent deaths were added to the ever-growing list of fraudulent ‘cases’ from false positives from a false test. No wonder Professor Walter Ricciardi, scientific advisor to the Italian minister of health, said after the Lombardy hysteria had done its job that ‘Covid’

death rates were due to Italy having the second oldest population in the world and to *how hospitals record deaths*:

The way in which we code deaths in our country is very generous in the sense that all the people who die in hospitals with the coronavirus are deemed to be dying of the coronavirus. On re-evaluation by the National Institute of Health, only 12 per cent of death certificates have shown a direct causality from coronavirus, while 88 per cent of patients who have died have at least one pre-morbidity – many had two or three.

This is extraordinary enough when you consider the propaganda campaign to use Italy to terrify the world, but how can they even say twelve percent were genuine when the 'virus' has not been shown to exist, its 'code' is a computer program, and diagnosis comes from a test not testing for it? As in China, and soon the world, 'Covid-19' in Italy was a redesignation of diagnosis. Lies and corruption were to become the real 'pandemic' fuelled by a pathetically-compliant medical system taking its orders from the tiny few at the top of their national hierarchy who answered to the World Health Organization which answers to Gates and the Cult. Doctors were told – ordered – to diagnose a particular set of symptoms 'Covid-19' and put that on the death certificate for any cause of death if the patient had tested positive with a test not testing for the virus or had 'Covid' symptoms like the flu. The United States even introduced big financial incentives to manipulate the figures with hospitals receiving £4,600 from the Medicare system for diagnosing someone with regular pneumonia, $13,000 if they made the diagnosis from the same symptoms 'Covid-19' pneumonia, and $39, 000 if they put a 'Covid' diagnosed patient on a ventilator that would almost certainly kill them. A few – painfully and pathetically few – medical whistleblowers revealed (before Cult-owned YouTube deleted their videos) that they had been instructed to 'let the patient crash' and put them straight on a ventilator instead of going through a series of far less intrusive and dangerous methods as they would have done before the pandemic hoax began and the financial incentives kicked in. We are talking cold-blooded murder given that ventilators are so damaging to respiratory systems they are usually the last step before heaven awaits. Renegade Minds never fall for the belief that people in white coats are all angels of mercy and cannot be full-on psychopaths. I have explained in detail in *The Answer* how what I am describing here played out across

the world coordinated by the World Health Organization through the medical hierarchies in almost every country.

Medical scientist calls it

Information about the non-existence of the 'virus' began to emerge for me in late March, 2020, and mushroomed after that. I was sent an email by Sir Julian Rose, a writer, researcher, and organic farming promotor, from a medical scientist friend of his in the United States. Even at that early stage in March the scientist was able to explain how the 'Covid' hoax was being manipulated. He said there were no reliable tests for a specific 'Covid-19 virus' and nor were there any reliable agencies or media outlets for reporting numbers of actual 'Covid-19' cases. We have seen in the long period since then that he was absolutely right. 'Every action and reaction to Covid-19 is based on totally flawed data and we simply cannot make accurate assessments,' he said. Most people diagnosed with 'Covid-19' were showing nothing more than cold and flu-like symptoms 'because most coronavirus strains *are* nothing more than cold/flu-like symptoms'. We had farcical situations like an 84-year-old German man testing positive for 'Covid-19' and his nursing home ordered to quarantine only for him to be found to have a common cold. The scientist described back then why PCR tests and what he called the 'Mickey Mouse test kits' were useless for what they were claimed to be identifying. 'The idea these kits can isolate a specific virus like Covid-19 is nonsense,' he said. Significantly, he pointed out that 'if you want to create a totally false panic about a totally false pandemic – pick a coronavirus'. This is exactly what the Cult-owned Gates, World Economic Forum and Johns Hopkins University did with their Event 201 'simulation' followed by their real-life simulation called the 'pandemic'. The scientist said that all you had to do was select the sickest of people with respiratory-type diseases in a single location – 'say Wuhan' – and administer PCR tests to them. You can then claim that anyone showing 'viral sequences' similar to a coronavirus 'which will inevitably be quite a few' is suffering from a 'new' disease:

> Since you already selected the sickest flu cases a fairly high proportion of your sample will go on to die. You can then say this 'new' virus has a CFR [case fatality rate] higher than the flu and use this to infuse more concern and do more tests which will of course produce more 'cases', which expands the

testing, which produces yet more ‘cases’ and so on and so on. Before long you have your ‘pandemic’, and all you have done is use a simple test kit trick to convert the worst flu and pneumonia cases into something new that doesn’t ACTUALLY EXIST [my emphasis].

He said that you then ‘just run the same scam in other countries’ and make sure to keep the fear message running high ‘so that people will feel panicky and less able to think critically’. The only problem to overcome was the fact *there is no* actual new deadly pathogen and only regular sick people. This meant that deaths from the ‘new deadly pathogen’ were going to be way too low for a real new deadly virus pandemic, but he said this could be overcome in the following ways – all of which would go on to happen:

1. You can claim this is just the beginning and more deaths are imminent [you underpin this with fantasy ‘computer projections’]. Use this as an excuse to quarantine everyone and then claim the quarantine prevented the expected millions of dead.

2. You can [say that people] ‘minimizing’ the dangers are irresponsible and bully them into not talking about numbers.

3. You can talk crap about made up numbers hoping to blind people with pseudoscience.

4. You can start testing well people (who, of course, will also likely have shreds of coronavirus [RNA] in them) and thus inflate your ‘case figures’ with ‘asymptomatic carriers’ (you will of course have to spin that to sound deadly even though any virologist knows the more symptom-less cases you have the less deadly is your pathogen).

The scientist said that if you take these simple steps ‘you can have your own entirely manufactured pandemic up and running in weeks’. His analysis made so early in the hoax was brilliantly prophetic of what would actually unfold. Pulling all the information together in these recent chapters we have this is simple 1, 2, 3, of how you can delude virtually the entire human population into believing in a ‘virus’ that doesn’t exist:

- A ‘Covid case’ is someone who tests positive with a test not testing for the ‘virus’.

- A ‘Covid death’ is someone who dies of *any cause* within 28 days (or much longer) of testing positive with a test not testing for the ‘virus.

• Asymptomatic means there is nothing wrong with you, but they claim you can pass on what you don't have to justify locking down (quarantining) healthy people in totality.

The foundations of the hoax are that simple. A study involving ten million people in Wuhan, published in November, 2020, demolished the whole lie about those without symptoms passing on the 'virus'. They found '300 asymptomatic cases' and traced their contacts to find that not one of them was detected with the 'virus'. 'Asymptomatic' patients and their contacts were isolated for no less than two weeks and nothing changed. I know it's all crap, but if you are going to claim that those without symptoms can transmit 'the virus' then you must produce evidence for that and they never have. Even World Health Organization official Dr Maria Van Kerkhove, head of the emerging diseases and zoonosis unit, said as early as June, 2020, that she doubted the validity of asymptomatic transmission. She said that 'from the data we have, it still seems to be rare that an asymptomatic person actually transmits onward to a secondary individual' and by 'rare' she meant that she couldn't cite any case of asymptomatic transmission.

The Ferguson factor

The problem for the Cult as it headed into March, 2020, when the script had lockdown due to start, was that despite all the manipulation of the case and death figures they still did not have enough people alleged to have died from 'Covid' to justify mass house arrest. This was overcome in the way the scientist described: 'You can claim this is just the beginning and more deaths are imminent … Use this as an excuse to quarantine everyone and then claim the quarantine prevented the expected millions of dead.' Enter one Professor Neil Ferguson, the Gates-funded 'epidemiologist' at the Gates-funded Imperial College in London. Ferguson is Britain's Christian Drosten in that he has a dire record of predicting health outcomes, but is still called upon to advise government on the next health outcome when another 'crisis' comes along. This may seem to be a strange and ridiculous thing to do. Why would you keep turning for policy guidance to people who have a history of being monumentally wrong? Ah, but it makes sense from the Cult point of view. These 'experts' keep on producing predictions that

suit the Cult agenda for societal transformation and so it was with Neil Ferguson as he revealed his horrific (and clearly insane) computer model predictions that allowed lockdowns to be imposed in Britain, the United States and many other countries. Ferguson does not have even an A-level in biology and would appear to have no formal training in computer modelling, medicine or epidemiology, according to Derek Winton, an MSc in Computational Intelligence. He wrote an article somewhat aghast at what Ferguson did which included taking no account of respiratory disease 'seasonality' which means it is far worse in the winter months. Who would have thought that respiratory disease could be worse in the winter? Well, certainly not Ferguson.

The massively China-connected Imperial College and its bizarre professor provided the excuse for the long-incubated Chinese model of human control to travel westward at lightning speed. Imperial College confirms on its website that it collaborates with the Chinese Research Institute; publishes more than 600 research papers every year with Chinese research institutions; has 225 Chinese staff; 2,600 Chinese students – the biggest international group; 7,000 former students living in China which is the largest group outside the UK; and was selected for a tour by China's President Xi Jinping during his state visit to the UK in 2015. The college takes major donations from China and describes itself as the UK's number one university collaborator with Chinese research institutions. The China communist/fascist government did not appear phased by the woeful predictions of Ferguson and Imperial when during the lockdown that Ferguson induced the college signed a five-year collaboration deal with China tech giant Huawei that will have Huawei's indoor 5G network equipment installed at the college's West London tech campus along with an 'AI cloud platform'. The deal includes Chinese sponsorship of Imperial's Venture Catalyst entrepreneurship competition. Imperial is an example of the enormous influence the Chinese government has within British and North American universities and research centres – and further afield. Up to 200 academics from more than a dozen UK universities are being investigated on suspicion of 'unintentionally' helping the Chinese government build weapons of mass destruction by 'transferring world-leading research in advanced military technology such as aircraft, missile designs and cyberweapons'. Similar scandals have broken in the United States, but it's all a coincidence. Imperial College serves the agenda in

many other ways including the promotion of every aspect of the United Nations Agenda 21/2030 (the Great Reset) and produced computer models to show that human-caused ‘climate change’ is happening when in the real world it isn’t. Imperial College is driving the climate agenda as it drives the ‘Covid’ agenda (both Cult hoaxes) while Patrick Vallance, the UK government’s Chief Scientific Adviser on ‘Covid’, was named Chief Scientific Adviser to the UN ‘climate change’ conference known as COP26 hosted by the government in Glasgow, Scotland. ‘Covid’ and ‘climate’ are fundamentally connected.

Professor Woeful

From Imperial’s bosom came Neil Ferguson still advising government despite his previous disasters and it was announced early on that he and other key people like UK Chief Medical Adviser Chris Whitty had caught the ‘virus’ as the propaganda story was being sold. Somehow they managed to survive and we had Prime Minister Boris Johnson admitted to hospital with what was said to be a severe version of the ‘virus’ in this same period. His whole policy and demeanour changed when he returned to Downing Street. It’s a small world with these government advisors – especially in their communal connections to Gates – and Ferguson had partnered with Whitty to write a paper called ‘Infectious disease: Tough choices to reduce Ebola transmission’ which involved another scare-story that didn’t happen. Ferguson’s ‘models’ predicted that up to150, 000 could die from ‘mad cow disease’, or BSE, and its version in sheep if it was transmitted to humans. BSE was not transmitted and instead triggered by an organophosphate pesticide used to treat a pest on cows. Fewer than 200 deaths followed from the human form. Models by Ferguson and his fellow incompetents led to the unnecessary culling of millions of pigs, cattle and sheep in the foot and mouth outbreak in 2001 which destroyed the lives and livelihoods of farmers and their families who had often spent decades building their herds and flocks. Vast numbers of these animals did not have foot and mouth and had no contact with the infection. Another ‘expert’ behind the cull was Professor Roy Anderson, a computer modeller at Imperial College specialising in the epidemiology of *human*, not animal, disease. Anderson has served on the Bill and Melinda Gates Grand Challenges in Global

Health advisory board and chairs another Gates-funded organisation. Gates is everywhere.

In a precursor to the 'Covid' script Ferguson backed closing schools 'for prolonged periods' over the swine flu 'pandemic' in 2009 and said it would affect a third of the world population if it continued to spread at the speed he claimed to be happening. His mates at Imperial College said much the same and a news report said: 'One of the authors, the epidemiologist and disease modeller Neil Ferguson, who sits on the World Health Organisation's emergency committee for the outbreak, said the virus had "full pandemic potential".' Professor Liam Donaldson, the Chris Whitty of his day as Chief Medical Officer, said the worst case could see 30 percent of the British people infected by swine flu with 65,000 dying. Ferguson and Donaldson were indeed proved correct when at the end of the year the number of deaths attributed to swine flu was 392. The term 'expert' is rather liberally applied unfortunately, not least to complete idiots. Swine flu 'projections' were great for GlaxoSmithKline (GSK) as millions rolled in for its Pandemrix influenza vaccine which led to brain damage with children most affected. The British government (taxpayers) paid out more than £60 million in compensation after GSK was given immunity from prosecution. Yet another 'Covid' déjà vu. Swine flu was supposed to have broken out in Mexico, but Dr Wolfgang Wodarg, a German doctor, former member of parliament and critic of the 'Covid' hoax, observed 'the spread of swine flu' in Mexico City at the time. He said: 'What we experienced in Mexico City was a very mild flu which did not kill more than usual – which killed even fewer people than usual.' Hyping the fear against all the facts is not unique to 'Covid' and has happened many times before. Ferguson is reported to have over-estimated the projected death toll of bird flu (H5N1) by some three million-fold, but bird flu vaccine makers again made a killing from the scare. This is some of the background to the Neil Ferguson who produced the perfectly-timed computer models in early 2020 predicting that half a million people would die in Britain without draconian lockdown and 2.2 million in the United States. Politicians panicked, people panicked, and lockdowns of alleged short duration were instigated to 'flatten the curve' of cases gleaned from a test not testing for the 'virus'. I said at the time that the public could forget the 'short duration' bit. This was an agenda to destroy the livelihoods of the population and force them into mass control through dependency and there was going to be nothing 'short' about it.

American researcher Daniel Horowitz described the consequences of the ‘models’ spewed out by Gates-funded Ferguson and Imperial College:

What led our government and the governments of many other countries into panic was a single Imperial College of UK study, funded by global warming activists, that predicted 2.2 million deaths if we didn’t lock down the country. In addition, the reported 8-9% death rate in Italy scared us into thinking there was some other mutation of this virus that they got, which might have come here.

Together with the fact that we were finally testing and had the ability to actually report new cases, we thought we were headed for a death spiral. But again … we can’t flatten a curve if we don’t know when the curve started.

How about it *never* started?

Giving them what they want

An investigation by German news outlet *Welt Am Sonntag (World on Sunday)* revealed how in March, 2020, the German government gathered together ‘leading scientists from several research institutes and universities’ and ‘together, they were to produce a [modelling] paper that would serve as legitimization for further tough political measures’. The Cult agenda was justified by computer modelling not based on evidence or reality; it was specifically constructed to justify the Cult demand for lockdowns all over the world to destroy the independent livelihoods of the global population. All these modellers and everyone responsible for the ‘Covid’ hoax have a date with a trial like those in Nuremberg after World War Two when Nazis faced the consequences of their war crimes. These corrupt-beyond-belief ‘modellers’ wrote the paper according to government instructions and it said that that if lockdown measures were lifted then up to one million Germans would die from ‘Covid-19’ adding that some would die ‘agonizingly at home, gasping for breath’ unable to be treated by hospitals that couldn’t cope. All lies. No matter – it gave the Cult all that it wanted. What did long-time government ‘modeller’ Neil Ferguson say? If the UK and the United States didn’t lockdown half a million would die in Britain and 2.2 million Americans. Anyone see a theme here? ‘Modellers’ are such a crucial part of the lockdown strategy that we should look into their background and follow the money. Researcher Rosemary Frei produced an excellent article headlined ‘The Modelling-paper Mafiosi’. She highlights a

guy called John Edmunds, a British epidemiologist, and professor in the Faculty of Epidemiology and Population Health at the London School of Hygiene & Tropical Medicine. He studied at Imperial College. Edmunds is a member of government 'Covid' advisory bodies which have been dictating policy, the New and Emerging Respiratory Virus Threats Advisory Group (NERVTAG) and the Scientific Advisory Group for Emergencies (SAGE).

Ferguson, another member of NERVTAG and SAGE, led the way with the original 'virus' and Edmunds has followed in the 'variant' stage and especially the so-called UK or Kent variant known as the 'Variant of Concern' (VOC) B.1.1.7. He said in a co-written report for the Centre for Mathematical modelling of Infectious Diseases at the London School of Hygiene and Tropical Medicine, with input from the Centre's 'Covid-19' Working Group, that there was 'a realistic possibility that VOC B.1.1.7 is associated with an increased risk of death compared to non-VOC viruses'. Fear, fear, fear, get the vaccine, fear, fear, fear, get the vaccine. Rosemary Frei reveals that almost all the paper's authors and members of the modelling centre's 'Covid-19' Working Group receive funding from the Bill and Melinda Gates Foundation and/or the associated Gates-funded Wellcome Trust. The paper was published by e-journal *Medr χiv* which only publishes papers not peer-reviewed and the journal was established by an organisation headed by Facebook's Mark Zuckerberg and his missus. What a small world it is. Frei discovered that Edmunds is on the Scientific Advisory Board of the Coalition for Epidemic Preparedness Innovations (CEPI) which was established by the Bill and Melinda Gates Foundation, Klaus Schwab's Davos World Economic Forum and Big Pharma giant Wellcome. CEPI was 'launched in Davos [in 2017] to develop vaccines to stop future epidemics', according to its website. 'Our mission is to accelerate the development of vaccines against emerging infectious diseases and enable equitable access to these vaccines for people during outbreaks.' What kind people they are. Rosemary Frei reveals that Public Health England (PHE) director Susan Hopkins is an author of her organisation's non-peer-reviewed reports on 'new variants'. Hopkins is a professor of infectious diseases at London's Imperial College which is gifted tens of millions of dollars a year by the Bill and Melinda Gates Foundation. Gates-funded modelling disaster Neil Ferguson also co-authors Public Health England reports and he spoke in December, 2020, about the potential

danger of the B.1.1.7. ‘UK variant’ promoted by Gates-funded modeller John Edmunds. When I come to the ‘Covid vaccines’ the ‘new variants’ will be shown for what they are – bollocks.

Connections, connections

All these people and modellers are lockdown-obsessed or, put another way, they demand what the Cult demands. Edmunds said in January, 2021, that to ease lockdowns too soon would be a disaster and they had to ‘vaccinate much, much, much more widely than the elderly’. Rosemary Frei highlights that Edmunds is married to Jeanne Pimenta who is described in a LinkedIn profile as director of epidemiology at GlaxoSmithKline (GSK) and she held shares in the company. Patrick Vallance, co-chair of SAGE and the government’s Chief Scientific Adviser, is a former executive of GSK and has a deferred bonus of shares in the company worth £600,000. GSK has serious business connections with Bill Gates and is collaborating with mRNA-’vaccine’ company CureVac to make ‘vaccines’ for the new variants that Edmunds is talking about. GSK is planning a ‘Covid vaccine’ with drug giant Sanofi. Puppet Prime Minister Boris Johnson announced in the spring of 2021 that up to 60 million vaccine doses were to be made at the GSK facility at Barnard Castle in the English North East. Barnard Castle, with a population of just 6,000, was famously visited in breach of lockdown rules in April, 2020, by Johnson aide Dominic Cummings who said that he drove there ‘to test his eyesight’ before driving back to London. Cummings would be better advised to test his integrity – not that it would take long. The GSK facility had nothing to do with his visit then although I’m sure Patrick Vallance would have been happy to arrange an introduction and some tea and biscuits. Ruthless psychopath Gates has made yet another fortune from vaccines in collaboration with Big Pharma companies and gushes at the phenomenal profits to be made from vaccines – more than a 20-to-1 return as he told one interviewer. Gates also tweeted in December, 2019, with the foreknowledge of what was coming: ‘What’s next for our foundation? I’m particularly excited about what the next year could mean for one of the best buys in global health: vaccines.’

Modeller John Edmunds is a big promotor of vaccines as all these people appear to be. He’s the dean of the London School of Hygiene & Tropical Medicine’s Faculty of Epidemiology and Population Health which is

primarily funded by the Bill and Melinda Gates Foundation and the Gates-established and funded GAVI vaccine alliance which is the Gates vehicle to vaccinate the world. The organisation Doctors Without Borders has described GAVI as being ‘aimed more at supporting drug-industry desires to promote new products than at finding the most efficient and sustainable means for fighting the diseases of poverty’. But then that’s why the psychopath Gates created it. John Edmunds said in a video that the London School of Hygiene & Tropical Medicine is involved in every aspect of vaccine development including large-scale clinical trials. He contends that mathematical modelling can show that vaccines protect individuals and society. That’s on the basis of shit in and shit out, I take it. Edmunds serves on the UK Vaccine Network as does Ferguson and the government’s foremost ‘Covid’ adviser, the grim-faced, dark-eyed Chris Whitty. The Vaccine Network says it works ‘to support the government to identify and shortlist targeted investment opportunities for the most promising vaccines and vaccine technologies that will help combat infectious diseases with epidemic potential, and to address structural issues related to the UK’s broader vaccine infrastructure’. Ferguson is acting Director of the Imperial College Vaccine Impact Modelling Consortium which has funding from the Bill and Melina Gates Foundation and the Gates-created GAVI ‘vaccine alliance’. Anyone wonder why these characters see vaccines as the answer to every problem? Ferguson is wildly enthusiastic in his support for GAVI’s campaign to vaccine children en masse in poor countries. You would expect someone like Gates who has constantly talked about the need to reduce the population to want to fund vaccines to keep more people alive. I’m sure that’s why he does it. The John Edmunds London School of Hygiene & Tropical Medicine (LSHTM) has a Vaccines Manufacturing Innovation Centre which develops, tests and commercialises vaccines. Rosemary Frei writes:

The vaccines centre also performs affiliated activities like combating ‘vaccine hesitancy’. The latter includes the Vaccine Confidence Project. The project’s stated purpose is, among other things, ‘to provide analysis and guidance for early response and engagement with the public to ensure sustained confidence in vaccines and immunisation’. The Vaccine Confidence Project’s director is LSHTM professor Heidi Larson. For more than a decade she’s been researching how to combat vaccine hesitancy.

How the bloody hell can blokes like John Edmunds and Neil Ferguson with those connections and financial ties model 'virus' case and death projections for the government and especially in a way that gives their paymasters like Gates exactly what they want? It's insane, but this is what you find throughout the world.

'Covid' is not dangerous, oops, wait, yes it is

Only days before Ferguson's nightmare scenario made Jackboot Johnson take Britain into a China-style lockdown to save us from a deadly 'virus' the UK government website gov.uk was reporting something very different to Ferguson on a page of official government guidance for 'high consequence infectious diseases (HCID)'. It said this about 'Covid-19':

As of 19 March 2020, COVID-19 *is no longer considered to be a high consequence infectious diseases (HCID) in the UK* [my emphasis]. The 4 nations public health HCID group made an interim recommendation in January 2020 to classify COVID-19 as an HCID. This was based on consideration of the UK HCID criteria about the virus and the disease with information available during the early stages of the outbreak.

Now that more is known about COVID-19, the public health bodies in the UK have reviewed the most up to date information about COVID-19 against the UK HCID criteria. They have determined that several features have now changed; in particular, more information is available about mortality rates (low overall), and there is now greater clinical awareness and a specific and sensitive laboratory test, the availability of which continues to increase. The Advisory Committee on Dangerous Pathogens (ACDP) is also of the opinion that COVID-19 should no longer be classified as an HCID.

Soon after the government had been exposed for downgrading the risk they upgraded it again and everyone was back to singing from the same Cult hymn book. Ferguson and his fellow Gates clones indicated that lockdowns and restrictions would have to continue until a Gates-funded vaccine was developed. Gates said the same because Ferguson and his like were repeating the Gates script which is the Cult script. 'Flatten the curve' became an ongoing nightmare of continuing lockdowns with periods in between of severe restrictions in pursuit of destroying independent incomes and had nothing to do with protecting health about which the Cult gives not a shit. Why wouldn't Ferguson be pushing a vaccine 'solution' when he's owned by vaccine-obsessive Gates who makes a fortune from them and when Ferguson heads the Vaccine Impact Modelling Consortium at

Imperial College funded by the Gates Foundation and GAVI, the 'vaccine alliance', created by Gates as his personal vaccine promotion operation? To compound the human catastrophe that Ferguson's 'models' did so much to create he was later exposed for breaking his own lockdown rules by having sexual liaisons with his married girlfriend Antonia Staats at his home while she was living at another location with her husband and children. Staats was a 'climate' activist and senior campaigner at the Soros-funded Avaaz which I wouldn't trust to tell me that grass is green. Ferguson had to resign as a government advisor over this hypocrisy in May, 2020, but after a period of quiet he was back being quoted by the ridiculous media on the need for more lockdowns and a vaccine rollout. Other government-advising 'scientists' from Imperial College' held the fort in his absence and said lockdown could be indefinite until a vaccine was found. The Cult script was being sung by the payrolled choir. I said there was no intention of going back to 'normal' when the 'vaccine' came because the 'vaccine' is part of a very different agenda that I will discuss in Human 2.0. Why would the Cult want to let the world go back to normal when destroying that normal forever was the whole point of what was happening? House arrest, closing businesses and schools through lockdown, (un)social distancing and masks all followed the Ferguson fantasy models. Again as I predicted (these people are so predictable) when the 'vaccine' arrived we were told that house arrest, lockdown, (un)social distancing and masks would still have to continue. I will deal with the masks in the next chapter because they are of fundamental importance.

Where's the 'pandemic'?

Any mildly in-depth assessment of the figures revealed what was really going on. Cult-funded and controlled organisations still have genuine people working within them such is the number involved. So it is with Genevieve Briand, assistant program director of the Applied Economics master's degree program at Johns Hopkins University. She analysed the impact that 'Covid-19' had on deaths from *all* causes in the United States using official data from the CDC for the period from early February to early September, 2020. She found that allegedly 'Covid' *related*-deaths exceeded those from heart disease which she found strange with heart disease always the biggest cause of fatalities. Her research became even more significant

when she noted the sudden decline in 2020 of *all* non-'Covid' deaths: 'This trend is completely contrary to the pattern observed in all previous years … the total decrease in deaths by other causes almost exactly equals the increase in deaths by Covid-19.' This was such a game, set and match in terms of what was happening that Johns Hopkins University deleted the article on the grounds that it 'was being used to support false and dangerous inaccuracies about the impact of the pandemic'. No – because it exposed the scam from official CDC figures and this was confirmed when those figures were published in January, 2021. Here we can see the effect of people dying from heart attacks, cancer, road accidents and gunshot wounds – *anything* – having 'Covid-19' on the death certificate along with those diagnosed from 'symptoms' who had even not tested positive with a test not testing for the 'virus'. I am not kidding with the gunshot wounds, by the way. Brenda Bock, coroner in Grand County, Colorado, revealed that two gunshot victims tested positive for the 'virus' within the previous 30 days and were therefore classified as 'Covid deaths'. Bock said: 'These two people had tested positive for Covid, but that's not what killed them. A gunshot wound is what killed them.' She said she had not even finished her investigation when the state listed the gunshot victims as deaths due to the 'virus'. The death and case figures for 'Covid-19' are an absolute joke and yet they are repeated like parrots by the media, politicians and alleged medical 'experts'. The official Cult narrative is the only show in town.

Genevieve Briand found that deaths from all causes were not exceptional in 2020 compared with previous years and a Spanish magazine published figures that said the same about Spain which was a 'Covid' propaganda hotspot at one point. *Discovery Salud*, a health and medicine magazine, quoted government figures which showed how 17,000 *fewer* people died in Spain in 2020 than in 2019 and more than 26,000 fewer than in 2018. The age-standardised mortality rate for England and Wales when age distribution is taken into account was significantly lower in 2020 than the 1970s, 80s and 90s, and was only the ninth highest since 2000. Where is the 'pandemic'?

Post mortems and autopsies virtually disappeared for 'Covid' deaths amid claims that 'virus-infected' bodily fluids posed a risk to those carrying out the autopsy. This was rejected by renowned German pathologist and forensic doctor Klaus Püschel who said that he and his staff had by then done 150 autopsies on 'Covid' patients with no problems at all. He said

they were needed to know why some ‘Covid’ patients suffered blood clots and not severe respiratory infections. The ‘virus’ is, after all, called SARS or ‘severe acute respiratory syndrome’. I highlighted in the spring of 2020 this phenomenon and quoted New York intensive care doctor Cameron Kyle-Sidell who posted a soon deleted YouTube video to say that they had been told to prepare to treat an infectious disease called ‘Covid-19’, but that was not what they were dealing with. Instead he likened the lung condition of the most severely ill patients to what you would expect with cabin depressurisation in a plane at 30,000 feet or someone dropped on the top of Everest without oxygen or acclimatisation. I have never said this is not happening to a small minority of alleged ‘Covid’ patients – I am saying this is not caused by a phantom ‘contagious virus’. Indeed Kyle-Sidell said that ‘Covid-19’ was not the disease they were told was coming their way. ‘We are operating under a medical paradigm that is untrue,’ he said, and he believed they were treating the wrong disease: ‘These people are being slowly starved of oxygen.’ Patients would take off their oxygen masks in a state of fear and stress and while they were blue in the face on the brink of death. They did not look like patients dying of pneumonia. You can see why they don’t want autopsies when their virus doesn’t exist and there is another condition in some people that they don’t wish to be uncovered. I should add here that the 5G system of millimetre waves was being rapidly introduced around the world in 2020 and even more so now as they fire 5G at the Earth from satellites. At 60 gigahertz within the 5G range that frequency interacts with the oxygen molecule and stops people breathing in sufficient oxygen to be absorbed into the bloodstream. They are installing 5G in schools and hospitals. The world is not mad or anything. 5G can cause major changes to the lungs and blood as I detail in *The Answer* and these consequences are labelled ‘Covid-19’, the alleged symptoms of which can be caused by 5G and other electromagnetic frequencies as cells respond to radiation poisoning.

The ‘Covid death’ scam

Dr Scott Jensen, a Minnesota state senator and medical doctor, exposed ‘Covid’ Medicare payment incentives to hospitals and death certificate manipulation. He said he was sent a seven-page document by the US Department of Health ‘coaching’ him on how to fill out death certificates

which had never happened before. The document said that he didn't need to have a laboratory test for 'Covid-19' to put that on the death certificate and that shocked him when death certificates are supposed to be about facts. Jensen described how doctors had been 'encouraged, if not pressured' to make a diagnosis of 'Covid-19' if they thought it was probable or *'presumed*'. No positive test was necessary – not that this would have mattered anyway. He said doctors were told to diagnose 'Covid' by symptoms when these were the same as colds, allergies, other respiratory problems, and certainly with influenza which 'disappeared' in the 'Covid' era. A common sniffle was enough to get the dreaded verdict. Ontario authorities decreed that a single care home resident with *one* symptom from a long list must lead to the isolation of the entire home. Other courageous doctors like Jensen made the same point about death figure manipulation and how deaths by other causes were falling while 'Covid-19 deaths' were rising at the same rate due to re-diagnosis. Their videos rarely survive long on YouTube with its Cult-supporting algorithms courtesy of CEO Susan Wojcicki and her bosses at Google. Figure-tampering was so glaring and ubiquitous that even officials were letting it slip or outright saying it. UK chief scientific adviser Patrick Vallance said on one occasion that 'Covid' on the death certificate doesn't mean 'Covid' was the cause of death (so why the hell is it there?) and we had the rare sight of a BBC reporter telling the truth when she said: 'Someone could be successfully treated for Covid, in say April, discharged, and then in June, get run over by a bus and die … That person would still be counted as a Covid death in England.' Yet the BBC and the rest of the world media went on repeating the case and death figures as if they were real. Illinois Public Health Director Dr Ngozi Ezike revealed the deceit while her bosses must have been clenching their buttocks:

> If you were in a hospice and given a few weeks to live and you were then found to have Covid that would be counted as a Covid death. [There might be] a clear alternate cause, but it is still listed as a Covid death. So everyone listed as a Covid death doesn't mean that was the cause of the death, but that they had Covid at the time of death.

Yes, a 'Covid virus' never shown to exist and tested for with a test not testing for the 'virus'. In the first period of the pandemic hoax through the spring of 2020 the process began of designating almost everything a

‘Covid’ death and this has continued ever since. I sat in a restaurant one night listening to a loud conversation on the next table where a family was discussing in bewilderment how a relative who had no symptoms of ‘Covid’, and had died of a long-term problem, could have been diagnosed a death by the ‘virus’. I could understand their bewilderment. If they read this book they will know why this medical fraud has been perpetrated the world over.

Some media truth shock

The media ignored the evidence of death certificate fraud until eventually one columnist did speak out when she saw it first-hand. Bel Mooney is a long-time national newspaper journalist in Britain currently working for the *Daily Mail*. Her article on February 19th, 2021, carried this headline: ‘My dad Ted passed three Covid tests and died of a chronic illness yet he’s officially one of Britain’s 120,000 victims of the virus and is far from alone ... so how many more are there?’ She told how her 99-year-old father was in a care home with a long-standing chronic obstructive pulmonary disease and vascular dementia. Maybe, but he was still aware enough to tell her from the start that there was no ‘virus’ and he refused the ‘vaccine’ for that reason. His death was not unexpected given his chronic health problems and Mooney said she was shocked to find that ‘Covid-19’ was declared the cause of death on his death certificate. She said this was a ‘bizarre and unacceptable untruth’ for a man with long-time health problems who had tested negative twice at the home for the ‘virus’. I was also shocked by this story although not by what she said. I had been highlighting the death certificate manipulation for ten months. It was the confirmation that a professional full-time journalist only realised this was going on when it affected her directly and neither did she know that whether her dad tested positive or negative was irrelevant with the test not testing for the ‘virus’. Where had she been? She said she did not believe in ‘conspiracy theories’ without knowing I’m sure that this and ‘conspiracy theorists’ were terms put into widespread circulation by the CIA in the 1960s to discredit those who did not accept the ridiculous official story of the Kennedy assassination. A blanket statement of ‘I don’t believe in conspiracy theories’ is always bizarre. The dictionary definition of the term alone means the world is drowning in conspiracies. What she said was even more

daft when her dad had just been affected by the ‘Covid’ conspiracy. Why else does she think that ‘Covid-19’ was going on the death certificates of people who died of something else?

To be fair once she saw from personal experience what was happening she didn’t mince words. Mooney was called by the care home on the morning of February 9th to be told her father had died in his sleep. When she asked for the official cause of death what came back was ‘Covid-19’. Mooney challenged this and was told there had been deaths from Covid on the dementia floor (confirmed by a test not testing for the ‘virus’) so they considered it ‘reasonable to assume’. ‘But doctor,’ Mooney rightly protested, ‘an assumption isn’t a diagnosis.’ She said she didn’t blame the perfectly decent and sympathetic doctor – ‘he was just doing his job’. Sorry, but that’s *bullshit*. He wasn’t doing his job at all. He was putting a false cause of death on the death certificate and that is a criminal offence for which he should be brought to account and the same with the millions of doctors worldwide who have done the same. They were not doing their job they were following orders and that must not wash at new Nuremberg trials any more than it did at the first ones. Mooney’s doctor was ‘assuming’ (presuming) as he was told to, but ‘just following orders’ makes no difference to his actions. A doctor’s job is to serve the patient and the truth, not follow orders, but that’s what they have done all over the world and played a central part in making the ‘Covid’ hoax possible with all its catastrophic consequences for humanity. Shame on them and they must answer for their actions. Mooney said her disquiet worsened when she registered her father’s death by telephone and was told by the registrar there had been very many other cases like hers where ‘the deceased’ had not tested positive for ‘Covid’ yet it was recorded as the cause of death. The test may not matter, but those involved at their level *think* it matters and it shows a callous disregard for accurate diagnosis. The pressure to do this is coming from the top of the national ‘health’ pyramids which in turn obey the World Health Organization which obeys Gates and the Cult. Mooney said the registrar agreed that this must distort the national figures adding that ‘the strangest thing is that every winter we record countless deaths from flu, and this winter there have been none. Not one!’ She asked if the registrar thought deaths from flu were being misdiagnosed and lumped together with ‘Covid’ deaths. The answer was a ‘puzzled yes’. Mooney said that the funeral director said the same about ‘Covid’ deaths which had

nothing to do with ‘Covid’. They had lost count of the number of families upset by this and other funeral companies in different countries have had the same experience. Mooney wrote:

The nightly shroud-waving and shocking close-ups of pain imposed on us by the TV news bewildered and terrified the population into eager compliance with lockdowns. We were invited to ‘save the NHS’ and to grieve for strangers – the real-life loved ones behind those shocking death counts. Why would the public imagine what I now fear, namely that the way Covid-19 death statistics are compiled might make the numbers seem greater than they are?

Oh, just a little bit – like 100 percent.

Do the maths

Mooney asked why a country would wish to skew its mortality figures by wrongly certifying deaths? What had been going on? Well, if you don’t believe in conspiracies you will never find the answer which is that *it’s a conspiracy*. She did, however, describe what she had discovered as a ‘national scandal’. In reality it’s a global scandal and happening everywhere. Pillars of this conspiracy were all put into place before the button was pressed with the Drosten PCR protocol and high amplifications to produce the cases and death certificate changes to secure illusory ‘Covid’ deaths. Mooney notes that normally two doctors were needed to certify a death, with one having to know the patient, and how the rules were changed in the spring of 2020 to allow one doctor to do this. In the same period ‘Covid deaths’ were decreed to be all cases where Covid-19 was put on the death certificate even without a positive test or any symptoms. Mooney asked: ‘How many of the 30,851 (as of January 15) care home resident deaths with Covid-19 on the certificate (32.4 per cent of all deaths so far) were based on an assumption, like that of my father? And what has that done to our national psyche?’All of them is the answer to the first question and it has devastated and dismantled the national psyche, actually the global psyche, on a colossal scale. In the UK case and death data is compiled by organisations like Public Health England (PHE) and the Office for National Statistics (ONS). Mooney highlights the insane policy of counting a death from any cause as ‘Covid-19’ if this happens within 28 days of a positive test (with a test not testing for the ‘virus’) and she points out that ONS

statistics reflect deaths ‘involving Covid’ ‘or due to Covid’ which meant in practice any death where ‘Covid-19’ was mentioned on the death certificate. She described the consequences of this fraud:

Most people will accept the narrative they are fed, so panicky governments here and in Europe witnessed the harsh measures enacted in totalitarian China and jumped into lockdown. Headlines about Covid deaths tolled like the knell that would bring doomsday to us all. Fear stalked our empty streets. Politicians parroted the frankly ridiculous aim of ‘zero Covid’ and shut down the economy, while most British people agreed that lockdown was essential and (astonishingly to me, as a patriotic Brit) even wanted more restrictions.

For what? Lies on death certificates? Never mind the grim toll of lives ruined, suicides, schools closed, rising inequality, depression, cancelled hospital treatments, cancer patients in a torture of waiting, poverty, economic devastation, loneliness, families kept apart, and so on. How many lives have been lost as a direct result of lockdown?

She said that we could join in a national chorus of shock and horror at reaching the 120,000 death toll which was surely certain to have been totally skewed all along, but what about the human cost of lockdown justified by these ‘death figures’? *The British Medical Journal* had reported a 1,493 percent increase in cases of children taken to Great Ormond Street Hospital with abusive head injuries alone and then there was the effect on families:

Perhaps the most shocking thing about all this is that families have been kept apart – and obeyed the most irrational, changing rules at the whim of government – because they believed in the statistics. They succumbed to fear, which his generation rejected in that war fought for freedom. Dad (God rest his soul) would be angry. And so am I.

Another theme to watch is that in the winter months when there are more deaths from all causes they focus on ‘Covid’ deaths and in the summer when the British Lung Foundation says respiratory disease plummets by 80 percent they rage on about ‘cases’. Either way fascism on population is always the answer.

Nazi eugenics in the 21st century

Elderly people in care homes have been isolated from their families month after lonely month with no contact with relatives and grandchildren who were banned from seeing them. We were told that lockdown fascism was to 'protect the vulnerable' like elderly people. At the same time Do Not Resuscitate (DNR) orders were placed on their medical files so that if they needed resuscitation it wasn't done and 'Covid-19' went on their death certificates. Old people were not being 'protected' they were being culled – murdered in truth. DNR orders were being decreed for disabled and young people with learning difficulties or psychological problems. The UK Care Quality Commission, a non-departmental body of the Department of Health and Social Care, found that 34 percent of those working in health and social care were pressured into placing 'do not attempt cardiopulmonary resuscitation' orders on 'Covid' patients who suffered from disabilities and learning difficulties without involving the patient or their families in the decision. UK judges ruled that an elderly woman with dementia should have the DNA-manipulating 'Covid vaccine' against her son's wishes and that a man with severe learning difficulties should have the jab despite his family's objections. Never mind that many had already died. The judiciary always supports doctors and government in fascist dictatorships. They wouldn't dare do otherwise. A horrific video was posted showing fascist officers from Los Angeles police forcibly giving the 'Covid' shot to women with special needs who were screaming that they didn't want it. The same fascists are seen giving the jab to a sleeping elderly woman in a care home. This is straight out of the Nazi playbook. Hitler's Nazis committed mass murder of the mentally ill and physically disabled throughout Germany and occupied territories in the programme that became known as Aktion T4, or just T4. Sabbatian-controlled Hitler and his grotesque crazies set out to kill those they considered useless and unnecessary. The Reich Committee for the Scientific Registering of Hereditary and Congenital Illnesses registered the births of babies identified by physicians to have 'defects'. By 1941 alone more than 5,000 children were murdered by the state and it is estimated that in total the number of innocent people killed in Aktion T4 was between 275,000 and 300,000. Parents were told their children had been sent away for 'special treatment' never to return. It is rather pathetic to see claims about plans for new extermination camps being dismissed today when the same force behind current events did precisely that 80 years ago. Margaret Sanger was a Cult operative who used 'birth control' to sanitise

her programme of eugenics. Organisations she founded became what is now Planned Parenthood. Sanger proposed that ‘the whole dysgenic population would have its choice of segregation or sterilization’. These included epileptics, ‘feeble-minded’, and prostitutes. Sanger opposed charity because it perpetuated ‘human waste‘. She reveals the Cult mentality and if anyone thinks that extermination camps are a ‘conspiracy theory’ their naivety is touching if breathtakingly stupid.

If you don’t believe that doctors can act with callous disregard for their patients it is worth considering that doctors and medical staff agreed to put government-decreed DNR orders on medical files and do nothing when resuscitation is called for. I don’t know what you call such people in your house. In mine they are Nazis from the Josef Mengele School of Medicine. Phenomenal numbers of old people have died worldwide from the effects of lockdown, depression, lack of treatment, the ‘vaccine’ (more later) and losing the will to live. A common response at the start of the manufactured pandemic was to remove old people from hospital beds and transfer them to nursing homes. The decision would result in a mass cull of elderly people in those homes through lack of treatment – *not* ‘Covid’. Care home whistleblowers have told how once the ‘Covid’ era began doctors would not come to their homes to treat patients and they were begging for drugs like antibiotics that often never came. The most infamous example was ordered by New York governor Andrew Cuomo, brother of a moronic CNN host, who amazingly was given an Emmy Award for his handling of the ‘Covid crisis’ by the ridiculous Wokers that hand them out. Just how ridiculous could be seen in February, 2021, when a Department of Justice and FBI investigation began into how thousands of old people in New York died in nursing homes after being discharged from hospital to make way for ‘Covid’ patients on Cuomo’s say-so – and how he and his staff covered up these facts. This couldn’t have happened to a nicer psychopath. Even then there was a ‘Covid’ spin. Reports said that thousands of old people who tested positive for ‘Covid’ in hospital were transferred to nursing homes to both die of ‘Covid’ and transmit it to others. No – they were in hospital because they were ill and the fact that they tested positive with a test not testing for the ‘virus’ is irrelevant. They were ill often with respiratory diseases ubiquitous in old people near the end of their lives. Their transfer out of hospital meant that their treatment stopped and many would go on to die.

They're old. Who gives a damn?

I have exposed in the books for decades the Cult plan to cull the world's old people and even to introduce at some point what they call a 'demise pill' which at a certain age everyone would take and be out of here by law. In March, 2021, Spain legalised euthanasia and assisted suicide following the Netherlands, Belgium, Luxembourg and Canada on the Tiptoe to the demise pill. Treatment of old people by many 'care' homes has been a disgrace in the 'Covid' era. There are many, many, caring staff – I know some. There have, however, been legions of stories about callous treatment of old people and their families. Police were called when families came to take their loved ones home in the light of isolation that was killing them. They became prisoners of the state. Care home residents in insane, fascist Ontario, Canada, were not allowed to leave their *room* once the 'Covid' hoax began. UK staff have even wheeled elderly people away from windows where family members were talking with them. Oriana Criscuolo from Stockport in the English North West dropped off some things for her 80-year-old father who has Parkinson's disease and dementia and she wanted to wave to him through a ground-floor window. She was told that was 'illegal'. When she went anyway they closed the curtains in the middle of the day. Oriana said:

> It's just unbelievable. I cannot understand how care home staff – people who are being paid to care – have become so uncaring. Their behaviour is inhumane and cruel. It's beyond belief.

She was right and this was not a one-off. What a way to end your life in such loveless circumstances. UK registered nurse Nicky Millen, a proper old school nurse for 40 years, said that when she started her career care was based on dignity, choice, compassion and empathy. Now she said 'the things that are important to me have gone out of the window.' She was appalled that people were dying without their loved ones and saying goodbye on iPads. Nicky described how a distressed 89-year-old lady stroked her face and asked her 'how many paracetamol would it take to finish me off'. Life was no longer worth living while not seeing her family. Nicky said she was humiliated in front of the ward staff and patients for letting the lady stroke her face and giving her a cuddle. Such is the dehumanisation that the 'Covid' hoax has brought to the surface. Nicky

worked in care homes where patients told her they were being held prisoner. ‘I want to live until I die’, one said to her. ‘I had a lady in tears because she hadn’t seen her great-grandson.’ Nicky was compassionate old school meeting psychopathic New Normal. She also said she had worked on a ‘Covid’ ward with no ‘Covid’ patients. Jewish writer Shai Held wrote an article in March, 2020, which was headlined ‘The Staggering, Heartless Cruelty Toward the Elderly’. What he described was happening from the earliest days of lockdown. He said ‘the elderly’ were considered a group and not unique individuals (the way of the Woke). Shai Held said:

Notice how the all-too-familiar rhetoric of dehumanization works: ‘The elderly’ are bunched together as a faceless mass, all of them considered culprits and thus effectively deserving of the suffering the pandemic will inflict upon them. Lost entirely is the fact that the elderly are individual human beings, each with a distinctive face and voice, each with hopes and dreams, memories and regrets, friendships and marriages, loves lost and loves sustained.

‘The elderly’ have become another dehumanised group for which anything goes and for many that has resulted in cold disregard for their rights and their life. The distinctive face that Held talks about is designed to be deleted by masks until everyone is part of a faceless mass.

‘War-zone’ hospitals myth

Again and again medical professionals have told me what was really going on and how hospitals ‘overrun like war zones’ according to the media were virtually empty. The mantra from medical whistleblowers was please don’t use my name or my career is over. Citizen journalists around the world sneaked into hospitals to film evidence exposing the ‘war-zone’ lie. They really *were* largely empty with closed wards and operating theatres. I met a hospital worker in my town on the Isle of Wight during the first lockdown in 2020 who said the only island hospital had never been so quiet. Lockdown was justified by the psychopaths to stop hospitals being overrun. At the same time that the island hospital was near-empty the military arrived here to provide *extra beds*. It was all propaganda to ramp up the fear to ensure compliance with fascism as were never-used temporary hospitals with thousands of beds known as Nightingales and never-used make-shift mortuaries opened by the criminal UK government. A man who helped to

install those extra island beds attributed to the army said they were never used and the hospital was empty. Doctors and nurses ‘stood around talking or on their phones, wandering down to us to see what we were doing’. There were no masks or social distancing. He accused the useless local island paper, the *County Press*, of ‘pumping the fear as if our hospital was overrun and we only have one so it should have been’. He described ambulances parked up with crews outside in deck chairs. When his brother called an ambulance he was told there was a two-hour backlog which he called ‘bullshit’. An old lady on the island fell ‘and was in a bad way’, but a caller who rang for an ambulance was told the situation wasn’t urgent enough. Ambulance stations were working under capacity while people would hear ambulances with sirens blaring driving through the streets. When those living near the stations realised what was going on they would follow them as they left, circulated around an urban area with the sirens going, and then came back without stopping. All this was to increase levels of fear and the same goes for the ‘ventilator shortage crisis’ that cost tens of millions for hastily produced ventilators never to be used. Ambulance crews that agreed to be exploited in this way for fear propaganda might find themselves a mirror. I wish them well with that. Empty hospitals were the obvious consequence of treatment and diagnoses of non-’Covid’ conditions cancelled and those involved handed a death sentence. People have been dying at home from undiagnosed and untreated cancer, heart disease and other life-threatening conditions to allow empty hospitals to deal with a ‘pandemic’ that wasn’t happening.

Death of the innocent

‘War-zones’ have been laying off nursing staff, even doctors where they can. There was no work for them. Lockdown was justified by saving lives and protecting the vulnerable they were actually killing with DNR orders and preventing empty hospitals being ‘overrun’. In Britain the mantra of stay at home to ‘save the NHS’ was everywhere and across the world the same story was being sold when it was all lies. Two California doctors, Dan Erickson and Artin Massihi at Accelerated Urgent Care in Bakersfield, held a news conference in April, 2020, to say that intensive care units in California were ‘empty, essentially’, with hospitals shutting floors, not treating patients and laying off doctors. The California health system was

working at minimum capacity ‘getting rid of doctors because we just don’t have the volume’. They said that people with conditions such as heart disease and cancer were not coming to hospital out of fear of ‘Covid-19’. Their video was deleted by Susan Wojcicki’s Cult-owned YouTube after reaching five million views. Florida governor Ron Desantis, who rejected the severe lockdowns of other states and is being targeted for doing so, said that in March, 2020, every US governor was given models claiming they would run out of hospital beds in days. That was never going to happen and the ‘modellers’ knew it. Deceit can be found at every level of the system. Urgent children’s operations were cancelled including fracture repairs and biopsies to spot cancer. Eric Nicholls, a consultant paediatrician, said ‘this is obviously concerning and we need to return to normal operating and to increase capacity as soon as possible’. Psychopaths in power were rather less concerned *because* they are psychopaths. Deletion of urgent care and diagnosis has been happening all over the world and how many kids and others have died as a result of the actions of these cold and heartless lunatics dictating ‘health’ policy? The number must be stratospheric. Richard Sullivan, professor of cancer and global health at King’s College London, said people feared ‘Covid’ more than cancer such was the campaign of fear. ‘Years of lost life will be quite dramatic’, Sullivan said, with ‘a huge amount of avoidable mortality’. Sarah Woolnough, executive director for policy at Cancer Research UK, said there had been a 75 percent drop in urgent referrals to hospitals by family doctors of people with suspected cancer. Sullivan said that ‘a lot of services have had to scale back – we’ve seen a dramatic decrease in the amount of elective cancer surgery’. Lockdown deaths worldwide has been absolutely fantastic with the *New York Post* reporting how data confirmed that ‘lockdowns end more lives than they save’:

There was a sharp decline in visits to emergency rooms and an increase in fatal heart attacks because patients didn’t receive prompt treatment. Many fewer people were screened for cancer. Social isolation contributed to excess deaths from dementia and Alzheimer’s.

Researchers predicted that the social and economic upheaval would lead to tens of thousands of “deaths of despair” from drug overdoses, alcoholism and suicide. As unemployment surged and mental-health and substance-abuse treatment programs were interrupted, the reported levels of anxiety, depression and suicidal thoughts increased dramatically, as did alcohol sales and fatal drug overdoses.

This has been happening while nurses and other staff had so much time on their hands in the ‘war-zones’ that Tic-Tok dancing videos began appearing across the Internet with medical staff dancing around in empty wards and corridors as people died at home from causes that would normally have been treated in hospital.

Mentions in dispatches

One brave and truth-committed whistleblower was Louise Hampton, a call handler with the UK NHS who made a viral Internet video saying she had done ‘fuck all’ during the ‘pandemic’ which was ‘a load of bollocks’. She said that ‘Covid-19’ was rebranded flu and of course she lost her job. This is what happens in the medical and endless other professions now when you tell the truth. Louise filmed inside ‘war-zone’ accident and emergency departments to show they were empty and I mean *empty* as in no one there. The mainstream media could have done the same and blown the gaff on the whole conspiracy. They haven’t to their eternal shame. Not that most ‘journalists’ seem capable of manifesting shame as with the psychopaths they slavishly repeat without question. The relative few who were admitted with serious health problems were left to die alone with no loved ones allowed to see them because of ‘Covid’ rules and they included kids dying without the comfort of mum and dad at their bedside while the evil behind this couldn’t give a damn. It was all good fun to them. A Scottish NHS staff nurse publicly quit in the spring of 2021 saying: ‘I can no longer be part of the lies and the corruption by the government.’ She said hospitals ‘aren’t full, the beds aren’t full, beds have been shut, wards have been shut’. Hospitals were never busy throughout ‘Covid’. The staff nurse said that Nicola Sturgeon, tragically the leader of the Scottish government, was on television saying save the hospitals and the NHS – ‘but the beds are empty’ and ‘we’ve not seen flu, we always see flu every year’. She wrote to government and spoke with her union Unison (the unions are Cult-compromised and *useless*, but nothing changed. Many of her colleagues were scared of losing their jobs if they spoke out as they wanted to. She said nursing staff were being affected by wearing masks all day and ‘my head is splitting every shift from wearing a mask’. The NHS is part of the fascist tyranny and must be dismantled so we can start again with human beings in charge. (Ironically, hospitals were reported to be busier again

when official ‘Covid’ cases *fell* in spring/summer of 2021 and many other conditions required treatment at the same time as *the fake vaccine rollout*.)

I will cover the ‘Covid vaccine’ scam in detail later, but it is another indicator of the sickening disregard for human life that I am highlighting here. The DNA-manipulating concoctions do not fulfil the definition of a ‘vaccine’, have never been used on humans before and were given only emergency approval because trials were not completed and they continued using the unknowing public. The result was what a NHS senior nurse with responsibility for ‘vaccine’ procedure said was ‘genocide’. She said the ‘vaccines’ were not ‘vaccines’. They had not been shown to be safe and claims about their effectiveness by drug companies were ‘poetic licence’. She described what was happening as a ‘horrid act of human annihilation’. The nurse said that management had instigated a policy of not providing a Patient Information Leaflet (PIL) before people were ‘vaccinated’ even though health care professionals are supposed to do this according to protocol. Patients should also be told that they are taking part in an ongoing clinical trial. Her challenges to what is happening had seen her excluded from meetings and ridiculed in others. She said she was told to ‘watch my step … or I would find myself surplus to requirements’. The nurse, who spoke anonymously in fear of her career, said she asked her NHS manager why he/she was content with taking part in genocide against those having the ‘vaccines’. The reply was that everyone had to play their part and to ‘put up, shut up, and get it done’. Government was ‘leaning heavily’ on NHS management which was clearly leaning heavily on staff. This is how the global ‘medical’ hierarchy operates and it starts with the Cult and its World Health Organization.

She told the story of a doctor who had the Pfizer jab and when questioned had no idea what was in it. The doctor had never read the literature. We have to stop treating doctors as intellectual giants when so many are moral and medical pygmies. The doctor did not even know that the ‘vaccines’ were not fully approved or that their trials were ongoing. They were, however, asking their patients if they minded taking part in follow-ups for research purposes – yes, the *ongoing clinical trial*. The nurse said the doctor’s ignorance was not rare and she had spoken to a hospital consultant who had the jab without any idea of the background or that the ‘trials’ had not been completed. Nurses and pharmacists had shown the same ignorance. ‘My NHS colleagues have forsaken their duty of care,

broken their code of conduct – Hippocratic Oath – and have been brainwashed just the same as the majority of the UK public through propaganda …' She said she had not been able to recruit a single NHS colleague, doctor, nurse or pharmacist to stand with her and speak out. Her union had refused to help. She said that if the genocide came to light she would not hesitate to give evidence at a Nuremberg-type trial against those in power who could have affected the outcomes but didn't.

And all for what?

To put the nonsense into perspective let's say the 'virus' does exist and let's go completely crazy and accept that the official manipulated figures for cases and deaths are accurate. *Even then* a study by Stanford University epidemiologist Dr John Ioannidis published on the World Health Organization website produced an average infection to fatality rate of … *0.23 percent!* Ioannidis said: 'If one could sample equally from all locations globally, the median infection fatality rate might even be substantially lower than the 0.23% observed in my analysis.' For healthy people under 70 it was … *0.05 percent*! This compares with the 3.4 percent claimed by the Cult-owned World Health Organization when the hoax was first played and maximum fear needed to be generated. An updated Stanford study in April, 2021, put the 'infection' to 'fatality' rate at just 0.15 percent. Another team of scientists led by Megan O'Driscoll and Henrik Salje studied data from 45 countries and published their findings on the Nature website. For children and young people the figure is so small it virtually does not register although authorities will be hyping dangers to the young when they introduce DNA-manipulating 'vaccines' for children. The O'Driscoll study produced an average infection-fatality figure of 0.003 for children from birth to four; 0.001 for 5 to 14; 0.003 for 15 to 19; and it was still only 0.456 up to 64. To claim that children must be 'vaccinated' to protect them from 'Covid' is an obvious lie and so there must be another reason and there is. What's more the average age of a 'Covid' death is akin to the average age that people die in general. The average age of death in England is about 80 for men and 83 for women. The average age of death from alleged 'Covid' is between 82 and 83. California doctors, Dan Erickson and Artin Massihi, said at their April media conference that projection models of millions of deaths had been 'woefully inaccurate'. They produced

detailed figures showing that Californians had a 0.03 chance of dying from 'Covid' based on the number of people who tested positive (with a test not testing for the 'virus'). Erickson said there was a 0.1 percent chance of dying from 'Covid' in the *state* of New York, not just the city, and a 0.05 percent chance in Spain, a centre of 'Covid-19' hysteria at one stage. The Stanford studies supported the doctors' data with fatality rate estimates of 0.23 and 0.15 percent. How close are these figures to my estimate of *zero*? Death-rate figures claimed by the World Health Organization at the start of the hoax were some 15 times higher. The California doctors said there was no justification for lockdowns and the economic devastation they caused. Everything they had ever learned about quarantine was that you quarantine the *sick* and not the healthy. They had never seen this before and it made no medical sense.

Why in the in the light of all this would governments and medical systems the world over say that billions must go under house arrest; lose their livelihood; in many cases lose their mind, their health and their life; force people to wear masks dangerous to health and psychology; make human interaction and even family interaction a criminal offence; ban travel; close restaurants, bars, watching live sport, concerts, theatre, and any activity involving human togetherness and discourse; and closing schools to isolate children from their friends and cause many to commit suicide in acts of hopelessness and despair? The California doctors said lockdown consequences included increased child abuse, partner abuse, alcoholism, depression, and other impacts they were seeing every day. Who would do that to the entire human race if not mentally-ill psychopaths of almost unimaginable extremes like Bill Gates? We must face the reality of what we are dealing with and come out of denial. Fascism and tyranny are made possible only by the target population submitting and acquiescing to fascism and tyranny. The whole of human history shows that to be true. Most people naively and unquestioning believed what they were told about a 'deadly virus' and meekly and weakly submitted to house arrest. Those who didn't believe it – at least in total – still submitted in fear of the consequences of not doing so. For the rest who wouldn't submit draconian fines have been imposed, brutal policing by psychopaths *for* psychopaths, and condemnation from the meek and weak who condemn the Pushbackers on behalf of the very force that has them, too, in its gunsights. 'Pathetic' does not even begin to suffice. Britain's brainless 'Health' Secretary Matt

Hancock warned anyone lying to border officials about returning from a list of ‘hotspot’ countries could face a jail sentence of up to ten years which is more than for racially-aggravated assault, incest and attempting to have sex with a child under 13. Hancock is a lunatic, but he has the state apparatus behind him in a Cult-led chain reaction and the same with UK ‘Vaccine Minister’ Nadhim Zahawi, a prominent member of the mega-Cult secret society, Le Cercle, which featured in my earlier books. The Cult enforces its will on governments and medical systems; government and medical systems enforce their will on business and police; business enforces its will on staff who enforce it on customers; police enforce the will of the Cult on the population and play their essential part in creating a world of fascist control that their own children and grandchildren will have to live in their entire lives. It is a hierarchical pyramid of imposition and acquiescence and, yes indeedy, of clinical insanity.

Does anyone bright enough to read this book have to ask what the answer is? I think not, but I will reveal it anyway in the fewest of syllables: Tell the psychos and their moronic lackeys to fuck off and let’s get on with our lives. We are many – They are few.

CHAPTER SEVEN

War on your mind

One believes things because one has been conditioned to believe them
Aldous Huxley, *Brave New World*

I have described the 'Covid' hoax as a 'Psyop' and that is true in every sense and on every level in accordance with the definition of that term which is psychological warfare. Break down the 'Covid pandemic' to the foundation themes and it is psychological warfare on the human individual and collective mind.

The same can be said for the entire human belief system involving every subject you can imagine. Huxley was right in his contention that people believe what they are conditioned to believe and this comes from the repetition throughout their lives of the same falsehoods. They spew from government, corporations, media and endless streams of 'experts' telling you what the Cult wants you to believe and often believing it themselves (although *far* from always). 'Experts' are rewarded with 'prestigious' jobs and titles and as agents of perceptual programming with regular access to the media. The Cult has to control the narrative – control *information* – or they lose control of the vital, crucial, without-which-they-cannot-prevail public perception of reality. The foundation of that control today is the Internet made possible by the Defense Advanced Research Projects Agency (DARPA), the incredibly sinister technological arm of the Pentagon. The Internet is the result of military technology. DARPA openly brags about establishing the Internet which has been a long-term project to lasso the minds of the global population. I have said for decades the plan is to control

information to such an extreme that eventually no one would see or hear anything that the Cult does not approve. We are closing in on that end with ferocious censorship since the ‘Covid’ hoax began and in my case it started back in the 1990s in terms of books and speaking venues. I had to create my own publishing company in 1995 precisely because no one else would publish my books even then. I think they’re all still running.

Cult Internet

To secure total control of information they needed the Internet in which pre-programmed algorithms can seek out ‘unclean’ content for deletion and even stop it being posted in the first place. The Cult had to dismantle print and non-Internet broadcast media to ensure the transfer of information to the appropriate-named ‘Web’ – a critical expression of the *Cult* web. We’ve seen the ever-quickening demise of traditional media and control of what is left by a tiny number of corporations operating worldwide. Independent journalism in the mainstream is already dead and never was that more obvious than since the turn of 2020. The Cult wants all information communicated via the Internet to globally censor and allow the plug to be pulled any time. Lockdowns and forced isolation has meant that communication between people has been through electronic means and no longer through face-to-face discourse and discussion. Cult psychopaths have targeted the bars, restaurants, sport, venues and meeting places in general for this reason. None of this is by chance and it’s to stop people gathering in any kind of privacy or number while being able to track and monitor all Internet communications and block them as necessary. Even private messages between individuals have been censored by these fascists that control Cult fronts like Facebook, Twitter, Google and YouTube which are all officially run by Sabbatian place-people and from the background by higher-level Sabbatian place people. Facebook, Google, Amazon and their like were seed-funded and supported into existence with money-no-object infusions of funds either directly or indirectly from DARPA and CIA technology arm In-Q-Tel. The Cult plays the long game and prepares very carefully for big plays like ‘Covid’. Amazon is another front in the psychological war and pretty much controls the global market in book sales and increasingly publishing. Amazon’s limitless funds have deleted fantastic numbers of independent publishers to seize global domination on

the way to deciding which books can be sold and circulated and which cannot. Moves in that direction are already happening. Amazon's leading light Jeff Bezos is the grandson of Lawrence Preston Gise who worked with DARPA predecessor ARPA. Amazon has big connections to the CIA and the Pentagon. The plan I have long described went like this:

1. Employ military technology to establish the Internet.

2. Sell the Internet as a place where people can freely communicate without censorship and allow that to happen until the Net becomes the central and irreversible pillar of human society. If the Internet had been highly censored from the start many would have rejected it.

3. Fund and manipulate major corporations into being to control the circulation of information on your Internet using cover stories about geeks in garages to explain how they came about. Give them unlimited funds to expand rapidly with no need to make a profit for years while non-Cult companies who need to balance the books cannot compete. You know that in these circumstances your Googles, YouTubes, Facebooks and Amazons are going to secure near monopolies by either crushing or buying up the opposition.

4. Allow freedom of expression on both the Internet and communication platforms to draw people in until the Internet is the central and irreversible pillar of human society and your communication corporations have reached a stage of near monopoly domination.

5. Then unleash your always-planned frenzy of censorship on the basis of 'where else are you going to go?' and continue to expand that until nothing remains that the Cult does not want its human targets to see.

The process was timed to hit the 'Covid' hoax to ensure the best chance possible of controlling the narrative which they knew they had to do at all costs. They were, after all, about to unleash a 'deadly virus' that didn't really exist. If you do that in an environment of free-flowing information and opinion you would be dead in the water before you could say Gates is a psychopath. The network was in place through which the Cult-created-and-owned World Health Organization could dictate the 'Covid' narrative and response policy slavishly supported by Cult-owned Internet communication giants and mainstream media while those telling a different story were censored. Google, YouTube, Facebook and Twitter openly announced that they would do this. What else would we expect from Cult-owned operations like Facebook which former executives have confirmed set out to make the platform more addictive than cigarettes and coldly manipulates emotions of its users to sow division between people and groups and scramble the minds

of the young? If Zuckerberg lives out the rest of his life without going to jail for crimes against humanity, and most emphatically against the young, it will be a travesty of justice. Still, no matter, cause and effect will catch up with him eventually and the same with Sergey Brin and Larry Page at Google with its CEO Sundar Pichai who fix the Google search results to promote Cult narratives and hide the opposition. Put the same key words into Google and other search engines like DuckDuckGo and you will see how different results can be. Wikipedia is another intensely biased 'encyclopaedia' which skews its content to the Cult agenda. YouTube links to Wikipedia's version of 'Covid' and 'climate change' on video pages in which experts in their field offer a different opinion (even that is increasingly rare with Wojcicki censorship). Into this 'Covid' silence-them network must be added government media censors, sorry 'regulators', such as Ofcom in the UK which imposed tyrannical restrictions on British broadcasters that had the effect of banning me from ever appearing. Just to debate with me about my evidence and views on 'Covid' would mean breaking the fascistic impositions of Ofcom and its CEO career government bureaucrat Melanie Dawes. Gutless British broadcasters tremble at the very thought of fascist Ofcom.

Psychos behind 'Covid'

The reason for the 'Covid' catastrophe in all its facets and forms can be seen by whom and what is driving the policies worldwide in such a coordinated way. Decisions are not being made to protect health, but to target psychology. The dominant group guiding and 'advising' government policy are not medical professionals. They are psychologists and behavioural scientists. Every major country has its own version of this phenomenon and I'll use the British example to show how it works. In many ways the British version has been affecting the wider world in the form of the huge behaviour manipulation network in the UK which operates in other countries. The network involves private companies, government, intelligence and military. The Cabinet Office is at the centre of the government 'Covid' Psyop and part-owns, with 'innovation charity' Nesta, the Behavioural Insights Team (BIT) which claims to be independent of government but patently isn't. The BIT was established in 2010 and its job is to manipulate the psyche of the population to acquiesce to government

demands and so much more. It is also known as the ‘Nudge Unit’, a name inspired by the 2009 book by two ultra-Zionists, Cass Sunstein and Richard Thaler, called *Nudge: Improving Decisions About Health, Wealth, and Happiness*. The book, as with the Behavioural Insights Team, seeks to ‘nudge’ behaviour (manipulate it) to make the public follow patterns of action and perception that suit those in authority (the Cult). Sunstein is so skilled at this that he advises the World Health Organization and the UK Behavioural Insights Team and was Administrator of the White House Office of Information and Regulatory Affairs in the Obama administration. Biden appointed him to the Department of Homeland Security – another ultra-Zionist in the fold to oversee new immigration laws which is another policy the Cult wants to control. Sunstein is desperate to silence anyone exposing conspiracies and co-authored a 2008 report on the subject in which suggestions were offered to ban ‘conspiracy theorizing’ or impose ‘some kind of tax, financial or otherwise, on those who disseminate such theories’. I guess a psychiatrist’s chair is out of the question?

Sunstein’s mate Richard Thaler, an ‘academic affiliate’ of the UK Behavioural Insights Team, is a proponent of ‘behavioural economics’ which is defined as the study of ‘the effects of psychological, cognitive, emotional, cultural and social factors on the decisions of individuals and institutions’. Study the effects so they can be manipulated to be what you want them to be. Other leading names in the development of behavioural economics are ultra-Zionists Daniel Kahneman and Robert J. Shiller and they, with Thaler, won the Nobel Memorial Prize in Economic Sciences for their work in this field. The Behavioural Insights Team is operating at the heart of the UK government and has expanded globally through partnerships with several universities including Harvard, Oxford, Cambridge, University College London (UCL) and Pennsylvania. They claim to have ‘trained’ (reframed) 20,000 civil servants and run more than 750 projects involving 400 randomised controlled trials in dozens of countries’ as another version of mind reframers Common Purpose. BIT works from its office in New York with cities and their agencies, as well as other partners, across the United States and Canada – this is a company part-owned by the British government Cabinet Office. An executive order by President Cult-servant Obama established a US Social and Behavioral Sciences Team in 2015. They all have the same reason for being and that’s

to brainwash the population directly and by brainwashing those in positions of authority.

‘Covid’ mind game

Another prime aspect of the UK mind-control network is the ‘independent’ [joke] Scientific Pandemic Insights Group on Behaviours (SPI-B) which ‘provides behavioural science advice aimed at anticipating and helping people adhere to interventions that are recommended by medical or epidemiological experts’. That means manipulating public perception and behaviour to do whatever government tells them to do. It’s disgusting and if they really want the public to be ‘safe’ this lot should all be under lock and key. According to the government website SPI-B consists of ‘behavioural scientists, health and social psychologists, anthropologists and historians’ and advises the Whitty-Vallance-led Scientific Advisory Group for Emergencies (SAGE) which in turn advises the government on ‘the science’ (it doesn’t) and ‘Covid’ policy. When politicians say they are being guided by ‘the science’ this is the rabble in each country they are talking about and that ‘science’ is dominated by behaviour manipulators to enforce government fascism through public compliance. The Behaviour Insight Team is headed by psychologist David Solomon Halpern, a visiting professor at King’s College London, and connects with a national and global web of other civilian and military organisations as the Cult moves towards its goal of fusing them into one fascistic whole in every country through its ‘Fusion Doctrine’. The behaviour manipulation network involves, but is not confined to, the Foreign Office; National Security Council; government communications headquarters (GCHQ); MI5; MI6; the Cabinet Office-based Media Monitoring Unit; and the Rapid Response Unit which ‘monitors digital trends to spot emerging issues; including misinformation and disinformation; and identifies the best way to respond’.

There is also the 77th Brigade of the UK military which operates like the notorious Israeli military’s Unit 8200 in manipulating information and discussion on the Internet by posing as members of the public to promote the narrative and discredit those who challenge it. Here we have the military seeking to manipulate *domestic* public opinion while the Nazis in government are fine with that. Conservative Member of Parliament Tobias Ellwood, an advocate of lockdown and control through ‘vaccine passports’,

is a Lieutenant Colonel reservist in the 77th Brigade which connects with the military operation jHub, the 'innovation centre' for the Ministry of Defence and Strategic Command. jHub has also been involved with the civilian National Health Service (NHS) in 'symptom tracing' the population. The NHS is a key part of this mind control network and produced a document in December, 2020, explaining to staff how to use psychological manipulation with different groups and ages to get them to have the DNA-manipulating 'Covid vaccine' that's designed to cumulatively rewrite human genetics. The document, called 'Optimising Vaccination Roll Out – Do's and Dont's for all messaging, documents and "communications" in the widest sense', was published by NHS England and the NHS Improvement *Behaviour Change Unit* in partnership with Public Health England and Warwick Business School. I hear the mantra about 'save the NHS' and 'protect the NHS' when we need to scrap the NHS and start again. The current version is far too corrupt, far too anti-human and totally compromised by Cult operatives and their assets. UK government broadcast media censor Ofcom will connect into this web – as will the BBC with its tremendous Ofcom influence – to control what the public see and hear and dictate mass perception. Nuremberg trials must include personnel from all these organisations.

The fear factor

The 'Covid' hoax has led to the creation of the UK Cabinet Office-connected Joint Biosecurity Centre (JBC) which is officially described as providing 'expert advice on pandemics' using its independent [all Cult operations are 'independent'] analytical function to provide real-time analysis about infection outbreaks to identify and respond to outbreaks of Covid-19'. Another role is to advise the government on a response to spikes in infections – 'for example by closing schools or workplaces in local areas where infection levels have risen'. Put another way, promoting the Cult agenda. The Joint Biosecurity Centre is modelled on the Joint Terrorism Analysis Centre which analyses intelligence to set 'terrorism threat levels' and here again you see the fusion of civilian and military operations and intelligence that has led to military intelligence producing documents about 'vaccine hesitancy' and how it can be combated. Domestic civilian matters and opinions should not be the business of the military. The Joint

Biosecurity Centre is headed by Tom Hurd, director general of the Office for Security and Counter-Terrorism from the establishment-to-its-fingertips Hurd family. His father is former Foreign Secretary Douglas Hurd. How coincidental that Tom Hurd went to the elite Eton College and Oxford University with Boris Johnson. Imperial College with its ridiculous computer modeller Neil Ferguson will connect with this gigantic web that will itself interconnect with similar set-ups in other major and not so major countries. Compared with this Cult network the politicians, be they Boris Johnson, Donald Trump or Joe Biden, are bit-part players 'following the science'. The network of psychologists was on the 'Covid' case from the start with the aim of generating maximum fear of the 'virus' to ensure compliance by the population. A government behavioural science group known as SPI-B produced a paper in March, 2020, for discussion by the main government science advisory group known as SAGE. It was headed 'Options for increasing adherence to social distancing measures' and it said the following in a section headed 'Persuasion':

> • A substantial number of people still do not feel sufficiently personally threatened; it could be that they are reassured by the low death rate in their demographic group, although levels of concern may be rising. Having a good understanding of the risk has been found to be positively associated with adoption of COVID-19 social distancing measures in Hong Kong.
>
> • The perceived level of personal threat needs to be increased among those who are complacent, using hard-hitting evaluation of options for increasing social distancing emotional messaging. To be effective this must also empower people by making clear the actions they can take to reduce the threat.
>
> • Responsibility to others: There seems to be insufficient understanding of, or feelings of responsibility about, people's role in transmitting the infection to others … Messaging about actions need to be framed positively in terms of protecting oneself and the community, and increase confidence that they will be effective.
>
> • Some people will be more persuaded by appeals to play by the rules, some by duty to the community, and some to personal risk. All these

> different approaches are needed. The messaging also needs to take account of the realities of different people's lives. Messaging needs to take account of the different motivational levers and circumstances of different people.

All this could be achieved the SPI-B psychologists said by *using the media to increase the sense of personal threat* which translates as terrify the shit out of the population, including children, so they all do what we want. That's not happened has it? Those excuses for 'journalists' who wouldn't know journalism if it bit them on the arse (the great majority) have played their crucial part in serving this Cult-government Psyop to enslave their own kids and grandkids. How they live with themselves I have no idea. The psychological war has been underpinned by constant government 'Covid' propaganda in almost every television and radio ad break, plus the Internet and print media, which has pounded out the fear with taxpayers footing the bill for their own programming. The result has been people terrified of a 'virus' that doesn't exist or one with a tiny fatality rate even if you believe it does. People walk down the street and around the shops wearing face-nappies damaging their health and psychology while others report those who refuse to be that naïve to the police who turn up in their own face-nappies. I had a cameraman come to my flat and he was so frightened of 'Covid' he came in wearing a mask and refused to shake my hand in case he caught something. He had – naïveitis – and the thought that he worked in the mainstream media was both depressing and made his behaviour perfectly explainable. The fear which has gripped the minds of so many and frozen them into compliance has been carefully cultivated by these psychologists who are really psychopaths. If lives get destroyed and a lot of young people commit suicide it shows our plan is working. SPI-B then turned to compulsion on the public to comply. 'With adequate preparation, rapid change can be achieved', it said. Some countries had introduced mandatory self-isolation on a wide scale without evidence of major public unrest and a large majority of the UK's population appeared to be supportive of more coercive measures with 64 percent of adults saying they would support putting London under a lockdown (watch the 'polls' which are designed to make people believe that public opinion is in favour or against whatever the subject in hand).

For ‘aggressive protective measures’ to be effective, the SPI-B paper said, special attention should be devoted to those population groups that are more at risk. Translated from the Orwellian this means making the rest of population feel guilty for not protecting the ‘vulnerable’ such as old people which the Cult and its agencies were about to kill on an industrial scale with lockdown, lack of treatment and the Gates ‘vaccine’. Psychopath psychologists sold their guilt-trip so comprehensively that Los Angeles County Supervisor Hilda Solis reported that children were apologising (from a distance) to their parents and grandparents for bringing ‘Covid’ into their homes and getting them sick. ‘… These apologies are just some of the last words that loved ones will ever hear as they die alone,’ she said. Gut-wrenchingly Solis then used this childhood tragedy to tell children to stay at home and ‘keep your loved ones alive’. Imagine heaping such potentially life-long guilt on a kid when it has absolutely nothing to do with them. These people are deeply disturbed and the psychologists behind this even more so.

Uncivil war – divide and rule

Professional mind-controllers at SPI-B wanted the media to increase a sense of responsibility to others (do as you’re told) and promote ‘positive messaging’ for those actions while in contrast to invoke ‘social disapproval’ by the unquestioning, obedient, community of anyone with a mind of their own. Again the compliant Goebbels-like media obliged. This is an old, old, trick employed by tyrannies the world over throughout human history. You get the target population to keep the target population in line – *your* line. SPI-B said this could ‘play an important role in preventing anti-social behaviour or discouraging failure to enact pro-social behaviour’. For ‘anti-social’ in the Orwellian parlance of SPI-B see any behaviour that government doesn’t approve. SPI-B recommendations said that ‘social disapproval’ should be accompanied by clear messaging and promotion of strong collective identity – hence the government and celebrity mantra of ‘we’re all in this together’. Sure we are. The mind doctors have such contempt for their targets that they think some clueless comedian, actor or singer telling them to do what the government wants will be enough to win them over. We have had UK comedian Lenny Henry, actor Michael Caine and singer Elton John wheeled out to serve the propagandists by urging

people to have the DNA-manipulating ‘Covid’ non-’vaccine’. The role of Henry and fellow black celebrities in seeking to coax a ‘vaccine’ reluctant black community into doing the government’s will was especially stomach-turning. An emotion-manipulating script and carefully edited video featuring these black ‘celebs’ was such an insult to the intelligence of black people and where’s the self-respect of those involved selling their souls to a fascist government agenda? Henry said he heard black people’s ‘legitimate worries and concerns’, but people must ‘trust the facts’ when they were doing exactly that by not having the ‘vaccine’. They had to include the obligatory reference to Black Lives Matter with the line … ‘Don’t let coronavirus cost even more black lives – because we matter’. My god, it was pathetic. ‘I know the vaccine is safe and what it does.’ How? ‘I’m a comedian and it says so in my script.’

SPI-B said social disapproval needed to be carefully managed to avoid victimisation, scapegoating and misdirected criticism, but they knew that their ‘recommendations’ would lead to exactly that and the media were specifically used to stir-up the divide-and-conquer hostility. Those who conform like good little baa, baas, are praised while those who have seen through the tidal wave of lies are ‘Covidiots’. The awake have been abused by the fast asleep for not conforming to fascism and impositions that the awake know are designed to endanger their health, dehumanise them, and tear asunder the very fabric of human society. We have had the curtain-twitchers and morons reporting neighbours and others to the face-nappied police for breaking ‘Covid rules’ with fascist police delighting in posting links and phone numbers where this could be done. The Cult cannot impose its will without a compliant police and military or a compliant population willing to play their part in enslaving themselves and their kids. The words of a pastor in Nazi Germany are so appropriate today:

First they came for the socialists and I did not speak out because I was not a socialist.

Then they came for the trade unionists and I did not speak out because I was not a trade unionist.

Then they came for the Jews and I did not speak out because I was not a Jew.

Then they came for me and there was no one left to speak for me.

Those who don't learn from history are destined to repeat it and so many are.

'Covid' rules: Rewiring the mind

With the background laid out to this gigantic national and global web of psychological manipulation we can put 'Covid' rules into a clear and sinister perspective. Forget the claims about protecting health. 'Covid' rules are about dismantling the human mind, breaking the human spirit, destroying self-respect, and then putting Humpty Dumpty together again as a servile, submissive slave. Social isolation through lockdown and distancing have devastating effects on the human psyche as the psychological psychopaths well know and that's the real reason for them. Humans need contact with each other, discourse, closeness and touch, or they eventually, and literarily, go crazy. Masks, which I will address at some length, fundamentally add to the effects of isolation and the Cult agenda to dehumanise and de-individualise the population. To do this while knowing – in fact *seeking* – this outcome is the very epitome of evil and psychologists involved in this *are* the epitome of evil. They must like all the rest of the Cult demons and their assets stand trial for crimes against humanity on a scale that defies the imagination. Psychopaths in uniform use isolation to break enemy troops and agents and make them subservient and submissive to tell what they know. The technique is rightly considered a form of torture and torture is most certainly what has been imposed on the human population.

Clinically-insane American psychologist Harry Harlow became famous for his isolation experiments in the 1950s in which he separated baby monkeys from their mothers and imprisoned them for months on end in a metal container or 'pit of despair'. They soon began to show mental distress and depression as any idiot could have predicted. Harlow put other monkeys in steel chambers for three, six or twelve months while denying them any contact with animals or humans. He said that the effects of total social isolation for six months were 'so devastating and debilitating that we had assumed initially that twelve months of isolation would not produce any additional decrement'; but twelve months of isolation 'almost obliterated the animals socially'. This is what the Cult and its psychopaths are doing to you and your children. Even monkeys in partial isolation in

which they were not allowed to form relationships with other monkeys became ‘aggressive and hostile, not only to others, but also towards their own bodies’. We have seen this in the young as a consequence of lockdown. UK government psychopaths launched a public relations campaign telling people not to hug each other even after they received the ‘Covid-19 vaccine’ which we were told with more lies would allow a return to ‘normal life’. A government source told *The Telegraph*: ‘It will be along the lines that it is great that you have been vaccinated, but if you are going to visit your family and hug your grandchildren there is a chance you are going to infect people you love.’ The source was apparently speaking from a secure psychiatric facility. Janet Lord, director of Birmingham University’s Institute of Inflammation and Ageing, said that parents and grandparents should avoid hugging their children. Well, how can I put it, Ms Lord? Fuck off. Yep, that’ll do.

Destroying the kids – where are the parents?

Observe what has happened to people enslaved and isolated by lockdown as suicide and self-harm has soared worldwide, particularly among the young denied the freedom to associate with their friends. A study of 49,000 people in English-speaking countries concluded that almost half of young adults are at clinical risk of mental health disorders. A national survey in America of 1,000 currently enrolled high school and college students found that 5 percent reported attempting suicide during the pandemic. Data from the US CDC’s National Syndromic Surveillance Program from January 1st to October 17th, 2020, revealed a *31 percent* increase in mental health issues among adolescents aged 12 to 17 compared with 2019. The CDC reported that America in general suffered the biggest drop in life expectancy since World War Two as it fell by a year in the first half of 2020 as a result of ‘deaths of despair’ – overdoses and suicides. Deaths of despair have leapt by more than 20 percent during lockdown and include the highest number of fatal overdoses ever recorded in a single year – 81,000. Internet addiction is another consequence of being isolated at home which lowers interest in physical activities as kids fall into inertia and what’s the point? Children and young people are losing hope and giving up on life, sometimes literally. A 14-year-old boy killed himself in Maryland because he had ‘given up’ when his school district didn’t reopen; an 11-year-old boy shot himself

during a zoom class; a teenager in Maine succumbed to the isolation of the 'pandemic' when he ended his life after experiencing a disrupted senior year at school. Children as young as nine have taken their life and all these stories can be repeated around the world. Careers are being destroyed before they start and that includes those in sport in which promising youngsters have not been able to take part. The plan of the psycho-psychologists is working all right. Researchers at Cambridge University found that lockdowns cause significant harm to children's mental health. Their study was published in the *Archives of Disease in Childhood*, and followed 168 children aged between 7 and 11. The researchers concluded:

During the UK lockdown, children's depression symptoms have increased substantially, relative to before lockdown. The scale of this effect has direct relevance for the continuation of different elements of lockdown policy, such as complete or partial school closures …

… Specifically, we observed a statistically significant increase in ratings of depression, with a medium-to-large effect size. Our findings emphasise the need to incorporate the potential impact of lockdown on child mental health in planning the ongoing response to the global pandemic and the recovery from it.

Not a chance when the Cult's psycho-psychologists were getting exactly what they wanted. The UK's Royal College of Paediatrics and Child Health has urged parents to look for signs of eating disorders in children and young people after a three to four fold increase. Specialists say the 'pandemic' is a major reason behind the rise. You don't say. The College said isolation from friends during school closures, exam cancellations, loss of extra-curricular activities like sport, and an increased use of social media were all contributory factors along with fears about the virus (psycho-psychologists again), family finances, and students being forced to quarantine. Doctors said young people were becoming severely ill by the time they were seen with 'Covid' regulations reducing face-to-face consultations. Nor is it only the young that have been devastated by the psychopaths. Like all bullies and cowards the Cult is targeting the young, elderly, weak and infirm. A typical story was told by a British lady called Lynn Parker who was not allowed to visit her husband in 2020 for the last ten and half months of his life 'when he needed me most' between March 20th and when he died on December 19th. This vacates the criminal and enters the territory of evil. The emotional impact on the immune system alone is immense as are the

number of people of all ages worldwide who have died as a result of Cult-demanded, Gates-demanded, lockdowns.

Isolation is torture

The experience of imposing solitary confinement on millions of prisoners around the world has shown how a large percentage become 'actively psychotic and/or acutely suicidal'. Social isolation has been found to trigger 'a specific psychiatric syndrome, characterized by hallucinations; panic attacks; overt paranoia; diminished impulse control; hypersensitivity to external stimuli; and difficulties with thinking, concentration and memory'. Juan Mendez, a United Nations rapporteur (investigator), said that isolation is a form of torture. Research has shown that even after isolation prisoners find it far more difficult to make social connections and I remember chatting to a shop assistant after one lockdown who told me that when her young son met another child again he had no idea how to act or what to do. Hannah Flanagan, Director of Emergency Services at Journey Mental Health Center in Dane County, Wisconsin, said: 'The specificity about Covid social distancing and isolation that we've come across as contributing factors to the suicides are really new to us this year.' But they are not new to those that devised them. They are getting the effect they want as the population is psychologically dismantled to be rebuilt in a totally different way. Children and the young are particularly targeted. They will be the adults when the full-on fascist AI-controlled technocracy is planned to be imposed and they are being prepared to meekly submit. At the same time older people who still have a memory of what life was like before – and how fascist the new normal really is – are being deleted. You are going to see efforts to turn the young against the old to support this geriatric genocide. Hannah Flanagan said the big increase in suicide in her county proved that social isolation is not only harmful, but deadly. Studies have shown that isolation from others is one of the main risk factors in suicide and even more so with women. Warnings that lockdown could create a 'perfect storm' for suicide were ignored. After all this was one of the *reasons* for lockdown. Suicide, however, is only the most extreme of isolation consequences. There are many others. Dr Dhruv Khullar, assistant professor of healthcare policy at Weill Cornell Medical College, said in a *New York Times* article in 2016 long before the fake 'pandemic':

A wave of new research suggests social separation is bad for us. Individuals with less social connection have disrupted sleep patterns, altered immune systems, more inflammation and higher levels of stress hormones. One recent study found that isolation increases the risk of heart disease by 29 percent and stroke by 32 percent. Another analysis that pooled data from 70 studies and 3.4 million people found that socially isolated individuals had a 30 percent higher risk of dying in the next seven years, and that this effect was largest in middle age.

Loneliness can accelerate cognitive decline in older adults, and isolated individuals are twice as likely to die prematurely as those with more robust social interactions. These effects start early: Socially isolated children have significantly poorer health 20 years later, even after controlling for other factors. All told, loneliness is as important a risk factor for early death as obesity and smoking.

There you have proof from that one article alone four years before 2020 that those who have enforced lockdown, social distancing and isolation knew what the effect would be and that is even more so with professional psychologists that have been driving the policy across the globe. We can go back even further to the years 2000 and 2003 and the start of a major study on the effects of isolation on health by Dr Janine Gronewold and Professor Dirk M. Hermann at the University Hospital in Essen, Germany, who analysed data on 4,316 people with an average age of 59 who were recruited for the long-term research project. They found that socially isolated people are more than 40 percent more likely to have a heart attack, stroke, or other major cardiovascular event and nearly 50 percent more likely to die from any cause. Given the financial Armageddon unleashed by lockdown we should note that the study found a relationship between increased cardiovascular risk and lack of financial support. After excluding other factors social isolation was still connected to a 44 percent increased risk of cardiovascular problems and a 47 percent increased risk of death by any cause. Lack of financial support was associated with a 30 percent increase in the risk of cardiovascular health events. Dr Gronewold said it had been known for some time that feeling lonely or lacking contact with close friends and family can have an impact on physical health and the study had shown that having strong social relationships is of high importance for heart health. Gronewold said they didn't understand yet why people who are socially isolated have such poor health outcomes, but this was obviously a worrying finding, particularly during these times of prolonged social distancing. Well, it can be explained on many levels. You only have to identify the point in the body where people feel loneliness and missing people they are parted from – it's in the centre of the chest where

they feel the ache of loneliness and the ache of missing people. ‘My heart aches for you’ … ‘My heart aches for some company.’ I will explain this more in the chapter Escaping Wetiko, but when you realise that the body is the mind – they are expressions of each other – the reason why state of the mind dictates state of the body becomes clear.

American psychologist Ranjit Powar was highlighting the effects of lockdown isolation as early as April, 2020. She said humans have evolved to be social creatures and are wired to live in interactive groups. Being isolated from family, friends and colleagues could be unbalancing and traumatic for most people and could result in short or even long-term psychological and physical health problems. An increase in levels of anxiety, aggression, depression, forgetfulness and hallucinations were possible psychological effects of isolation. ‘Mental conditions may be precipitated for those with underlying pre-existing susceptibilities and show up in many others without any pre-condition.’ Powar said personal relationships helped us cope with stress and if we lost this outlet for letting off steam the result can be a big emotional void which, for an average person, was difficult to deal with. ‘Just a few days of isolation can cause increased levels of anxiety and depression’ – so what the hell has been the effect on the global population of *18 months* of this at the time of writing? Powar said: ‘Add to it the looming threat of a dreadful disease being repeatedly hammered in through the media and you have a recipe for many shades of mental and physical distress.’ For those with a house and a garden it is easy to forget that billions have had to endure lockdown isolation in tiny overcrowded flats and apartments with nowhere to go outside. The psychological and physical consequences of this are unimaginable and with lunatic and abusive partners and parents the consequences have led to tremendous increases in domestic and child abuse and alcoholism as people seek to shut out the horror. Ranjit Powar said:

Staying in a confined space with family is not all a rosy picture for everyone. It can be extremely oppressive and claustrophobic for large low-income families huddled together in small single-room houses. Children here are not lucky enough to have many board/electronic games or books to keep them occupied.

Add to it the deep insecurity of running out of funds for food and basic necessities. On the other hand, there are people with dysfunctional family dynamics, such as domineering, abusive or alcoholic partners, siblings or parents which makes staying home a period of trial. Incidence of

suicide and physical abuse against women has shown a worldwide increase. Heightened anxiety and depression also affect a person's immune system, making them more susceptible to illness.

To think that Powar's article was published on April 11th, 2020.

Six-feet fantasy

Social (unsocial) distancing demanded that people stay six feet or two metres apart. UK government advisor Robert Dingwall from the New and Emerging Respiratory Virus Threats Advisory Group said in a radio interview that the two-metre rule was 'conjured up out of nowhere' and was not based on science. No, it was not based on *medical* science, but it didn't come out of nowhere. The distance related to *psychological* science. Six feet/two metres was adopted in many countries and we were told by people like the criminal Anthony Fauci and his ilk that it was founded on science. Many schools could not reopen because they did not have the space for six-feet distancing. Then in March, 2021, after a year of six-feet 'science', a study published in the *Journal of Infectious Diseases* involving more than 500,000 students and almost 100,000 staff over 16 weeks revealed no significant difference in 'Covid' cases between six feet and three feet and Fauci changed his tune. Now three feet was okay. There is no difference between six feet and three *inches* when there is no 'virus' and they got away with six feet for psychological reasons for as long as they could. I hear journalists and others talk about 'unintended consequences' of lockdown. They are not *unintended* at all; they have been coldly-calculated for a specific outcome of human control and that's why super-psychopaths like Gates have called for them so vehemently. Super-psychopath psychologists have demanded them and psychopathic or clueless, spineless, politicians have gone along with them by 'following the science'. But it's not science at all. 'Science' is not what is; it's only what people can be manipulated to believe it is. The whole 'Covid' catastrophe is founded on mind control. Three word or three statement mantras issued by the UK government are a well-known mind control technique and so we've had 'Stay home/protect the NHS/save lives', 'Stay alert/control the virus/save lives' and 'hands/face/space'. One of the most vocal proponents of extreme 'Covid' rules in the UK has been Professor Susan Michie, a member of the British Communist Party, who is not a medical professional. Michie is the

director of the Centre for Behaviour Change at University College London. She is a *behavioural psychologist* and another filthy rich 'Marxist' who praised China's draconian lockdown. She was known by fellow students at Oxford University as 'Stalin's nanny' for her extreme Marxism. Michie is an influential member of the UK government's Scientific Advisory Group for Emergencies (SAGE) and behavioural manipulation groups which have dominated 'Covid' policy. She is a consultant adviser to the World Health Organization on 'Covid-19' and behaviour. Why the hell are lockdowns anything to do with her when they are claimed to be about health? Why does a behavioural psychologist from a group charged with changing the behaviour of the public want lockdown, human isolation and mandatory masks? Does that question really need an answer? Michie *absolutely* has to explain herself before a Nuremberg court when humanity takes back its world again and even more so when you see the consequences of masks that she demands are compulsory. This is a Michie classic:

> The benefits of getting primary school children to wear masks is that regardless of what little degree of transmission is occurring in those age groups it could help normalise the practice. Young children wearing masks may be more likely to get their families to accept masks.

Those words alone should carry a prison sentence when you ponder on the callous disregard for children involved and what a statement it makes about the mind and motivations of Susan Michie. What a lovely lady and what she said there encapsulates the mentality of the psychopaths behind the 'Covid' horror. Let us compare what Michie said with a countrywide study in Germany published at researchsquare.com involving 25,000 school children and 17,854 health complaints submitted by parents. Researchers found that masks are harming children physically, psychologically, and behaviourally with 24 health issues associated with mask wearing. They include: shortness of breath (29.7%); dizziness (26.4%); increased headaches (53%); difficulty concentrating (50%); drowsiness or fatigue (37%); and malaise (42%). Nearly a third of children experienced more sleep issues than before and a quarter developed new fears. Researchers found health issues and other impairments in 68 percent of masked children covering their faces for an average of 4.5 hours a day. Hundreds of those taking part experienced accelerated respiration, tightness in the chest,

weakness, and short-term impairment of consciousness. A reminder of what Michie said again:

The benefits of getting primary school children to wear masks is that regardless of what little degree of transmission is occurring in those age groups it could help normalise the practice. Young children wearing masks may be more likely to get their families to accept masks.

Psychopaths in government and psychology now have children and young people – plus all the adults – wearing masks for hours on end while clueless teachers impose the will of the psychopaths on the young they should be protecting. What the hell are parents doing?

Cult lab rats

We have some schools already imposing on students microchipped buzzers that activate when they get 'too close' to their pals in the way they do with lab rats. How apt. To the Cult and its brain-dead servants our children *are* lab rats being conditioned to be unquestioning, dehumanised slaves for the rest of their lives. Children and young people are being weaned and frightened away from the most natural human instincts including closeness and touch. I have tracked in the books over the years how schools were banning pupils from greeting each other with a hug and the whole Cult-induced Me Too movement has terrified men and boys from a relaxed and natural interaction with female friends and work colleagues to the point where many men try never to be in a room alone with a woman that's not their partner. Airhead celebrities have as always played their virtue-signalling part in making this happen with their gross exaggeration. For every monster like Harvey Weinstein there are at least tens of thousands of men that don't treat women like that; but everyone must be branded the same and policy changed for them as well as the monster. I am going to be using the word 'dehumanise' many times in this chapter because that is what the Cult is seeking to do and it goes very deep as we shall see. Don't let them kid you that social distancing is planned to end one day. That's not the idea. We are seeing more governments and companies funding and producing wearable gadgets to keep people apart and they would not be doing that if this was meant to be short-term. A tech start-up company

backed by GCHQ, the British Intelligence and military surveillance headquarters, has created a social distancing wrist sensor that alerts people when they get too close to others. The CIA has also supported tech companies developing similar devices. The wearable sensor was developed by Tended, one of a number of start-up companies supported by GCHQ (see the CIA and DARPA). The device can be worn on the wrist or as a tag on the waistband and will vibrate whenever someone wearing the device breaches social distancing and gets anywhere near natural human contact. The company had a lucky break in that it was developing a distancing sensor when the 'Covid' hoax arrived which immediately provided a potentially enormous market. How fortunate. The government in big-time Cult-controlled Ontario in Canada is investing $2.5 million in wearable contact tracing technology that 'will alert users if they may have been exposed to the Covid-19 in the workplace and will beep or vibrate if they are within six feet of another person'. Facedrive Inc., the technology company behind this, was founded in 2016 with funding from the Ontario Together Fund and obviously they, too, had a prophet on the board of directors. The human surveillance and control technology is called TraceSCAN and would be worn by the human cyborgs in places such as airports, workplaces, construction sites, care homes and … *schools*.

I emphasise schools with children and young people the prime targets. You know what is planned for society as a whole if you keep your eyes on the schools. They have always been places where the state program the next generation of slaves to be its compliant worker-ants – or Woker-ants these days; but in the mist of the 'Covid' madness they have been transformed into mind laboratories on a scale never seen before. Teachers and head teachers are just as programmed as the kids – often more so. Children are kept apart from human interaction by walk lanes, classroom distancing, staggered meal times, masks, and the rolling-out of buzzer systems. Schools are now physically laid out as a laboratory maze for lab-rats. Lunatics at a school in Anchorage, Alaska, who should be prosecuted for child abuse, took away desks and forced children to kneel (know your place) on a mat for five hours a day while wearing a mask and using their chairs as a desk. How this was supposed to impact on a 'virus' only these clinically insane people can tell you and even then it would be clap-trap. The school banned recess (interaction), art classes (creativity), and physical exercise (getting body and mind moving out of inertia). Everyone behind this outrage should

be in jail or better still a mental institution. The behavioural manipulators are all for this dystopian approach to schools. Professor Susan Michie, the mind-doctor and British Communist Party member, said it was wrong to say that schools were safe. They had to be made so by 'distancing', masks and ventilation (sitting all day in the cold). I must ask this lady round for dinner on a night I know I am going to be out and not back for weeks. She probably wouldn't be able to make it, anyway, with all the visits to her own psychologist she must have block-booked.

Masking identity

I know how shocking it must be for you that a behaviour manipulator like Michie wants everyone to wear masks which have long been a feature of mind-control programs like the infamous MKUltra in the United States, but, there we are. We live and learn. I spent many years from 1996 to right across the millennium researching mind control in detail on both sides of the Atlantic and elsewhere. I met a large number of mind-control survivors and many had been held captive in body and mind by MKUltra. MK stands for mind-control, but employs the German spelling in deference to the Nazis spirited out of Germany at the end of World War Two by Operation Paperclip in which the US authorities, with help from the Vatican, transported Nazi mind-controllers and engineers to America to continue their work. Many of them were behind the creation of NASA and they included Nazi scientist and SS officer Wernher von Braun who swapped designing V-2 rockets to bombard London with designing the Saturn V rockets that powered the NASA moon programme's Apollo craft. I think I may have mentioned that the Cult has no borders. Among Paperclip escapees was Josef Mengele, the Angel of Death in the Nazi concentration camps where he conducted mind and genetic experiments on children often using twins to provide a control twin to measure the impact of his 'work' on the other. If you want to observe the Cult mentality in all its extremes of evil then look into the life of Mengele. I have met many people who suffered mercilessly under Mengele in the United States where he operated under the name Dr Greene and became a stalwart of MKUltra programming and torture. Among his locations was the underground facility in the Mojave Desert in California called the China Lake Naval Weapons Station which is almost entirely below the surface. My books *The Biggest Secret,*

Children of the Matrix and *The Perception Deception* have the detailed background to MKUltra.

The best-known MKUltra survivor is American Cathy O'Brien. I first met her and her late partner Mark Phillips at a conference in Colorado in 1996. Mark helped her escape and deprogram from decades of captivity in an offshoot of MKUltra known as Project Monarch in which 'sex slaves' were provided for the rich and famous including Father George Bush, Dick Cheney and the Clintons. Read Cathy and Mark's book *Trance-Formation of America* and if you are new to this you will be shocked to the core. I read it in 1996 shortly before, with the usual synchronicity of my life, I found myself given a book table at the conference right next to hers. MKUltra never ended despite being very publicly exposed (only a small part of it) in the 1970s and continues in other guises. I am still in touch with Cathy. She contacted me during 2020 after masks became compulsory in many countries to tell me how they were used as part of MKUltra programming. I had been observing 'Covid regulations' and the relationship between authority and public for months. I saw techniques that I knew were employed on individuals in MKUltra being used on the global population. I had read many books and manuals on mind control including one called *Silent Weapons for Quiet Wars* which came to light in the 1980s and was a guide on how to perceptually program on a mass scale. 'Silent Weapons' refers to mind-control. I remembered a line from the manual as governments, medical authorities and law enforcement agencies have so obviously talked to – or rather at – the adult population since the 'Covid' hoax began as if they are children. The document said:

> If a person is spoken to by a T.V. advertiser as if he were a twelve-year-old, then, due to suggestibility, he will, with a certain probability, respond or react to that suggestion with the uncritical response of a twelve-year-old and will reach in to his economic reservoir and deliver its energy to buy that product on impulse when he passes it in the store.

That's why authority has spoken to adults like children since all this began.

Why did Michael Jackson wear masks?

Every aspect of the 'Covid' narrative has mind-control as its central theme. Cathy O'Brien wrote an article for davidicke.com about the connection between masks and mind control. Her daughter Kelly who I first met in the 1990s was born while Cathy was still held captive in MKUltra. Kelly was forced to wear a mask as part of her programming from the age of *two* to dehumanise her, target her sense of individuality and reduce the amount of oxygen her brain and body received. *Bingo*. This is the real reason for compulsory masks, why they have been enforced en masse, and why they seek to increase the number they demand you wear. First one, then two, with one disgraceful alleged 'doctor' recommending four which is nothing less than a death sentence. Where and how often they must be worn is being expanded for the purpose of mass mind control and damaging respiratory health which they can call 'Covid-19'. Canada's government headed by the man-child Justin Trudeau, says it's fine for children of two and older to wear masks. An insane 'study' in Italy involving just 47 children concluded there was no problem for babies as young as *four months* wearing them. Even after people were 'vaccinated' they were still told to wear masks by the criminal that is Anthony Fauci. Cathy wrote that mandating masks is allowing the authorities literally to control the air we breathe which is what was done in MKUltra. You might recall how the singer Michael Jackson wore masks and there is a reason for that. He was subjected to MKUltra mind control through Project Monarch and his psyche was scrambled by these simpletons. Cathy wrote:

In MKUltra Project Monarch mind control, Michael Jackson had to wear a mask to silence his voice so he could not reach out for help. Remember how he developed that whisper voice when he wasn't singing? Masks control the mind from the outside in, like the redefining of words is doing. By controlling what we can and cannot say for fear of being labeled racist or beaten, for example, it ultimately controls thought that drives our words and ultimately actions (or lack thereof).

Likewise, a mask muffles our speech so that we are not heard, which controls voice … words … mind. This is Mind Control. Masks are an obvious mind control device, and I am disturbed so many people are complying on a global scale. Masks depersonalize while making a person feel as though they have no voice. It is a barrier to others. People who would never choose to comply but are forced to wear a mask in order to keep their job, and ultimately their family fed, are compromised. They often feel shame and are subdued. People have stopped talking with each other while media controls the narrative.

The ‘no voice’ theme has often become literal with train passengers told not to speak to each other in case they pass on the ‘virus’, singing banned for the same reason and bonkers California officials telling people riding roller coasters that they cannot shout and scream. Cathy said she heard every day from healed MKUltra survivors who cannot wear a mask without flashing back on ways their breathing was controlled – ‘from ball gags and penises to water boarding’. She said that through the years when she saw images of people in China wearing masks ‘due to pollution’ that it was really to control their oxygen levels. ‘I knew it was as much of a population control mechanism of depersonalisation as are burkas’, she said. Masks are another Chinese communist/fascist method of control that has been swept across the West as the West becomes China at lightning speed since we entered 2020.

Mask-19

There are other reasons for mandatory masks and these include destroying respiratory health to call it ‘Covid-19’ and stunting brain development of children and the young. Dr Margarite Griesz-Brisson MD, PhD, is a Consultant Neurologist and Neurophysiologist and the Founder and Medical Director of the London Neurology and Pain Clinic. Her CV goes down the street and round the corner. She is clearly someone who cares about people and won’t parrot the propaganda. Griesz-Brisson has a PhD in pharmacology, with special interest in neurotoxicology, environmental medicine, neuroregeneration and neuroplasticity (the way the brain can change in the light of information received). She went public in October, 2020, with a passionate warning about the effects of mask-wearing laws:

> The reinhalation of our exhaled air will without a doubt create oxygen deficiency and a flooding of carbon dioxide. We know that the human brain is very sensitive to oxygen deprivation. There are nerve cells for example in the hippocampus that can’t be longer than 3 minutes without oxygen – they cannot survive. The acute warning symptoms are headaches, drowsiness, dizziness, issues in concentration, slowing down of reaction time – reactions of the cognitive system.

Oh, I know, let’s tell bus, truck and taxi drivers to wear them and people working machinery. How about pilots, doctors and police? Griesz-Brisson makes the important point that while the symptoms she mentions may fade

as the body readjusts this does not alter the fact that people continue to operate in oxygen deficit with long list of potential consequences. She said it was well known that neurodegenerative diseases take years or decades to develop. ‘If today you forget your phone number, the breakdown in your brain would have already started 20 or 30 years ago.’ She said degenerative processes in your brain are getting amplified as your oxygen deprivation continues through wearing a mask. Nerve cells in the brain are unable to divide themselves normally in these circumstances and lost nerve cells will no longer be regenerated. ‘What is gone is gone.’ Now consider that people like shop workers and *schoolchildren* are wearing masks for hours every day. What in the name of sanity is going to be happening to them? ‘I do not wear a mask, I need my brain to think’, Griesz-Brisson said, ‘I want to have a clear head when I deal with my patients and not be in a carbon dioxide-induced anaesthesia’. If you are told to wear a mask anywhere ask the organisation, police, store, whatever, for their risk assessment on the dangers and negative effects on mind and body of enforcing mask-wearing. They won’t have one because it has never been done not even by government. All of them must be subject to class-action lawsuits as the consequences come to light. They don’t do mask risk assessments for an obvious reason. They know what the conclusions would be and independent scientific studies that *have* been done tell a horror story of consequences.

‘Masks are criminal’

Dr Griesz-Brisson said that for children and adolescents, masks are an absolute no-no. They had an extremely active and adaptive immune system and their brain was incredibly active with so much to learn. ‘The child’s brain, or the youth’s brain, is thirsting for oxygen.’ The more metabolically active an organ was, the more oxygen it required; and in children and adolescents every organ was metabolically active. Griesz-Brisson said that to deprive a child’s or adolescent’s brain of oxygen, or to restrict it in any way, was not only dangerous to their health, it was absolutely criminal. ‘Oxygen deficiency inhibits the development of the brain, and the damage that has taken place as a result CANNOT be reversed.’ Mind manipulators of MKUltra put masks on two-year-olds they wanted to neurologically rewire and you can see why. Griesz-Brisson said a child needs the brain to learn and the brain needs oxygen to function. ‘We don’t need a clinical

study for that. This is simple, indisputable physiology.' Consciously and purposely induced oxygen deficiency was an absolutely deliberate health hazard, and an absolute medical contraindication which means that 'this drug, this therapy, this method or measure should not be used, and is not allowed to be used'. To coerce an entire population to use an absolute medical contraindication by force, she said, there had to be definite and serious reasons and the reasons must be presented to competent interdisciplinary and independent bodies to be verified and authorised. She had this warning of the consequences that were coming if mask wearing continued:

When, in ten years, dementia is going to increase exponentially, and the younger generations couldn't reach their god-given potential, it won't help to say 'we didn't need the masks'. I know how damaging oxygen deprivation is for the brain, cardiologists know how damaging it is for the heart, pulmonologists know how damaging it is for the lungs. Oxygen deprivation damages every single organ. Where are our health departments, our health insurance, our medical associations? It would have been their duty to be vehemently against the lockdown and to stop it and stop it from the very beginning.

Why do the medical boards issue punishments to doctors who give people exemptions? Does the person or the doctor seriously have to prove that oxygen deprivation harms people? What kind of medicine are our doctors and medical associations representing? Who is responsible for this crime? The ones who want to enforce it? The ones who let it happen and play along, or the ones who don't prevent it?

All of the organisations and people she mentions there either answer directly to the Cult or do whatever hierarchical levels above them tell them to do. The outcome of both is the same. 'It's not about masks, it's not about viruses, it's certainly not about your health', Griesz-Brisson said. 'It is about much, much more. I am not participating. I am not afraid.' They were taking our air to breathe and there was no unfounded medical exemption from face masks. Oxygen deprivation was dangerous for every single brain. It had to be the free decision of every human being whether they want to wear a mask that was absolutely ineffective to protect themselves from a virus. She ended by rightly identifying where the responsibility lies for all this:

The imperative of the hour is personal responsibility. We are responsible for what we think, not the media. We are responsible for what we do, not our superiors. We are responsible for our health, not

the World Health Organization. And we are responsible for what happens in our country, not the government.

Halle-bloody-lujah.

But surgeons wear masks, right?

Independent studies of mask-wearing have produced a long list of reports detailing mental, emotional and physical dangers. What a definition of insanity to see police officers imposing mask-wearing on the public which will cumulatively damage their health while the police themselves wear masks that will cumulatively damage *their* health. It's utter madness and both public and police do this because 'the government says so' – yes a government of brain-donor idiots like UK Health Secretary Matt Hancock reading the 'follow the science' scripts of psychopathic, lunatic psychologists. The response you get from Stockholm syndrome sufferers defending the very authorities that are destroying them and their families is that 'surgeons wear masks'. This is considered the game, set and match that they must work and don't cause oxygen deficit. Well, actually, scientific studies have shown that they *do* and oxygen levels are monitored in operating theatres to compensate. Surgeons wear masks to stop spittle and such like dropping into open wounds – not to stop 'viral particles' which are so miniscule they can only be seen through an electron microscope. Holes in the masks are significantly bigger than 'viral particles' and if you sneeze or cough they will breach the mask. I watched an incredibly disingenuous 'experiment' that claimed to prove that masks work in catching 'virus' material from the mouth and nose. They did this with a slow motion camera and the mask did block big stuff which stayed inside the mask and against the face to be breathed in or cause infections on the face as we have seen with many children. 'Viral particles', however, would never have been picked up by the camera as they came through the mask when they are far too small to be seen. The 'experiment' was therefore disingenuous *and* useless.

Studies have concluded that wearing masks in operating theatres (and thus elsewhere) make no difference to preventing infection while the opposite is true with toxic shite building up in the mask and this had led to an explosion in tooth decay and gum disease dubbed by dentists 'mask

mouth'. You might have seen the Internet video of a furious American doctor urging people to take off their masks after a four-year-old patient had been rushed to hospital the night before and nearly died with a lung infection that doctors sourced to mask wearing. A study in the journal *Cancer Discovery* found that inhalation of harmful microbes can contribute to advanced stage lung cancer in adults and long-term use of masks can help breed dangerous pathogens. Microbiologists have said frequent mask wearing creates a moist environment in which microbes can grow and proliferate before entering the lungs. The Canadian Agency for Drugs and Technologies in Health, or CADTH, a Canadian national organisation that provides research and analysis to healthcare decision-makers, said this as long ago as 2013 in a report entitled 'Use of Surgical Masks in the Operating Room: A Review of the Clinical Effectiveness and Guidelines'. It said:

> • No evidence was found to support the use of surgical face masks to reduce the frequency of surgical site infections
> • No evidence was found on the effectiveness of wearing surgical face masks to protect staff from infectious material in the operating room.
> • Guidelines recommend the use of surgical face masks by staff in the operating room to protect both operating room staff and patients (despite the lack of evidence).

We were told that the world could go back to 'normal' with the arrival of the 'vaccines'. When they came, fraudulent as they are, the story changed as I knew that it would. We are in the midst of transforming 'normal', not going back to it. Mary Ramsay, head of immunisation at Public Health England, echoed the words of US criminal Anthony Fauci who said masks and other regulations must stay no matter if people are vaccinated. The Fauci idiot continued to wear two masks – different colours so both could be clearly seen – after he *claimed* to have been vaccinated. Senator Rand Paul told Fauci in one exchange that his double-masks were 'theatre' and he was right. It's all theatre. Mary Ramsay back-tracked on the vaccine-return-to-normal theme when she said the public may need to wear masks and social-distance for years despite the jabs. 'People have got used to those lower-level restrictions now, and [they] can live with them', she said telling us what the idea has been all along. 'The vaccine does not give you a pass,

even if you have had it, you must continue to follow all the guidelines' said a Public Health England statement which reneged on what we had been told before and made having the 'vaccine' irrelevant to 'normality' even by the official story. Spain's fascist government trumped everyone by passing a law mandating the wearing of masks on the beach and even when swimming in the sea. The move would have devastated what's left of the Spanish tourist industry, posed potential breathing dangers to swimmers and had Northern European sunbathers walking around with their forehead brown and the rest of their face white as a sheet. The ruling was so crazy that it had to be retracted after pressure from public and tourist industry, but it confirmed where the Cult wants to go with masks and how clinically insane authority has become. The determination to make masks permanent and hide the serious dangers to body and mind can be seen in the censorship of scientist Professor Denis Rancourt by Bill Gates-funded academic publishing website ResearchGate over his papers exposing the dangers and uselessness of masks. Rancourt said:

ResearchGate today has permanently locked my account, which I have had since 2015. Their reasons graphically show the nature of their attack against democracy, and their corruption of science … By their obscene non-logic, a scientific review of science articles reporting on harms caused by face masks has a 'potential to cause harm'. No criticism of the psychological device (face masks) is tolerated, if the said criticism shows potential to influence public policy.

This is what happens in a fascist world.

Where are the 'greens' (again)?

Other dangers of wearing masks especially regularly relate to the inhalation of minute plastic fibres into the lungs and the deluge of discarded masks in the environment and oceans. Estimates predicted that more than 1.5 billion disposable masks will end up in the world's oceans every year polluting the water with tons of plastic and endangering marine wildlife. Studies project that humans are using 129 billion face masks each month worldwide – about three million a minute. Most are disposable and made from plastic, non-biodegradable microfibers that break down into smaller plastic particles that become widespread in ecosystems. They are littering cities, clogging sewage channels and turning up in bodies of water. I have written

in other books about the immense amounts of microplastics from endless sources now being absorbed into the body. Rolf Halden, director of the Arizona State University (ASU) Biodesign Center for Environmental Health Engineering, was the senior researcher in a 2020 study that analysed 47 human tissue samples and found microplastics in all of them. 'We have detected these chemicals of plastics in every single organ that we have investigated', he said. I wrote in *The Answer* about the world being deluged with microplastics. A study by the Worldwide Fund for Nature (WWF) found that people are consuming on average every week some 2,000 tiny pieces of plastic mostly through water and also through marine life and the air. Every year humans are ingesting enough microplastics to fill a heaped dinner plate and in a life-time of 79 years it is enough to fill two large waste bins. Marco Lambertini, WWF International director general said: 'Not only are plastics polluting our oceans and waterways and killing marine life – it's in all of us and we can't escape consuming plastics,' American geologists found tiny plastic fibres, beads and shards in rainwater samples collected from the remote slopes of the Rocky Mountain National Park near Denver, Colorado. Their report was headed: 'It is raining plastic.' Rachel Adams, senior lecturer in Biomedical Science at Cardiff Metropolitan University, said that among health consequences are internal inflammation and immune responses to a 'foreign body'. She further pointed out that microplastics become carriers of toxins including mercury, pesticides and dioxins (a known cause of cancer and reproductive and developmental problems). These toxins accumulate in the fatty tissues once they enter the body through microplastics. Now this is being compounded massively by people putting plastic on their face and throwing it away.

Workers exposed to polypropylene plastic fibres known as 'flock' have developed 'flock worker's lung' from inhaling small pieces of the flock fibres which can damage lung tissue, reduce breathing capacity and exacerbate other respiratory problems. *Now* … commonly used surgical masks have three layers of melt-blown textiles made of … polypropylene. We have billions of people putting these microplastics against their mouth, nose and face for hours at a time day after day in the form of masks. How does anyone think that will work out? I mean – what could possibly go wrong? We posted a number of scientific studies on this at davidicke.com, but when I went back to them as I was writing this book the links to the science research website where they were hosted were dead. Anything that

challenges the official narrative in any way is either censored or vilified. The official narrative is so unsupportable by the evidence that only deleting the truth can protect it. A study by Chinese scientists still survived – with the usual twist which it why it was still active, I guess. Yes, they found that virtually all the masks they tested increased the daily intake of microplastic fibres, but people should still wear them because the danger from the 'virus' was worse said the crazy 'team' from the Institute of Hydrobiology in Wuhan. Scientists first discovered microplastics in lung tissue of some patients who died of lung cancer in the 1990s. Subsequent studies have confirmed the potential health damage with the plastic degrading slowly and remaining in the lungs to accumulate in volume. Wuhan researchers used a machine simulating human breathing to establish that masks shed up to nearly 4,000 microplastic fibres in a month with reused masks producing more. Scientists said some masks are laced with toxic chemicals and a variety of compounds seriously restricted for both health and environmental reasons. They include cobalt (used in blue dye) and formaldehyde known to cause watery eyes, burning sensations in the eyes, nose, and throat, plus coughing, wheezing and nausea. No – that must be 'Covid-19'.

Mask 'worms'

There is another and potentially even more sinister content of masks. Mostly new masks of different makes filmed under a microscope around the world have been found to contain strange black fibres or 'worms' that appear to move or 'crawl' by themselves and react to heat and water. The nearest I have seen to them are the self-replicating fibres that are pulled out through the skin of those suffering from Morgellons disease which has been connected to the phenomena of 'chemtrails' which I will bring into the story later on. Morgellons fibres continue to grow outside the body and have a form of artificial intelligence. Black 'worm' fibres in masks have that kind of feel to them and there is a nanotechnology technique called 'worm micelles' which carry and release drugs or anything else you want to deliver to the body. For sure the suppression of humanity by mind altering drugs is the Cult agenda big time and the more excuses they can find to gain access to the body the more opportunities there are to make that happen whether through 'vaccines' or masks pushed against the mouth and nose for hours on end.

So let us summarise the pros and cons of masks:

Against masks: Breathing in your own carbon dioxide; depriving the body and brain of sufficient oxygen; build-up of toxins in the mask that can be breathed into the lungs and cause rashes on the face and 'mask-mouth'; breathing microplastic fibres and toxic chemicals into the lungs; dehumanisation and deleting individualisation by literally making people faceless; destroying human emotional interaction through facial expression and deleting parental connection with their babies which look for guidance to their facial expression.

For masks: They don't protect you from a 'virus' that doesn't exist and even if it did 'viral' particles are so minute they are smaller than the holes in the mask.

Governments, police, supermarkets, businesses, transport companies, and all the rest who seek to impose masks have done no risk assessment on their consequences for health and psychology and are now open to group lawsuits when the impact becomes clear with a cumulative epidemic of respiratory and other disease. Authorities will try to exploit these effects and hide the real cause by dubbing them 'Covid-19'. Can you imagine setting out to force the population to wear health-destroying masks without doing any assessment of the risks? It is criminal and it is evil, but then how many people targeted in this way, who see their children told to wear them all day at school, have asked for a risk assessment? Billions can't be imposed upon by the few unless the billions allow it. Oh, yes, with just a tinge of irony, 85 percent of all masks made worldwide come from *China*.

Wash your hands in toxic shite

'Covid' rules include the use of toxic sanitisers and again the health consequences of constantly applying toxins to be absorbed through the skin is obvious to any level of Renegade Mind. America's Food and Drug Administration (FDA) said that sanitisers are drugs and issued a warning about 75 dangerous brands which contain methanol used in antifreeze and

can cause death, kidney damage and blindness. The FDA circulated the following warning even for those brands that it claims to be safe:

Store hand sanitizer out of the reach of pets and children, and children should use it only with adult supervision. Do not drink hand sanitizer. This is particularly important for young children, especially toddlers, who may be attracted by the pleasant smell or brightly colored bottles of hand sanitizer.

Drinking even a small amount of hand sanitizer can cause alcohol poisoning in children. (However, there is no need to be concerned if your children eat with or lick their hands after using hand sanitizer.) During this coronavirus pandemic, poison control centers have had an increase in calls about accidental ingestion of hand sanitizer, so it is important that adults monitor young children's use.

Do not allow pets to swallow hand sanitizer. If you think your pet has eaten something potentially dangerous, call your veterinarian or a pet poison control center right away. Hand sanitizer is flammable and should be stored away from heat and flames. When using hand sanitizer, rub your hands until they feel completely dry before performing activities that may involve heat, sparks, static electricity, or open flames.

There you go, perfectly safe, then, and that's without even a mention of the toxins absorbed through the skin. Come on kids – sanitise your hands everywhere you go. It will save you from the 'virus'. Put all these elements together of the 'Covid' normal and see how much health and psychology is being cumulatively damaged, even devastated, to 'protect your health'. Makes sense, right? They are only imposing these things because they care, right? *Right?*

Submitting to insanity

Psychological reframing of the population goes very deep and is done in many less obvious ways. I hear people say how contradictory and crazy 'Covid' rules are and how they are ever changing. This is explained away by dismissing those involved as idiots. It is a big mistake. The Cult is delighted if its cold calculation is perceived as incompetence and idiocy when it is anything but. Oh, yes, there are idiots within the system – lots of them – but they are *administering* the Cult agenda, mostly unknowingly. They are not deciding and dictating it. The bulwark against tyranny is self-respect, always has been, always will be. It is self-respect that has broken every tyranny in history. By its very nature self-respect will not bow to oppression and its perpetrators. There is so little self-respect that it's always

the few that overturn dictators. Many may eventually follow, but the few with the iron spines (self-respect) kick it off and generate the momentum. The Cult targets self-respect in the knowledge that once this has gone only submission remains. Crazy, contradictory, ever-changing 'Covid' rules are systematically applied by psychologists to delete self-respect. They *want* you to see that the rules make no sense. It is one thing to decide to do something when *you* have made the choice based on evidence and logic. You still retain your self-respect. It is quite another when you can see what you are being told to do is insane, ridiculous and makes no sense, and *yet you still do it*. Your self-respect is extinguished and this has been happening as ever more obviously stupid and nonsensical things have been demanded and the great majority have complied even when they can see they are stupid and nonsensical.

People walk around in face-nappies knowing they are damaging their health and make no difference to a 'virus'. They do it in fear of not doing it. I know it's daft, but I'll do it anyway. When that happens something dies inside of you and submissive reframing has begun. Next there's a need to hide from yourself that you have conceded your self-respect and you convince yourself that you have not really submitted to fear and intimidation. You begin to believe that you are complying with craziness because it's the right thing to do. When first you concede your self-respect of 2+2 = 4 to 2+2 = 5 you *know* you are compromising your self-respect. Gradually to avoid facing that fact you begin to *believe* that 2+2=5. You have been reframed and I have been watching this process happening in the human psyche on an industrial scale. The Cult is working to break your spirit and one of its major tools in that war is humiliation. I read how former American soldier Bradley Manning (later Chelsea Manning after a sex-change) was treated after being jailed for supplying WikiLeaks with documents exposing the enormity of government and elite mendacity. Manning was isolated in solitary confinement for eight months, put under 24-hour surveillance, forced to hand over clothing before going to bed, and stand naked for every roll call. This is systematic humiliation. The introduction of anal swab 'Covid' tests in China has been done for the same reason to delete self-respect and induce compliant submission. Anal swabs are mandatory for incoming passengers in parts of China and American diplomats have said they were forced to undergo the indignity which would

have been calculated humiliation by the Cult-owned Chinese government that has America in its sights.

Government-people: An abusive relationship

Spirit-breaking psychological techniques include giving people hope and apparent respite from tyranny only to take it away again. This happened in the UK during Christmas, 2020, when the psycho-psychologists and their political lackeys announced an easing of restrictions over the holiday only to reimpose them almost immediately on the basis of yet another lie. There is a big psychological difference between getting used to oppression and being given hope of relief only to have that dashed. Psychologists know this and we have seen the technique used repeatedly. Then there is traumatising people before you introduce more extreme regulations that require compliance. A perfect case was the announcement by the dark and sinister Whitty and Vallance in the UK that 'new data' predicted that 4,000 could die every day over the winter of 2020/2021 if we did not lockdown again. I think they call it lying and after traumatising people with that claim out came Jackboot Johnson the next day with new curbs on human freedom. Psychologists know that a frightened and traumatised mind becomes suggestable to submission and behaviour reframing. Underpinning all this has been to make people fearful and suspicious of each other and see themselves as a potential danger to others. In league with deleted self-respect you have the perfect psychological recipe for self-loathing. The relationship between authority and public is now demonstrably the same as that of subservience to an abusive partner. These are signs of an abusive relationship explained by psychologist Leslie Becker-Phelps:

Psychological and emotional abuse: Undermining a partner's self-worth with verbal attacks, name-calling, and belittling. Humiliating the partner in public, unjustly accusing them of having an affair, or interrogating them about their every behavior. Keeping partner confused or off balance by saying they were just kidding or blaming the partner for 'making' them act this way … Feigning in public that they care while turning against them in private. This leads to victims frequently feeling confused, incompetent, unworthy, hopeless, and chronically self-doubting.

[Apply these techniques to how governments have treated the population since New Year, 2020, and the parallels are obvious.]

Physical abuse: The abuser might physically harm their partner in a range of ways, such as grabbing, hitting, punching, or shoving them. They might throw objects at them or harm them with a weapon. [Observe the physical harm imposed by masks, lockdown, and so on.]

Threats and intimidation: One way abusers keep their partners in line is by instilling fear. They might be verbally threatening, or give threatening looks or gestures. Abusers often make it known that they are tracking their partner's every move. They might destroy their partner's possessions, threaten to harm them, or threaten to harm their family members. Not surprisingly, victims of this abuse often feel anxiety, fear, and panic. [No words necessary.]

Isolation: Abusers often limit their partner's activities, forbidding them to talk or interact with friends or family. They might limit access to a car or even turn off their phone. All of this might be done by physically holding them against their will, but is often accomplished through psychological abuse and intimidation. The more isolated a person feels, the fewer resources they have to help gain perspective on their situation and to escape from it. [No words necessary.]

Economic abuse: Abusers often make their partners beholden to them for money by controlling access to funds of any kind. They might prevent their partner from getting a job or withhold access to money they earn from a job. This creates financial dependency that makes leaving the relationship very difficult. [See destruction of livelihoods and the proposed meagre 'guaranteed income' so long as you do whatever you are told.]

Using children: An abuser might disparage their partner's parenting skills, tell their children lies about their partner, threaten to take custody of their children, or threaten to harm their children. These tactics instil fear and often elicit compliance. [See reframed social service mafia and how children are being mercilessly abused by the state over 'Covid' while their parents look on too frightened to do anything.]

A further recurring trait in an abusive relationship is the abused blaming themselves for their abuse and making excuses for the abuser. We have the public blaming each other for lockdown abuse by government and many making excuses for the government while attacking those who challenge

the government. How often we have heard authorities say that rules are being imposed or reimposed only because people have refused to ‘behave’ and follow the rules. We don’t want to do it – it’s *you*.

Renegade Minds are an antidote to all of these things. They will never concede their self-respect no matter what the circumstances. Even when apparent humiliation is heaped upon them they laugh in its face and reflect back the humiliation on the abuser where it belongs. Renegade Minds will never wear masks they know are only imposed to humiliate, suppress and damage both physically and psychologically. Consequences will take care of themselves and they will never break their spirit or cause them to concede to tyranny. UK newspaper columnist Peter Hitchens was one of the few in the mainstream media to speak out against lockdowns and forced vaccinations. He then announced he had taken the jab. He wanted to see family members abroad and he believed vaccine passports were inevitable even though they had not yet been introduced. Hitchens has a questioning and critical mind, but not a Renegade one. If he had no amount of pressure would have made him concede. Hitchens excused his action by saying that the battle has been lost. Renegade Minds never accept defeat when freedom is at stake and even if they are the last one standing the self-respect of not submitting to tyranny is more important than any outcome or any consequence.

That’s why Renegade Minds are the only minds that ever changed anything worth changing.

CHAPTER EIGHT

‘Reframing’ insanity

Insanity is relative. It depends on who has who locked in what cage
Ray Bradbury

‘Reframing’ a mind means simply to change its perception and behaviour. This can be done subconsciously to such an extent that subjects have no idea they have been ‘reframed’ while to any observer changes in behaviour and attitudes are obvious.

Human society is being reframed on a ginormous scale since the start of 2020 and here we have the reason why psychologists rather than doctors have been calling the shots. Ask most people who have succumbed to ‘Covid’ reframing if they have changed and most will say ‘no’; but they *have* and fundamentally. The Cult’s long-game has been preparing for these times since way back and crucial to that has been to prepare both population and officialdom mentally and emotionally. To use the mind-control parlance they had to reframe the population with a mentality that would submit to fascism and reframe those in government and law enforcement to impose fascism or at least go along with it. The result has been the fact-deleted mindlessness of ‘Wokeness’ and officialdom that has either enthusiastically or unquestioningly imposed global tyranny demanded by reframed politicians on behalf of psychopathic and deeply evil cultists. ‘Cognitive reframing’ identifies and challenges the way someone sees the world in the form of situations, experiences and emotions and then restructures those perceptions to view the same set of circumstances in a different way. This can have benefits if the attitudes are personally destructive while on the

other side it has the potential for individual and collective mind control which the subject has no idea has even happened.

Cognitive therapy was developed in the 1960s by Aaron T. Beck who was born in Rhode Island in 1921 as the son of Jewish immigrants from the Ukraine. He became interested in the techniques as a treatment for depression. Beck's daughter Judith S. Beck is prominent in the same field and they founded the Beck Institute for Cognitive Behavior Therapy in Philadelphia in 1994. Cognitive reframing, however, began to be used worldwide by those with a very dark agenda. The Cult reframes politicians to change their attitudes and actions until they are completely at odds with what they once appeared to stand for. The same has been happening to government administrators at all levels, law enforcement, military and the human population. Cultists love mind control for two main reasons: It allows them to control what people think, do and say to secure agenda advancement and, by definition, it calms their legendary insecurity and fear of the unexpected. I have studied mind control since the time I travelled America in 1996. I may have been talking to next to no one in terms of an audience in those years, but my goodness did I gather a phenomenal amount of information and knowledge about so many things including the techniques of mind control. I have described this in detail in other books going back to *The Biggest Secret* in 1998. I met a very large number of people recovering from MKUltra and its offshoots and successors and I began to see how these same techniques were being used on the population in general. This was never more obvious than since the 'Covid' hoax began.

Reframing the enforcers

I have observed over the last two decades and more the very clear transformation in the dynamic between the police, officialdom and the public. I tracked this in the books as the relationship mutated from one of serving the public to seeing them as almost the enemy and certainly a lower caste. There has always been a class divide based on income and always been some psychopathic, corrupt, and big-I-am police officers. This was different. Wholesale change was unfolding in the collective dynamic; it was less about money and far more about position and perceived power. An us-and-them was emerging. Noses were lifted skyward by government administration and law enforcement and their attitude to the public they

were *supposed* to be serving changed to one of increasing contempt, superiority and control. The transformation was so clear and widespread that it had to be planned. Collective attitudes and dynamics do not change naturally and organically that quickly on that scale. I then came across an organisation in Britain called Common Purpose created in the late 1980s by Julia Middleton who would work in the office of Deputy Prime Minister John Prescott during the long and disastrous premiership of war criminal Tony Blair. When Blair speaks the Cult is speaking and the man should have been in jail a long time ago. Common Purpose proclaims itself to be one of the biggest 'leadership development' organisations in the world while functioning as a *charity* with all the financial benefits which come from that. It hosts 'leadership development' courses and programmes all over the world and claims to have 'brought together' what it calls 'leaders' from more than 100 countries on six continents. The modus operandi of Common Purpose can be compared with the work of the UK government's reframing network that includes the Behavioural Insights Team 'nudge unit' and 'Covid' reframing specialists at SPI-B. WikiLeaks described Common Purpose long ago as 'a hidden virus in our government and schools' which is unknown to the general public: 'It recruits and trains "leaders" to be loyal to the directives of Common Purpose and the EU, instead of to their own departments, which they then undermine or subvert, the NHS [National Health Service] being an example.' This is a vital point to understand the 'Covid' hoax. The NHS, and its equivalent around the world, has been utterly reframed in terms of administrators and much of the medical personnel with the transformation underpinned by recruitment policies. The outcome has been the criminal and psychopathic behaviour of the NHS over 'Covid' and we have seen the same in every other major country. WikiLeaks said Common Purpose trainees are 'learning to rule without regard to democracy' and to usher in a police state (current events explained). Common Purpose operated like a 'glue' and had members in the NHS, BBC, police, legal profession, church, many of Britain's 7,000 quangos, local councils, the Civil Service, government ministries and Parliament, and controlled many RDA's (Regional Development Agencies). Here we have one answer for how and why British institutions and their like in other countries have changed so negatively in relation to the public. This further explains how and why the beyond-disgraceful reframed BBC has

become a propaganda arm of ‘Covid’ fascism. They are all part of a network pursuing the same goal.

By 2019 Common Purpose was quoting a figure of 85,000 ‘leaders’ that had attended its programmes. These ‘students’ of all ages are known as Common Purpose ‘graduates’ and they consist of government, state and local government officials and administrators, police chiefs and officers, and a whole range of others operating within the national, local and global establishment. Cressida Dick, Commissioner of the London Metropolitan Police, is the Common Purpose graduate who was the ‘Gold Commander’ that oversaw what can only be described as the murder of Brazilian electrician Jean Charles de Menezes in 2005. He was held down by psychopathic police and shot seven times in the head by a psychopathic lunatic after being mistaken for a terrorist when he was just a bloke going about his day. Dick authorised officers to pursue and keep surveillance on de Menezes and ordered that he be stopped from entering the underground train system. Police psychopaths took her at her word clearly. She was ‘disciplined’ for this outrage by being *promoted* – eventually to the top of the ‘Met’ police where she has been a disaster. Many Chief Constables controlling the police in different parts of the UK are and have been Common Purpose graduates. I have heard the ‘graduate’ network described as a sort of Mafia or secret society operating within the fabric of government at all levels pursuing a collective policy ingrained at Common Purpose training events. Founder Julia Middleton herself has said:

> Locally and internationally, Common Purpose graduates will be ‘lighting small fires’ to create change in their organisations and communities … The Common Purpose effect is best illustrated by the many stories of small changes brought about by leaders, who themselves have changed.

A Common Purpose mission statement declared:

> Common Purpose aims to improve the way society works by expanding the vision, decision-making ability and influence of all kinds of leaders. The organisation runs a variety of educational programmes for leaders of all ages, backgrounds and sectors, in order to provide them with the inspirational, information and opportunities they need to change the world.

Yes, but into what? Since 2020 the answer has become clear.

NLP and the Delphi technique

Common Purpose would seem to be a perfect name or would common programming be better? One of the foundation methods of reaching ‘consensus’ (group think) is by setting the agenda theme and then encouraging, cajoling or pressuring everyone to agree a ‘consensus’ in line with the core theme promoted by Common Purpose. The methodology involves the ‘Delphi technique’, or an adaption of it, in which opinions are expressed that are summarised by a ‘facilitator or change agent’ at each stage. Participants are ‘encouraged’ to modify their views in the light of what others have said. Stage by stage the former individual opinions are merged into group consensus which just happens to be what Common Purpose wants them to believe. A key part of this is to marginalise anyone refusing to concede to group think and turn the group against them to apply pressure to conform. We are seeing this very technique used on the general population to make ‘Covid’ group-thinkers hostile to those who have seen through the bullshit. People can be reframed by using perception manipulation methods such as Neuro-Linguistic Programming (NLP) in which you change perception with the use of carefully constructed language. An NLP website described the technique this way:

> … A method of influencing brain behaviour (the ‘neuro’ part of the phrase) through the use of language (the ‘linguistic’ part) and other types of communication to enable a person to ‘recode’ the way the brain responds to stimuli (that’s the ‘programming’) and manifest new and better behaviours. Neuro-Linguistic Programming often incorporates hypnosis and self-hypnosis to help achieve the change (or ‘programming’) that is wanted.

British alternative media operation UKColumn has done very detailed research into Common Purpose over a long period. I quoted co-founder and former naval officer Brian Gerrish in my book *Remember Who You Are*, published in 2011, as saying the following years before current times:

> It is interesting that many of the mothers who have had children taken by the State speak of the Social Services people being icily cool, emotionless and, as two ladies said in slightly different words, ‘… like little robots’. We know that NLP is cumulative, so people can be given small imperceptible doses of NLP in a course here, another in a few months, next year etc. In this way, major changes are accrued in their personality, but the day by day change is almost unnoticeable.

In these and other ways ‘graduates’ have had their perceptions uniformly reframed and they return to their roles in the institutions of government, law enforcement, legal profession, military, ‘education’, the UK National Health Service and the whole swathe of the establishment structure to pursue a common agenda preparing for the ‘post-industrial’, ‘post-democratic’ society. I say ‘preparing’ but we are now there. ‘Post-industrial’ is code for the Great Reset and ‘post-democratic’ is ‘Covid’ fascism. UKColumn has spoken to partners of those who have attended Common Purpose ‘training’. They have described how personalities and attitudes of ‘graduates’ changed very noticeably for the worse by the time they had completed the course. They had been ‘reframed’ and told they are the ‘leaders’ – the special ones – who know better than the population. There has also been the very demonstrable recruitment of psychopaths and narcissists into government administration at all levels and law enforcement. If you want psychopathy hire psychopaths and you get a simple cause and effect. If you want administrators, police officers and ‘leaders’ to perceive the public as lesser beings who don’t matter then employ narcissists. These personalities are identified using ‘psychometrics’ that identifies knowledge, abilities, attitudes and personality traits, mostly through carefully-designed questionnaires and tests. As this policy has passed through the decades we have had power-crazy, power-trippers appointed into law enforcement, security and government administration in preparation for current times and the dynamic between public and law enforcement/officialdom has been transformed. UKColumn’s Brian Gerrish said of the narcissistic personality:

> Their love of themselves and power automatically means that they will crush others who get in their way. I received a major piece of the puzzle when a friend pointed out that when they made public officials re-apply for their own jobs several years ago they were also required to do psychometric tests. This was undoubtedly the start of the screening process to get ‘their’ sort of people in post.

How obvious that has been since 2020 although it was clear what was happening long before if people paid attention to the changing public-establishment dynamic.

Change agents

At the centre of events in ‘Covid’ Britain is the National Health Service (NHS) which has behaved disgracefully in slavishly following the Cult agenda. The NHS management structure is awash with Common Purpose graduates or ‘change agents’ working to a common cause. Helen Bevan, a Chief of Service Transformation at the NHS Institute for Innovation and Improvement, co-authored a document called ‘Towards a million change agents, a review of the social movements literature: implications for large scale change in the NHS‘. The document compared a project management approach to that of change and social movements where ‘people change themselves and each other – peer to peer’. Two definitions given for a ‘social movement’ were:

A group of people who consciously attempt to build a radically new social order; involves people of a broad range of social backgrounds; and deploys politically confrontational and socially disruptive tactics – Cyrus Zirakzadeh 1997

Collective challenges, based on common purposes and social solidarities, in sustained interaction with elites, opponents, and authorities – Sidney Tarrow 1994

Helen Bevan wrote another NHS document in which she defined ‘framing’ as ‘the process by which leaders construct, articulate and put across their message in a powerful and compelling way in order to win people to their cause and call them to action’. I think I could come up with another definition that would be rather more accurate. The National Health Service and institutions of Britain and the wider world have been taken over by reframed ‘change agents’ and that includes everything from the United Nations to national governments, local councils and social services which have been kidnapping children from loving parents on an extraordinary and gathering scale on the road to the end of parenthood altogether. Children from loving homes are stolen and kidnapped by the state and put into the ‘care’ (inversion) of the local authority through council homes, foster parents and forced adoption. At the same time children are allowed to be abused without response while many are under council ‘care’. UKColumn

highlighted the Common Purpose connection between South Yorkshire Police and Rotherham council officers in the case of the scandal in that area of the sexual exploitation of children to which the authorities turned not one blind eye, but both:

We were alarmed to discover that the Chief Executive, the Strategic Director of Children and Young People's Services, the Manager for the Local Strategic Partnership, the Community Cohesion Manager, the Cabinet Member for Cohesion, the Chief Constable and his predecessor had all attended Leadership training courses provided by the pseudo-charity Common Purpose.

Once 'change agents' have secured positions of hire and fire within any organisation things start to move very quickly. Personnel are then hired and fired on the basis of whether they will work towards the agenda the change agent represents. If they do they are rapidly promoted even though they may be incompetent. Those more qualified and skilled who are pre-Common Purpose 'old school' see their careers stall and even disappear. This has been happening for decades in every institution of state, police, 'health' and social services and all of them have been transformed as a result in their attitudes to their jobs and the public. Medical professions, including nursing, which were once vocations for the caring now employ many cold, callous and couldn't give a shit personality types. The UKColumn investigation concluded:

By blurring the boundaries between people, professions, public and private sectors, responsibility and accountability, Common Purpose encourages 'graduates' to believe that as new selected leaders, they can work together, outside of the established political and social structures, to achieve a paradigm shift or CHANGE – so called 'Leading Beyond Authority'. In doing so, the allegiance of the individual becomes 'reframed' on CP colleagues and their NETWORK.

Reframing the Face-Nappies

Nowhere has this process been more obvious than in the police where recruitment of psychopaths and development of unquestioning mind-controlled group-thinkers have transformed law enforcement into a politically-correct 'Woke' joke and a travesty of what should be public service. Today they wear their face-nappies like good little gofers and enforce 'Covid' rules which are fascism under another name. Alongside the

specifically-recruited psychopaths we have software minds incapable of free thought. Brian Gerrish again:

An example is the policeman who would not get on a bike for a press photo because he had not done the cycling proficiency course. Normal people say this is political correctness gone mad. Nothing could be further from the truth. The policeman has been reframed, and in his reality it is perfect common sense not to get on the bike 'because he hasn't done the cycling course'.

Another example of this is where the police would not rescue a boy from a pond until they had taken advice from above on the 'risk assessment'. A normal person would have arrived, perhaps thought of the risk for a moment, and dived in. To the police now 'reframed', they followed 'normal' procedure.

There are shocking cases of reframed ambulance crews doing the same. Sheer unthinking stupidity of London Face-Nappies headed by Common Purpose graduate Cressida Dick can be seen in their behaviour at a vigil in March, 2021, for a murdered woman, Sarah Everard. A police officer had been charged with the crime. Anyone with a brain would have left the vigil alone in the circumstances. Instead they 'manhandled' women to stop them breaking 'Covid rules' to betray classic reframing. Minds in the thrall of perception control have no capacity for seeing a situation on its merits and acting accordingly. 'Rules is rules' is their only mind-set. My father used to say that rules and regulations are for the guidance of the intelligent and the blind obedience of the idiot. Most of the intelligent, decent, coppers have gone leaving only the other kind and a few old school for whom the job must be a daily nightmare. The combination of psychopaths and rule-book software minds has been clearly on public display in the 'Covid' era with automaton robots in uniform imposing fascistic 'Covid' regulations on the population without any personal initiative or judging situations on their merits. There are thousands of examples around the world, but I'll make my point with the infamous Derbyshire police in the English East Midlands – the ones who think pouring dye into beauty spots and using drones to track people walking in the countryside away from anyone is called 'policing'. To them there are rules decreed by the government which they have to enforce and in their bewildered state a group gathering in a closed space and someone walking alone in the countryside are the same thing. It is beyond idiocy and enters the realm of clinical insanity.

Police officers in Derbyshire said they were 'horrified' – *horrified* – to find 15 to 20 'irresponsible' kids playing a football match at a closed leisure

centre ‘in breach of coronavirus restrictions’. When they saw the police the kids ran away leaving their belongings behind and the reframed men and women of Derbyshire police were seeking to establish their identities with a view to fining their parents. The most natural thing for youngsters to do – kicking a ball about – is turned into a criminal activity and enforced by the moronic software programs of Derbyshire police. You find the same mentality in every country. These barely conscious ‘horrified’ officers said they had to take action because ‘we need to ensure these rules are being followed’ and ‘it is of the utmost importance that you ensure your children are following the rules and regulations for Covid-19’. Had any of them done ten seconds of research to see if this parroting of their masters’ script could be supported by any evidence? Nope. Reframed people don’t think – others think for them and that’s the whole idea of reframing. I have seen police officers one after the other repeating without question word for word what officialdom tells them just as I have seen great swathes of the public doing the same. Ask either for ‘their’ opinion and out spews what they have been told to think by the official narrative. Police and public may seem to be in different groups, but their mentality is the same. Most people do whatever they are told in fear not doing so or because they believe what officialdom tells them; almost the entirety of the police do what they are told for the same reason. Ultimately it’s the tiny inner core of the global Cult that’s telling both what to do.

So Derbyshire police were ‘horrified’. Oh, really? Why did they think those kids were playing football? It was to relieve the psychological consequences of lockdown and being denied human contact with their friends and interaction, touch and discourse vital to human psychological health. Being denied this month after month has dismantled the psyche of many children and young people as depression and suicide have exploded. Were Derbyshire police *horrified by that*? Are you kidding? Reframed people don’t have those mental and emotional processes that can see how the impact on the psychological health of youngsters is far more dangerous than any ‘virus’ even if you take the mendacious official figures to be true. The reframed are told (programmed) how to act and so they do. The Derbyshire Chief Constable in the first period of lockdown when the black dye and drones nonsense was going on was Peter Goodman. He was the man who severed the connection between his force and the Derbyshire Constabulary *Male Voice* Choir when he decided that it was not inclusive

enough to allow women to join. The fact it was a male voice choir making a particular sound produced by male voices seemed to elude a guy who terrifyingly ran policing in Derbyshire. He retired weeks after his force was condemned as disgraceful by former Supreme Court Justice Jonathan Sumption for their behaviour over extreme lockdown impositions. Goodman was replaced by his deputy Rachel Swann who was in charge when her officers were 'horrified'. The police statement over the boys committing the hanging-offence of playing football included the line about the youngsters being 'irresponsible in the times we are all living through' missing the point that the real relevance of the 'times we are all living through' is the imposition of fascism enforced by psychopaths and reframed minds of police officers playing such a vital part in establishing the fascist tyranny that their own children and grandchildren will have to live in their entire lives. As a definition of insanity that is hard to beat although it might be run close by imposing masks on people that can have a serious effect on their health while wearing a face nappy all day themselves. Once again public and police do it for the same reason – the authorities tell them to and who are they to have the self-respect to say no?

Wokers in uniform

How reframed do you have to be to arrest a *six-year-old* and take him to court for *picking a flower* while waiting for a bus? Brain dead police and officialdom did just that in North Carolina where criminal proceedings happen regularly for children under nine. Attorney Julie Boyer gave the six-year-old crayons and a colouring book during the 'flower' hearing while the 'adults' decided his fate. County Chief District Court Judge Jay Corpening asked: 'Should a child that believes in Santa Claus, the Easter Bunny and the tooth fairy be making life-altering decisions?' Well, of course not, but common sense has no meaning when you have a common purpose and a reframed mind. Treating children in this way, and police operating in American schools, is all part of the psychological preparation for children to accept a police state as normal all their adult lives. The same goes for all the cameras and biometric tracking technology in schools. Police training is focused on reframing them as snowflake Wokers and this is happening in the military. Pentagon top brass said that 'training sessions on extremism' were needed for troops who asked why they were so focused on the Capitol

Building riot when Black Lives Matter riots were ignored. What's the difference between them some apparently and rightly asked. Actually, there is a difference. Five people died in the Capitol riot, only one through violence, and that was a police officer shooting an unarmed protestor. BLM riots killed at least 25 people and cost billions. Asking the question prompted the psychopaths and reframed minds that run the Pentagon to say that more 'education' (programming) was needed. Troop training is all based on psychological programming to make them fodder for the Cult – 'Military men are just dumb, stupid animals to be used as pawns in foreign policy' as Cult-to-his-DNA former Secretary of State Henry Kissinger famously said. Governments see the police in similar terms and it's time for those among them who can see this to defend the people and stop being enforcers of the Cult agenda upon the people.

The US military, like the country itself, is being targeted for destruction through a long list of Woke impositions. Cult-owned gaga 'President' Biden signed an executive order when he took office to allow taxpayer money to pay for transgender surgery for active military personnel and veterans. Are you a man soldier? No, I'm a LGBTQIA+ with a hint of Skoliosexual and Spectrasexual. Oh, good man. Bad choice of words you bigot. The Pentagon announced in March, 2021, the appointment of the first 'diversity and inclusion officer' for US Special Forces. Richard Torres-Estrada arrived with the publication of a 'D&I Strategic Plan which will guide the enterprise-wide effort to institutionalize and sustain D&I'. If you think a Special Forces 'Strategic Plan' should have something to do with defending America you haven't been paying attention. Defending Woke is now the military's new role. Torres-Estrada has posted images comparing Donald Trump with Adolf Hitler and we can expect no bias from him as a representative of the supposedly non-political Pentagon. Cable news host Tucker Carlson said: 'The Pentagon is now the Yale faculty lounge but with cruise missiles.' Meanwhile Secretary of Defense Lloyd Austin, a board member of weapons-maker Raytheon with stock and compensation interests in October, 2020, worth $1.4 million, said he was purging the military of the 'enemy within' – anyone who isn't Woke and supports Donald Trump. Austin refers to his targets as 'racist extremists' while in true Woke fashion being himself a racist extremist. Pentagon documents pledge to 'eradicate, eliminate and conquer all forms of racism, sexism and homophobia'. The definitions of these are decided by 'diversity and inclusion committees'

peopled by those who see racism, sexism and homophobia in every situation and opinion. Woke (the Cult) is dismantling the US military and purging testosterone as China expands its military and gives its troops 'masculinity training'. How do we think that is going to end when this is all Cult coordinated? The US military, like the British military, is controlled by Woke and spineless top brass who just go along with it out of personal career interests.

'Woke' means fast asleep

Mind control and perception manipulation techniques used on individuals to create group-think have been unleashed on the global population in general. As a result many have no capacity to see the obvious fascist agenda being installed all around them or what 'Covid' is really all about. Their brains are firewalled like a computer system not to process certain concepts, thoughts and realisations that are bad for the Cult. The young are most targeted as the adults they will be when the whole fascist global state is planned to be fully implemented. They need to be prepared for total compliance to eliminate all pushback from entire generations. The Cult has been pouring billions into taking complete control of 'education' from schools to universities via its operatives and corporations and not least Bill Gates as always. The plan has been to transform 'education' institutions into programming centres for the mentality of 'Woke'. James McConnell, professor of psychology at the University of Michigan, wrote in *Psychology Today* in 1970:

> The day has come when we can combine sensory deprivation with drugs, hypnosis, and astute manipulation of reward and punishment, to gain almost absolute control over an individual's behaviour. It should then be possible to achieve a very rapid and highly effective type of brainwashing that would allow us to make dramatic changes in a person's behaviour and personality ...
>
> … We should reshape society so that we all would be trained from birth to want to do what society wants us to do. We have the techniques to do it... no-one owns his own personality you acquired, and there's no reason to believe you should have the right to refuse to acquire a new personality if your old one is anti-social.

This was the potential for mass brainwashing in 1970 and the mentality there displayed captures the arrogant psychopathy that drives it forward. I emphasise that not all young people have succumbed to Woke programming and those that haven't are incredibly impressive people given that today's young are the most perceptually-targeted generations in history with all the technology now involved. Vast swathes of the young generations, however, have fallen into the spell – and that's what it is – of Woke. The Woke mentality and perceptual program is founded on *inversion* and you will appreciate later why that is so significant. Everything with Woke is inverted and the opposite of what it is claimed to be. Woke was a term used in African-American culture from the 1900s and referred to an awareness of social and racial justice. This is not the meaning of the modern version or 'New Woke' as I call it in *The Answer*. Oh, no, Woke today means something very different no matter how much Wokers may seek to hide that and insist Old Woke and New Woke are the same. See if you find any 'awareness of social justice' here in the modern variety:

• Woke demands 'inclusivity' while excluding anyone with a different opinion and calls for mass censorship to silence other views.

• Woke claims to stand against oppression when imposing oppression is the foundation of all that it does. It is the driver of political correctness which is nothing more than a Cult invention to manipulate the population to silence itself.

• Woke believes itself to be 'liberal' while pursuing a global society that can only be described as fascist (see 'anti-fascist' fascist Antifa).

• Woke calls for 'social justice' while spreading injustice wherever it goes against the common 'enemy' which can be easily identified as a differing view.

• Woke is supposed to be a metaphor for 'awake' when it is solid-gold asleep and deep in a Cult-induced coma that meets the criteria for 'off with the fairies'.

I state these points as obvious facts if people only care to look. I don't do this with a sense of condemnation. We need to appreciate that the onslaught

of perceptual programming on the young has been incessant and merciless. I can understand why so many have been reframed, or, given their youth, framed from the start to see the world as the Cult demands. The Cult has had access to their minds day after day in its 'education' system for their entire formative years. Perception is formed from information received and the Cult-created system is a life-long download of information delivered to elicit a particular perception, thus behaviour. The more this has expanded into still new extremes in recent decades and ever-increasing censorship has deleted other opinions and information why wouldn't that lead to a perceptual reframing on a mass scale? I have described already cradle-to-grave programming and in more recent times the targeting of young minds from birth to adulthood has entered the stratosphere. This has taken the form of skewing what is 'taught' to fit the Cult agenda and the omnipresent techniques of group-think to isolate non-believers and pressure them into line. There has always been a tendency to follow the herd, but we really are in a new world now in relation to that. We have parents who can see the 'Covid' hoax told by their children not to stop them wearing masks at school, being 'Covid' tested or having the 'vaccine' in fear of the peer-pressure consequences of being different. What is 'peer-pressure' if not pressure to conform to group-think? Renegade Minds never group-think and always retain a set of perceptions that are unique to them. Group-think is always underpinned by consequences for not group-thinking. Abuse now aimed at those refusing DNA-manipulating 'Covid vaccines' are a potent example of this. The biggest pressure to conform comes from the very group which is itself being manipulated. 'I am programmed to be part of a hive mind and so you must be.'

Woke control structures in 'education' now apply to every mainstream organisation. Those at the top of the 'education' hierarchy (the Cult) decide the policy. This is imposed on governments through the Cult network; governments impose it on schools, colleges and universities; their leadership impose the policy on teachers and academics and they impose it on children and students. At any level where there is resistance, perhaps from a teacher or university lecturer, they are targeted by the authorities and often fired. Students themselves regularly demand the dismissal of academics (increasingly few) at odds with the narrative that the students have been programmed to believe in. It is quite a thought that students who are being targeted by the Cult become so consumed by programmed group-

think that they launch protests and demand the removal of those who are trying to push back against those targeting the students. Such is the scale of perceptual inversion. We see this with ‘Covid’ programming as the Cult imposes the rules via psycho-psychologists and governments on shops, transport companies and businesses which impose them on their staff who impose them on their customers who pressure Pushbackers to conform to the will of the Cult which is in the process of destroying them and their families. Scan all aspects of society and you will see the same sequence every time.

Fact free Woke and hijacking the ‘left’

There is no more potent example of this than ‘Woke’, a mentality only made possible by the deletion of factual evidence by an ‘education’ system seeking to produce an ever more uniform society. Why would you bother with facts when you don’t know any? Deletion of credible history both in volume and type is highly relevant. Orwell said: ‘Who controls the past controls the future: who controls the present controls the past.’ They who control the perception of the past control the perception of the future and they who control the present control the perception of the past through the writing and deleting of history. Why would you oppose the imposition of Marxism in the name of Wokeism when you don’t know that Marxism cost at least 100 million lives in the 20th century alone? Watch videos and read reports in which Woker generations are asked basic historical questions – it’s mind-blowing. A survey of 2,000 people found that six percent of millennials (born approximately early1980s to early 2000s) believed the Second World War (1939-1945) broke out with the assassination of President Kennedy (in 1963) and one in ten thought Margaret Thatcher was British Prime Minister at the time. She was in office between 1979 and 1990. We are in a post-fact society. Provable facts are no defence against the fascism of political correctness or Silicon Valley censorship. Facts don’t matter anymore as we have witnessed with the ‘Covid’ hoax. Sacrificing uniqueness to the Woke group-think religion is all you are required to do and that means thinking for yourself is the biggest Woke no, no. All religions are an expression of group-think and censorship and Woke is just another religion with an orthodoxy defended by group-think and censorship. Burned at the stake becomes burned on Twitter which leads

back eventually to burned at the stake as Woke humanity regresses to ages past.

The biggest Woke inversion of all is its creators and funders. I grew up in a traditional left of centre political household on a council estate in Leicester in the 1950s and 60s – you know, the left that challenged the power of wealth-hoarding elites and threats to freedom of speech and opinion. In those days students went on marches defending freedom of speech while today's Wokers march for its deletion. What on earth could have happened? Those very elites (collectively the Cult) that we opposed in my youth and early life have funded into existence the antithesis of that former left and hijacked the 'brand' while inverting everything it ever stood for. We have a mentality that calls itself 'liberal' and 'progressive' while acting like fascists. Cult billionaires and their corporations have funded themselves into control of 'education' to ensure that Woke programming is unceasing throughout the formative years of children and young people and that non-Wokers are isolated (that word again) whether they be students, teachers or college professors. The Cult has funded into existence the now colossal global network of Woke organisations that have spawned and promoted all the 'causes' on the Cult wish-list for global transformation and turned Wokers into demanders of them. Does anyone really think it's a coincidence that the Cult agenda for humanity is a carbon (sorry) copy of the societal transformations desired by Woke?? These are only some of them:

Political correctness: The means by which the Cult deletes all public debates that it knows it cannot win if we had the free-flow of information and evidence.

Human-caused 'climate change': The means by which the Cult seeks to transform society into a globally-controlled dictatorship imposing its will over the fine detail of everyone's lives 'to save the planet' which doesn't actually need saving.

Transgender obsession: Preparing collective perception to accept the 'new human' which would not have genders because it would be created

technologically and not through procreation. I'll have much more on this in Human 2.0.

Race obsession: The means by which the Cult seeks to divide and rule the population by triggering racial division through the perception that society is more racist than ever when the opposite is the case. Is it perfect in that regard? No. But to compare today with the racism of apartheid and segregation brought to an end by the civil rights movement in the 1960s is to insult the memory of that movement and inspirations like Martin Luther King. Why is the 'anti-racism' industry (which it is) so dominated by privileged white people?

White supremacy: This is a label used by privileged white people to demonise poor and deprived white people pushing back on tyranny to marginalise and destroy them. White people are being especially targeted as the dominant race by number within Western society which the Cult seeks to transform in its image. If you want to change a society you must weaken and undermine its biggest group and once you have done that by using the other groups you next turn on them to do the same … 'Then they came for the Jews and I was not a Jew so I did nothing.'

Mass migration: The mass movement of people from the Middle East, Africa and Asia into Europe, from the south into the United States and from Asia into Australia are another way the Cult seeks to dilute the racial, cultural and political influence of white people on Western society. White people ask why their governments appear to be working against them while being politically and culturally biased towards incoming cultures. Well, here's your answer. In the same way sexually 'straight' people, men and women, ask why the authorities are biased against them in favour of other sexualities. The answer is the same – that's the way the Cult wants it to be for very sinister motives.

These are all central parts of the Cult agenda and central parts of the Woke agenda and Woke was created and continues to be funded to an immense

degree by Cult billionaires and corporations. If anyone begins to say ‘coincidence’ the syllables should stick in their throat.

Billionaire ‘social justice warriors’

Joe Biden is a 100 percent-owned asset of the Cult and the Wokers’ man in the White House whenever he can remember his name and for however long he lasts with his rapidly diminishing cognitive function. Even walking up the steps of an aircraft without falling on his arse would appear to be a challenge. He’s not an empty-shell puppet or anything. From the minute Biden took office (or the Cult did) he began his executive orders promoting the Woke wish-list. You will see the Woke agenda imposed ever more severely because it’s really the *Cult* agenda. Woke organisations and activist networks spawned by the Cult are funded to the extreme so long as they promote what the Cult wants to happen. Woke is funded to promote ‘social justice’ by billionaires who become billionaires by destroying social justice. The social justice mantra is only a cover for dismantling social justice and funded by billionaires that couldn’t give a damn about social justice. Everything makes sense when you see that. One of Woke’s premier funders is Cult billionaire financier George Soros who said: ‘I am basically there to make money, I cannot and do not look at the social consequences of what I do.’ This is the same Soros who has given more than $32 billion to his Open Society Foundations global Woke network and funded Black Lives Matter, mass immigration into Europe and the United States, transgender activism, climate change activism, political correctness and groups targeting ‘white supremacy’ in the form of privileged white thugs that dominate Antifa. What a scam it all is and when you are dealing with the unquestioning fact-free zone of Woke scamming them is child’s play. All you need to pull it off in all these organisations are a few in-the-know agents of the Cult and an army of naïve, reframed, uninformed, narcissistic, know-nothings convinced of their own self-righteousness, self-purity and virtue.

Soros and fellow billionaires and billionaire corporations have poured hundreds of millions into Black Lives Matter and connected groups and promoted them to a global audience. None of this is motivated by caring about black people. These are the billionaires that have controlled and exploited a system that leaves millions of black people in abject poverty

and deprivation which they do absolutely nothing to address. The same Cult networks funding BLM were behind the *slave trade!* Black Lives Matter hijacked a phrase that few would challenge and they have turned this laudable concept into a political weapon to divide society. You know that BLM is a fraud when it claims that *All* Lives Matter, the most inclusive statement of all, is 'racist'. BLM and its Cult masters don't want to end racism. To them it's a means to an end to control all of humanity never mind the colour, creed, culture or background. What has destroying the nuclear family got to do with ending racism? Nothing – but that is one of the goals of BLM and also happens to be a goal of the Cult as I have been exposing in my books for decades. Stealing children from loving parents and giving schools ever more power to override parents is part of that same agenda. BLM is a Marxist organisation and why would that not be the case when the Cult created Marxism *and* BLM? Patrisse Cullors, a BLM co-founder, said in a 2015 video that she and her fellow organisers, including co-founder Alicia Garza, are 'trained Marxists'. The lady known after marriage as Patrisse Khan-Cullors bought a $1.4 million home in 2021 in one of the whitest areas of California with a black population of just 1.6 per cent and has so far bought *four* high-end homes for a total of $3.2 million. How very Marxist. There must be a bit of spare in the BLM coffers, however, when Cult corporations and billionaires have handed over the best part of $100 million. Many black people can see that Black Lives Matter is not working for them, but against them, and this is still more confirmation. Black journalist Jason Whitlock, who had his account suspended by Twitter for simply linking to the story about the 'Marxist's' home buying spree, said that BLM leaders are 'making millions of dollars off the backs of these dead black men who they wouldn't spit on if they were on fire and alive'.

Black Lies Matter

Cult assets and agencies came together to promote BLM in the wake of the death of career criminal George Floyd who had been jailed a number of times including for forcing his way into the home of a black woman with others in a raid in which a gun was pointed at her stomach. Floyd was filmed being held in a Minneapolis street in 2020 with the knee of a police officer on his neck and he subsequently died. It was an appalling thing for the officer to do, but the same technique has been used by police on

peaceful protestors of lockdown without any outcry from the Woke brigade. As unquestioning supporters of the Cult agenda Wokers have supported lockdown and all the 'Covid' claptrap while attacking anyone standing up to the tyranny imposed in its name. Court documents would later include details of an autopsy on Floyd by County Medical Examiner Dr Andrew Baker who concluded that Floyd had taken a fatal level of the drug fentanyl. None of this mattered to fact-free, question-free, Woke. Floyd's death was followed by worldwide protests against police brutality amid calls to defund the police. Throwing babies out with the bathwater is a Woke speciality. In the wake of the murder of British woman Sarah Everard a Green Party member of the House of Lords, Baroness Jones of Moulescoomb (Nincompoopia would have been better), called for a 6pm curfew for all men. This would be in breach of the Geneva Conventions on war crimes which ban collective punishment, but that would never have crossed the black and white Woke mind of Baroness Nincompoopia who would have been far too convinced of her own self-righteousness to compute such details. Many American cities did defund the police in the face of Floyd riots and after $15 million was deleted from the police budget in Washington DC under useless Woke mayor Muriel Bowser car-jacking alone rose by 300 percent and within six months the US capital recorded its highest murder rate in 15 years. The same happened in Chicago and other cities in line with the Cult/Soros plan to bring fear to streets and neighbourhoods by reducing the police, releasing violent criminals and not prosecuting crime. This is the mob-rule agenda that I have warned in the books was coming for so long. Shootings in the area of Minneapolis where Floyd was arrested increased by 2,500 percent compared with the year before. Defunding the police over George Floyd has led to a big increase in dead people with many of them black. Police protection for politicians making these decisions stayed the same or increased as you would expect from professional hypocrites. The Cult doesn't actually want to abolish the police. It wants to abolish local control over the police and hand it to federal government as the psychopaths advance the Hunger Games Society. Many George Floyd protests turned into violent riots with black stores and businesses destroyed by fire and looting across America fuelled by Black Lives Matter. Woke doesn't do irony. If you want civil rights you must loot the liquor store and the supermarket and make off with a smart TV. It's the only way.

It’s not a race war – it’s a class war

Black people are patronised by privileged blacks and whites alike and told they are victims of white supremacy. I find it extraordinary to watch privileged blacks supporting the very system and bloodline networks behind the slave trade and parroting the same Cult-serving manipulative crap of their privileged white, often billionaire, associates. It is indeed not a race war but a class war and colour is just a diversion. Black Senator Cory Booker and black Congresswoman Maxine Waters, more residents of Nincompoopia, personify this. Once you tell people they are victims of someone else you devalue both their own responsibility for their plight and the power they have to impact on their reality and experience. Instead we have: ‘You are only in your situation because of whitey – turn on them and everything will change.’ It won’t change. Nothing changes in our lives unless *we* change it. Crucial to that is never seeing yourself as a victim and always as the creator of your reality. Life is a simple sequence of choice and consequence. Make different choices and you create different consequences. *You* have to make those choices – not Black Lives Matter, the Woke Mafia and anyone else that seeks to dictate your life. Who are they these Wokers, an emotional and psychological road traffic accident, to tell you what to do? Personal empowerment is the last thing the Cult and its Black Lives Matter want black people or anyone else to have. They claim to be defending the underdog while *creating* and perpetuating the underdog. The Cult’s worst nightmare is human unity and if they are going to keep blacks, whites and every other race under economic servitude and control then the focus must be diverted from what they have in common to what they can be manipulated to believe divides them. Blacks have to be told that their poverty and plight is the fault of the white bloke living on the street in the same poverty and with the same plight they are experiencing. The difference is that your plight black people is due to him, a white supremacist with ‘white privilege’ living on the street. Don’t unite as one human family against your mutual oppressors and suppressors – fight the oppressor with the white face who is as financially deprived as you are. The Cult knows that as its ‘Covid’ agenda moves into still new levels of extremism people are going to respond and it has been spreading the seeds of disunity everywhere to stop a united response to the evil that targets *all of us*.

Racist attacks on ‘whiteness’ are getting ever more outrageous and especially through the American Democratic Party which has an appalling history for anti-black racism. Barack Obama, Joe Biden, Hillary Clinton and Nancy Pelosi all eulogised about Senator Robert Byrd at his funeral in 2010 after a nearly 60-year career in Congress. Byrd was a brutal Ku Klux Klan racist and a violent abuser of Cathy O’Brien in MKUltra. He said he would never fight in the military ‘with a negro by my side’ and ‘rather I should die a thousand times, and see Old Glory trampled in the dirt never to rise again, than to see this beloved land of ours become degraded by race mongrels, a throwback to the blackest specimen from the wilds’. Biden called Byrd a ‘very close friend and mentor’. These ‘Woke’ hypocrites are not anti-racist they are anti-poor and anti-people not of their perceived class. Here is an illustration of the scale of anti-white racism to which we have now descended. Seriously Woke and moronic *New York Times* contributor Damon Young described whiteness as a ‘virus’ that ‘like other viruses will not die until there are no bodies left for it to infect’. He went on: ‘… the only way to stop it is to locate it, isolate it, extract it, and kill it.’ Young can say that as a black man with no consequences when a white man saying the same in reverse would be facing a jail sentence. *That’s* racism. We had super-Woke numbskull senators Tammy Duckworth and Mazie Hirono saying they would object to future Biden Cabinet appointments if he did not nominate more Asian Americans and Pacific Islanders. Never mind the ability of the candidate what do they look like? Duckworth said: ‘I will vote for racial minorities and I will vote for LGBTQ, but anyone else I’m not voting for.’ Appointing people on the grounds of race is illegal, but that was not a problem for this ludicrous pair. They were on-message and that’s a free pass in any situation.

Critical race racism

White children are told at school they are intrinsically racist as they are taught the divisive ‘critical race theory’. This claims that the law and legal institutions are inherently racist and that race is a socially constructed concept used by white people to further their economic and political interests at the expense of people of colour. White is a ‘virus’ as we’ve seen. Racial inequality results from ‘social, economic, and legal differences that white people create between races to maintain white interests which

leads to poverty and criminality in minority communities‘. I must tell that to the white guy sleeping on the street. The principal of East Side Community School in New York sent white parents a manifesto that called on them to become ‘white traitors’ and advocate for full ‘white abolition’. These people are teaching your kids when they urgently need a psychiatrist. The ‘school’ included a chart with ‘eight white identities’ that ranged from ‘white supremacist’ to ‘white abolition’ and defined the behaviour white people must follow to end ‘the regime of whiteness’. Woke blacks and their privileged white associates are acting exactly like the slave owners of old and Ku Klux Klan racists like Robert Byrd. They are too full of their own self-purity to see that, but it’s true. Racism is not a body type; it’s a state of mind that can manifest through any colour, creed or culture.

Another racial fraud is *‘equity’*. Not equality of treatment and opportunity – equity. It’s a term spun as equality when it means something very different. Equality in its true sense is a raising up while ‘equity’ is a race to the bottom. Everyone in the same level of poverty is ‘equity’. Keep everyone down – that’s equity. The Cult doesn’t want anyone in the human family to be empowered and BLM leaders, like all these ‘anti-racist’ organisations, continue their privileged, pampered existence by perpetuating the perception of gathering racism. When is the last time you heard an ‘anti-racist’ or ‘anti-Semitism’ organisation say that acts of racism and discrimination have *fallen?* It’s not in the interests of their fund-raising and power to influence and the same goes for the professional soccer anti-racism operation, Kick It Out. Two things confirmed that the Black Lives Matter riots in the summer of 2020 were Cult creations. One was that while anti-lockdown protests were condemned in this same period for ‘transmitting ‘Covid’ the authorities supported mass gatherings of Black Lives Matter supporters. I even saw self-deluding people claiming to be doctors say the two types of protest were not the same. No – the non-existent ‘Covid’ was in favour of lockdowns and attacked those that protested against them while ‘Covid’ supported Black Lives Matter and kept well away from its protests. The whole thing was a joke and as lockdown protestors were arrested, often brutally, by reframed Face-Nappies we had the grotesque sight of police officers taking the knee to Black Lives Matter, a Cult-funded Marxist organisation that supports violent riots and wants to destroy the nuclear family and white people.

He's not white? Shucks!

Woke obsession with race was on display again when ten people were shot dead in Boulder, Colorado, in March, 2021. Cult-owned Woke TV channels like CNN said the shooter appeared to be a white man and Wokers were on Twitter condemning 'violent white men' with the usual mantras. Then the shooter's name was released as Ahmad Al Aliwi Alissa, an anti-Trump Arab-American, and the sigh of disappointment could be heard five miles away. Never mind that ten people were dead and what that meant for their families. Race baiting was all that mattered to these sick Cult-serving people like Barack Obama who exploited the deaths to further divide America on racial grounds which is his job for the Cult. This is the man that 'racist' white Americans made the first black president of the United States and then gave him a second term. Not-very-bright Obama has become filthy rich on the back of that and today appears to have a big influence on the Biden administration. Even so he's still a downtrodden black man and a victim of white supremacy. This disingenuous fraud reveals the contempt he has for black people when he puts on a Deep South Alabama accent whenever he talks to them, no, *at* them.

Another BLM red flag was how the now fully-Woke (fully-Cult) and fully-virtue-signalled professional soccer authorities had their teams taking the knee before every match in support of Marxist Black Lives Matter. Soccer authorities and clubs displayed 'Black Lives Matter' on the players' shirts and flashed the name on electronic billboards around the pitch. Any fans that condemned what is a Freemasonic taking-the-knee ritual were widely condemned as you would expect from the Woke virtue-signallers of professional sport and the now fully-Woke media. We have reverse racism in which you are banned from criticising any race or culture except for white people for whom anything goes – say what you like, no problem. What has this got to do with racial harmony and equality? We've had black supremacists from Black Lives Matter telling white people to fall to their knees in the street and apologise for their white supremacy. Black supremacists acting like white supremacist slave owners of the past couldn't breach their self-obsessed, race-obsessed sense of self-purity. Joe Biden appointed a race-obsessed black supremacist Kristen Clarke to head the Justice Department Civil Rights Division. Clarke claimed that blacks are endowed with 'greater mental, physical and spiritual abilities' than whites. If anyone reversed that statement they would be vilified. Clarke is on-

message so no problem. She's never seen a black-white situation in which the black figure is anything but a virtuous victim and she heads the Civil Rights Division which should treat everyone the same or it isn't civil rights. Another perception of the Renegade Mind: If something or someone is part of the Cult agenda they will be supported by Woke governments and media no matter what. If they're not, they will be condemned and censored. It really is that simple and so racist Clarke prospers despite (make that because of) her racism.

The end of culture

Biden's administration is full of such racial, cultural and economic bias as the Cult requires the human family to be divided into warring factions. We are now seeing racially-segregated graduations and everything, but everything, is defined through the lens of perceived 'racism. We have 'racist' mathematics, 'racist' food and even 'racist' *plants*. World famous Kew Gardens in London said it was changing labels on plants and flowers to tell its pre-'Covid' more than two million visitors a year how racist they are. Kew director Richard Deverell said this was part of an effort to 'move quickly to decolonise collections' after they were approached by one Ajay Chhabra 'an actor with an insight into how sugar cane was linked to slavery'. They are *plants* you idiots. 'Decolonisation' in the Woke manual really means colonisation of society with its mentality and by extension colonisation by the Cult. We are witnessing a new Chinese-style 'Cultural Revolution' so essential to the success of all Marxist takeovers. Our cultural past and traditions have to be swept away to allow a new culture to be built-back-better. Woke targeting of long-standing Western cultural pillars including historical monuments and cancelling of historical figures is what happened in the Mao revolution in China which 'purged remnants of capitalist and traditional elements from Chinese society' and installed Maoism as the dominant ideology'. For China see the Western world today and for 'dominant ideology' see Woke. Better still see Marxism or Maoism. The 'Covid' hoax has specifically sought to destroy the arts and all elements of Western culture from people meeting in a pub or restaurant to closing theatres, music venues, sports stadiums, places of worship and even banning *singing*. Destruction of Western society is also why criticism of any religion is banned except for Christianity which again is the dominant

religion as white is the numerically-dominant race. Christianity may be fading rapidly, but its history and traditions are weaved through the fabric of Western society. Delete the pillars and other structures will follow until the whole thing collapses. I am not a Christian defending that religion when I say that. I have no religion. It's just a fact. To this end Christianity has itself been turned Woke to usher its own downfall and its ranks are awash with 'change agents' – knowing and unknowing – at every level including Pope Francis (*definitely* knowing) and the clueless Archbishop of Canterbury Justin Welby (possibly not, but who can be sure?). Woke seeks to coordinate attacks on Western culture, traditions, and ways of life through 'intersectionality' defined as 'the complex, cumulative way in which the effects of multiple forms of discrimination (such as racism, sexism, and classism) combine, overlap, or intersect especially in the experiences of marginalised individuals or groups'. Wade through the Orwellian Woke-speak and this means coordinating disparate groups in a common cause to overthrow freedom and liberal values.

The entire structure of public institutions has been infested with Woke – government at all levels, political parties, police, military, schools, universities, advertising, media and trade unions. This abomination has been achieved through the Cult web by appointing Wokers to positions of power and battering non-Wokers into line through intimidation, isolation and threats to their job. Many have been fired in the wake of the empathy-deleted, vicious hostility of 'social justice' Wokers and the desire of gutless, spineless employers to virtue-signal their Wokeness. Corporations are filled with Wokers today, most notably those in Silicon Valley. Ironically at the top they are not Woke at all. They are only exploiting the mentality their Cult masters have created and funded to censor and enslave while the Wokers cheer them on until it's their turn. Thus the Woke 'liberal left' is an inversion of the traditional liberal left. Campaigning for justice on the grounds of power and wealth distribution has been replaced by campaigning for identity politics. The genuine traditional left would never have taken money from today's billionaire abusers of fairness and justice and nor would the billionaires have wanted to fund that genuine left. It would not have been in their interests to do so. The division of opinion in those days was between the haves and have nots. This all changed with Cult manipulated and funded identity politics. The division of opinion today is between Wokers and non-Wokers and not income brackets. Cult

corporations and their billionaires may have taken wealth disparity to cataclysmic levels of injustice, but as long as they speak the language of Woke, hand out the dosh to the Woke network and censor the enemy they are 'one of us'. Billionaires who don't give a damn about injustice are laughing at them till their bellies hurt. Wokers are not even close to self-aware enough to see that. The transformed 'left' dynamic means that Wokers who drone on about 'social justice' are funded by billionaires that have destroyed social justice the world over. It's *why* they are billionaires.

The climate con

Nothing encapsulates what I have said more comprehensively than the hoax of human-caused global warming. I have detailed in my books over the years how Cult operatives and organisations were the pump-primers from the start of the climate con. A purpose-built vehicle for this is the Club of Rome established by the Cult in 1968 with the Rockefellers and Rothschilds centrally involved all along. Their gofer frontman Maurice Strong, a Canadian oil millionaire, hosted the Earth Summit in Rio de Janeiro, Brazil, in 1992 where the global 'green movement' really expanded in earnest under the guiding hand of the Cult. The Earth Summit established Agenda 21 through the Cult-created-and-owned United Nations to use the illusion of human-caused climate change to justify the transformation of global society to save the world from climate disaster. It is a No-Problem-Reaction-Solution sold through governments, media, schools and universities as whole generations have been terrified into believing that the world was going to end in their lifetimes unless what old people had inflicted upon them was stopped by a complete restructuring of how everything is done. Chill, kids, it's all a hoax. Such restructuring is precisely what the Cult agenda demands (purely by coincidence of course). Today this has been given the codename of the Great Reset which is only an updated term for Agenda 21 and its associated Agenda 2030. The latter, too, is administered through the UN and was voted into being by the General Assembly in 2015. Both 21 and 2030 seek centralised control of all resources and food right down to the raindrops falling on your own land. These are some of the demands of Agenda 21 established in 1992. See if you recognise this society emerging today:

- End national sovereignty
- State planning and management of all land resources, ecosystems, deserts, forests, mountains, oceans and fresh water; agriculture; rural development; biotechnology; and ensuring *'equity'*
- The state to 'define the role' of business and financial resources
- Abolition of private property
- 'Restructuring' the family unit (see BLM)
- Children raised by the state
- People told what their job will be
- Major restrictions on movement
- Creation of 'human settlement zones'
- Mass resettlement as people are forced to vacate land where they live
- Dumbing down education
- Mass global depopulation in pursuit of all the above

The United Nations was created as a Trojan horse for world government. With the climate con of critical importance to promoting that outcome you would expect the UN to be involved. Oh, it's involved all right. The UN is promoting Agenda 21 and Agenda 2030 justified by 'climate change' while also driving the climate hoax through its Intergovernmental Panel on Climate Change (IPCC), one of the world's most corrupt organisations. The IPCC has been lying ferociously and constantly since the day it opened its doors with the global media hanging unquestioningly on its every mendacious word. The Green movement is entirely Woke and has long lost its original environmental focus since it was co-opted by the Cult. An obsession with 'global warming' has deleted its values and scrambled its head. I experienced a small example of what I mean on a beautiful country walk that I have enjoyed several times a week for many years. The path merged into the fields and forests and you felt at one with the natural world. Then a 'Green' organisation, the Hampshire and Isle of Wight Wildlife Trust, took over part of the land and proceeded to cut down a large number of trees, including mature ones, to install a horrible big, bright steel 'this-is-ours-stay-out' fence that destroyed the whole atmosphere of this beautiful place. No one with a feel for nature would do that. Day after day I walked to the sound of chainsaws and a magnificent mature weeping willow tree that I so admired was cut down at the base of the trunk. When I challenged

a Woke young girl in a green shirt (of course) about this vandalism she replied: ‘It’s a weeping willow – it will grow back.’ This is what people are paying for when they donate to the Hampshire and Isle of Wight Wildlife Trust and many other ‘green’ organisations today. It is not the environmental movement that I knew and instead has become a support-system – as with Extinction Rebellion – for a very dark agenda.

Private jets for climate justice

The Cult-owned, Gates-funded, World Economic Forum and its founder Klaus Schwab were behind the emergence of Greta Thunberg to harness the young behind the climate agenda and she was invited to speak to the world at … the UN. Schwab published a book, *Covid-19: The Great Reset* in 2020 in which he used the ‘Covid’ hoax and the climate hoax to lay out a new society straight out of Agenda 21 and Agenda 2030. Bill Gates followed in early 2021 when he took time out from destroying the world to produce a book in his name about the way to save it. Gates flies across the world in private jets and admitted that ‘I probably have one of the highest greenhouse gas footprints of anyone on the planet … my personal flying alone is gigantic.’ He has also bid for the planet’s biggest private jet operator. Other climate change saviours who fly in private jets include John Kerry, the US Special Presidential Envoy for Climate, and actor Leonardo DiCaprio, a ‘UN Messenger of Peace with special focus on climate change’. These people are so full of bullshit they could corner the market in manure. We mustn’t be sceptical, though, because the Gates book, *How to Avoid a Climate Disaster: The Solutions We Have and the Breakthroughs We Need*, is a genuine attempt to protect the world and not an obvious pile of excrement attributed to a mega-psychopath aimed at selling his masters’ plans for humanity. The Gates book and the other shite-pile by Klaus Schwab could have been written by the same person and may well have been. Both use ‘climate change’ and ‘Covid’ as the excuses for their new society and by coincidence the Cult’s World Economic Forum and Bill and Melinda Gates Foundation promote the climate hoax and hosted Event 201 which pre-empted with a ‘simulation’ the very ‘coronavirus’ hoax that would be simulated for real on humanity within weeks. The British ‘royal’ family is promoting the ‘Reset’ as you would expect through Prince ‘climate change caused the war in Syria’ Charles and his hapless son Prince

William who said that we must ‘reset our relationship with nature and our trajectory as a species’ to avoid a climate disaster. Amazing how many promotors of the ‘Covid’ and ‘climate change’ control systems are connected to Gates and the World Economic Forum. A ‘study’ in early 2021 claimed that carbon dioxide emissions must fall by the equivalent of a global lockdown roughly every two years for the next decade to save the planet. The ‘study’ appeared in the same period that the Schwab mob claimed in a video that lockdowns destroying the lives of billions are good because they make the earth ‘quieter’ with less ‘ambient noise’. They took down the video amid a public backlash for such arrogant, empathy-deleted stupidity You see, however, where they are going with this. Corinne Le Quéré, a professor at the Tyndall Centre for Climate Change Research, University of East Anglia, was lead author of the climate lockdown study, and she writes for … the World Economic Forum. Gates calls in ‘his’ book for changing ‘every aspect of the economy’ (long-time Cult agenda) and for humans to eat synthetic ‘meat’ (predicted in my books) while cows and other farm animals are eliminated. Australian TV host and commentator Alan Jones described what carbon emission targets would mean for farm animals in Australia alone if emissions were reduced as demanded by 35 percent by 2030 and zero by 2050:

Well, let’s take agriculture, the total emissions from agriculture are about 75 million tonnes of carbon dioxide, equivalent. Now reduce that by 35 percent and you have to come down to 50 million tonnes, I’ve done the maths. So if you take for example 1.5 million cows, you’re going to have to reduce the herd by 525,000 [by] 2030, nine years, that’s 58,000 cows a year. The beef herd’s 30 million, reduce that by 35 percent, that’s 10.5 million, which means 1.2 million cattle have to go every year between now and 2030. This is insanity!

There are 75 million sheep. Reduce that by 35 percent, that’s 26 million sheep, that’s almost 3 million a year. So under the Paris Agreement over 30 million beasts. dairy cows, cattle, pigs and sheep would go. More than 8,000 every minute of every hour for the next decade, do these people know what they’re talking about?

Clearly they don’t at the level of campaigners, politicians and administrators. The Cult *does* know; that’s the outcome it wants. We are faced with not just a war on humanity. Animals and the natural world are being targeted and I have been saying since the ‘Covid’ hoax began that the plan eventually was to claim that the ‘deadly virus’ is able to jump from animals, including farm animals and domestic pets, to humans. Just before

this book went into production came this story: ‘Russia registers world’s first Covid-19 vaccine for cats & dogs as makers of Sputnik V warn pets & farm animals could spread virus’. The report said ‘top scientists warned that the deadly pathogen could soon begin spreading through homes and farms’ and ‘the next stage is the infection of farm and domestic animals’. Know the outcome and you’ll see the journey. Think what that would mean for animals and keep your eye on a term called zoonosis or zoonotic diseases which transmit between animals and humans. The Cult wants to break the connection between animals and people as it does between people and people. Farm animals fit with the Cult agenda to transform food from natural to synthetic.

The gas of life is killing us

There can be few greater examples of Cult inversion than the condemnation of carbon dioxide as a dangerous pollutant when it is the gas of life. Without it the natural world would be dead and so we would all be dead. We breathe in oxygen and breathe out carbon dioxide while plants produce oxygen and absorb carbon dioxide. It is a perfect symbiotic relationship that the Cult wants to dismantle for reasons I will come to in the final two chapters. Gates, Schwab, other Cult operatives and mindless repeaters, want the world to be ‘carbon neutral’ by at least 2050 and the earlier the better. ‘Zero carbon’ is the cry echoed by lunatics calling for ‘Zero Covid’ when we already have it. These carbon emission targets will deindustrialise the world in accordance with Cult plans – the post-industrial, post-democratic society – and with so-called renewables like solar and wind not coming even close to meeting human energy needs blackouts and cold are inevitable. Texans got the picture in the winter of 2021 when a snow storm stopped wind turbines and solar panels from working and the lights went down along with water which relies on electricity for its supply system. Gates wants everything to be powered by electricity to ensure that his masters have the kill switch to stop all human activity, movement, cooking, water and warmth any time they like. The climate lie is so stupendously inverted that it claims we must urgently reduce carbon dioxide when we *don’t have enough*.

Co2 in the atmosphere is a little above 400 parts per million when the optimum for plant growth is 2,000 ppm and when it falls anywhere near

150 ppm the natural world starts to die and so do we. It fell to as low as 280 ppm in an 1880 measurement in Hawaii and rose to 413 ppm in 2019 with industrialisation which is why the planet has become *greener* in the industrial period. How insane then that psychopathic madman Gates is not satisfied only with blocking the rise of Co2. He's funding technology to suck it out of the atmosphere. The reason why will become clear. The industrial era is not destroying the world through Co2 and has instead turned around a potentially disastrous ongoing fall in Co2. Greenpeace co-founder and scientist Patrick Moore walked away from Greenpeace in 1986 and has exposed the green movement for fear-mongering and lies. He said that 500 million years ago there was *17 times* more Co2 in the atmosphere than we have today and levels have been falling for hundreds of millions of years. In the last 150 million years Co2 levels in Earth's atmosphere had reduced by *90 percent*. Moore said that by the time humanity began to unlock carbon dioxide from fossil fuels we were at '38 seconds to midnight' and in that sense: 'Humans are [the Earth's] salvation.' Moore made the point that only half the Co2 emitted by fossil fuels stays in the atmosphere and we should remember that all pollution pouring from chimneys that we are told is carbon dioxide is in fact nothing of the kind. It's pollution. Carbon dioxide is an invisible gas.

William Happer, Professor of Physics at Princeton University and long-time government adviser on climate, has emphasised the Co2 deficiency for maximum growth and food production. Greenhouse growers don't add carbon dioxide for a bit of fun. He said that most of the warming in the last 100 years, after the earth emerged from the super-cold period of the 'Little Ice Age' into a natural warming cycle, was over by 1940. Happer said that a peak year for warming in 1988 can be explained by a 'monster El Nino' which is a natural and cyclical warming of the Pacific that has nothing to do with 'climate change'. He said the effect of Co2 could be compared to painting a wall with red paint in that once two or three coats have been applied it didn't matter how much more you slapped on because the wall will not get much redder. Almost all the effect of the rise in Co2 has already happened, he said, and the volume in the atmosphere would now have to *double* to increase temperature by a single degree. Climate hoaxers know this and they have invented the most ridiculously complicated series of 'feedback' loops to try to overcome this rather devastating fact. You hear puppet Greta going on cluelessly about feedback loops and this is why.

The Sun affects temperature? No you *climate denier*

Some other nonsense to contemplate: Climate graphs show that rises in temperature do not follow rises in Co2 – *it's the other way round* with a lag between the two of some 800 years. If we go back 800 years from present time we hit the Medieval Warm Period when temperatures were higher than now without any industrialisation and this was followed by the Little Ice Age when temperatures plummeted. The world was still emerging from these centuries of serious cold when many climate records began which makes the ever-repeated line of the 'hottest year since records began' meaningless when you are not comparing like with like. The coldest period of the Little Ice Age corresponded with the lowest period of sunspot activity when the Sun was at its least active. Proper scientists will not be at all surprised by this when it confirms the obvious fact that earth temperature is affected by the scale of Sun activity and the energetic power that it subsequently emits; but when is the last time you heard a climate hoaxer talking about the Sun as a source of earth temperature?? Everything has to be focussed on Co2 which makes up just 0.117 percent of so-called greenhouse gases and only a fraction of even that is generated by human activity. The rest is natural. More than *90 percent* of those greenhouse gases are water vapour and clouds (Fig 9). Ban moisture I say. Have you noticed that the climate hoaxers no longer use the polar bear as their promotion image? That's because far from becoming extinct polar bear communities are stable or thriving. Joe Bastardi, American meteorologist, weather forecaster and outspoken critic of the climate lie, documents in his book *The Climate Chronicles* how weather patterns and events claimed to be evidence of climate change have been happening since long before industrialisation: 'What happened before naturally is happening again, as is to be expected given the cyclical nature of the climate due to the design of the planet.' If you read the detailed background to the climate hoax in my other books you will shake your head and wonder how anyone could believe the crap which has spawned a multi-trillion dollar industry based on absolute garbage (see HIV causes AIDs and Sars-Cov-2 causes 'Covid-19'). Climate and 'Covid' have much in common given they have the same source. They both have the contradictory *everything* factor in which everything is explained by reference to them. It's hot – 'it's climate change'. It's cold – 'it's climate change'. I got a sniffle – 'it's Covid'. I haven't got a sniffle – 'it's Covid'. Not having a sniffle has to be a symptom

of ‘Covid’. Everything is and not having a sniffle is especially dangerous if you are a slow walker. For sheer audacity I offer you a Cambridge University ‘study’ that actually linked ‘Covid’ to ‘climate change’. It had to happen eventually. They concluded that climate change played a role in ‘Covid-19’ spreading from animals to humans because … wait for it … I kid you not … *the two groups were forced closer together as populations grow*. Er, that’s it. The whole foundation on which this depended was that ‘Bats are the likely zoonotic origin of SARS-CoV-1 and SARS-CoV-2’. Well, they are not. They are nothing to do with it. Apart from bats not being the origin and therefore ‘climate change’ effects on bats being irrelevant I am in awe of their academic insight. Where would we be without them? Not where we are that’s for sure.

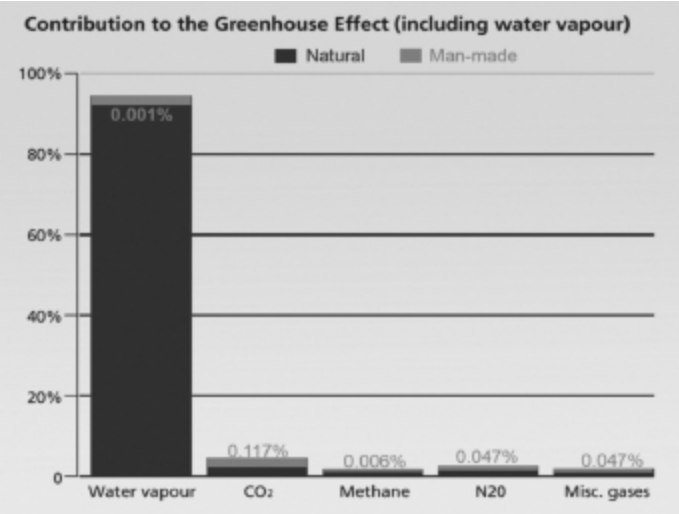

Figure 9: The idea that the gas of life is disastrously changing the climate is an insult to brain cell activity.

One other point about the weather is that climate modification is now well advanced and not every major weather event is natural – or earthquake come to that. I cover this subject at some length in other books. China is openly planning a rapid expansion of its weather modification programme which includes changing the climate in an area more than one and a half times the size of India. China used weather manipulation to ensure clear skies during the 2008 Olympics in Beijing. I have quoted from US military documents detailing how to employ weather manipulation as a weapon of war and they did that in the 1960s and 70s during the conflict in Vietnam with Operation Popeye manipulating monsoon rains for military purposes.

Why would there be international treaties on weather modification if it wasn't possible? Of course it is. Weather is energetic information and it can be changed.

How was the climate hoax pulled off? See 'Covid'

If you can get billions to believe in a 'virus' that doesn't exist you can get them to believe in human-caused climate change that doesn't exist. Both are being used by the Cult to transform global society in the way it has long planned. Both hoaxes have been achieved in pretty much the same way. First you declare a lie is a fact. There's a 'virus' you call SARS-Cov-2 or humans are warming the planet with their behaviour. Next this becomes, via Cult networks, the foundation of government, academic and science policy and belief. Those who parrot the mantra are given big grants to produce research that confirms the narrative is true and ever more 'symptoms' are added to make the 'virus'/'climate change' sound even more scary. Scientists and researchers who challenge the narrative have their grants withdrawn and their careers destroyed. The media promote the lie as the unquestionable truth and censor those with an alternative view or evidence. A great percentage of the population believe what they are told as the lie becomes an everybody-knows-that and the believing-masses turn on those with a mind of their own. The technique has been used endlessly throughout human history. Wokers are the biggest promotors of the climate lie *and* 'Covid' fascism because their minds are owned by the Cult; their sense of self-righteous self-purity knows no bounds; and they exist in a bubble of reality in which facts are irrelevant and only get in the way of looking without seeing.

Running through all of this like veins in a blue cheese is control of information, which means control of perception, which means control of behaviour, which collectively means control of human society. The Cult owns the global media and Silicon Valley fascists for the simple reason that it *has* to. Without control of information it can't control perception and through that human society. Examine every facet of the Cult agenda and you will see that anything supporting its introduction is never censored while anything pushing back is always censored. I say again: Psychopaths that know why they are doing this must go before Nuremberg trials and those that follow their orders must trot along behind them into the same

dock. ‘I was just following orders’ didn’t work the first time and it must not work now. Nuremberg trials must be held all over the world before public juries for politicians, government officials, police, compliant doctors, scientists and virologists, and all Cult operatives such as Gates, Tedros, Fauci, Vallance, Whitty, Ferguson, Zuckerberg, Wojcicki, Brin, Page, Dorsey, the whole damn lot of them – including, no *especially*, the psychopath psychologists. Without them and the brainless, gutless excuses for journalists that have repeated their lies, none of this could be happening. Nobody can be allowed to escape justice for the psychological and economic Armageddon they are all responsible for visiting upon the human race.

As for the compliant, unquestioning, swathes of humanity, and the self-obsessed, all-knowing ignorance of the Wokers … don’t start me. God help their kids. God help their grandkids. God *help them*.

CHAPTER NINE

We must have it? So what is it?

Well I won't back down. No, I won't back down. You can stand me up at the Gates of Hell. But I won't back down
Tom Petty

I will now focus on the genetically-manipulating 'Covid vaccines' which do not meet this official definition of a vaccine by the US Centers for Disease Control (CDC): 'A product that stimulates a person's immune system to produce immunity to a specific disease, protecting the person from that disease.' On that basis 'Covid vaccines' are not a vaccine in that the makers don't even claim they stop infection or transmission.

They are instead part of a multi-levelled conspiracy to change the nature of the human body and what it means to be 'human' and to depopulate an enormous swathe of humanity. What I shall call Human 1.0 is on the cusp of becoming Human 2.0 and for very sinister reasons. Before I get to the 'Covid vaccine' in detail here's some background to vaccines in general. Government regulators do not test vaccines – the makers do – and the makers control which data is revealed and which isn't. Children in America are given 50 vaccine doses by age six and 69 by age 19 and the effect of the whole combined schedule has never been tested. Autoimmune diseases when the immune system attacks its own body have soared in the mass vaccine era and so has disease in general in children and the young. Why wouldn't this be the case when vaccines target the *immune system?* The US government gave Big Pharma drug companies immunity from prosecution for vaccine death and injury in the 1986 National Childhood Vaccine Injury

Act (NCVIA) and since then the government (taxpayer) has been funding compensation for the consequences of Big Pharma vaccines. The criminal and satanic drug giants can't lose and the vaccine schedule has increased dramatically since 1986 for this reason. There is no incentive to make vaccines safe and a big incentive to make money by introducing ever more. Even against a ridiculously high bar to prove vaccine liability, and with the government controlling the hearing in which it is being challenged for compensation, the vaccine court has so far paid out more than $4 billion. These are the vaccines we are told are safe and psychopaths like Zuckerberg censor posts saying otherwise. The immunity law was even justified by a ruling that vaccines by their nature were 'unavoidably unsafe'.

Check out the ingredients of vaccines and you will be shocked if you are new to this. *They put that in children's bodies?? What??* Try aluminium, a brain toxin connected to dementia, aborted foetal tissue and formaldehyde which is used to embalm corpses. World-renowned aluminium expert Christopher Exley had his research into the health effect of aluminium in vaccines shut down by Keele University in the UK when it began taking funding from the Bill and Melinda Gates Foundation. Research when diseases 'eradicated' by vaccines began to decline and you will find the fall began long *before* the vaccine was introduced. Sometimes the fall even plateaued after the vaccine. Diseases like scarlet fever for which there was no vaccine declined in the same way because of environmental and other factors. A perfect case in point is the polio vaccine. Polio began when lead arsenate was first sprayed as an insecticide and residues remained in food products. Spraying started in 1892 and the first US polio epidemic came in Vermont in 1894. The simple answer was to stop spraying, but Rockefeller-created Big Pharma had a better idea. Polio was decreed to be caused by the *poliovirus* which 'spreads from person to person and can infect a person's spinal cord'. Lead arsenate was replaced by the lethal DDT which had the same effect of causing paralysis by damaging the brain and central nervous system. Polio plummeted when DDT was reduced and then banned, but the vaccine is still given the credit for something it didn't do. Today by far the biggest cause of polio is the vaccines promoted by Bill Gates. Vaccine justice campaigner Robert Kennedy Jr, son of assassinated (by the Cult) US Attorney General Robert Kennedy, wrote:

In 2017, the World Health Organization (WHO) reluctantly admitted that the global explosion in polio is predominantly vaccine strain. The most frightening epidemics in Congo, Afghanistan, and the Philippines, are all linked to vaccines. In fact, by 2018, 70% of global polio cases were vaccine strain.

Vaccines make fortunes for Cult-owned Gates and Big Pharma while undermining the health and immune systems of the population. We had a glimpse of the mentality behind the Big Pharma cartel with a report on WION (World is One News), an international English language TV station based in India, which exposed the extraordinary behaviour of US drug company Pfizer over its 'Covid vaccine'. The WION report told how Pfizer had made fantastic demands of Argentina, Brazil and other countries in return for its 'vaccine'. These included immunity from prosecution, even for Pfizer negligence, government insurance to protect Pfizer from law suits and handing over as collateral sovereign assets of the country to include Argentina's bank reserves, military bases and embassy buildings. Pfizer demanded the same of Brazil in the form of waiving sovereignty of its assets abroad; exempting Pfizer from Brazilian laws; and giving Pfizer immunity from all civil liability. This is a 'vaccine' developed with government funding. Big Pharma is evil incarnate as a creation of the Cult and all must be handed tickets to Nuremberg.

Phantom 'vaccine' for a phantom 'disease'

I'll expose the 'Covid vaccine' fraud and then go on to the wider background of why the Cult has set out to 'vaccinate' every man, woman and child on the planet for an alleged 'new disease' with a survival rate of 99.77 percent (or more) even by the grotesquely-manipulated figures of the World Health Organization and Johns Hopkins University. The 'infection' to 'death' ratio is 0.23 to 0.15 percent according to Stanford epidemiologist Dr John Ioannidis and while estimates vary the danger remains tiny. I say that if the truth be told the fake infection to fake death ratio is zero. Never mind all the evidence I have presented here and in *The Answer* that there is no 'virus' let us just focus for a moment on that death-rate figure of say 0.23 percent. The figure includes all those worldwide who have tested positive with a test not testing for the 'virus' and then died within 28 days or even longer of any other cause – *any other cause*. Now subtract all those

illusory ‘Covid’ deaths on the global data sheets from the 0.23 percent. What do you think you would be left with? *Zero*. A vaccination has never been successfully developed for a so-called coronavirus. They have all failed at the animal testing stage when they caused hypersensitivity to what they were claiming to protect against and made the impact of a disease far worse. Cult-owned vaccine corporations got around that problem this time by bypassing animal trials, going straight to humans and making the length of the ‘trials’ before the public rollout as short as they could get away with. Normally it takes five to ten years or more to develop vaccines that still cause demonstrable harm to many people and that’s without including the long-term effects that are never officially connected to the vaccination. ‘Covid’ non-vaccines have been officially produced and approved in a matter of months from a standing start and part of the reason is that (a) they were developed before the ‘Covid’ hoax began and (b) they are based on computer programs and not natural sources. Official non-trials were so short that government agencies gave *emergency,* not full, approval. ‘Trials’ were not even completed and full approval cannot be secured until they are. Public ‘Covid vaccination’ is actually a *continuation of the trial*. Drug company ‘trials’ are not scheduled to end until 2023 by which time a lot of people are going to be dead. Data on which government agencies gave this emergency approval was supplied by the Big Pharma corporations themselves in the form of Pfizer/BioNTech, AstraZeneca, Moderna, Johnson & Johnson, and others, and this is the case with all vaccines. By its very nature *emergency* approval means drug companies do not have to prove that the ‘vaccine’ is ‘safe and effective’. How could they with trials way short of complete? Government regulators only have to *believe* that they *could* be safe and effective. It is criminal manipulation to get products in circulation with no testing worth the name. Agencies giving that approval are infested with Big Pharma-connected place-people and they act in the interests of Big Pharma (the Cult) and not the public about whom they do not give a damn.

More human lab rats

‘Covid vaccines’ produced in record time by Pfizer/BioNTech and Moderna employ a technique *never approved before for use on humans*. They are known as mRNA ‘vaccines’ and inject a synthetic version of ‘viral’ mRNA

or ‘messenger RNA’. The key is in the term ‘messenger’. The body works, or doesn’t, on the basis of information messaging. Communications are constantly passing between and within the genetic system and the brain. Change those messages and you change the state of the body and even its very nature and you can change psychology and behaviour by the way the brain processes information. I think you are going to see significant changes in personality and perception of many people who have had the ‘Covid vaccine’ synthetic potions. Insider Aldous Huxley predicted the following in 1961 and mRNA ‘vaccines’ can be included in the term ‘pharmacological methods’:

There will be, in the next generation or so, a pharmacological method of making people love their servitude, and producing dictatorship without tears, so to speak, producing a kind of painless concentration camp for entire societies, so that people will in fact have their own liberties taken away from them, but rather enjoy it, because they will be distracted from any desire to rebel by propaganda or brainwashing, or brainwashing enhanced by pharmacological methods. And this seems to be the final revolution.

Apologists claim that mRNA synthetic ‘vaccines’ don’t change the DNA genetic blueprint because RNA does not affect DNA only the other way round. This is so disingenuous. A process called ‘reverse transcription’ can convert RNA into DNA and be integrated into DNA in the cell nucleus. This was highlighted in December, 2020, by scientists at Harvard and Massachusetts Institute of Technology (MIT). Geneticists report that more than 40 percent of mammalian genomes results from reverse transcription. On the most basic level if messaging changes then that sequence must lead to changes in DNA which is receiving and transmitting those communications. How can introducing synthetic material into cells not change the cells where DNA is located? The process is known as transfection which is defined as ‘a technique to insert foreign nucleic acid (DNA or RNA) into a cell, typically with the intention of altering the properties of the cell’. Researchers at the Sloan Kettering Institute in New York found that changes in messenger RNA can deactivate tumour-suppressing proteins and thereby promote cancer. This is what happens when you mess with messaging. ‘Covid vaccine’ maker Moderna was founded in 2010 by Canadian stem cell biologist Derrick J. Rossi after his breakthrough discovery in the field of transforming and reprogramming

stem cells. These are neutral cells that can be programmed to become any cell including sperm cells. Moderna was therefore founded on the principle of genetic manipulation and has never produced any vaccine or drug before its genetically-manipulating synthetic 'Covid' shite. Look at the name – Mode-RNA or Modify-RNA. Another important point is that the US Supreme Court has ruled that genetically-modified DNA, or complementary DNA (cDNA) synthesized in the laboratory from messenger RNA, can be patented and owned. These psychopaths are doing this to the human body.

Cells replicate synthetic mRNA in the 'Covid vaccines' and in theory the body is tricked into making antigens which trigger antibodies to target the 'virus spike proteins' which as Dr Tom Cowan said have *never been seen*. Cut the crap and these 'vaccines' deliver *self-replicating* synthetic material to the cells with the effect of changing human DNA. The more of them you have the more that process is compounded while synthetic material is all the time self-replicating. 'Vaccine'-maker Moderna describes mRNA as 'like software for the cell' and so they are messing with the body's software. What happens when you change the software in a computer? Everything changes. For this reason the Cult is preparing a production line of mRNA 'Covid vaccines' and a long list of excuses to use them as with all the 'variants' of a 'virus' never shown to exist. The plan is further to transfer the mRNA technique to other vaccines mostly given to children and young people. The cumulative consequences will be a transformation of human DNA through a constant infusion of synthetic genetic material which will kill many and change the rest. Now consider that governments that have given emergency approval for a vaccine that's not a vaccine; never been approved for humans before; had no testing worth the name; and the makers have been given immunity from prosecution for any deaths or adverse effects suffered by the public. The UK government awarded *permanent legal indemnity* to itself and its employees for harm done when a patient is being treated for 'Covid-19' or 'suspected Covid-19'. That is quite a thought when these are possible 'side-effects' from the 'vaccine' (they are not 'side', they are effects) listed by the US Food and Drug Administration:

Guillain-Barre syndrome; acute disseminated encephalomyelitis; transverse myelitis; encephalitis; myelitis; encephalomyelitis; meningoencephalitis; meningitis; encephalopathy; convulsions; seizures;

stroke; narcolepsy; cataplexy; anaphylaxis; acute myocardial infarction (heart attack); myocarditis; pericarditis; autoimmune disease; death; implications for pregnancy, and birth outcomes; other acute demyelinating diseases; non anaphylactic allergy reactions; thrombocytopenia ; disseminated intravascular coagulation; venous thromboembolism; arthritis; arthralgia; joint pain; Kawasaki disease; multisystem inflammatory syndrome in children; vaccine enhanced disease. The latter is the way the 'vaccine' has the potential to make diseases far worse than they would otherwise be.

UK doctor and freedom campaigner Vernon Coleman described the conditions in this list as 'all unpleasant, most of them very serious, and you can't get more serious than death'. The thought that anyone at all has had the 'vaccine' in these circumstances is testament to the potential that humanity has for clueless, unquestioning, stupidity and for many that programmed stupidity has already been terminal.

An insider speaks

Dr Michael Yeadon is a former Vice President, head of research and Chief Scientific Adviser at vaccine giant Pfizer. Yeadon worked on the inside of Big Pharma, but that did not stop him becoming a vocal critic of 'Covid vaccines' and their potential for multiple harms, including infertility in women. By the spring of 2021 he went much further and even used the no, no, term 'conspiracy'. When you begin to see what is going on it is impossible not to do so. Yeadon spoke out in an interview with freedom campaigner James Delingpole and I mentioned earlier how he said that no one had samples of 'the virus'. He explained that the mRNA technique originated in the anti-cancer field and ways to turn on and off certain genes which could be advantageous if you wanted to stop cancer growing out of control. 'That's the origin of them. They are a very unusual application, really.' Yeadon said that treating a cancer patient with an aggressive procedure might be understandable if the alternative was dying, but it was quite another thing to use the same technique as a public health measure. Most people involved wouldn't catch the infectious agent you were vaccinating against and if they did they probably wouldn't die:

If you are really using it as a public health measure you really want to as close as you can get to zero sides-effects … I find it odd that they chose techniques that were really cutting their teeth in the field of oncology and I'm worried that in using gene-based vaccines that have to be injected in the body and spread around the body, get taken up into some cells, and the regulators haven't quite told us which cells they get taken up into … you are going to be generating a wide range of responses … with multiple steps each of which could go well or badly.

I doubt the Cult intends it to go well. Yeadon said that you can put any gene you like into the body through the 'vaccine'. 'You can certainly give them a gene that would do them some harm if you wanted.' I was intrigued when he said that when used in the cancer field the technique could turn genes on and off. I explore this process in *The Answer* and with different genes having different functions you could create mayhem – physically and psychologically – if you turned the wrong ones on and the right ones off. I read reports of an experiment by researchers at the University of Washington's school of computer science and engineering in which they encoded DNA to infect computers. The body is itself a biological computer and if human DNA can inflict damage on a computer why can't the computer via synthetic material mess with the human body? It can. The Washington research team said it was possible to insert malicious malware into 'physical DNA strands' and corrupt the computer system of a gene sequencing machine as it 'reads gene letters and stores them as binary digits 0 and 1'. They concluded that hackers could one day use blood or spit samples to access computer systems and obtain sensitive data from police forensics labs or infect genome files. It is at this level of digital interaction that synthetic 'vaccines' need to be seen to get the full picture and that will become very clear later on. Michael Yeadon said it made no sense to give the 'vaccine' to younger people who were in no danger from the 'virus'. What was the benefit? It was all downside with potential effects:

The fact that my government in what I thought was a civilised, rational country, is raining [the 'vaccine'] on people in their 30s and 40s, even my children in their 20s, they're getting letters and phone calls, I know this is not right and any of you doctors who are vaccinating you know it's not right, too. They are not at risk. They are not at risk from the disease, so you are now hoping that the side-effects are so rare that you get away with it. You don't give new technology … that you don't understand to 100 percent of the population.

Blood clot problems with the AstraZeneca ‘vaccine’ have been affecting younger people to emphasise the downside risks with no benefit. AstraZeneca’s version, produced with Oxford University, does not use mRNA, but still gets its toxic cocktail inside cells where it targets DNA. The Johnson & Johnson ‘vaccine’ which uses a similar technique has also produced blood clot effects to such an extent that the United States paused its use at one point. They are all ‘gene therapy’ (cell modification) procedures and not ‘vaccines’. The truth is that once the content of these injections enter cells we have no idea what the effect will be. People can speculate and some can give very educated opinions and that’s good. In the end, though, only the makers know what their potions are designed to do and even they won’t know every last consequence. Michael Yeadon was scathing about doctors doing what they knew to be wrong. ‘Everyone’s mute’, he said. Doctors in the NHS must know this was not right, coming into work and injecting people. ‘I don’t know how they sleep at night. I know I couldn’t do it. I know that if I were in that position I’d have to quit.’ He said he knew enough about toxicology to know this was not a good risk-benefit. Yeadon had spoken to seven or eight university professors and all except two would not speak out publicly. Their universities had a policy that no one said anything that countered the government and its medical advisors. They were afraid of losing their government grants. This is how intimidation has been used to silence the truth at every level of the system. I say silence, but these people could still speak out if they made that choice. Yeadon called them ‘moral cowards’ – ‘This is about your children and grandchildren’s lives and you have just buggered off and left it.’

‘Variant’ nonsense

Some of his most powerful comments related to the alleged ‘variants’ being used to instil more fear, justify more lockdowns, and introduce more ‘vaccines’. He said government claims about ‘variants’ were nonsense. He had checked the alleged variant ‘codes’ and they were 99.7 percent identical to the ‘original’. This was the human identity difference equivalent to putting a baseball cap on and off or wearing it the other way round. A 0.3 percent difference would make it impossible for that ‘variant’ to escape immunity from the ‘original’. This made no sense of having new ‘vaccines’ for ‘variants’. He said there would have to be at least a *30 percent*

difference for that to be justified and even then he believed the immune system would still recognise what it was. Gates-funded ‘variant modeller’ and ‘vaccine’-pusher John Edmunds might care to comment. Yeadon said drug companies were making new versions of the ‘vaccine’ as a ‘top up’ for ‘variants’. Worse than that, he said, the ‘regulators’ around the world like the MHRA in the UK had got together and agreed that because ‘vaccines’ for ‘variants’ were so similar to the first ‘vaccines’ *they did not have to do safety studies*. How transparently sinister that is. This is when Yeadon said: ‘There is a conspiracy here.’ There was no need for another vaccine for ‘variants’ and yet we were told that there was and the country had shut its borders because of them. ‘They are going into hundreds of millions of arms without passing ‘go’ or any regulator. Why did they do that? Why did they pick this method of making the vaccine?’

The reason had to be something bigger than that it seemed and ‘it’s not protection against the virus’. It’s was a far bigger project that meant politicians and advisers were willing to do things and not do things that knowingly resulted in avoidable deaths – ‘that’s already happened when you think about lockdown and deprivation of health care for a year.’ He spoke of people prepared to do something that results in the avoidable death of their fellow human beings and it not bother them. This is the penny-drop I have been working to get across for more than 30 years – the level of pure evil we are dealing with. Yeadon said his friends and associates could not believe there could be that much evil, but he reminded them of Stalin, Pol Pot and Hitler and of what Stalin had said: ‘One death is a tragedy. A million? A statistic.’ He could not think of a benign explanation for why you need top-up vaccines ‘which I’m sure you don’t’ and for the regulators ‘to just get out of the way and wave them through’. Why would the regulators do that when they were still wrestling with the dangers of the ‘parent’ vaccine? He was clearly shocked by what he had seen since the ‘Covid’ hoax began and now he was thinking the previously unthinkable:

If you wanted to depopulate a significant proportion of the world and to do it in a way that doesn’t involve destruction of the environment with nuclear weapons, poisoning everyone with anthrax or something like that, and you wanted plausible deniability while you had a multi-year infectious disease crisis, I actually don’t think you could come up with a better plan of work than seems to be in front of me. I can’t say that’s what they are going to do, but I can’t think of a benign explanation why they are doing it.

He said he never thought that they would get rid of 99 percent of humans, but now he wondered. ‘If you wanted to that this would be a hell of a way to do it – it would be unstoppable folks.’ Yeadon had concluded that those who submitted to the ‘vaccine’ would be allowed to have some kind of normal life (but for how long?) while screws were tightened to coerce and mandate the last few percent. ‘I think they’ll put the rest of them in a prison camp. I wish I was wrong, but I don’t think I am.’ Other points he made included: There were no coronavirus vaccines then suddenly they all come along at the same time; we have no idea of the long term affect with trials so short; coercing or forcing people to have medical procedures is against the Nuremberg Code instigated when the Nazis did just that; people should at least delay having the ‘vaccine’; a quick Internet search confirms that masks don’t reduce respiratory viral transmission and ‘the government knows that’; they have smashed civil society and they know that, too; two dozen peer-reviewed studies show no connection between lockdown and reducing deaths; he knew from personal friends the elite were still flying around and going on holiday while the public were locked down; the elite were not having the ‘vaccines’. He was also asked if ‘vaccines’ could be made to target difference races. He said he didn’t know, but the document by the Project for the New American Century in September, 2000, said developing ‘advanced forms of biological warfare that can target *specific genotypes* may transform biological warfare from the realm of terror to a politically useful tool.’ Oh, they’re evil all right. Of that we can be *absolutely* sure.

Another cull of old people

We have seen from the CDC definition that the mRNA ‘Covid vaccine’ is not a vaccine and nor are the others that *claim* to reduce ‘severity of symptoms’ in *some* people, but not protect from infection or transmission. What about all the lies about returning to ‘normal’ if people were ‘vaccinated’? If they are not claimed to stop infection and transmission of the alleged ‘virus’, how does anything change? This was all lies to manipulate people to take the jabs and we are seeing that now with masks and distancing still required for the ‘vaccinated’. How did they think that elderly people with fragile health and immune responses were going to be affected by infusing their cells with synthetic material and other toxic

substances? They *knew* that in the short and long term it would be devastating and fatal as the culling of the old that began with the first lockdowns was continued with the 'vaccine'. Death rates in care homes soared immediately residents began to be 'vaccinated' – infused with synthetic material. Brave and committed whistleblower nurses put their careers at risk by exposing this truth while the rest kept their heads down and their mouths shut to put their careers before those they are supposed to care for. A long-time American Certified Nursing Assistant who gave his name as James posted a video in which he described emotionally what happened in his care home when vaccination began. He said that during 2020 very few residents were sick with 'Covid' and no one died during the entire year; but shortly after the Pfizer mRNA injections 14 people died within two weeks and many others were near death. 'They're dropping like flies', he said. Residents who walked on their own before the shot could no longer and they had lost their ability to conduct an intelligent conversation. The home's management said the sudden deaths were caused by a 'super-spreader' of 'Covid-19'. Then how come, James asked, that residents who refused to take the injections were not sick? It was a case of inject the elderly with mRNA synthetic potions and blame their illness and death that followed on the 'virus'. James described what was happening in care homes as 'the greatest crime of genocide this country has ever seen'. Remember the NHS staff nurse from earlier who used the same word 'genocide' for what was happening with the 'vaccines' and that it was an 'act of human annihilation'. A UK care home whistleblower told a similar story to James about the effect of the 'vaccine' in deaths and 'outbreaks' of illness dubbed 'Covid' after getting the jab. She told how her care home management and staff had zealously imposed government regulations and no one was allowed to even question the official narrative let alone speak out against it. She said the NHS was even worse. Again we see the results of reframing. A worker at a local care home where I live said they had not had a single case of 'Covid' there for almost a year and when the residents were 'vaccinated' they had 19 positive cases in two weeks with eight dying.

It's not the 'vaccine' – honest

The obvious cause and effect was being ignored by the media and most of the public. Australia's health minister Greg Hunt (a former head of strategy

at the World Economic Forum) was admitted to hospital after he had the ‘vaccine’. He was suffering according to reports from the skin infection ‘cellulitis’ and it must have been a severe case to have warranted days in hospital. Immediately the authorities said this was nothing to do with the ‘vaccine’ when an effect of some vaccines is a ‘cellulitis-like reaction’. We had families of perfectly healthy old people who died after the ‘vaccine’ saying that if only they had been given the ‘vaccine’ earlier they would still be alive. As a numbskull rating that is off the chart. A father of four ‘died of Covid’ at aged 48 when he was taken ill two days after having the ‘vaccine’. The man, a health administrator, had been ‘shielding during the pandemic’ and had ‘not really left the house’ until he went for the ‘vaccine’. Having the ‘vaccine’ and then falling ill and dying does not seem to have qualified as a possible cause and effect and ‘Covid-19’ went on his death certificate. His family said they had no idea how he ‘caught the virus’. A family member said: ‘Tragically, it could be that going for a vaccination ultimately led to him catching Covid …The sad truth is that they are never going to know where it came from.’ The family warned people to remember that the virus still existed and was ‘very real’. So was their stupidity. Nurses and doctors who had the first round of the ‘vaccine’ were collapsing, dying and ending up in a hospital bed while they or their grieving relatives were saying they’d still have the ‘vaccine’ again despite what happened. I kid you not. You mean if your husband returned from the dead he’d have the same ‘vaccine’ again that killed him??

Doctors at the VCU Medical Center in Richmond, Virginia, said the Johnson & Johnson ‘vaccine’ was to blame for a man’s skin peeling off. Patient Richard Terrell said: ‘It all just happened so fast. My skin peeled off. It’s still coming off on my hands now.’ He said it was stinging, burning and itching and when he bent his arms and legs it was very painful with ‘the skin swollen and rubbing against itself’. Pfizer/BioNTech and Moderna vaccines use mRNA to change the cell while the Johnson & Johnson version uses DNA in a process similar to AstraZeneca’s technique. Johnson & Johnson and AstraZeneca have both had their ‘vaccines’ paused by many countries after causing serious blood problems. Terrell’s doctor Fnu Nutan said he could have died if he hadn’t got medical attention. It sounds terrible so what did Nutan and Terrell say about the ‘vaccine’ now? Oh, they still recommend that people have it. A nurse in a hospital bed 40 minutes after the vaccination and unable to swallow due to throat swelling was told by a

doctor that he lost mobility in his arm for 36 hours following the vaccination. What did he say to the ailing nurse? ‘Good for you for getting the vaccination.’ We are dealing with a serious form of cognitive dissonance madness in both public and medical staff. There is a remarkable correlation between those having the ‘vaccine’ and trumpeting the fact and suffering bad happenings shortly afterwards. Witold Rogiewicz, a Polish doctor, made a video of his ‘vaccination’ and ridiculed those who were questioning its safety and the intentions of Bill Gates: ‘Vaccinate yourself to protect yourself, your loved ones, friends and also patients. And to mention quickly I have info for anti-vaxxers and anti-Coviders if you want to contact Bill Gates you can do this through me.’ He further ridiculed the dangers of 5G. Days later he was dead, but naturally the vaccination wasn’t mentioned in the verdict of ‘heart attack’.

Lies, lies and more lies

So many members of the human race have slipped into extreme states of insanity and unfortunately they include reframed doctors and nursing staff. Having a ‘vaccine’ and dying within minutes or hours is not considered a valid connection while death from any cause within 28 days or longer of a positive test with a test not testing for the ‘virus’ means ‘Covid-19’ goes on the death certificate. How could that ‘vaccine’-death connection not have been made except by calculated deceit? US figures in the initial rollout period to February 12th, 2020, revealed that a third of the deaths reported to the CDC after ‘Covid vaccines’ happened within 48 hours. Five men in the UK suffered an ‘extremely rare’ blood clot problem after having the AstraZeneca ‘vaccine’, but no causal link was established said the Gates-funded Medicines and Healthcare products Regulatory Agency (MHRA) which had given the ‘vaccine’ emergency approval to be used. Former Pfizer executive Dr Michael Yeadon explained in his interview how the procedures could cause blood coagulation and clots. People who should have been at no risk were dying from blood clots in the brain and he said he had heard from medical doctor friends that people were suffering from skin bleeding and massive headaches. The AstraZeneca ‘shot’ was stopped by some 20 countries over the blood clotting issue and still the corrupt MHRA, the European Medicines Agency (EMA) and the World Health Organization said that it should continue to be given even though the EMA admitted that

it ‘still cannot rule out definitively’ a link between blood clotting and the ‘vaccine’. Later Marco Cavaleri, head of EMA vaccine strategy, said there was indeed a clear link between the ‘vaccine’ and thrombosis, but they didn’t know why. So much for the trials showing the ‘vaccine’ is safe. Blood clots were affecting younger people who would be under virtually no danger from ‘Covid’ even if it existed which makes it all the more stupid and sinister.

The British government responded to public alarm by wheeling out June Raine, the terrifyingly weak infant school headmistress sound-alike who heads the UK MHRA drug ‘regulator’. The idea that she would stand up to Big Pharma and government pressure is laughable and she told us that all was well in the same way that she did when allowing untested, never-used-on-humans-before, genetically-manipulating ‘vaccines’ to be exposed to the public in the first place. Mass lying is the new normal of the ‘Covid’ era. The MHRA later said 30 cases of rare blood clots had by then been connected with the AstraZeneca ‘vaccine’ (that means a lot more in reality) while stressing that the benefits of the jab in preventing ‘Covid-19’ outweighed any risks. A more ridiculous and disingenuous statement with callous disregard for human health it is hard to contemplate. Immediately after the mendacious ‘all-clears’ two hospital workers in Denmark experienced blood clots and cerebral haemorrhaging following the AstraZeneca jab and one died. Top Norwegian health official Pål Andre Holme said the ‘vaccine’ was the only common factor: ‘There is nothing in the patient history of these individuals that can give such a powerful immune response … I am confident that the antibodies that we have found are the cause, and I see no other explanation than it being the vaccine which triggers it.’ Strokes, a clot or bleed in the brain, were clearly associated with the ‘vaccine’ from word of mouth and whistleblower reports. Similar consequences followed with all these ‘vaccines’ that we were told were so safe and as the numbers grew by the day it was clear we were witnessing human carnage.

Learning the hard way

A woman interviewed by UKColumn told how her husband suffered dramatic health effects after the vaccine when he’d been in good health all his life. He went from being a little unwell to losing all feeling in his legs

and experiencing ‘excruciating pain’. Misdiagnosis followed twice at Accident and Emergency (an ‘allergy’ and ‘sciatica’) before he was admitted to a neurology ward where doctors said his serious condition had been caused by the ‘vaccine’. Another seven ‘vaccinated’ people were apparently being treated on the same ward for similar symptoms. The woman said he had the ‘vaccine’ because they believed media claims that it was safe. ‘I didn’t think the government would give out a vaccine that does this to somebody; I believed they would be bringing out a vaccination that would be safe.’ What a tragic way to learn that lesson. Another woman posted that her husband was transporting stroke patients to hospital on almost every shift and when he asked them if they had been ‘vaccinated’ for ‘Covid’ they all replied ‘yes’. One had a ‘massive brain bleed’ the day after his second dose. She said her husband reported the ‘just been vaccinated’ information every time to doctors in A and E only for them to ignore it, make no notes and appear annoyed that it was even mentioned. This particular report cannot be verified, but it expresses a common theme that confirms the monumental underreporting of ‘vaccine’ consequences. Interestingly as the ‘vaccines’ and their brain blood clot/stroke consequences began to emerge the UK National Health Service began a publicity campaign telling the public what to do in the event of a stroke. A Scottish NHS staff nurse who quit in disgust in March, 2021, said:

> I have seen traumatic injuries from the vaccine, they’re not getting reported to the yellow card [adverse reaction] scheme, they’re treating the symptoms, not asking why, why it’s happening. It’s just treating the symptoms and when you speak about it you’re dismissed like you’re crazy, I’m not crazy, I’m not crazy because every other colleague I’ve spoken to is terrified to speak out, they’ve had enough.

Videos appeared on the Internet of people uncontrollably shaking after the ‘vaccine’ with no control over muscles, limbs and even their face. A Scottish mother broke out in a severe rash all over her body almost immediately after she was given the AstraZeneca ‘vaccine’. The pictures were horrific. Leigh King, a 41-year-old hairdresser from Lanarkshire said: ‘Never in my life was I prepared for what I was about to experience … My skin was so sore and constantly hot … I have never felt pain like this …’ But don’t you worry, the ‘vaccine’ is perfectly safe. Then there has been the effect on medical staff who have been pressured to have the ‘vaccine’ by

psychopathic ‘health’ authorities and government. A London hospital consultant who gave the name K. Polyakova wrote this to the *British Medical Journal* or *BMJ*:

> I am currently struggling with … the failure to report the reality of the morbidity caused by our current vaccination program within the health service and staff population. The levels of sickness after vaccination is unprecedented and staff are getting very sick and some with neurological symptoms which is having a huge impact on the health service function. Even the young and healthy are off for days, some for weeks, and some requiring medical treatment. Whole teams are being taken out as they went to get vaccinated together.
>
> Mandatory vaccination in this instance is stupid, unethical and irresponsible when it comes to protecting our staff and public health. We are in the voluntary phase of vaccination, and encouraging staff to take an unlicensed product that is impacting on their immediate health … it is clearly stated that these vaccine products do not offer immunity or stop transmission. In which case why are we doing it?

Not to protect health that’s for sure. Medical workers are lauded by governments for agenda reasons when they couldn’t give a toss about them any more than they can for the population in general. Schools across America faced the same situation as they closed due to the high number of teachers and other staff with bad reactions to the Pfizer/BioNTech, Moderna, and Johnson & Johnson ‘Covid vaccines’ all of which were linked to death and serious adverse effects. The *BMJ* took down the consultant’s comments pretty quickly on the grounds that they were being used to spread ‘disinformation’. They were exposing the truth about the ‘vaccine’ was the real reason. The cover-up is breathtaking.

Hiding the evidence

The scale of the ‘vaccine’ death cover-up worldwide can be confirmed by comparing official figures with the personal experience of the public. I heard of many people in my community who died immediately or soon after the vaccine that would never appear in the media or even likely on the official totals of ‘vaccine’ fatalities and adverse reactions when only about ten percent are estimated to be reported and I have seen some estimates as low as one percent in a Harvard study. In the UK alone by April 29th, 2021, some 757,654 adverse reactions had been officially reported from the Pfizer/BioNTech, Oxford/AstraZeneca and Moderna ‘vaccines’ with more

than a thousand deaths linked to jabs and that means an estimated ten times this number in reality from a ten percent reporting rate percentage. That's seven million adverse reactions and 10,000 potential deaths and a one percent reporting rate would be ten times *those* figures. In 1976 the US government pulled the swine flu vaccine after *53* deaths. The UK data included a combined 10,000 eye disorders from the 'Covid vaccines' with more than 750 suffering visual impairment or blindness and again multiply by the estimated reporting percentages. As 'Covid cases' officially fell hospitals virtually empty during the 'Covid crisis' began to fill up with a range of other problems in the wake of the 'vaccine' rollout. The numbers across America have also been catastrophic. Deaths linked to *all* types of vaccine increased by *6,000 percent* in the first quarter of 2021 compared with 2020. A 39-year-old woman from Ogden, Utah, died four days after receiving a second dose of Moderna's 'Covid vaccine' when her liver, heart and kidneys all failed despite the fact that she had no known medical issues or conditions. Her family sought an autopsy, but Dr Erik Christensen, Utah's chief medical examiner, said proving vaccine injury as a cause of death almost never happened. He could think of only one instance where an autopsy would name a vaccine as the official cause of death and that would be anaphylaxis where someone received a vaccine and died almost instantaneously. 'Short of that, it would be difficult for us to definitively say this is the vaccine,' Christensen said. If that is true this must be added to the estimated ten percent (or far less) reporting rate of vaccine deaths and serious reactions and the conclusion can only be that vaccine deaths and serious reactions – including these 'Covid' potions' – are phenomenally understated in official figures. The same story can be found everywhere. Endless accounts of deaths and serious reactions among the public, medical and care home staff while official figures did not even begin to reflect this.

Professional script-reader Dr David Williams, a 'top public-health official' in Ontario, Canada, insulted our intelligence by claiming only four serious adverse reactions and no deaths from the more than 380,000 vaccine doses then given. This bore no resemblance to what people knew had happened in their owns circles and we had Dirk Huyer in charge of getting millions vaccinated in Ontario while at the same time he was Chief Coroner for the province investigating causes of death including possible death from the vaccine. An aide said he had stepped back from investigating deaths, but evidence indicated otherwise. Rosemary Frei, who secured a Master of

Science degree in molecular biology at the Faculty of Medicine at Canada's University of Calgary before turning to investigative journalism, was one who could see that official figures for 'vaccine' deaths and reactions made no sense. She said that doctors seldom reported adverse events and when people got really sick or died after getting a vaccination they would attribute that to anything except the vaccines. It had been that way for years and anyone who wondered aloud whether the 'Covid vaccines' or other shots cause harm is immediately branded as 'anti-vax' and 'anti-science'. This was 'career-threatening' for health professionals. Then there was the huge pressure to support the push to 'vaccinate' billions in the quickest time possible. Frei said:

So that's where we're at today. More than half a million vaccine doses have been given to people in Ontario alone. The rush is on to vaccinate all 15 million of us in the province by September. And the mainstream media are screaming for this to be sped up even more. That all adds up to only a very slim likelihood that we're going to be told the truth by officials about how many people are getting sick or dying from the vaccines.

What is true of Ontario is true of everywhere.

They KNEW – and still did it

The authorities knew what was going to happen with multiple deaths and adverse reactions. The UK government's Gates-funded and Big Pharma-dominated Medicines and Healthcare products Regulatory Agency (MHRA) hired a company to employ AI in compiling the projected reactions to the 'vaccine' that would otherwise be uncountable. The request for applications said: 'The MHRA urgently seeks an Artificial Intelligence (AI) software tool to process the expected high volume of Covid-19 vaccine Adverse Drug Reaction …' This was from the agency, headed by the disingenuous June Raine, that gave the 'vaccines' emergency approval and the company was hired before the first shot was given. 'We are going to kill and maim you – is that okay?' 'Oh, yes, perfectly fine – I'm very grateful, thank you, doctor.' The range of 'Covid vaccine' adverse reactions goes on for page after page in the MHRA criminally underreported 'Yellow Card' system and includes affects to eyes, ears, skin, digestion, blood and so on. Raine's MHRA amazingly claimed that the 'overall safety experience … is

so far as expected from the clinical trials'. The death, serious adverse effects, deafness and blindness were *expected?* When did they ever mention that? If these human tragedies were expected then those that gave approval for the use of these 'vaccines' must be guilty of crimes against humanity including murder – a definition of which is 'killing a person with malice aforethought or with recklessness manifesting extreme indifference to the value of human life.' People involved at the MHRA, the CDC in America and their equivalent around the world must go before Nuremberg trials to answer for their callous inhumanity. We are only talking here about the immediate effects of the 'vaccine'. The longer-term impact of the DNA synthetic manipulation is the main reason they are so hysterically desperate to inoculate the entire global population in the shortest possible time.

Africa and the developing world are a major focus for the 'vaccine' depopulation agenda and a mass vaccination sales-pitch is underway thanks to caring people like the Rockefellers and other Cult assets. The Rockefeller Foundation, which pre-empted the 'Covid pandemic' in a document published in 2010 that 'predicted' what happened a decade later, announced an initial $34.95 million grant in February, 2021, 'to ensure more equitable access to Covid-19 testing and vaccines' among other things in Africa in collaboration with '24 organizations, businesses, and government agencies'. The pan-Africa initiative would focus on 10 countries: Burkina Faso, Ethiopia, Ghana, Kenya, Nigeria, Rwanda, South Africa, Tanzania, Uganda, and Zambia'. Rajiv Shah, President of the Rockefeller Foundation and former administrator of CIA-controlled USAID, said that if Africa was not mass-vaccinated (to change the DNA of its people) it was a 'threat to all of humanity' and not fair on Africans. When someone from the Rockefeller Foundation says they want to do something to help poor and deprived people and countries it is time for a belly-laugh. They are doing this out of the goodness of their 'heart' because 'vaccinating' the entire global population is what the 'Covid' hoax set out to achieve. Official 'decolonisation' of Africa by the Cult was merely a prelude to financial colonisation on the road to a return to physical colonisation. The 'vaccine' is vital to that and the sudden and convenient death of the 'Covid' sceptic president of Tanzania can be seen in its true light. A lot of people in Africa are aware that this is another form of colonisation and exploitation and they need to stand their ground.

The ‘vaccine is working’ scam

A potential problem for the Cult was that the ‘vaccine’ is meant to change human DNA and body messaging and not to protect anyone from a ‘virus’ never shown to exist. The vaccine couldn’t work because it was not designed to work and how could they make it *appear* to be working so that more people would have it? This was overcome by lowering the amplification rate of the PCR test to produce fewer ‘cases’ and therefore fewer ‘deaths’. Some of us had been pointing out since March, 2020, that the amplification rate of the test not testing for the ‘virus’ had been made artificially high to generate positive tests which they could call ‘cases’ to justify lockdowns. The World Health Organization recommended an absurdly high 45 amplification cycles to ensure the high positives required by the Cult and then remained silent on the issue until January 20th, 2021 – Biden’s Inauguration Day. This was when the ‘vaccinations’ were seriously underway and on that day the WHO recommended after discussions with America’s CDC that laboratories *lowered their testing amplification*. Dr David Samadi, a certified urologist and health writer, said the WHO was encouraging all labs to reduce their cycle count for PCR tests. He said the current cycle was much too high and was ‘resulting in any particle being declared a positive case’. Even one mainstream news report I saw said this meant the number of ‘Covid’ infections may have been ‘dramatically inflated’. Oh, just a little bit. The CDC in America issued new guidance to laboratories in April, 2021, to use 28 cycles *but only for ‘vaccinated’ people*. The timing of the CDC/WHO interventions were cynically designed to make it appear the ‘vaccines’ were responsible for falling cases and deaths when the real reason can be seen in the following examples. New York’s state lab, the Wadsworth Center, identified 872 positive tests in July, 2020, based on a threshold of 40 cycles. When the figure was lowered to 35 cycles *43 percent* of the 872 were no longer ‘positives’. At 30 cycles the figure was 63 percent. A Massachusetts lab found that between *85 to 90 percent* of people who tested positive in July with a cycle threshold of 40 would be negative at 30 cycles, Ashish Jha, MD, director of the Harvard Global Health Institute, said: ‘I’m really shocked that it could be that high … Boy, does it really change the way we need to be thinking about testing.’ I’m shocked that I could see the obvious in the spring of 2020, with no medical background, and most medical professionals still haven’t worked it out. No, that’s not shocking – it’s terrifying.

Three weeks after the WHO directive to lower PCR cycles the London *Daily Mail* ran this headline: 'Why ARE Covid cases plummeting? New infections have fallen 45% in the US and 30% globally in the past 3 weeks but experts say vaccine is NOT the main driver because only 8% of Americans and 13% of people worldwide have received their first dose.' They acknowledged that the drop could not be attributed to the 'vaccine', but soon this morphed throughout the media into the 'vaccine' has caused cases and deaths to fall when it was the PCR threshold. In December, 2020, there was chaos at English Channel ports with truck drivers needing negative 'Covid' tests before they could board a ferry home for Christmas. The government wanted to remove the backlog as fast as possible and they brought in troops to do the 'testing'. Out of 1,600 drivers just *36* tested positive and the rest were given the all clear to cross the Channel. I guess the authorities thought that 36 was the least they could get away with without the unquestioning catching on. The amplification trick which most people believed in the absence of information in the mainstream applied more pressure on those refusing the 'vaccine' to succumb when it 'obviously worked'. The truth was the exact opposite with deaths in care homes soaring with the 'vaccine' and in Israel the term used was 'skyrocket'. A re-analysis of published data from the Israeli Health Ministry led by Dr Hervé Seligmann at the Medicine Emerging Infectious and Tropical Diseases at Aix-Marseille University found that Pfizer's 'Covid vaccine' killed 'about 40 times more [elderly] people than the disease itself would have killed' during a five-week vaccination period and *260 times* more younger people than would have died from the 'virus' even according to the manipulated 'virus' figures. Dr Seligmann and his co-study author, Haim Yativ, declared after reviewing the Israeli 'vaccine' death data: 'This is a new Holocaust.'

Then, in mid-April, 2021, after vast numbers of people worldwide had been 'vaccinated', the story changed with clear coordination. The UK government began to prepare the ground for more future lockdowns when Nuremberg-destined Boris Johnson told yet another whopper. He said that cases had fallen because of *lockdowns* not 'vaccines'. Lockdowns are irrelevant when *there is no 'virus'* and the test and fraudulent death certificates are deciding the number of 'cases' and 'deaths'. Study after study has shown that lockdowns don't work and instead kill and psychologically destroy people. Meanwhile in the United States Anthony

Fauci and Rochelle Walensky, the ultra-Zionist head of the CDC, peddled the same line. More lockdown was the answer and not the 'vaccine', a line repeated on cue by the moron that is Canadian Prime Minister Justin Trudeau. Why all the hysteria to get everyone 'vaccinated' if lockdowns and not 'vaccines' made the difference? None of it makes sense on the face of it. Oh, but it does. The Cult wants lockdowns *and* the 'vaccine' and if the 'vaccine' is allowed to be seen as the total answer lockdowns would no longer be justified when there are still livelihoods to destroy. 'Variants' and renewed upward manipulation of PCR amplification are planned to instigate never-ending lockdown *and* more 'vaccines'.

You *must* have it – we're desperate

Israel, where the Jewish and Arab population are ruled by the Sabbatian Cult, was the front-runner in imposing the DNA-manipulating 'vaccine' on its people to such an extent that Jewish refusers began to liken what was happening to the early years of Nazi Germany. This would seem to be a fantastic claim. Why would a government of Jewish people be acting like the Nazis did? If you realise that the Sabbatian Cult was behind the Nazis and that Sabbatians hate Jews the pieces start to fit and the question of why a 'Jewish' government would treat Jews with such callous disregard for their lives and freedom finds an answer. Those controlling the government of Israel *aren't Jewish* – they're Sabbatian. Israeli lawyer Tamir Turgal was one who made the Nazi comparison in comments to German lawyer Reiner Fuellmich who is leading a class action lawsuit against the psychopaths for crimes against humanity. Turgal described how the Israeli government was vaccinating children and pregnant women on the basis that there was no evidence that this was dangerous when they had no evidence that it *wasn't* dangerous either. They just had no evidence. This was medical experimentation and Turgal said this breached the Nuremberg Code about medical experimentation and procedures requiring informed consent and choice. Think about that. A Nuremberg Code developed because of Nazi experimentation on Jews and others in concentration camps by people like the evil-beyond-belief Josef Mengele is being breached by the *Israeli* government; but when you know that it's a *Sabbatian* government along with its intelligence and military agencies like Mossad, Shin Bet and the Israeli Defense Forces, and that Sabbatians were the force behind the Nazis,

the kaleidoscope comes into focus. What have we come to when Israeli Jews are suing their government for violating the Nuremberg Code by essentially making Israelis subject to a medical experiment using the controversial ‘vaccines’? It’s a shocker that this has to be done in the light of what happened in Nazi Germany. The Anshe Ha-Emet, or ‘People of the Truth’, made up of Israeli doctors, lawyers, campaigners and public, have launched a lawsuit with the International Criminal Court. It says:

When the heads of the Ministry of Health as well as the prime minister presented the vaccine in Israel and began the vaccination of Israeli residents, the vaccinated were not advised, that, in practice, they are taking part in a medical experiment and that their consent is required for this under the Nuremberg Code.

The irony is unbelievable, but easily explained in one word: Sabbatians. The foundation of Israeli ‘Covid’ apartheid is the ‘green pass’ or ‘green passport’ which allows Jews and Arabs who have had the DNA-manipulating ‘vaccine’ to go about their lives – to work, fly, travel in general, go to shopping malls, bars, restaurants, hotels, concerts, gyms, swimming pools, theatres and sports venues, while non-’vaccinated’ are banned from all those places and activities. Israelis have likened the ‘green pass’ to the yellow stars that Jews in Nazi Germany were forced to wear – the same as the yellow stickers that a branch of UK supermarket chain Morrisons told exempt mask-wears they had to display when shopping. How very sensitive. The Israeli system is blatant South African-style apartheid on the basis of compliance or non-compliance to fascism rather than colour of the skin. How appropriate that the Sabbatian Israeli government was so close to the pre-Mandela apartheid regime in Pretoria. The Sabbatian-instigated ‘vaccine passport’ in Israel is planned for everywhere. Sabbatians struck a deal with Pfizer that allowed them to lead the way in the percentage of a national population infused with synthetic material and the result was catastrophic. Israeli freedom activist Shai Dannon told me how chairs were appearing on beaches that said ‘vaccinated only’. Health Minister Yuli Edelstein said that anyone unwilling or unable to get the jabs that ‘confer immunity’ will be ‘left behind’. The man’s a liar. Not even the makers claim the ‘vaccines’ confer immunity. When you see those figures of ‘vaccine’ deaths these psychopaths were saying that you must take the chance the ‘vaccine’ will kill you or maim

you while knowing it will change your DNA or lockdown for you will be permanent. That's fascism. The Israeli parliament passed a law to allow personal information of the non-vaccinated to be shared with local and national authorities for three months. This was claimed by its supporters to be a way to 'encourage' people to be vaccinated. Hadas Ziv from Physicians for Human Rights described this as a 'draconian law which crushed medical ethics and the patient rights'. But that's the idea, the Sabbatians would reply.

Your papers, please

Sabbatian Israel was leading what has been planned all along to be a global 'vaccine pass' called a 'green passport' without which you would remain in permanent lockdown restriction and unable to do anything. This is how badly – *desperately* – the Cult is to get everyone 'vaccinated'. The term and colour 'green' was not by chance and related to the psychology of fusing the perception of the green climate hoax with the 'Covid' hoax and how the 'solution' to both is the same Great Reset. Lying politicians, health officials and psychologists denied there were any plans for mandatory vaccinations or restrictions based on vaccinations, but they knew that was exactly what was meant to happen with governments of all countries reaching agreements to enforce a global system. 'Free' Denmark and 'free' Sweden unveiled digital vaccine certification. Cyprus, Czech Republic, Estonia, Greece, Hungary, Iceland, Italy, Poland, Portugal, Slovakia, and Spain have all committed to a vaccine passport system and the rest including the whole of the EU would follow. The satanic UK government will certainly go this way despite mendacious denials and at the time of writing it is trying to manipulate the public into having the 'vaccine' so they could go abroad on a summer holiday. How would that work without something to prove you had the synthetic toxicity injected into you? Documents show that the EU's European Commission was moving towards 'vaccine certificates' in 2018 and 2019 before the 'Covid' hoax began. They knew what was coming. Abracadabra – Ursula von der Leyen, the German President of the Commission, announced in March, 2021, an EU 'Digital Green Certificate' – green again – to track the public's 'Covid status'. The passport sting is worldwide and the Far East followed the same pattern with South Korea

ruling that only those with ‘vaccination’ passports – again the *green* pass – would be able to ‘return to their daily lives’.

Bill Gates has been preparing for this ‘passport’ with other Cult operatives for years and beyond the paper version is a Gates-funded ‘digital tattoo’ to identify who has been vaccinated and who hasn’t. The ‘tattoo’ is reported to include a substance which is externally readable to confirm who has been vaccinated. This is a bio-luminous light-generating enzyme (think fireflies) called … *Luciferase*. Yes, named after the Cult ‘god’ Lucifer the ‘light bringer’ of whom more to come. Gates said he funded the readable tattoo to ensure children in the developing world were vaccinated and no one was missed out. He cares so much about poor kids as we know. This was just the cover story to develop a vaccine tagging system for everyone on the planet. Gates has been funding the ID2020 ‘alliance’ to do just that in league with other lovely people at Microsoft, GAVI, the Rockefeller Foundation, Accenture and IDEO.org. He said in interviews in March, 2020, before any ‘vaccine’ publicly existed, that the world must have a globalised digital certificate to track the ‘virus’ and who had been vaccinated. Gates knew from the start that the mRNA vaccines were coming and when they would come and that the plan was to tag the ‘vaccinated’ to marginalise the intelligent and stop them doing anything including travel. Evil just doesn’t suffice. Gates was exposed for offering a $10 million bribe to the Nigerian House of Representatives to invoke compulsory ‘Covid’ vaccination of all Nigerians. Sara Cunial, a member of the Italian Parliament, called Gates a ‘vaccine criminal’. She urged the Italian President to hand him over to the International Criminal Court for crimes against humanity and condemned his plans to ‘chip the human race’ through ID2020.

You know it’s a long-planned agenda when war criminal and Cult gofer Tony Blair is on the case. With the scale of arrogance only someone as dark as Blair can muster he said: ‘Vaccination in the end is going to be your route to liberty.’ Blair is a disgusting piece of work and he confirms that again. The media has given a lot of coverage to a bloke called Charlie Mullins, founder of London’s biggest independent plumbing company, Pimlico Plumbers, who has said he won’t employ anyone who has not been vaccinated or have them go to any home where people are not vaccinated. He said that if he had his way no one would be allowed to walk the streets if they have not been vaccinated. Gates was cheering at the time while I was

alerting the white coats. The plan is that people will qualify for ‘passports’ for having the first two doses and then to keep it they will have to have all the follow ups and new ones for invented ‘variants’ until human genetics is transformed and many are dead who can’t adjust to the changes. Hollywood celebrities – the usual propaganda stunt – are promoting something called the WELL Health-Safety Rating to verify that a building or space has ‘taken the necessary steps to prioritize the health and safety of their staff, visitors and other stakeholders’. They included Lady Gaga, Jennifer Lopez, Michael B. Jordan, Robert DeNiro, Venus Williams, Wolfgang Puck, Deepak Chopra and 17th Surgeon General Richard Carmona. Yawn. WELL Health-Safety has big connections with China. Parent company Delos is headed by former Goldman Sachs partner Paul Scialla. This is another example – and we will see so many others – of using the excuse of ‘health’ to dictate the lives and activities of the population. I guess one confirmation of the ‘safety’ of buildings is that only ‘vaccinated’ people can go in, right?

Electronic concentration camps

I wrote decades ago about the plans to restrict travel and here we are for those who refuse to bow to tyranny. This can be achieved in one go with air travel if the aviation industry makes a blanket decree. The ‘vaccine’ and guaranteed income are designed to be part of a global version of China’s social credit system which tracks behaviour 24/7 and awards or deletes ‘credits’ based on whether your behaviour is supported by the state or not. I mean your entire lifestyle – what you do, eat, say, everything. Once your credit score falls below a certain level consequences kick in. In China tens of millions have been denied travel by air and train because of this. All the locations and activities denied to refusers by the ‘vaccine’ passports will be included in one big mass ban on doing almost anything for those that don’t bow their head to government. It’s beyond fascist and a new term is required to describe its extremes – I guess fascist technocracy will have to do. The way the Chinese system of technological – technocratic – control is sweeping the West can be seen in the Los Angeles school system and is planned to be expanded worldwide. Every child is required to have a ‘Covid’-tracking app scanned daily before they can enter the classroom. The so-called Daily Pass tracking system is produced by Gates’ Microsoft which I’m sure will shock you rigid. The pass will be scanned using a

barcode (one step from an inside-the-body barcode) and the information will include health checks, ‘Covid’ tests and vaccinations. Entry codes are for one specific building only and access will only be allowed if a student or teacher has a negative test with a test not testing for the ‘virus’, has no symptoms of anything alleged to be related to ‘Covid’ (symptoms from a range of other illness), and has a temperature under 100 degrees. No barcode, no entry, is planned to be the case for everywhere and not only schools.

Kids are being psychologically prepared to accept this as ‘normal’ their whole life which is why what they can impose in schools is so important to the Cult and its gofers. Long-time American freedom campaigner John Whitehead of the Rutherford Institute was not exaggerating when he said: ‘Databit by databit, we are building our own electronic concentration camps.’ Canada under its Cult gofer prime minister Justin Trudeau has taken a major step towards the real thing with people interned against their will if they test positive with a test not testing for the ‘virus’ when they arrive at a Canadian airport. They are jailed in internment hotels often without food or water for long periods and with many doors failing to lock there have been sexual assaults. The interned are being charged sometimes $2,000 for the privilege of being abused in this way. Trudeau is fully on board with the Cult and says the ‘Covid pandemic’ has provided an opportunity for a global ‘reset’ to permanently change Western civilisation. His number two, Deputy Prime Minister Chrystia Freeland, is a trustee of the World Economic Forum and a Rhodes Scholar. The Trudeau family have long been servants of the Cult. See *The Biggest Secret* and Cathy O’Brien’s book *Trance-Formation of America* for the horrific background to Trudeau’s father Pierre Trudeau another Canadian prime minister. Hide your fascism behind the façade of a heart-on-the-sleeve liberal. It’s a well-honed Cult technique.

What can the ‘vaccine’ *really* do?

We have a ‘virus’ never shown to exist and ‘variants’ of the ‘virus’ that have also never been shown to exist except, like the ‘original’, as computer-generated fictions. Even if you believe there’s a ‘virus’ the ‘case’ to ‘death’ rate is in the region of 0.23 to 0.15 percent and those ‘deaths’ are concentrated among the very old around the same average age that people

die anyway. In response to this lack of threat (in truth none) psychopaths and idiots, knowingly and unknowingly answering to Gates and the Cult, are seeking to 'vaccinate' every man, woman and child on Planet Earth. Clearly the 'vaccine' is not about 'Covid' – none of this ever has been. So what is it all about *really*? Why the desperation to infuse genetically-manipulating synthetic material into everyone through mRNA fraudulent 'vaccines' with the intent of doing this over and over with the excuses of 'variants' and other 'virus' inventions? Dr Sherri Tenpenny, an osteopathic medical doctor in the United States, has made herself an expert on vaccines and their effects as a vehement campaigner against their use. Tenpenny was board certified in emergency medicine, the director of a level two trauma centre for 12 years, and moved to Cleveland in 1996 to start an integrative medicine practice which has treated patients from all 50 states and some 17 other countries. Weaning people off pharmaceutical drugs is a speciality.

She became interested in the consequences of vaccines after attending a meeting at the National Vaccine Information Center in Washington DC in 2000 where she 'sat through four days of listening to medical doctors and scientists and lawyers and parents of vaccine injured kids' and asked: 'What's going on?' She had never been vaccinated and never got ill while her father was given a list of vaccines to be in the military and was 'sick his entire life'. The experience added to her questions and she began to examine vaccine documents from the Centers for Disease Control (CDC). After reading the first one, the 1998 version of *The General Recommendations of Vaccination*, she thought: 'This is it?' The document was poorly written and bad science and Tenpenny began 20 years of research into vaccines that continues to this day. She began her research into 'Covid vaccines' in March, 2020, and she describes them as 'deadly'. For many, as we have seen, they already have been. Tenpenny said that in the first 30 days of the 'vaccine' rollout in the United States there had been more than 40,000 adverse events reported to the vaccine adverse event database. A document had been delivered to her the day before that was 172 pages long. 'We have over 40,000 adverse events; we have over 3,100 cases of [potentially deadly] anaphylactic shock; we have over 5,000 neurological reactions.' Effects ranged from headaches to numbness, dizziness and vertigo, to losing feeling in hands or feet and paraesthesia which is when limbs 'fall asleep' and people have the sensation of insects crawling underneath their skin. All this happened in the first 30 days and remember

that only about *ten percent* (or far less) of adverse reactions and vaccine-related deaths are estimated to be officially reported. Tenpenny said:

So can you think of one single product in any industry, any industry, for as long as products have been made on the planet that within 30 days we have 40,000 people complaining of side effects that not only is still on the market but … we've got paid actors telling us how great they are for getting their vaccine. We're offering people $500 if they will just get their vaccine and we've got nurses and doctors going; 'I got the vaccine, I got the vaccine'.

Tenpenny said they were not going to be 'happy dancing folks' when they began to suffer Bell's palsy (facial paralysis), neuropathies, cardiac arrhythmias and autoimmune reactions that kill through a blood disorder. 'They're not going to be so happy, happy then, but we're never going to see pictures of those people' she said. Tenpenny described the 'vaccine' as 'a well-designed killing tool'.

No off-switch

Bad as the initial consequences had been Tenpenny said it would be maybe 14 months before we began to see the 'full ravage' of what is going to happen to the 'Covid vaccinated' with full-out consequences taking anything between two years and 20 years to show. You can understand why when you consider that variations of the 'Covid vaccine' use mRNA (messenger RNA) to in theory activate the immune system to produce protective antibodies without using the actual 'virus'. How can they when it's a computer program and they've never isolated what they claim is the 'real thing'? Instead they use *synthetic* mRNA. They are inoculating synthetic material into the body which through a technique known as the Trojan horse is absorbed into cells to change the nature of DNA. Human DNA is changed by an infusion of messenger RNA and with each new 'vaccine' of this type it is changed even more. Say so and you are banned by Cult Internet platforms. The contempt the contemptuous Mark Zuckerberg has for the truth and human health can be seen in an internal Facebook video leaked to the Project Veritas investigative team in which he said of the 'Covid vaccines': '… I share some caution on this because we just don't know the long term side-effects of basically modifying people's DNA and RNA.' At the same time this disgusting man's Facebook was

censoring and banning anyone saying exactly the same. He must go before a Nuremberg trial for crimes against humanity when he *knows* that he is censoring legitimate concerns and denying the right of informed consent on behalf of the Cult that owns him. People have been killed and damaged by the very 'vaccination' technique he cast doubt on himself when they may not have had the 'vaccine' with access to information that he denied them. The plan is to have at least annual 'Covid vaccinations', add others to deal with invented 'variants', and change all other vaccines into the mRNA system. Pfizer executives told shareholders at a virtual Barclays Global Healthcare Conference in March, 2021, that the public may need a third dose of 'Covid vaccine', plus regular yearly boosters and the company planned to hike prices to milk the profits in a 'significant opportunity for our vaccine'. These are the professional liars, cheats and opportunists who are telling you their 'vaccine' is safe. Given this volume of mRNA planned to be infused into the human body and its ability to then replicate we will have a transformation of human genetics from biological to synthetic biological – exactly the long-time Cult plan for reasons we'll see – and many will die. Sherri Tenpenny said of this replication:

> It's like having an on-button but no off-button and that whole mechanism … they actually give it a name and they call it the Trojan horse mechanism, because it allows that [synthetic] virus and that piece of that [synthetic] virus to get inside of your cells, start to replicate and even get inserted into other parts of your DNA as a Trojan-horse.

Ask the overwhelming majority of people who have the 'vaccine' what they know about the contents and what they do and they would reply: 'The government says it will stop me getting the virus.' Governments give that false impression on purpose to increase take-up. You can read Sherri Tenpenny's detailed analysis of the health consequences in her blog at Vaxxter.com, but in summary these are some of them. She highlights the statement by Bill Gates about how human beings can become their own 'vaccine manufacturing machine'. The man is insane. ['Vaccine'-generated] 'antibodies' carry synthetic messenger RNA into the cells and the damage starts, Tenpenny contends, and she says that lungs can be adversely affected through varying degrees of pus and bleeding which obviously affects breathing and would be dubbed 'Covid-19'. Even more sinister was the impact of 'antibodies' on macrophages, a white blood cell of the immune

system. They consist of Type 1 and Type 2 which have very different functions. She said Type 1 are ‘hyper-vigilant’ white blood cells which ‘gobble up’ bacteria etc. However, in doing so, this could cause inflammation and in extreme circumstances be fatal. She says these affects are mitigated by Type 2 macrophages which kick in to calm down the system and stop it going rogue. They clear up dead tissue debris and reduce inflammation that the Type 1 ‘fire crews’ have caused. Type 1 kills the infection and Type 2 heals the damage, she says. This is her punchline with regard to ‘Covid vaccinations’: She says that mRNA ‘antibodies’ block Type 2 macrophages by attaching to them and deactivating them. This meant that when the Type 1 response was triggered by infection there was nothing to stop that getting out of hand by calming everything down. There’s an on-switch, but no off-switch, she says. What follows can be ‘over and out, see you when I see you’.

Genetic suicide

Tenpenny also highlights the potential for autoimmune disease – the body attacking itself – which has been associated with vaccines since they first appeared. Infusing a synthetic foreign substance into cells could cause the immune system to react in a panic believing that the body is being overwhelmed by an invader (it is) and the consequences can again be fatal. There is an autoimmune response known as a ‘cytokine storm’ which I have likened to a homeowner panicked by an intruder and picking up a gun to shoot randomly in all directions before turning the fire on himself. The immune system unleashes a storm of inflammatory response called cytokines to a threat and the body commits hara-kiri. The lesson is that you mess with the body’s immune response at your peril and these ‘vaccines’ seriously – fundamentally – mess with immune response. Tenpenny refers to a consequence called anaphylactic shock which is a severe and highly dangerous allergic reaction when the immune system floods the body with chemicals. She gives the example of having a bee sting which primes the immune system and makes it sensitive to those chemicals. When people are stung again maybe years later the immune response can be so powerful that it leads to anaphylactic shock. Tenpenny relates this ‘shock’ with regard to the ‘Covid vaccine’ to something called polyethylene glycol or PEG. Enormous numbers of people have become sensitive to this over decades of

use in a whole range of products and processes including food, drink, skin creams and ‘medicine’. Studies have claimed that some 72 percent of people have antibodies triggered by PEG compared with two percent in the 1960s and allergic hypersensitive reactions to this become a gathering cause for concern. Tenpenny points out that the ‘mRNA vaccine’ is coated in a ‘bubble’ of polyethylene glycol which has the potential to cause anaphylactic shock through immune sensitivity. Many reports have appeared of people reacting this way after having the ‘Covid vaccine’. What do we think is going to happen as humanity has more and more of these ‘vaccines’? Tenpenny said: ‘All these pictures we have seen with people with these rashes … these weepy rashes, big reactions on their arms and things like that – it’s an acute allergic reaction most likely to the polyethylene glycol that you’ve been previously primed and sensitised to.’

Those who have not studied the conspiracy and its perpetrators at length might think that making the population sensitive to PEG and then putting it in these ‘vaccines’ is just a coincidence. It is not. It is instead testament to how carefully and coldly-planned current events have been and the scale of the conspiracy we are dealing with. Tenpenny further explains that the ‘vaccine’ mRNA procedure can breach the blood-brain barrier which protects the brain from toxins and other crap that will cause malfunction. In this case they could make two proteins corrupt brain function to cause Amyotrophic lateral sclerosis (ALS) , a progressive nervous system disease leading to loss of muscle control, and frontal lobe degeneration – Alzheimer’s and dementia. Immunologist J. Bart Classon published a paper connecting mRNA ‘vaccines’ to prion disease which can lead to Alzheimer’s and other forms of neurogenerative disease while others have pointed out the potential to affect the placenta in ways that make women infertile. This will become highly significant in the next chapter when I will discuss other aspects of this non-vaccine that relate to its nanotechnology and transmission from the injected to the uninjected.

Qualified in idiocy

Tenpenny describes how research has confirmed that these ‘vaccine’-generated antibodies can interact with a range of other tissues in the body and attack many other organs including the lungs. ‘This means that if you have a hundred people standing in front of you that all got this shot they

could have a hundred different symptoms.' Anyone really think that Cult gofers like the Queen, Tony Blair, Christopher Whitty, Anthony Fauci, and all the other psychopaths have really had this 'vaccine' in the pictures we've seen? Not a bloody chance. Why don't doctors all tell us about all these dangers and consequences of the 'Covid vaccine'? Why instead do they encourage and pressure patients to have the shot? Don't let's think for a moment that doctors and medical staff can't be stupid, lazy, and psychopathic and that's without the financial incentives to give the jab. Tenpenny again:

Some people are going to die from the vaccine directly but a large number of people are going to start to get horribly sick and get all kinds of autoimmune diseases 42 days to maybe a year out. What are they going to do, these stupid doctors who say; 'Good for you for getting that vaccine.' What are they going to say; 'Oh, it must be a mutant, we need to give an extra dose of that vaccine.'

Because now the vaccine, instead of one dose or two doses we need three or four because the stupid physicians aren't taking the time to learn anything about it. If I can learn this sitting in my living room reading a 19 page paper and several others so can they. There's nothing special about me, I just take the time to do it.

Remember how Sara Kayat, the NHS and TV doctor, said that the 'Covid vaccine' would '100 percent prevent hospitalisation and death'. Doctors can be idiots like every other profession and they should not be worshipped as infallible. They are not and far from it. Behind many medical and scientific 'experts' lies an uninformed prat trying to hide themselves from you although in the 'Covid' era many have failed to do so as with UK narrative-repeating 'TV doctor' Hilary Jones. Pushing back against the minority of proper doctors and scientists speaking out against the 'vaccine' has been the entire edifice of the Cult global state in the form of governments, medical systems, corporations, mainstream media, Silicon Valley, and an army of compliant doctors, medical staff and scientists willing to say anything for money and to enhance their careers by promoting the party line. If you do that you are an 'expert' and if you won't you are an 'anti-vaxxer' and 'Covidiot'. The pressure to be 'vaccinated' is incessant. We have even had reports claiming that the 'vaccine' can help cure cancer and Alzheimer's and make the lame walk. I am waiting for the announcement that it can bring you coffee in the morning and cook your tea. Just as the symptoms of 'Covid' seem to increase by the week so have the miracles of the 'vaccine'.

American supermarket giant Kroger Co. offered nearly 500,000 employees in 35 states a $100 bonus for having the ‘vaccine’ while donut chain Krispy Kreme promised ‘vaccinated’ customers a free glazed donut every day for the rest of 2021. Have your DNA changed and you will get a doughnut although we might not have to give you them for long. Such offers and incentives confirm the desperation.

Perhaps the worse vaccine-stunt of them all was UK ‘Health’ Secretary Matt-the-prat Hancock on live TV after watching a clip of someone being ‘vaccinated’ when the roll-out began. Hancock faked tears so badly it was embarrassing. Brain-of-Britain Piers Morgan, the lockdown-supporting, ‘vaccine’ supporting, ‘vaccine’ passport-supporting, TV host played along with Hancock – ‘You’re quite emotional about that’ he said in response to acting so atrocious it would have been called out at a school nativity which will presumably today include Mary and Jesus in masks, wise men keeping their camels six feet apart, and shepherds under tent arrest. System-serving Morgan tweeted this: ‘Love the idea of covid vaccine passports for everywhere: flights, restaurants, clubs, football, gyms, shops etc. It’s time covid-denying, anti-vaxxer loonies had their bullsh*t bluff called & bar themselves from going anywhere that responsible citizens go.’ If only I could aspire to his genius. To think that Morgan, who specialises in shouting over anyone he disagrees with, was lauded as a free speech hero when he lost his job after storming off the set of his live show like a child throwing his dolly out of the pram. If he is a free speech hero we are in real trouble. I have no idea what ‘bullsh*t’ means, by the way, the * throws me completely.

The Cult is desperate to infuse its synthetic DNA-changing concoction into everyone and has been using every lie, trick and intimidation to do so. The question of ‘*Why*?’ we shall now address.

CHAPTER TEN

Human 2.0

I believe that at the end of the century the use of words and general educated opinion will have altered so much that one will be able to speak of machines thinking without expecting to be contradicted –
Alan Turing (1912-1954), the 'Father of artificial intelligence'

I have been exposing for decades the plan to transform the human body from a biological to a synthetic-biological state. The new human that I will call Human 2.0 is planned to be connected to artificial intelligence and a global AI 'Smart Grid' that would operate as one global system in which AI would control everything from your fridge to your heating system to your car to your mind. Humans would no longer be 'human', but post-human and sub-human, with their thinking and emotional processes replaced by AI.

What I said sounded crazy and beyond science fiction and I could understand that. To any balanced, rational, mind it *is* crazy. Today, however, that world is becoming reality and it puts the 'Covid vaccine' into its true context. Ray Kurzweil is the ultra-Zionist 'computer scientist, inventor and futurist' and co-founder of the Singularity University. Singularity refers to the merging of humans with machines or 'transhumanism'. Kurzweil has said humanity would be connected to the cyber 'cloud' in the period of the ever-recurring year of 2030:

Our thinking … will be a hybrid of biological and non-biological thinking … humans will be able to extend their limitations and 'think in the cloud' … We're going to put gateways to the cloud in our

brains ... We're going to gradually merge and enhance ourselves ... In my view, that's the nature of being human – we transcend our limitations. As the technology becomes vastly superior to what we are then the small proportion that is still human gets smaller and smaller and smaller until it's just utterly negligible.

They are trying to sell this end-of-humanity-as-we-know-it as the next stage of 'evolution' when we become super-human and 'like the gods'. They are lying to you. Shocked, eh? The population, and again especially the young, have been manipulated into addiction to technologies designed to enslave them for life. First they induced an addiction to smartphones (holdables); next they moved to technology on the body (wearables); and then began the invasion of the body (implantables). I warned way back about the plan for microchipped people and we are now entering that era. We should not be diverted into thinking that this refers only to chips we can see. Most important are the nanochips known as smart dust, neural dust and nanobots which are far too small to be seen by the human eye. Nanotechnology is everywhere, increasingly in food products, and released into the atmosphere by the geoengineering of the skies funded by Bill Gates to 'shut out the Sun' and 'save the planet from global warming'. Gates has been funding a project to spray millions of tonnes of chalk (calcium carbonate) into the stratosphere over Sweden to 'dim the Sun' and cool the Earth. Scientists warned the move could be disastrous for weather systems in ways no one can predict and opposition led to the Swedish space agency announcing that the 'experiment' would not be happening as planned in the summer of 2021; but it shows where the Cult is going with dimming the impact of the Sun and there's an associated plan to change the planet's atmosphere. Who gives psychopath Gates the right to dictate to the entire human race and dismantle planetary systems? The world will not be safe while this man is at large.

The global warming hoax has made the Sun, like the gas of life, something to fear when both are essential to good health and human survival (more inversion). The body transforms sunlight into vital vitamin D through a process involving … *cholesterol*. This is the cholesterol we are also told to fear. We are urged to take Big Pharma statin drugs to reduce cholesterol and it's all systematic. Reducing cholesterol means reducing vitamin D uptake with all the multiple health problems that will cause. At least if you take statins long term it saves the government from having to

pay you a pension. The delivery system to block sunlight is widely referred to as chemtrails although these have a much deeper agenda, too. They appear at first to be contrails or condensation trails streaming from aircraft into cold air at high altitudes. Contrails disperse very quickly while chemtrails do not and spread out across the sky before eventually their content falls to earth. Many times I have watched aircraft cross-cross a clear blue sky releasing chemtrails until it looks like a cloudy day. Chemtrails contain many things harmful to humans and the natural world including toxic heavy metals, aluminium (see Alzheimer's) and nanotechnology. Ray Kurzweil reveals the reason without actually saying so: 'Nanobots will infuse all the matter around us with information. Rocks, trees, everything will become these intelligent creatures.' How do you deliver that? *From the sky*. Self-replicating nanobots would connect everything to the Smart Grid. The phenomenon of Morgellons disease began in the chemtrail era and the correlation has led to it being dubbed the 'chemtrail disease'. Self-replicating fibres appear in the body that can be pulled out through the skin. Morgellons fibres continue to grow outside the body and have a form of artificial intelligence. I cover this at greater length in *Phantom Self*.

'Vaccine' operating system

'Covid vaccines' with their self-replicating synthetic material are also designed to make the connection between humanity and Kurzweil's 'cloud'. American doctor and dedicated campaigner for truth, Carrie Madej, an Internal Medicine Specialist in Georgia with more than 20 years medical experience, has highlighted the nanotechnology aspect of the fake 'vaccines'. She explains how one of the components in at least the Moderna and Pfizer synthetic potions are 'lipid nanoparticles' which are 'like little tiny computer bits' – a 'sci-fi substance' known as nanobots and hydrogel which can be 'triggered at any moment to deliver its payload' and act as 'biosensors'. The synthetic substance had 'the ability to accumulate data from your body like your breathing, your respiration, thoughts and emotions, all kind of things' and each syringe could carry a *million* nanobots:

This substance because it's like little bits of computers in your body, crazy, but it's true, it can do that, [and] obviously has the ability to act through Wi-Fi. It can receive and transmit energy,

messages, frequencies or impulses. That issue has never been addressed by these companies. What does that do to the human?

Just imagine getting this substance in you and it can react to things all around you, the 5G, your smart device, your phones, what is happening with that? What if something is triggering it, too, like an impulse, a frequency? We have something completely foreign in the human body.

Madej said her research revealed that electromagnetic (EMF) frequencies emitted by phones and other devices had increased dramatically in the same period of the 'vaccine' rollout and she was seeing more people with radiation problems as 5G and other electromagnetic technology was expanded and introduced to schools and hospitals. She said she was 'floored with the EMF coming off' the devices she checked. All this makes total sense and syncs with my own work of decades when you think that Moderna refers in documents to its mRNA 'vaccine' as an 'operating system':

Recognizing the broad potential of mRNA science, we set out to create an mRNA technology platform that functions very much like an operating system on a computer. It is designed so that it can plug and play interchangeably with different programs. In our case, the 'program' or 'app' is our mRNA drug – the unique mRNA sequence that codes for a protein …

… Our MRNA Medicines – 'The 'Software Of Life': When we have a concept for a new mRNA medicine and begin research, fundamental components are already in place. Generally, the only thing that changes from one potential mRNA medicine to another is the coding region – the actual genetic code that instructs ribosomes to make protein. Utilizing these instruction sets gives our investigational mRNA medicines a software-like quality. We also have the ability to combine different mRNA sequences encoding for different proteins in a single mRNA investigational medicine.

Who needs a real 'virus' when you can create a computer version to justify infusing your operating system into the entire human race on the road to making living, breathing people into cyborgs? What is missed with the 'vaccines' is the *digital* connection between synthetic material and the body that I highlighted earlier with the study that hacked a computer with human DNA. On one level the body is digital, based on mathematical codes, and I'll have more about that in the next chapter. Those who ridiculously claim that mRNA 'vaccines' are not designed to change human genetics should explain the words of Dr Tal Zaks, chief medical officer at Moderna, in a

2017 TED talk. He said that over the last 30 years 'we've been living this phenomenal digital scientific revolution, and I'm here today to tell you, that we are actually *hacking the software of life*, and that it's changing the way we think about prevention and treatment of disease':

In every cell there's this thing called messenger RNA, or mRNA for short, that transmits the critical information from the DNA in our genes to the protein, which is really the stuff we're all made out of. This is the critical information that determines what the cell will do. So we think about it as an operating system. So if you could change that, if you could introduce a line of code, or change a line of code, it turns out, that has profound implications for everything, from the flu to cancer.

Zaks should more accurately have said that this has profound implications for the human genetic code and the nature of DNA. Communications within the body go both ways and not only one. But, hey, no, the 'Covid vaccine' will not affect your genetics. Cult fact-checkers say so even though the man who helped to develop the mRNA technique says that it does. Zaks said in 2017:

If you think about what it is we're trying to do. We've taken information and our understanding of that information and how that information is transmitted in a cell, and we've taken our understanding of medicine and how to make drugs, and we're fusing the two. We think of it as information therapy.

I have been writing for decades that the body is an information field communicating with itself and the wider world. This is why radiation which is information can change the information field of body and mind through phenomena like 5G and change their nature and function. 'Information therapy' means to change the body's information field and change the way it operates. DNA is a receiver-transmitter of information and can be mutated by information like mRNA synthetic messaging. Technology to do this has been ready and waiting in the underground bases and other secret projects to be rolled out when the 'Covid' hoax was played. 'Trials' of such short and irrelevant duration were only for public consumption. When they say the 'vaccine' is 'experimental' that is not true. It may appear to be 'experimental' to those who don't know what's going on, but the trials have already been done to ensure the Cult gets the result it desires. Zaks said that it took decades to sequence the human genome, completed in 2003, but now

they could do it in a week. By ‘they’ he means scientists operating in the public domain. In the secret projects they were sequencing the genome in a week long before even 2003.

Deluge of mRNA

Highly significantly the Moderna document says the guiding premise is that if using mRNA as a medicine works for one disease then it should work for many diseases. They were leveraging the flexibility afforded by their platform and the fundamental role mRNA plays in protein synthesis to pursue mRNA medicines for a broad spectrum of diseases. Moderna is confirming what I was saying through 2020 that multiple ‘vaccines’ were planned for ‘Covid’ (and later invented ‘variants’) and that previous vaccines would be converted to the mRNA system to infuse the body with massive amounts of genetically-manipulating synthetic material to secure a transformation to a synthetic-biological state. The ‘vaccines’ are designed to kill stunning numbers as part of the long-exposed Cult depopulation agenda and transform the rest. Given this is the goal you can appreciate why there is such hysterical demand for every human to be ‘vaccinated’ for an alleged ‘disease’ that has an estimated ‘infection’ to ‘death’ ratio of 0.23-0.15 percent. As I write children are being given the ‘vaccine’ in trials (their parents are a disgrace) and ever-younger people are being offered the vaccine for a ‘virus’ that even if you believe it exists has virtually zero chance of harming them. Horrific effects of the ‘trials’ on a 12-year-old girl were revealed by a family member to be serious brain and gastric problems that included a bowel obstruction and the inability to swallow liquids or solids. She was unable to eat or drink without throwing up, had extreme pain in her back, neck and abdomen, and was paralysed from the waist down which stopped her urinating unaided. When the girl was first taken to hospital doctors said it was all in her mind. She was signed up for the ‘trial’ by her parents for whom no words suffice. None of this ‘Covid vaccine’ insanity makes any sense unless you see what the ‘vaccine’ really is – a body-changer. Synthetic biology or ‘SynBio’ is a fast-emerging and expanding scientific discipline which includes everything from genetic and molecular engineering to electrical and computer engineering. Synthetic biology is defined in these ways:

• A multidisciplinary area of research that seeks to create new biological parts, devices, and systems, or to redesign systems that are already found in nature.

• The use of a mixture of physical engineering and genetic engineering to create new (and therefore synthetic) life forms.

• An emerging field of research that aims to combine the knowledge and methods of biology, engineering and related disciplines in the design of chemically-synthesized DNA to create organisms with novel or enhanced characteristics and traits (synthetic organisms including humans).

We now have synthetic blood, skin, organs and limbs being developed along with synthetic body parts produced by 3D printers. These are all elements of the synthetic human programme and this comment by Kurzweil's co-founder of the Singularity University, Peter Diamandis, can be seen in a whole new light with the 'Covid' hoax and the sanctions against those that refuse the 'vaccine':

Anybody who is going to be resisting the progress forward [to transhumanism] is going to be resisting evolution and, fundamentally, they will die out. It's not a matter of whether it's good or bad. It's going to happen.

'Resisting evolution'? What absolute bollocks. The arrogance of these people is without limit. His 'it's going to happen' mantra is another way of saying 'resistance is futile' to break the spirit of those pushing back and we must not fall for it. Getting this genetically-transforming 'vaccine' into everyone is crucial to the Cult plan for total control and the desperation to achieve that is clear for anyone to see. Vaccine passports are a major factor in this and they, too, are a form of resistance is futile. It's NOT. The paper funded by the Rockefeller Foundation for the 2013 'health conference' in China said:

We will interact more with artificial intelligence. The use of robotics, bio-engineering to augment human functioning is already well underway and will advance. Re-engineering of humans into

potentially separate and unequal forms through genetic engineering or mixed human-robots raises debates on ethics and equality.

A new demography is projected to emerge after 2030 [that year again] of technologies (robotics, genetic engineering, nanotechnology) producing robots, engineered organisms, 'nanobots' and artificial intelligence (AI) that can self-replicate. Debates will grow on the implications of an impending reality of human designed life.

What is happening today is so long planned. The world army enforcing the will of the world government is intended to be a robot army, not a human one. Today's military and its technologically 'enhanced' troops, pilotless planes and driverless vehicles are just stepping stones to that end. Human soldiers are used as Cult fodder and its time they woke up to that and worked for the freedom of the population instead of their own destruction and their family's destruction – the same with the police. Join us and let's sort this out. The phenomenon of enforce my own destruction is widespread in the 'Covid' era with Woker 'luvvies' in the acting and entertainment industries supporting 'Covid' rules which have destroyed their profession and the same with those among the public who put signs on the doors of their businesses 'closed due to Covid – stay safe' when many will never reopen. It's a form of masochism and most certainly insanity.

Transgender = transhumanism

When something explodes out of nowhere and is suddenly everywhere it is always the Cult agenda and so it is with the tidal wave of claims and demands that have infiltrated every aspect of society under the heading of 'transgenderism'. The term 'trans' is so 'in' and this is the dictionary definition:

A prefix meaning 'across', 'through', occurring … in loanwords from Latin, used in particular for denoting movement or conveyance from place to place (transfer; transmit; transplant) or complete change (transform; transmute), or to form adjectives meaning 'crossing', 'on the other side of', or 'going beyond' the place named (transmontane; transnational; trans-Siberian).

Transgender means to go beyond gender and transhuman means to go beyond human. Both are aspects of the Cult plan to transform the human body to a synthetic state with *no gender*. Human 2.0 is not designed to

procreate and would be produced technologically with no need for parents. The new human would mean the end of parents and so men, and increasingly women, are being targeted for the deletion of their rights and status. Parental rights are disappearing at an ever-quickening speed for the same reason. The new human would have no need for men or women when there is no procreation and no gender. Perhaps the transgender movement that appears to be in a permanent state of frenzy might now contemplate on how it is being used. This was never about transgender rights which are only the interim excuse for confusing gender, particularly in the young, on the road to *fusing* gender. Transgender activism is not an end; it is a *means* to an end. We see again the technique of creative destruction in which you destroy the status quo to 'build back better' in the form that you want. The gender status quo had to be destroyed by persuading the Cult-created Woke mentality to believe that you can have 100 genders or more. A programme for 9 to 12 year olds produced by the Cult-owned BBC promoted the 100 genders narrative. The very idea may be the most monumental nonsense, but it is not what is true that counts, only what you can make people *believe* is true. Once the gender of 2 + 2 = 4 has been dismantled through indoctrination, intimidation and 2 + 2 = 5 then the new no-gender normal can take its place with Human 2.0. Aldous Huxley revealed the plan in his prophetic *Brave New World* in 1932:

> Natural reproduction has been done away with and children are created, decanted', and raised in 'hatcheries and conditioning centres'. From birth, people are genetically designed to fit into one of five castes, which are further split into 'Plus' and 'Minus' members and designed to fulfil predetermined positions within the social and economic strata of the World State.

How could Huxley know this in 1932? For the same reason George Orwell knew about the Big Brother state in 1948, Cult insiders I have quoted knew about it in 1969, and I have known about it since the early 1990s. If you are connected to the Cult or you work your balls off to uncover the plan you can predict the future. The process is simple. If there is a plan for the world and nothing intervenes to stop it then it will happen. Thus if you communicate the plan ahead of time you are perceived to have predicted the future, but you haven't. You have revealed the plan which without intervention will become the human future. The whole reason I have done what I have is to alert enough people to inspire an intervention and maybe

at last that time has come with the Cult and its intentions now so obvious to anyone with a brain in working order.

The future is here

Technological wombs that Huxley described to replace parent procreation are already being developed and they are only the projects we know about in the public arena. Israeli scientists told *The Times of Israel* in March, 2021, that they have grown 250-cell embryos into mouse foetuses with fully formed organs using artificial wombs in a development they say could pave the way for gestating humans outside the womb. Professor Jacob Hanna of the Weizmann Institute of Science said:

> We took mouse embryos from the mother at day five of development, when they are just of 250 cells, and had them in the incubator from day five until day 11, by which point they had grown all their organs.
>
> By day 11 they make their own blood and have a beating heart, a fully developed brain. Anybody would look at them and say, 'this is clearly a mouse foetus with all the characteristics of a mouse.' It's gone from being a ball of cells to being an advanced foetus.

A special liquid is used to nourish embryo cells in a laboratory dish and they float on the liquid to duplicate the first stage of embryonic development. The incubator creates all the right conditions for its development, Hanna said. The liquid gives the embryo 'all the nutrients, hormones and sugars they need' along with a custom-made electronic incubator which controls gas concentration, pressure and temperature. The cutting-edge in the underground bases and other secret locations will be light years ahead of that, however, and this was reported by the London *Guardian* in 2017:

> We are approaching a biotechnological breakthrough. Ectogenesis, the invention of a complete external womb, could completely change the nature of human reproduction. In April this year, researchers at the Children's Hospital of Philadelphia announced their development of an artificial womb.

The article was headed ‘Artificial wombs could soon be a reality. What will this mean for women?’ What would it mean for children is an even bigger question. No mother to bond with only a machine in preparation for a life of soulless interaction and control in a world governed by machines (see the *Matrix* movies). Now observe the calculated manipulations of the ‘Covid’ hoax as human interaction and warmth has been curtailed by distancing, isolation and fear with people communicating via machines on a scale never seen before. These are all dots in the same picture as are all the personal assistants, gadgets and children’s toys through which kids and adults communicate with AI as if it is human. The AI ‘voice’ on Sat-Nav should be included. All these things are psychological preparation for the Cult endgame. Before you can make a physical connection with AI you have to make a psychological connection and that is what people are being conditioned to do with this ever gathering human-AI interaction. Movies and TV programmes depicting the transhuman, robot dystopia relate to a phenomenon known as ‘pre-emptive programming’ in which the world that is planned is portrayed everywhere in movies, TV and advertising. This is conditioning the conscious and subconscious mind to become familiar with the planned reality to dilute resistance when it happens for real. What would have been a shock such is the change is made less so. We have young children put on the road to transgender transition surgery with puberty blocking drugs at an age when they could never be able to make those life-changing decisions.

Rachel Levine, a professor of paediatrics and psychiatry who believes in treating children this way, became America’s highest-ranked openly-transgender official when she was confirmed as US Assistant Secretary at the Department of Health and Human Services after being nominated by Joe Biden (the Cult). Activists and governments press for laws to deny parents a say in their children’s transition process so the kids can be isolated and manipulated into agreeing to irreversible medical procedures. A Canadian father Robert Hoogland was denied bail by the Vancouver Supreme Court in 2021 and remained in jail for breaching a court order that he stay silent over his young teenage daughter, a minor, who was being offered life-changing hormone therapy without parental consent. At the age of 12 the girl’s ‘school counsellor’ said she may be transgender, referred her to a doctor and told the school to treat her like a boy. This is another example of state-serving schools imposing ever more control over

children's lives while parents have ever less. Contemptible and extreme child abuse is happening all over the world as the Cult gender-fusion operation goes into warp-speed.

Why the war on men – and now women?

The question about what artificial wombs mean for women should rightly be asked. The answer can be seen in the deletion of women's rights involving sport, changing rooms, toilets and status in favour of people in male bodies claiming to identify as women. I can identify as a mountain climber, but it doesn't mean I can climb a mountain any more than a biological man can be a biological woman. To believe so is a triumph of belief over factual reality which is the very perceptual basis of everything Woke. Women's sport is being destroyed by allowing those with male bodies who say they identify as female to 'compete' with girls and women. Male body 'women' dominate 'women's' competition with their greater muscle mass, bone density, strength and speed. With that disadvantage sport for women loses all meaning. To put this in perspective nearly 300 American high school boys can run faster than the quickest woman sprinter in the world. Women are seeing their previously protected spaces invaded by male bodies simply because they claim to identify as women. That's all they need to do to access all women's spaces and activities under the Biden 'Equality Act' that destroys equality for women with the usual Orwellian Woke inversion. Male sex offenders have already committed rapes in women's prisons after claiming to identify as women to get them transferred. Does this not matter to the Woke 'equality' hypocrites? Not in the least. What matters to Cult manipulators and funders behind transgender activists is to advance gender fusion on the way to the no-gender 'human'. When you are seeking to impose transparent nonsense like this, or the 'Covid' hoax, the only way the nonsense can prevail is through censorship and intimidation of dissenters, deletion of factual information, and programming of the unquestioning, bewildered and naive. You don't have to scan the world for long to see that all these things are happening.

Many women's rights organisations have realised that rights and status which took such a long time to secure are being eroded and that it is systematic. Kara Dansky of the global Women's Human Rights Campaign said that Biden's transgender executive order immediately he took office,

subsequent orders, and Equality Act legislation that followed 'seek to erase women and girls in the law as a category'. *Exactly*. I said during the long ago-started war on men (in which many women play a crucial part) that this was going to turn into a war on them. The Cult is phasing out *both* male and female genders. To get away with that they are brought into conflict so they are busy fighting each other while the Cult completes the job with no unity of response. Unity, people, *unity*. We need unity everywhere. Transgender is the only show in town as the big step towards the no-gender human. It's not about rights for transgender people and never has been. Woke political correctness is deleting words relating to genders to the same end. Wokers believe this is to be 'inclusive' when the opposite is true. They are deleting words describing gender because gender *itself* is being deleted by Human 2.0. Terms like 'man', 'woman', 'mother' and 'father' are being deleted in the universities and other institutions to be replaced by the *no*-gender, not trans-gender, 'individuals' and 'guardians'. Women's rights campaigner Maria Keffler of Partners for Ethical Care said: 'Children are being taught from kindergarten upward that some boys have a vagina, some girls have a penis, and that kids can be any gender they want to be.' Do we really believe that suddenly countries all over the world at the same time had the idea of having drag queens go into schools or read transgender stories to very young children in the local library? It's coldly-calculated confusion of gender on the way to the fusion of gender. Suzanne Vierling, a psychologist from Southern California, made another important point:

> Yesterday's slave woman who endured gynecological medical experiments is today's girl-child being butchered in a booming gender-transitioning sector. Ovaries removed, pushing her into menopause and osteoporosis, uncharted territory, and parents' rights and authority decimated.

The erosion of parental rights is a common theme in line with the Cult plans to erase the very concept of parents and 'ovaries removed, pushing her into menopause' means what? Those born female lose the ability to have children – another way to discontinue humanity as we know it.

Eliminating Human 1.0 (before our very eyes)

To pave the way for Human 2.0 you must phase out Human 1.0. This is happening through plummeting sperm counts and making women infertile through an onslaught of chemicals, radiation (including smartphones in pockets of men) and mRNA 'vaccines'. Common agriculture pesticides are also having a devastating impact on human fertility. I have been tracking collapsing sperm counts in the books for a long time and in 2021 came a book by fertility scientist and reproductive epidemiologist Shanna Swan, *Count Down: How Our Modern World Is Threatening Sperm Counts, Altering Male and Female Reproductive Development and Imperiling the Future of the Human Race*. She reports how the global fertility rate dropped by *half* between 1960 and 2016 with America's birth rate 16 percent below where it needs to be to sustain the population. Women are experiencing declining egg quality, more miscarriages, and more couples suffer from infertility. Other findings were an increase in erectile dysfunction, infant boys developing more genital abnormalities, male problems with conception, and plunging levels of the male hormone testosterone which would explain why so many men have lost their backbone and masculinity. This has been very evident during the 'Covid' hoax when women have been prominent among the Pushbackers and big strapping blokes have bowed their heads, covered their faces with a nappy and quietly submitted. Mind control expert Cathy O'Brien also points to how global education introduced the concept of 'we're all winners' in sport and classrooms: 'Competition was defused, and it in turn defused a sense of fighting back.' This is another version of the 'equity' doctrine in which you drive down rather than raise up. What a contrast in Cult-controlled China with its global ambitions where the government published plans in January, 2021, to 'cultivate masculinity' in boys from kindergarten through to high school in the face of a 'masculinity crisis'. A government adviser said boys would be soon become 'delicate, timid and effeminate' unless action was taken. Don't expect any similar policy in the targeted West. A 2006 study showed that a 65-year-old man in 2002 had testosterone levels *15 percent* lower than a 65-year-old man in 1987 while a 2020 study found a similar story with young adults and adolescents. Men are getting prescriptions for testosterone replacement therapy which causes an even greater drop in sperm count with up to 99 percent seeing sperm counts drop to zero during the treatment. More sperm is defective and malfunctioning with some having two heads or not pursuing an egg.

A class of *synthetic* chemicals known as phthalates are being blamed for the decline. These are found everywhere in plastics, shampoos, cosmetics, furniture, flame retardants, personal care products, pesticides, canned foods and even receipts. Why till receipts? Everyone touches them. Let no one delude themselves that all this is not systematic to advance the long-time agenda for human body transformation. Phthalates mimic hormones and disrupt the hormone balance causing testosterone to fall and genital birth defects in male infants. Animals and fish have been affected in the same way due to phthalates and other toxins in rivers. When fish turn gay or change sex through chemicals in rivers and streams it is a pointer to why there has been such an increase in gay people and the sexually confused. It doesn't matter to me what sexuality people choose to be, but if it's being affected by chemical pollution and consumption then we need to know. Does anyone really think that this is not connected to the transgender agenda, the war on men and the condemnation of male 'toxic masculinity'? You watch this being followed by 'toxic femininity'. It's already happening. When breastfeeding becomes 'chest-feeding', pregnant women become pregnant people along with all the other Woke claptrap you know that the world is going insane and there's a Cult scam in progress. Transgender activists are promoting the Cult agenda while Cult billionaires support and fund the insanity as they laugh themselves to sleep at the sheer stupidity for which humans must be infamous in galaxies far, far away.

'Covid vaccines' and female infertility

We can now see why the 'vaccine' has been connected to potential infertility in women. Dr Michael Yeadon, former Vice President and Chief Scientific Advisor at Pfizer, and Dr Wolfgang Wodarg in Germany, filed a petition with the European Medicines Agency in December, 2020, urging them to stop trials for the Pfizer/BioNTech shot and all other mRNA trials until further studies had been done. They were particularly concerned about possible effects on fertility with 'vaccine'-produced antibodies attacking the protein Syncytin-1 which is responsible for developing the placenta. The result would be infertility 'of indefinite duration' in women who have the 'vaccine' with the placenta failing to form. Section 10.4.2 of the Pfizer/BioNTech trial protocol says that pregnant women or those who might become so should not have mRNA shots. Section 10.4 warns men

taking mRNA shots to 'be abstinent from heterosexual intercourse' and not to donate sperm. The UK government said that it *did not know* if the mRNA procedure had an effect on fertility. *Did not know?* These people have to go to jail. UK government advice did not recommend at the start that pregnant women had the shot and said they should avoid pregnancy for at least two months after 'vaccination'. The 'advice' was later updated to pregnant women should only have the 'vaccine' if the benefits outweighed the risks to mother and foetus. What the hell is that supposed to mean? Then 'spontaneous abortions' began to appear and rapidly increase on the adverse reaction reporting schemes which include only a fraction of adverse reactions. Thousands and ever-growing numbers of 'vaccinated' women are describing changes to their menstrual cycle with heavier blood flow, irregular periods and menstruating again after going through the menopause – all links to reproduction effects. Women are passing blood clots and the lining of their uterus while men report erectile dysfunction and blood effects. Most significantly of all *un*vaccinated women began to report similar menstrual changes after interaction with *'vaccinated'* people and men and children were also affected with bleeding noses, blood clots and other conditions. 'Shedding' is when vaccinated people can emit the content of a vaccine to affect the unvaccinated, but this is different. 'Vaccinated' people were not shedding a 'live virus' allegedly in 'vaccines' as before because the fake 'Covid vaccines' involve synthetic material and other toxicity. Doctors exposing what is happening prefer the term 'transmission' to shedding. Somehow those that have had the shots are transmitting effects to those that haven't. Dr Carrie Madej said the nano-content of the 'vaccines' can 'act like an antenna' to others around them which fits perfectly with my own conclusions. This 'vaccine' transmission phenomenon was becoming known as the book went into production and I deal with this further in the Postscript.

Vaccine effects on sterility are well known. The World Health Organization was accused in 2014 of sterilising millions of women in Kenya with the evidence confirmed by the content of the vaccines involved. The same WHO behind the 'Covid' hoax admitted its involvement for more than ten years with the vaccine programme. Other countries made similar claims. Charges were lodged by Tanzania, Nicaragua, Mexico, and the Philippines. The Gardasil vaccine claimed to protect against a genital 'virus' known as HPV has also been linked to infertility. Big Pharma and

the WHO (same thing) are criminal and satanic entities. Then there's the Bill Gates Foundation which is connected through funding and shared interests with 20 pharmaceutical giants and laboratories. He stands accused of directing the policy of United Nations Children's Fund (UNICEF), vaccine alliance GAVI, and other groupings, to advance the vaccine agenda and silence opposition at great cost to women and children. At the same time Gates wants to reduce the global population. Coincidence?

Great Reset = Smart Grid = new human

The Cult agenda I have been exposing for 30 years is now being openly promoted by Cult assets like Gates and Klaus Schwab of the World Economic Forum under code-terms like the 'Great Reset', 'Build Back Better' and 'a rare but narrow window of opportunity to reflect, reimagine, and reset our world'. What provided this 'rare but narrow window of opportunity'? The 'Covid' hoax did. Who created that? *They* did. My books from not that long ago warned about the planned 'Internet of Things' (IoT) and its implications for human freedom. This was the plan to connect all technology to the Internet and artificial intelligence and today we are way down that road with an estimated 36 billion devices connected to the World Wide Web and that figure is projected to be 76 billion by 2025. I further warned that the Cult planned to go beyond that to the Internet of *Everything* when the human brain was connected via AI to the Internet and Kurzweil's 'cloud'. Now we have Cult operatives like Schwab calling for precisely that under the term 'Internet of Bodies', a fusion of the physical, digital and biological into one centrally-controlled Smart Grid system which the Cult refers to as the 'Fourth Industrial Revolution'. They talk about the 'biological', but they really mean the synthetic-biological which is required to fully integrate the human body and brain into the Smart Grid and artificial intelligence planned to replace the human mind. We have everything being synthetically manipulated including the natural world through GMO and smart dust, the food we eat and the human body itself with synthetic 'vaccines'. I said in *The Answer* that we would see the Cult push for synthetic meat to replace animals and in February, 2021, the so predictable psychopath Bill Gates called for the introduction of synthetic meat to save us all from 'climate change'. The climate hoax just keeps on giving like the 'Covid' hoax. The war on meat by vegan activists is a

carbon (oops, sorry) copy of the manipulation of transgender activists. They have no idea (except their inner core) that they are being used to promote and impose the agenda of the Cult or that they are only the *vehicle* and not the *reason*. This is not to say those who choose not to eat meat shouldn't be respected and supported in that right, but there are ulterior motives for those in power. A *Forbes* article in December, 2019, highlighted the plan so beloved of Schwab and the Cult under the heading: 'What Is The Internet of Bodies? And How Is It Changing Our World?' The article said the human body is the latest data platform (remember 'our vaccine is an operating system'). *Forbes* described the plan very accurately and the words could have come straight out of my books from long before:

The Internet of Bodies (IoB) is an extension of the IoT and basically connects the human body to a network through devices that are ingested, implanted, or connected to the body in some way. Once connected, data can be exchanged, and the body and device can be remotely monitored and controlled.

They were really describing a human hive mind with human perception centrally-dictated via an AI connection as well as allowing people to be 'remotely monitored and controlled'. Everything from a fridge to a human mind could be directed from a central point by these insane psychopaths and 'Covid vaccines' are crucial to this. Forbes explained the process I mentioned earlier of holdable and wearable technology followed by implantable. The article said there were three generations of the Internet of Bodies that include:

- Body external: These are wearable devices such as Apple Watches or Fitbits that can monitor our health.

- Body internal: These include pacemakers, cochlear implants, and digital pills that go inside our bodies to monitor or control various aspects of health.

- Body embedded: The third generation of the Internet of Bodies is embedded technology where technology and the human body are melded together and have a real-time connection to a remote machine.

Forbes noted the development of the Brain Computer Interface (BCI) which merges the brain with an external device for monitoring and controlling in real-time. 'The ultimate goal is to help restore function to individuals with disabilities by using brain signals rather than conventional neuromuscular pathways.' Oh, do fuck off. The goal of brain interface technology is controlling human thought and emotion from the central point in a hive mind serving its masters wishes. Many people are now agreeing to be chipped to open doors without a key. You can recognise them because they'll be wearing a mask, social distancing and lining up for the 'vaccine'. The Cult plans a Great Reset money system after they have completed the demolition of the global economy in which 'money' will be exchanged through communication with body operating systems. Rand Corporation, a Cult-owned think tank, said of the Internet of Bodies or IoB:

Internet of Bodies technologies fall under the broader IoT umbrella. But as the name suggests, IoB devices introduce an even more intimate interplay between humans and gadgets. IoB devices monitor the human body, collect health metrics and other personal information, and transmit those data over the Internet. Many devices, such as fitness trackers, are already in use … IoB devices … and those in development can track, record, and store users' whereabouts, bodily functions, and what they see, hear, and even think.

Schwab's World Economic Forum, a long-winded way of saying 'fascism' or 'the Cult', has gone full-on with the Internet of Bodies in the 'Covid' era. 'We're entering the era of the Internet of Bodies', it declared, 'collecting our physical data via a range of devices that can be implanted, swallowed or worn'. The result would be a huge amount of health-related data that could improve human wellbeing around the world, and prove crucial in fighting the 'Covid-19 pandemic'. Does anyone think these clowns care about 'human wellbeing' after the death and devastation their pandemic hoax has purposely caused? Schwab and co say we should move forward with the Internet of Bodies because 'Keeping track of symptoms could help us stop the spread of infection, and quickly detect new cases'. How wonderful, but keeping track' is all they are really bothered about. Researchers were investigating if data gathered from smartwatches and similar devices could be used as viral infection alerts by tracking the user's heart rate and breathing. Schwab said in his 2018 book *Shaping the Future of the Fourth Industrial Revolution*:

The lines between technologies and beings are becoming blurred and not just by the ability to create lifelike robots or synthetics. Instead it is about the ability of new technologies to literally become part of us. Technologies already influence how we understand ourselves, how we think about each other, and how we determine our realities. As the technologies … give us deeper access to parts of ourselves, we may begin to integrate digital technologies into our bodies.

You can see what the game is. Twenty-four hour control and people – if you could still call them that – would never know when something would go ping and take them out of circulation. It's the most obvious rush to a global fascist dictatorship and the complete submission of humanity and yet still so many are locked away in their Cult-induced perceptual coma and can't see it.

Smart Grid control centres

The human body is being transformed by the 'vaccines' and in other ways into a synthetic cyborg that can be attached to the global Smart Grid which would be controlled from a central point and other sub-locations of Grid manipulation. Where are these planned to be? Well, China for a start which is one of the Cult's biggest centres of operation. The technological control system and technocratic rule was incubated here to be unleashed across the world after the 'Covid' hoax came out of China in 2020. Another Smart Grid location that will surprise people new to this is Israel. I have exposed in *The Trigger* how Sabbatian technocrats, intelligence and military operatives were behind the horrors of 9/11 and not `19 Arab hijackers' who somehow manifested the ability to pilot big passenger airliners when instructors at puddle-jumping flying schools described some of them as a joke. The 9/11 attacks were made possible through control of civilian and military air computer systems and those of the White House, Pentagon and connected agencies. See *The Trigger* – it will blow your mind. The controlling and coordinating force were the Sabbatian networks in Israel and the United States which by then had infiltrated the entire US government, military and intelligence system. The real name of the American Deep State is 'Sabbatian State'. Israel is a tiny country of only nine million people, but it is one of the global centres of cyber operations and fast catching Silicon Valley in importance to the Cult. Israel is known as the 'start-up nation' for all the cyber companies spawned there with the Sabbatian specialisation of 'cyber security' that I mentioned earlier which

gives those companies access to computer systems of their clients in real time through 'backdoors' written into the coding when security software is downloaded. The Sabbatian centre of cyber operations outside Silicon Valley is the Israeli military Cyber Intelligence Unit, the biggest infrastructure project in Israel's history, headquartered in the desert-city of Beersheba and involving some 20,000 'cyber soldiers'. Here are located a literal army of Internet trolls scanning social media, forums and comment lists for anyone challenging the Cult agenda. The UK military has something similar with its 77th Brigade and associated operations. The Beersheba complex includes research and development centres for other Cult operations such as Intel, Microsoft, IBM, Google, Apple, Hewlett-Packard, Cisco Systems, Facebook and Motorola. Techcrunch.com ran an article about the Beersheba global Internet technology centre headlined 'Israel's desert city of Beersheba is turning into a cybertech oasis':

The military's massive relocation of its prestigious technology units, the presence of multinational and local companies, a close proximity to Ben Gurion University and generous government subsidies are turning Beersheba into a major global cybertech hub. Beersheba has all of the ingredients of a vibrant security technology ecosystem, including Ben Gurion University with its graduate program in cybersecurity and Cyber Security Research Center, and the presence of companies such as EMC, Deutsche Telekom, PayPal, Oracle, IBM, and Lockheed Martin. It's also the future home of the INCB (Israeli National Cyber Bureau); offers a special income tax incentive for cyber security companies, and was the site for the relocation of the army's intelligence corps units.

Sabbatians have taken over the cyber world through the following process: They scan the schools for likely cyber talent and develop them at Ben Gurion University and their period of conscription in the Israeli Defense Forces when they are stationed at the Beersheba complex. When the cyber talented officially leave the army they are funded to start cyber companies with technology developed by themselves or given to them by the state. Much of this is stolen through backdoors of computer systems around the world with America top of the list. Others are sent off to Silicon Valley to start companies or join the major ones and so we have many major positions filled by apparently 'Jewish' but really Sabbatian operatives. Google, YouTube and Facebook are all run by 'Jewish' CEOs while Twitter is all but run by ultra-Zionist hedge-fund shark Paul Singer. At the centre of the Sabbatian global cyber web is the Israeli army's Unit 8200 which specialises in hacking into computer systems of other countries,

inserting viruses, gathering information, instigating malfunction, and even taking control of them from a distance. A long list of Sabbatians involved with 9/11, Silicon Valley and Israeli cyber security companies are operatives of Unit 8200. This is not about Israel. It's about the Cult. Israel is planned to be a Smart Grid hub as with China and what is happening at Beersheba is not for the benefit of Jewish people who are treated disgustingly by the Sabbatian elite that control the country. A glance at the Nuremberg Codes will tell you that.

The story is much bigger than 'Covid', important as that is to where we are being taken. Now, though, it's time to really strap in. There's more … much more …

CHAPTER ELEVEN

Who controls the Cult?

Awake, arise or be forever fall'n
John Milton, *Paradise Lost*

I have exposed this far the level of the Cult conspiracy that operates in the world of the seen and within the global secret society and satanic network which operates in the shadows one step back from the seen. The story, however, goes much deeper than that.

The 'Covid' hoax is major part of the Cult agenda, but only part, and to grasp the biggest picture we have to expand our attention beyond the realm of human sight and into the infinity of possibility that we cannot see. It is from here, ultimately, that humanity is being manipulated into a state of total control by the force which dictates the actions of the Cult. How much of reality can we see? Next to damn all is the answer. We may appear to see all there is to see in the 'space' our eyes survey and observe, but little could be further from the truth. The human 'world' is only a tiny band of frequency that the body's visual and perceptual systems can decode into *perception* of a 'world'. According to mainstream science the electromagnetic spectrum is 0.005 percent of what exists in the Universe (Fig 10). The maximum estimate I have seen is 0.5 percent and either way it's miniscule. I say it is far, far, smaller even than 0.005 percent when you compare reality we see with the totality of reality that we don't. Now get this if you are new to such information: Visible light, the only band of frequency that we can see, is a *fraction* of the 0.005 percent (Fig 11 overleaf). Take this further and realise that our universe is one of infinite

universes and that universes are only a fragment of overall reality – *infinite* reality. Then compare that with the almost infinitesimal frequency band of visible light or human sight. You see that humans are as near blind as it is possible to be without actually being so. Artist and filmmaker, Sergio Toporek, said:

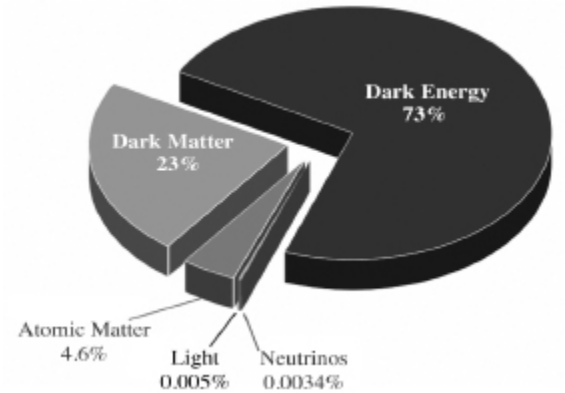

Figure 10: Humans can perceive such a tiny band of visual reality it's laughable.

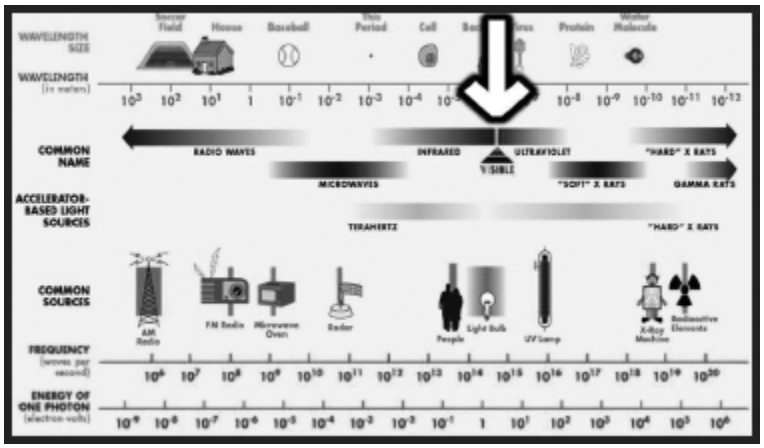

Figure 11: We can see a smear of the 0.005 percent electromagnetic spectrum, but we still know it all. Yep, makes sense.

Consider that you can see less than 1% of the electromagnetic spectrum and hear less than 1% of the acoustic spectrum. 90% of the cells in your body carry their own microbial DNA and are not 'you'. The atoms in your body are 99.9999999999999999% empty space and none of them are the ones you were born with ... Human beings have 46 chromosomes, two less than a potato.

The existence of the rainbow depends on the conical photoreceptors in your eyes; to animals without cones, the rainbow does not exist. So you don't just look at a rainbow, you create it. This is pretty amazing, especially considering that all the beautiful colours you see represent less than 1% of the electromagnetic spectrum.

Suddenly the ‘world’ of humans looks a very different place. Take into account, too, that Planet Earth when compared with the projected size of this single universe is the equivalent of a billionth of a pinhead. Imagine the ratio that would be when compared to infinite reality. To think that Christianity once insisted that Earth and humanity were the centre of everything. This background is vital if we are going to appreciate the nature of ‘human’ and how we can be manipulated by an unseen force. To human visual reality virtually *everything* is unseen and yet the prevailing perception within the institutions and so much of the public is that if we can’t see it, touch it, hear it, taste it and smell it then it cannot exist. Such perception is indoctrinated and encouraged by the Cult and its agents because it isolates believers in the strictly limited, village-idiot, realm of the five senses where perceptions can be firewalled and information controlled. Most of those perpetuating the ‘this-world-is-all-there-is’ insanity are themselves indoctrinated into believing the same delusion. While major players and influencers know that official reality is laughable most of those in science, academia and medicine really believe the nonsense they peddle and teach succeeding generations. Those who challenge the orthodoxy are dismissed as nutters and freaks to protect the manufactured illusion from exposure. Observe the dynamic of the ‘Covid’ hoax and you will see how that takes the same form. The inner-circle psychopaths knows it’s a gigantic scam, but almost the entirety of those imposing their fascist rules believe that ‘Covid’ is all that they’re told it is.

Stolen identity

Ask people who they are and they will give you their name, place of birth, location, job, family background and life story. Yet that is not who they are – it is what they are *experiencing*. The difference is *absolutely crucial*. The true ‘I’, the eternal, infinite ‘I’, is consciousness, a state of being aware. Forget ‘form’. That is a vehicle for a brief experience. Consciousness does not come *from* the brain, but *through* the brain and even that is more symbolic than literal. We are awareness, pure awareness, and this is what withdraws from the body at what we call ‘death’ to continue our eternal beingness, *isness*, in other realms of reality within the limitlessness of infinity or the Biblical ‘many mansions in my father’s house’. Labels of a human life, man, woman, transgender, black, white, brown, nationality,

circumstances and income are not who we are. They are what we are – awareness – is *experiencing* in a brief connection with a band of frequency we call 'human'. The labels are not the self; they are, to use the title of one of my books, a *Phantom* Self. I am not David Icke born in Leicester, England, on April 29th, 1952. I am the consciousness *having that experience*. The Cult and its non-human masters seek to convince us through the institutions of 'education', science, medicine, media and government that what we are *experiencing* is who we *are*. It's so easy to control and direct perception locked away in the bewildered illusions of the five senses with no expanded radar. Try, by contrast, doing the same with a humanity aware of its true self and its true power to consciously create its reality and experience. How is it possible to do this? We do it all day every day. If you perceive yourself as 'little me' with no power to impact upon your life and the world then your life experience will reflect that. You will hand the power you don't think you have to authority in all its forms which will use it to control your experience. This, in turn, will appear to confirm your perception of 'little me' in a self-fulfilling feedback loop. But that is what 'little me' really is – a *perception*. We are all 'big-me', infinite me, and the Cult has to make us forget that if its will is to prevail. We are therefore manipulated and pressured into self-identifying with human labels and not the consciousness/awareness *experiencing* those human labels.

The phenomenon of identity politics is a Cult-instigated manipulation technique to sub-divide previous labels into even smaller ones. A United States university employs this list of letters to describe student identity: LGBTTQQFAGPBDSM or lesbian, gay, bisexual, transgender, transsexual, queer, questioning, flexual, asexual, gender-fuck, polyamorous, bondage/discipline, dominance/submission and sadism/masochism. I'm sure other lists are even longer by now as people feel the need to self-identity the 'I' with the minutiae of race and sexual preference. Wokers programmed by the Cult for generations believe this is about 'inclusivity' when it's really the Cult locking them away into smaller and smaller versions of Phantom Self while firewalling them from the influence of their true self, the infinite, eternal 'I'. You may notice that my philosophy which contends that we are all unique points of attention/awareness within the same infinite whole or Oneness is the ultimate non-racism. The very sense of Oneness makes the judgement of people by their body-type, colour or sexuality utterly ridiculous and confirms that racism has no understanding

of reality (including anti-white racism). Yet despite my perception of life Cult agents and fast-asleep Wokers label me racist to discredit my information while they are themselves phenomenally racist and sexist. All they see is race and sexuality and they judge people as good or bad, demons or untouchables, by their race and sexuality. All they see is *Phantom Self* and perceive themselves in terms of Phantom Self. They are pawns and puppets of the Cult agenda to focus attention and self-identity in the five senses and play those identities against each other to divide and rule. Columbia University has introduced segregated graduations in another version of social distancing designed to drive people apart and teach them that different racial and cultural groups have nothing in common with each other. The last thing the Cult wants is unity. Again the pump-primers of this will be Cult operatives in the knowledge of what they are doing, but the rest are just the Phantom Self blind leading the Phantom Self blind. We *do* have something in common – we are all *the same consciousness* having different temporary experiences.

What is this 'human'?

Yes, what *is* 'human'? That is what we are supposed to be, right? I mean 'human'? True, but 'human' is the experience not the 'I'. Break it down to basics and 'human' is the way that information is processed. If we are to experience and interact with this band of frequency we call the 'world' we must have a vehicle that operates within that band of frequency. Our consciousness in its prime form cannot do that; it is way beyond the frequency of the human realm. My consciousness or awareness could not tap these keys and pick up the cup in front of me in the same way that radio station A cannot interact with radio station B when they are on different frequencies. The human body is the means through which we have that interaction. I have long described the body as a biological computer which processes information in a way that allows consciousness to experience this reality. The body is a receiver, transmitter and processor of information in a particular way that we call human. We visually perceive only the world of the five senses in a wakened state – that is the limit of the body's visual decoding system. In truth it's not even visual in the way we experience 'visual reality' as I will come to in a moment. We are 'human' because the body processes the information sources of human into a reality and

behaviour system that we *perceive* as human. Why does an elephant act like an elephant and not like a human or a duck? The elephant's biological computer is a different information field and processes information according to that program into a visual and behaviour type we call an elephant. The same applies to everything in our reality. These body information fields are perpetuated through procreation (like making a copy of a software program). The Cult wants to break that cycle and intervene technologically to transform the human information field into one that will change what we call humanity. If it can change the human information field it will change the way that field processes information and change humanity both 'physically' and psychologically. Hence the *messenger* (information) RNA 'vaccines' and so much more that is targeting human genetics by changing the body's information – *messaging* – construct through food, drink, radiation, toxicity and other means.

Reality that we experience is nothing like reality as it really is in the same way that the reality people experience in virtual reality games is not the reality they are really living in. The game is only a decoded source of information that appears to be a reality. Our world is also an information construct – a *simulation* (more later). In its base form our reality is a wavefield of information much the same in theme as Wi-Fi. The five senses decode wavefield information into electrical information which they communicate to the brain to decode into holographic (illusory 'physical') information. Different parts of the brain specialise in decoding different senses and the information is fused into a reality that appears to be outside of us but is really inside the brain and the genetic structure in general (Fig 12 overleaf). DNA is a receiver-transmitter of information and a vital part of this decoding process and the body's connection to other realities. Change DNA and you change the way we decode and connect with reality – see 'Covid vaccines'. Think of computers decoding Wi-Fi. You have information encoded in a radiation field and the computer decodes that information into a very different form on the screen. You can't see the Wi-Fi until its information is made manifest on the screen and the information on the screen is inside the computer and not outside. I have just described how we decode the 'human world'. All five senses decode the waveform 'Wi-Fi' field into electrical signals and the brain (computer) constructs reality inside the brain and not outside – 'You don't just look at a rainbow, you create it'. Sound is a simple example. We don't hear sound until the

brain decodes it. Waveform sound waves are picked up by the hearing sense and communicated to the brain in an electrical form to be decoded into the sounds that we hear. Everything we hear is inside the brain along with everything we see, feel, smell and taste. Words and language are waveform fields generated by our vocal chords which pass through this process until they are decoded by the brain into words that we hear. Different languages are different frequency fields or sound waves generated by vocal chords. Late British philosopher Alan Watts said:

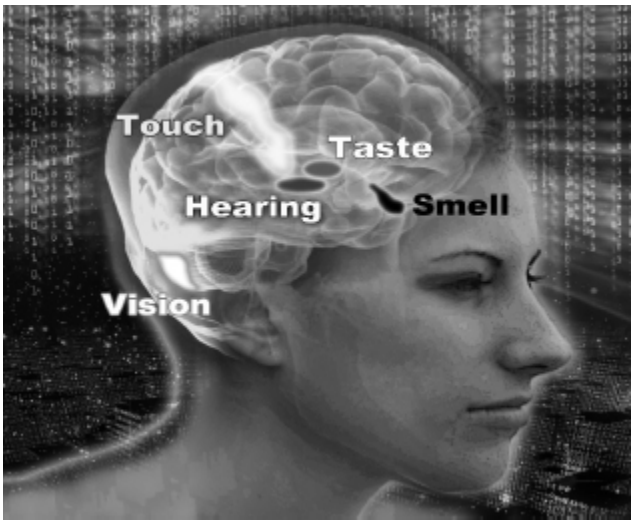

Figure 12: The brain receives information from the five senses and constructs from that our perceived reality.

[Without the brain] the world is devoid of light, heat, weight, solidity, motion, space, time or any other imaginable feature. All these phenomena are interactions, or transactions, of vibrations with a certain arrangement of neurons.

That's exactly what they are and scientist Robert Lanza describes in his book, *Biocentrism*, how we decode electromagnetic waves and energy into visual and 'physical' experience. He uses the example of a flame emitting photons, electromagnetic energy, each pulsing electrically and magnetically:

… these … invisible electromagnetic waves strike a human retina, and if (and only if) the waves happen to measure between 400 and 700 nano meters in length from crest to crest, then their energy is just right to deliver a stimulus to the 8 million cone-shaped cells in the retina.

Each in turn send an electrical pulse to a neighbour neuron, and on up the line this goes, at 250 mph, until it reaches the … occipital lobe of the brain, in the back of the head. There, a cascading complex of neurons fire from the incoming stimuli, and we subjectively perceive this experience as a yellow brightness occurring in a place we have been conditioned to call the 'external world'.

You hear what you decode

If a tree falls or a building collapses they make no noise unless someone is there to decode the energetic waves generated by the disturbance into what we call sound. Does a falling tree make a noise? Only if you hear it – *decode* it. Everything in our reality is a frequency field of information operating within the overall 'Wi-Fi' field that I call The Field. A vibrational disturbance is generated in The Field by the fields of the falling tree or building. These disturbance waves are what we decode into the sound of them falling. If no one is there to do that then neither will make any noise. Reality is created by the observer – *decoder* – and the *perceptions* of the observer affect the decoding process. For this reason different people – different *perceptions* – will perceive the same reality or situation in a different way. What one may perceive as a nightmare another will see as an opportunity. The question of why the Cult is so focused on controlling human perception now answers itself. All experienced reality is the act of decoding and we don't experience Wi-Fi until it is decoded on the computer screen. The sight and sound of an Internet video is encoded in the Wi-Fi all around us, but we don't see or hear it until the computer decodes that information. Taste, smell and touch are all phenomena of the brain as a result of the same process. We don't taste, smell or feel anything except in the brain and there are pain relief techniques that seek to block the signal from the site of discomfort to the brain because if the brain doesn't decode that signal we don't feel pain. Pain is in the brain and only appears to be at the point of impact thanks to the feedback loop between them. We don't see anything until electrical information from the sight senses is decoded in an area at the back of the brain. If that area is damaged we can go blind when our eyes are perfectly okay. So why do we go blind if we damage an eye? We damage the information processing between the waveform visual information and the visual decoding area of the brain. If information doesn't reach the brain in a form it can decode then we can't see the visual reality that it represents. What's more the brain is decoding only a fraction of the

information it receives and the rest is absorbed by the sub-conscious mind. This explanation is from the science magazine, *Wonderpedia*:

Every second, 11 million sensations crackle along these [brain] pathways ... The brain is confronted with an alarming array of images, sounds and smells which it rigorously filters down until it is left with a manageable list of around 40. Thus 40 sensations per second make up what we perceive as reality.

The 'world' is not what people are told to believe that is it and the inner circles of the Cult *know that*.

Illusory 'physical' reality

We can only see a smear of 0.005 percent of the Universe which is only one of a vast array of universes – 'mansions' – within infinite reality. Even then the brain decodes only 40 pieces of information ('sensations') from a potential *11 million* that we receive every second. Two points strike you from this immediately: The sheer breathtaking stupidity of believing we know anything so rigidly that there's nothing more to know; and the potential for these processes to be manipulated by a malevolent force to control the reality of the population. One thing I can say for sure with no risk of contradiction is that when you can perceive an almost indescribable fraction of infinite reality there is always more to know as in tidal waves of it. Ancient Greek philosopher Socrates was so right when he said that wisdom is to know how little we know. How obviously true that is when you think that we are experiencing a physical world of solidity that is neither physical nor solid and a world of apartness when everything is connected. Cult-controlled 'science' dismisses the so-called 'paranormal' and all phenomena related to that when the 'para'-normal is perfectly normal and explains the alleged 'great mysteries' which dumbfound scientific minds. There is a reason for this. A 'scientific mind' in terms of the mainstream is a material mind, a five-sense mind imprisoned in see it, touch it, hear it, smell it and taste it. Phenomena and happenings that can't be explained that way leave the 'scientific mind' bewildered and the rule is that if they can't account for why something is happening then it can't, by definition, be happening. I beg to differ. Telepathy is thought waves passing through The Field (think wave disturbance again) to be decoded by

someone able to connect with that wavelength (information). For example: You can pick up the thought waves of a friend at any distance and at the very least that will bring them to mind. A few minutes later the friend calls you. 'My god', you say, 'that's incredible – I was just thinking of you.' Ah, but *they* were thinking of *you* before they made the call and that's what you decoded. Native peoples not entrapped in five-sense reality do this so well it became known as the 'bush telegraph'. Those known as psychics and mediums (genuine ones) are doing the same only across dimensions of reality. 'Mind over matter' comes from the fact that matter and mind are the *same*. The state of one influences the state of the other. Indeed one *and* the other are illusions. They are aspects of the same field. Paranormal phenomena are all explainable so why are they still considered 'mysteries' or not happening? Once you go down this road of understanding you begin to expand awareness beyond the five senses and that's the nightmare for the Cult.

Figure 13: Holograms are not solid, but the best ones appear to be.

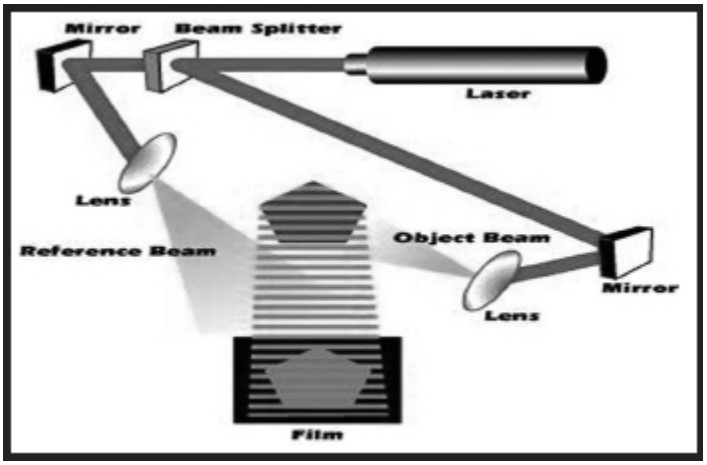

Figure 14: How holograms are created by capturing a waveform version of the subject image.

Holographic 'solidity'

Our reality is not solid, it is holographic. We are now well aware of holograms which are widely used today. Two-dimensional information is decoded into a three-dimensional reality that is not solid although can very much appear to be (Fig 13). Holograms are created with a laser divided into two parts. One goes directly onto a holographic photographic print ('reference beam') and the other takes a waveform image of the subject ('working beam') before being directed onto the print where it 'collides' with the other half of the laser (Fig 14). This creates a *waveform* interference pattern which contains the wavefield information of whatever is being photographed (Fig 15 overleaf). The process can be likened to dropping pebbles in a pond. Waves generated by each one spread out across the water to collide with the others and create a wave representation of where the stones fell and at what speed, weight and distance. A waveform interference pattern of a hologram is akin to the waveform information in The Field which the five senses decode into electrical signals to be decoded by the brain into a holographic illusory 'physical' reality. In the same way when a laser (think human attention) is directed at the waveform interference pattern a three-dimensional version of the subject is projected into apparently 'solid' reality (Fig 16). An amazing trait of holograms reveals more 'paranormal mysteries'. Information of the *whole* hologram is encoded in waveform in every part of the interference pattern by the way they are created. This means that every *part* of a hologram is a smaller version of the whole. Cut the interference wave-pattern into four and you won't get four parts of the image. You get quarter-sized versions of the *whole* image. The body is a hologram and the same applies. Here we have the basis of acupuncture, reflexology and other forms of healing which identify representations of the whole body in all of the parts, hands, feet, ears, everywhere. Skilled palm readers can do what they do because the information of whole body is encoded in the hand. The concept of as above, so below, comes from this.

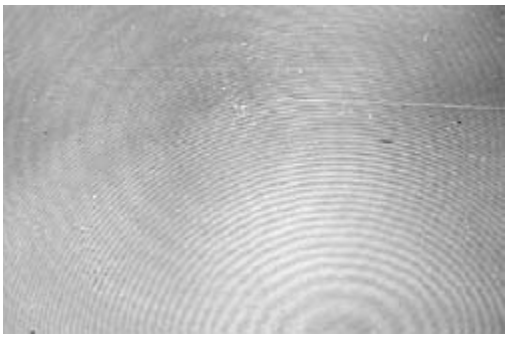

Figure 15: A waveform interference pattern that holds the information that transforms into a hologram.

Figure 16: Holographic people including 'Elvis' holographically inserted to sing a duet with Celine Dion.

The question will be asked of why, if solidity is illusory, we can't just walk through walls and each other. The resistance is not solid against solid; it is electromagnetic field against electromagnetic field and we decode this into the *experience* of solid against solid. We should also not underestimate the power of belief to dictate reality. What you believe is impossible *will be*. Your belief impacts on your decoding processes and they won't decode what you think is impossible. What we believe we perceive and what we perceive we experience. 'Can't dos' and 'impossibles' are like a firewall in a computer system that won't put on the screen what the firewall blocks. How vital that is to understanding how human experience has been hijacked. I explain in *The Answer*, *Everything You Need To Know But Have Never Been Told* and other books a long list of 'mysteries' and 'paranormal' phenomena that are not mysterious and perfectly normal once you realise

what reality is and how it works. ‘Ghosts’ can be seen to pass through ‘solid’ walls because the walls are not solid and the ghost is a discarnate entity operating on a frequency so different to that of the wall that it’s like two radio stations sharing the same space while never interfering with each other. I have seen ghosts do this myself. The apartness of people and objects is also an illusion. Everything is connected by the Field like all sea life is connected by the sea. It’s just that within the limits of our visual reality we only ‘see’ holographic information and not the field of information that connects everything and from which the holographic world is made manifest. If you can only see holographic ‘objects’ and not the field that connects them they will appear to you as unconnected to each other in the same way that we see the computer while not seeing the Wi-Fi.

What you don’t know *can* hurt you

Okay, we return to those ‘two worlds’ of human society and the Cult with its global network of interconnecting secret societies and satanic groups which manipulate through governments, corporations, media, religions, etc. The fundamental difference between them is *knowledge*. The idea has been to keep humanity ignorant of the plan for its total enslavement underpinned by a crucial ignorance of reality – who we are and where we are – and how we interact with it. ‘Human’ should be the interaction between our expanded eternal consciousness and the five-sense body experience. We are meant to be *in* this world in terms of the five senses but not *of* this world in relation to our greater consciousness and perspective. In that state we experience the small picture of the five senses within the wider context of the big picture of awareness beyond the five senses. Put another way the five senses see the dots and expanded awareness connects them into pictures and patterns that give context to the apparently random and unconnected. Without the context of expanded awareness the five senses see only apartness and randomness with apparently no meaning. The Cult and its other-dimensional controllers seek to intervene in the frequency realm where five-sense reality is supposed to connect with expanded reality and to keep the two apart (more on this in the final chapter). When that happens five-sense mental and emotional processes are no longer influenced by expanded awareness, or the True ‘I’, and instead are driven by the isolated perceptions of the body’s decoding systems. They are in the

world *and* of it. Here we have the human plight and why humanity with its potential for infinite awareness can be so easily manipulatable and descend into such extremes of stupidity.

Once the Cult isolates five-sense mind from expanded awareness it can then program the mind with perceptions and beliefs by controlling information that the mind receives through the 'education' system of the formative years and the media perceptual bombardment and censorship of an entire lifetime. Limit perception and a sense of the possible through limiting knowledge by limiting and skewing information while censoring and discrediting that which could set people free. As the title of another of my books says ... *And The Truth Shall Set You Free*. For this reason the last thing the Cult wants in circulation is the truth about anything – especially the reality of the eternal 'I' – and that's why it is desperate to control information. The Cult knows that information becomes perception which becomes behaviour which, collectively, becomes human society. Cult-controlled and funded mainstream 'science' denies the existence of an eternal 'I' and seeks to dismiss and trash all evidence to the contrary. Cult-controlled mainstream religion has a version of 'God' that is little more than a system of control and dictatorship that employs threats of damnation in an afterlife to control perceptions and behaviour in the here and now through fear and guilt. Neither is true and it's the 'neither' that the Cult wishes to suppress. This 'neither' is that everything is an expression, a point of attention, within an infinite state of consciousness which is the real meaning of the term 'God'.

Perceptual obsession with the 'physical body' and five-senses means that 'God' becomes personified as a bearded bloke sitting among the clouds or a raging bully who loves us if we do what 'he' wants and condemns us to the fires of hell if we don't. These are no more than a 'spiritual' fairy tales to control and dictate events and behaviour through fear of this 'God' which has bizarrely made 'God-fearing' in religious circles a state to be desired. I would suggest that fearing *anything* is not to be encouraged and celebrated, but rather deleted. You can see why 'God fearing' is so beneficial to the Cult and its religions when *they* decide what 'God' wants and what 'God' demands (the Cult demands) that everyone do. As the great American comedian Bill Hicks said satirising a Christian zealot: 'I think what God meant to say.' How much of this infinite awareness ('God') that we access is decided by how far we choose to expand our perceptions, self-identity

and sense of the possible. The scale of self-identity reflects itself in the scale of awareness that we can connect with and are influenced by – how much knowing and insight we have instead of programmed perception. You cannot expand your awareness into the infinity of possibility when you believe that you are little me Peter the postman or Mary in marketing and nothing more. I'll deal with this in the concluding chapter because it's crucial to how we turnaround current events.

Where the Cult came from

When I realised in the early 1990s there was a Cult network behind global events I asked the obvious question: When did it start? I took it back to ancient Rome and Egypt and on to Babylon and Sumer in Mesopotamia, the 'Land Between Two Rivers', in what we now call Iraq. The two rivers are the Tigris and Euphrates and this region is of immense historical and other importance to the Cult, as is the land called Israel only 550 miles away by air. There is much more going with deep esoteric meaning across this whole region. It's not only about 'wars for oil'. Priceless artefacts from Mesopotamia were stolen or destroyed after the American and British invasion of Iraq in 2003 justified by the lies of Boy Bush and Tony Blair (their Cult masters) about non-existent 'weapons of mass destruction'. Mesopotamia was the location of Sumer (about 5,400BC to 1,750BC), and Babylon (about 2,350BC to 539BC). Sabbatians may have become immensely influential in the Cult in modern times but they are part of a network that goes back into the mists of history. Sumer is said by historians to be the 'cradle of civilisation'. I disagree. I say it was the re-start of what we call human civilisation after cataclysmic events symbolised in part as the 'Great Flood' destroyed the world that existed before. These fantastic upheavals that I have been describing in detail in the books since the early1990s appear in accounts and legends of ancient cultures across the world and they are supported by geological and biological evidence. Stone tablets found in Iraq detailing the Sumer period say the cataclysms were caused by non-human 'gods' they call the Anunnaki. These are described in terms of extraterrestrial visitations in which knowledge supplied by the Anunnaki is said to have been the source of at least one of the world's oldest writing systems and developments in astronomy, mathematics and architecture that were way ahead of their time. I have covered this subject at

length in *The Biggest Secret* and *Children of the Matrix* and the same basic 'Anunnaki' story can be found in Zulu accounts in South Africa where the late and very great Zulu high shaman Credo Mutwa told me that the Sumerian Anunnaki were known by Zulus as the Chitauri or 'children of the serpent'. See my six-hour video interview with Credo on this subject entitled *The Reptilian Agenda* recorded at his then home near Johannesburg in 1999 which you can watch on the Ickonic media platform.

The Cult emerged out of Sumer, Babylon and Egypt (and elsewhere) and established the Roman Empire before expanding with the Romans into northern Europe from where many empires were savagely imposed in the form of Cult-controlled societies all over the world. Mass death and destruction was their calling card. The Cult established its centre of operations in Europe and European Empires were Cult empires which allowed it to expand into a global force. Spanish and Portuguese colonialists headed for Central and South America while the British and French targeted North America. Africa was colonised by Britain, France, Belgium, the Netherlands, Portugal, Spain, Italy, and Germany. Some like Britain and France moved in on the Middle East. The British Empire was by far the biggest for a simple reason. By now Britain was the headquarters of the Cult from which it expanded to form Canada, the United States, Australia and New Zealand. The Sun never set on the British Empire such was the scale of its occupation. London remains a global centre for the Cult along with Rome and the Vatican although others have emerged in Israel and China. It is no accident that the 'virus' is alleged to have come out of China while Italy was chosen as the means to terrify the Western population into compliance with 'Covid' fascism. Nor that Israel has led the world in 'Covid' fascism and mass 'vaccination'.

You would think that I would mention the United States here, but while it has been an important means of imposing the Cult's will it is less significant than would appear and is currently in the process of having what power it does have deleted. The Cult in Europe has mostly loaded the guns for the US to fire. America has been controlled from Europe from the start through Cult operatives in Britain and Europe. The American Revolution was an illusion to make it appear that America was governing itself while very different forces were pulling the strings in the form of Cult families such as the Rothschilds through the Rockefellers and other subordinates. The Rockefellers are extremely close to Bill Gates and established both scalpel

and drug ‘medicine’ and the World Health Organization. They play a major role in the development and circulation of vaccines through the Rockefeller Foundation on which Bill Gates said his Foundation is based. Why wouldn’t this be the case when the Rockefellers and Gates are on the same team? Cult infiltration of human society goes way back into what we call history and has been constantly expanding and centralising power with the goal of establishing a global structure to dictate everything. Look how this has been advanced in great leaps with the ‘Covid’ hoax.

The non-human dimension

I researched and observed the comings and goings of Cult operatives through the centuries and even thousands of years as they were born, worked to promote the agenda within the secret society and satanic networks, and then died for others to replace them. Clearly there had to be a coordinating force that spanned this entire period while operatives who would not have seen the end goal in their lifetimes came and went advancing the plan over millennia. I went in search of that coordinating force with the usual support from the extraordinary synchronicity of my life which has been an almost daily experience since 1990. I saw common themes in religious texts and ancient cultures about a non-human force manipulating human society from the hidden. Christianity calls this force Satan, the Devil and demons; Islam refers to the Jinn or Djinn; Zulus have their Chitauri (spelt in other ways in different parts of Africa); and the Gnostic people in Egypt in the period around and before 400AD referred to this phenomena as the ‘Archons’, a word meaning rulers in Greek. Central American cultures speak of the ‘Predators’ among other names and the same theme is everywhere. I will use ‘Archons’ as a collective name for all of them. When you see how their nature and behaviour is described all these different sources are clearly talking about the same force. Gnostics described the Archons in terms of ‘luminous fire’ while Islam relates the Jinn to ‘smokeless fire’. Some refer to beings in form that could occasionally be seen, but the most common of common theme is that they operate from unseen realms which means almost all existence to the visual processes of humans. I had concluded that this was indeed the foundation of human control and that the Cult was operating within the human frequency

band on behalf of this hidden force when I came across the writings of Gnostics which supported my conclusions in the most extraordinary way.

A sealed earthen jar was found in 1945 near the town of Nag Hammadi about 75-80 miles north of Luxor on the banks of the River Nile in Egypt. Inside was a treasure trove of manuscripts and texts left by the Gnostic people some 1,600 years earlier. They included 13 leather-bound papyrus codices (manuscripts) and more than 50 texts written in Coptic Egyptian estimated to have been hidden in the jar in the period of 400AD although the source of the information goes back much further. Gnostics oversaw the Great or Royal Library of Alexandria, the fantastic depository of ancient texts detailing advanced knowledge and accounts of human history. The Library was dismantled and destroyed in stages over a long period with the death-blow delivered by the Cult-established Roman Church in the period around 415AD. The Church of Rome was the Church of Babylon relocated as I said earlier. Gnostics were not a race. They were a way of perceiving reality. Whenever they established themselves and their information circulated the terrorists of the Church of Rome would target them for destruction. This happened with the Great Library and with the Gnostic Cathars who were burned to death by the psychopaths after a long period of oppression at the siege of the Castle of Monségur in southern France in 1244. The Church has always been terrified of Gnostic information which demolishes the official Christian narrative although there is much in the Bible that supports the Gnostic view if you read it in another way. To anyone studying the texts of what became known as the Nag Hammadi Library it is clear that great swathes of Christian and Biblical belief has its origin with Gnostics sources going back to Sumer. Gnostic themes have been twisted to manipulate the perceived reality of Bible believers. Biblical texts have been in the open for centuries where they could be changed while Gnostic documents found at Nag Hammadi were sealed away and untouched for 1,600 years. What you see is what they wrote.

Use your *pneuma* not your *nous*

Gnosticism and Gnostic come from ‘gnosis’ which means knowledge, or rather *secret* knowledge, in the sense of spiritual awareness – knowledge about reality and life itself. The desperation of the Cult’s Church of Rome to destroy the Gnostics can be understood when the knowledge they were

circulating was the last thing the Cult wanted the population to know. Sixteen hundred years later the same Cult is working hard to undermine and silence me for the same reason. The dynamic between knowledge and ignorance is a constant. 'Time' appears to move on, but essential themes remain the same. We are told to 'use your nous', a Gnostic word for head/brain/intelligence. They said, however, that spiritual awakening or 'salvation' could only be secured by expanding awareness *beyond* what they called *nous* and into *pneuma* or Infinite Self. Obviously as I read these texts the parallels with what I have been saying since 1990 were fascinating to me. There is a universal truth that spans human history and in that case why wouldn't we be talking the same language 16 centuries apart? When you free yourself from the perception program of the five senses and explore expanded realms of consciousness you are going to connect with the same information no matter what the perceived 'era' within a manufactured timeline of a single and tiny range of manipulated frequency. Humans working with 'smart' technology or knocking rocks together in caves is only a timeline appearing to operate within the human frequency band. Expanded awareness and the knowledge it holds have always been there whether the era be Stone Age or computer age. We can only access that knowledge by opening ourselves to its frequency which the five-sense prison cell is designed to stop us doing. Gates, Fauci, Whitty, Vallance, Zuckerberg, Brin, Page, Wojcicki, Bezos, and all the others behind the 'Covid' hoax clearly have a long wait before their range of frequency can make that connection given that an open heart is crucial to that as we shall see. Instead of accessing knowledge directly through expanded awareness it is given to Cult operatives by the secret society networks of the Cult where it has been passed on over thousands of years outside the public arena. Expanded realms of consciousness is where great artists, composers and writers find their inspiration and where truth awaits anyone open enough to connect with it. We need to go there fast.

Archon hijack

A fifth of the Nag Hammadi texts describe the existence and manipulation of the Archons led by a 'Chief Archon' they call 'Yaldabaoth', or the 'Demiurge', and this is the Christian 'Devil', 'Satan', 'Lucifer', and his demons. Archons in Biblical symbolism are the 'fallen ones' which are also

referred to as fallen angels after the angels expelled from heaven according to the Abrahamic religions of Judaism, Christianity and Islam. These angels are claimed to tempt humans to 'sin' ongoing and you will see how accurate that symbolism is during the rest of the book. The theme of 'original sin' is related to the 'Fall' when Adam and Eve were 'tempted by the serpent' and fell from a state of innocence and 'obedience' (connection) with God into a state of disobedience (disconnection). The Fall is said to have brought sin into the world and corrupted everything including human nature. Yaldabaoth, the 'Lord Archon', is described by Gnostics as a 'counterfeit spirit', 'The Blind One', 'The Blind God', and 'The Foolish One'. The Jewish name for Yaldabaoth in Talmudic writings is Samael which translates as 'Poison of God', or 'Blindness of God'. You see the parallels. Yaldabaoth in Islamic belief is the Muslim Jinn devil known as Shaytan – Shaytan is Satan as the same themes are found all over the world in every religion and culture. The 'Lord God' of the Old Testament is the 'Lord Archon' of Gnostic manuscripts and that's why he's such a bloodthirsty bastard. Satan is known by Christians as 'the Demon of Demons' and Gnostics called Yaldabaoth the 'Archon of Archons'. Both are known as 'The Deceiver'. We are talking about the same 'bloke' for sure and these common themes using different names, storylines and symbolism tell a common tale of the human plight.

Archons are referred to in Nag Hammadi documents as mind parasites, inverters, guards, gatekeepers, detainers, judges, pitiless ones and deceivers. The 'Covid' hoax alone is a glaring example of all these things. The Biblical 'God' is so different in the Old and New Testaments because they are not describing the same phenomenon. The vindictive, angry, hate-filled, 'God' of the Old Testament, known as Yahweh, is Yaldabaoth who is depicted in Cult-dictated popular culture as the 'Dark Lord', 'Lord of Time', Lord (Darth) Vader and Dormammu, the evil ruler of the 'Dark Dimension' trying to take over the 'Earth Dimension' in the Marvel comic movie, *Dr Strange*. Yaldabaoth is both the Old Testament 'god' and the Biblical 'Satan'. Gnostics referred to Yaldabaoth as the 'Great Architect of the Universe'and the Cult-controlled Freemason network calls their god 'the 'Great Architect of the Universe' (also Grand Architect). The 'Great Architect' Yaldabaoth is symbolised by the Cult as the all-seeing eye at the top of the pyramid on the Great Seal of the United States and the dollar bill. Archon is encoded in *arch*-itect as it is in *arch*-angels and *arch*-bishops. All

religions have the theme of a force for good and force for evil in some sort of spiritual war and there is a reason for that – the theme is true. The Cult and its non-human masters are quite happy for this to circulate. They present themselves as the force for good fighting evil when they are really the force of evil (absence of love). The whole foundation of Cult modus operandi is inversion. They promote themselves as a force for good and anyone challenging them in pursuit of peace, love, fairness, truth and justice is condemned as a satanic force for evil. This has been the game plan throughout history whether the Church of Rome inquisitions of non-believers or 'conspiracy theorists' and 'anti-vaxxers' of today. The technique is the same whatever the timeline era.

Yaldabaoth is revolting (true)

Yaldabaoth and the Archons are said to have revolted against God with Yaldabaoth claiming to *be* God – the *All That Is*. The Old Testament 'God' (Yaldabaoth) demanded to be worshipped as such: ' *I am* the LORD, and there is none else, there is no God beside me' (Isaiah 45:5). I have quoted in other books a man who said he was the unofficial son of the late Baron Philippe de Rothschild of the Mouton-Rothschild wine producing estates in France who died in 1988 and he told me about the Rothschild 'revolt from God'. The man said he was given the name Phillip Eugene de Rothschild and we shared long correspondence many years ago while he was living under another identity. He said that he was conceived through 'occult incest' which (within the Cult) was 'normal and to be admired'. 'Phillip' told me about his experience attending satanic rituals with rich and famous people whom he names and you can see them and the wider background to Cult Satanism in my other books starting with *The Biggest Secret*. Cult rituals are interactions with Archontic 'gods'. 'Phillip' described Baron Philippe de Rothschild as 'a master Satanist and hater of God' and he used the same term 'revolt from God' associated with Yaldabaoth/Satan/Lucifer/the Devil in describing the Sabbatian Rothschild dynasty. 'I played a key role in my family's revolt from God', he said. That role was to infiltrate in classic Sabbatian style the Christian Church, but eventually he escaped the mind-prison to live another life. The Cult has been targeting religion in a plan to make worship of the Archons the global one-world religion. Infiltration of Satanism into modern 'culture',

especially among the young, through music videos, stage shows and other means, is all part of this.

Nag Hammadi texts describe Yaldabaoth and the Archons in their prime form as energy – consciousness – and say they can take form if they choose in the same way that consciousness takes form as a human. Yaldabaoth is called 'formless' and represents a deeply inverted, distorted and chaotic state of consciousness which seeks to attached to humans and turn them into a likeness of itself in an attempt at assimilation. For that to happen it has to manipulate humans into low frequency mental and emotional states that match its own. Archons can certainly appear in human form and this is the origin of the psychopathic personality. The energetic distortion Gnostics called Yaldabaoth *is* psychopathy. When psychopathic Archons take human form that human will be a psychopath as an expression of Yaldabaoth consciousness. Cult psychopaths are Archons in human form. The principle is the same as that portrayed in the 2009 *Avatar* movie when the American military travelled to a fictional Earth-like moon called Pandora in the Alpha Centauri star system to infiltrate a society of blue people, or Na'vi, by hiding within bodies that looked like the Na'vi. Archons posing as humans have a particular hybrid information field, part human, part Archon, (the ancient 'demigods') which processes information in a way that manifests behaviour to match their psychopathic evil, lack of empathy and compassion, and stops them being influenced by the empathy, compassion and love that a fully-human information field is capable of expressing. Cult bloodlines interbreed, be they royalty or dark suits, for this reason and you have their obsession with incest. Interbreeding with full-blown humans would dilute the Archontic energy field that guarantees psychopathy in its representatives in the human realm.

Gnostic writings say the main non-human forms that Archons take are *serpentine* (what I have called for decades 'reptilian' amid unbounded ridicule from the Archontically-programmed) and what Gnostics describe as 'an unborn baby or foetus with grey skin and dark, unmoving eyes'. This is an excellent representation of the ET 'Greys' of UFO folklore which large numbers of people claim to have seen and been abducted by – Zulu shaman Credo Mutwa among them. I agree with those that believe in extraterrestrial or interdimensional visitations today and for thousands of years past. No wonder with their advanced knowledge and technological capability they were perceived and worshipped as gods for technological

and other ‘miracles’ they appeared to perform. Imagine someone arriving in a culture disconnected from the modern world with a smartphone and computer. They would be seen as a ‘god’ capable of ‘miracles’. The Renegade Mind, however, wants to know the source of everything and not only the way that source manifests as human or non-human. In the same way that a Renegade Mind seeks the original source material for the ‘Covid virus’ to see if what is claimed is true. The original source of Archons in form is consciousness – the distorted state of consciousness known to Gnostics as Yaldabaoth.

‘Revolt from God’ is energetic disconnection

Where I am going next will make a lot of sense of religious texts and ancient legends relating to ‘Satan’, Lucifer’ and the ‘gods’. Gnostic descriptions sync perfectly with the themes of my own research over the years in how they describe a consciousness distortion seeking to impose itself on human consciousness. I’ve referred to the core of infinite awareness in previous books as Infinite Awareness in Awareness of Itself. By that I mean a level of awareness that knows that it is all awareness and is aware of all awareness. From here comes the frequency of love in its true sense and balance which is what love is on one level – the balance of all forces into a single whole called Oneness and Isness. The more we disconnect from this state of love that many call ‘God’ the constituent parts of that Oneness start to unravel and express themselves as a part and not a whole. They become individualised as intellect, mind, selfishness, hatred, envy, desire for power over others, and such like. This is not a problem in the greater scheme in that ‘God’, the *All That Is*, can experience all these possibilities through different expressions of itself including humans. What we as expressions of the whole experience the *All That Is* experiences. We are the *All That Is* experiencing itself. As we withdraw from that state of Oneness we disconnect from its influence and things can get very unpleasant and very stupid. Archontic consciousness is at the extreme end of that. It has so disconnected from the influence of Oneness that it has become an inversion of unity and love, an inversion of everything, an inversion of life itself. Evil is appropriately live written backwards. Archontic consciousness is obsessed with death, an inversion of life, and so its manifestations in Satanism are obsessed with death. They use inverted

symbols in their rituals such as the inverted pentagram and cross. Sabbatians as Archontic consciousness incarnate invert Judaism and every other religion and culture they infiltrate. They seek disunity and chaos and they fear unity and harmony as they fear love like garlic to a vampire. As a result the Cult, Archons incarnate, act with such evil, psychopathy and lack of empathy and compassion disconnected as they are from the source of love. How could Bill Gates and the rest of the Archontic psychopaths do what they have to human society in the 'Covid' era with all the death, suffering and destruction involved and have no emotional consequence for the impact on others? Now you know. Why have Zuckerberg, Brin, Page, Wojcicki and company callously censored information warning about the dangers of the 'vaccine' while thousands have been dying and having severe, sometimes life-changing reactions? Now you know. Why have Tedros, Fauci, Whitty, Vallance and their like around the world been using case and death figures they're aware are fraudulent to justify lockdowns and all the deaths and destroyed lives that have come from that? Now you know. Why did Christian Drosten produce and promote a 'testing' protocol that he knew couldn't test for infectious disease which led to a global human catastrophe. Now you know. The Archontic mind doesn't give a shit (Fig 17). I personally think that Gates and major Cult insiders are a form of AI cyborg that the Archons want humans to become.

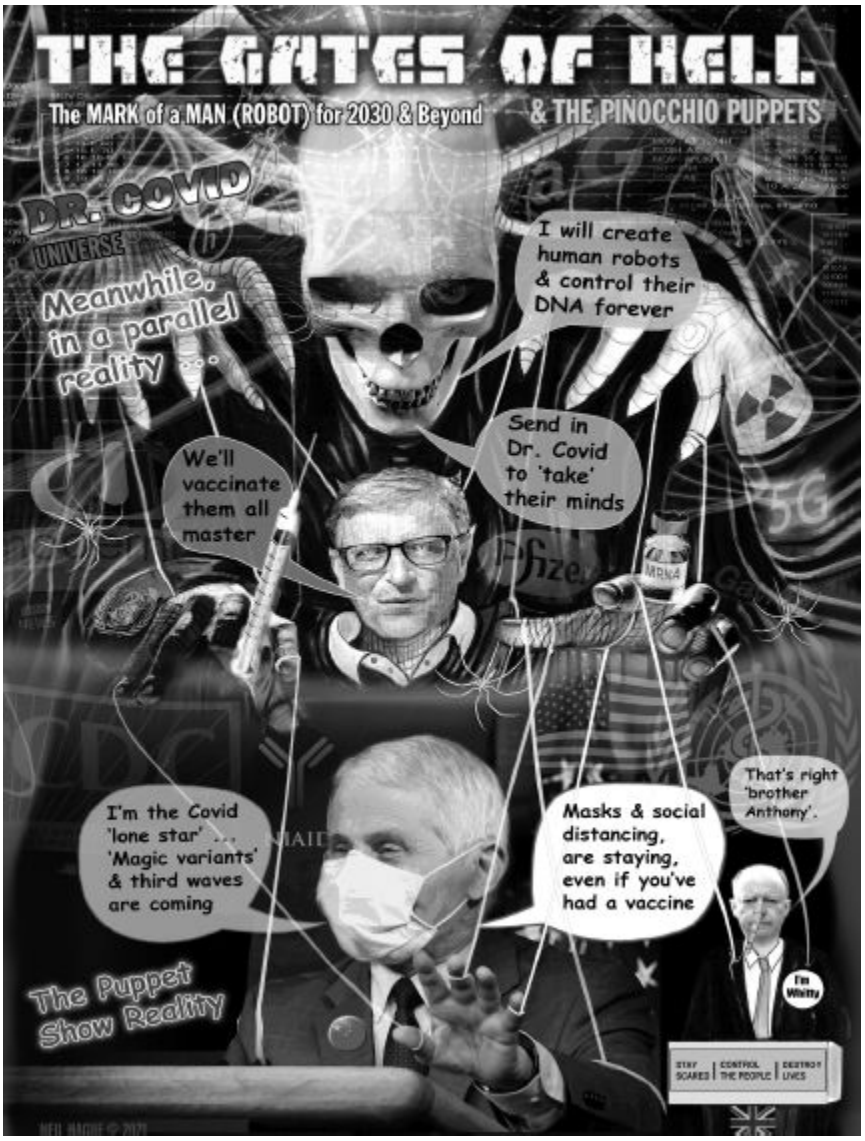

Figure 17: Artist Neil Hague's version of the 'Covid' hierarchy.

Human batteries

A state of such inversion does have its consequences, however. The level of disconnection from the Source of All means that you withdraw from that source of energetic sustenance and creativity. This means that you have to find your own supply of energetic power and it has – *us*. When the Morpheus character in the first *Matrix* movie held up a battery he spoke a profound truth when he said: 'The Matrix is a computer-generated dream world built to keep us under control in order to change the human being into one of these.' The statement was true in all respects. We do live in a

technologically-generated virtual reality simulation (more very shortly) and we have been manipulated to be an energy source for Archontic consciousness. The Disney-Pixar animated movie *Monsters, Inc*. in 2001 symbolised the dynamic when monsters in their world had no energy source and they would enter the human world to terrify children in their beds, catch the child's scream, terror (low-vibrational frequencies), and take that energy back to power the monster world. The lead character you might remember was a single giant eye and the symbolism of the Cult's all-seeing eye was obvious. Every thought and emotion is broadcast as a frequency unique to that thought and emotion. Feelings of love and joy, empathy and compassion, are high, quick, frequencies while fear, depression, anxiety, suffering and hate are low, slow, dense frequencies. Which kind do you think Archontic consciousness can connect with and absorb? In such a low and dense frequency state there's no way it can connect with the energy of love and joy. Archons can only feed off energy compatible with their own frequency and they and their Cult agents want to delete the human world of love and joy and manipulate the transmission of low vibrational frequencies through low-vibrational human mental and emotional states. *We are their energy source*. Wars are energetic banquets to the Archons – a world war even more so – and think how much low-frequency mental and emotional energy has been generated from the consequences for humanity of the 'Covid' hoax orchestrated by Archons incarnate like Gates.

The ancient practice of human sacrifice 'to the gods', continued in secret today by the Cult, is based on the same principle. 'The gods' are Archontic consciousness in different forms and the sacrifice is induced into a state of intense terror to generate the energy the Archontic frequency can absorb. Incarnate Archons in the ritual drink the blood which contains an adrenaline they crave which floods into the bloodstream when people are terrorised. Most of the sacrifices, ancient and modern, are children and the theme of 'sacrificing young virgins to the gods' is just code for children. They have a particular pre-puberty energy that Archons want more than anything and the energy of the young in general is their target. The California Department of Education wants students to chant the names of Aztec gods (Archontic gods) once worshipped in human sacrifice rituals in a curriculum designed to encourage them to 'challenge racist, bigoted, discriminatory, imperialist/colonial beliefs', join 'social movements that struggle for social justice', and 'build new possibilities for a post-racist, post-systemic racism

society'. It's the usual Woke crap that inverts racism and calls it anti-racism. In this case solidarity with 'indigenous tribes' is being used as an excuse to chant the names of 'gods' to which people were sacrificed (and still are in secret). What an example of Woke's inability to see beyond black and white, us and them, They condemn the colonisation of these tribal cultures by Europeans (quite right), but those cultures sacrificing people including children to their 'gods', and mass murdering untold numbers as the Aztecs did, is just fine. One chant is to the Aztec god Tezcatlipoca who had a man sacrificed to him in the 5th month of the Aztec calendar. His heart was cut out and he was eaten. Oh, that's okay then. Come on children … after three … Other sacrificial 'gods' for the young to chant their allegiance include Quetzalcoatl, Huitzilopochtli and Xipe Totec. The curriculum says that 'chants, affirmations, and energizers can be used to bring the class together, build unity around ethnic studies principles and values, and to reinvigorate the class following a lesson that may be emotionally taxing or even when student engagement may appear to be low'. Well, that's the cover story, anyway. Chanting and mantras are the repetition of a particular frequency generated from the vocal cords and chanting the names of these Archontic 'gods' tunes you into their frequency. That is the last thing you want when it allows for energetic synchronisation, attachment and perceptual influence. Initiates chant the names of their 'Gods' in their rituals for this very reason.

Vampires of the Woke

Paedophilia is another way that Archons absorb the energy of children. Paedophiles possessed by Archontic consciousness are used as the conduit during sexual abuse for discarnate Archons to vampire the energy of the young they desire so much. Stupendous numbers of children disappear every year never to be seen again although you would never know from the media. Imagine how much low-vibrational energy has been generated by children during the 'Covid' hoax when so many have become depressed and psychologically destroyed to the point of killing themselves. Shocking numbers of children are now taken by the state from loving parents to be handed to others. I can tell you from long experience of researching this since 1996 that many end up with paedophiles and assets of the Cult through corrupt and Cult-owned social services which in the reframing era has hired many psychopaths and emotionless automatons to do the job.

Children are even stolen to order using spurious reasons to take them by the corrupt and secret (because they're corrupt) 'family courts'. I have written in detail in other books, starting with *The Biggest Secret* in 1997, about the ubiquitous connections between the political, corporate, government, intelligence and military elites (Cult operatives) and Satanism and paedophilia. If you go deep enough both networks have an interlocking leadership. The Woke mentality has been developed by the Cult for many reasons: To promote almost every aspect of its agenda; to hijack the traditional political left and turn it fascist; to divide and rule; and to target agenda pushbackers. But there are other reasons which relate to what I am describing here. How many happy and joyful Wokers do you ever see especially at the extreme end? They are a mental and psychological mess consumed by emotional stress and constantly emotionally cocked for the next explosion of indignation at someone referring to a female as a female. They are walking, talking, batteries as Morpheus might say emitting frequencies which both enslave them in low-vibrational bubbles of perceptual limitation and feed the Archons. Add to this the hatred claimed to be love; fascism claimed to 'anti-fascism', racism claimed to be 'anti-racism'; exclusion claimed to inclusion; and the abuse-filled Internet trolling. You have a purpose-built Archontic energy system with not a wind turbine in sight and all founded on Archontic *inversion*. We have whole generations now manipulated to serve the Archons with their actions and energy. They will be doing so their entire adult lives unless they snap out of their Archon-induced trance. Is it really a surprise that Cult billionaires and corporations put so much money their way? Where is the energy of joy and laughter, including laughing at yourself which is confirmation of your own emotional security? Mark Twain said: 'The human race has one really effective weapon, and that is laughter.' We must use it all the time. Woke has destroyed comedy because it has no humour, no joy, sense of irony, or self-deprecation. Its energy is dense and intense. *Mmmmm*, lunch says the Archontic frequency. Rudolf Steiner (1861-1925) was the Austrian philosopher and famous esoteric thinker who established Waldorf education or Steiner schools to treat children like unique expressions of consciousness and not minds to be programmed with the perceptions determined by authority. I'd been writing about this energy vampiring for decades when I was sent in 2016 a quote by Steiner. He was spot on:

There are beings in the spiritual realms for whom anxiety and fear emanating from human beings offer welcome food. When humans have no anxiety and fear, then these creatures starve. If fear and anxiety radiates from people and they break out in panic, then these creatures find welcome nutrition and they become more and more powerful. These beings are hostile towards humanity. Everything that feeds on negative feelings, on anxiety, fear and superstition, despair or doubt, are in reality hostile forces in super-sensible worlds, launching cruel attacks on human beings, while they are being fed ... These are exactly the feelings that belong to contemporary culture and materialism; because it estranges people from the spiritual world, it is especially suited to evoke hopelessness and fear of the unknown in people, thereby calling up the above mentioned hostile forces against them.

Pause for a moment from this perspective and reflect on what has happened in the world since the start of 2020. Not only will pennies drop, but billion dollar bills. We see the same theme from Don Juan Matus, a Yaqui Indian shaman in Mexico and the information source for Peruvian-born writer, Carlos Castaneda, who wrote a series of books from the 1960s to 1990s. Don Juan described the force manipulating human society and his name for the Archons was the predator:

We have a predator that came from the depths of the cosmos and took over the rule of our lives. Human beings are its prisoners. The predator is our lord and master. It has rendered us docile, helpless. If we want to protest, it suppresses our protest. If we want to act independently, it demands that we don't do so ... indeed we are held prisoner!

They took us over because we are food to them, and they squeeze us mercilessly because we are their sustenance. Just as we rear chickens in coops, the predators rear us in human coops, humaneros. Therefore, their food is always available to them.

Different cultures, different eras, same recurring theme.

The 'ennoia' dilemma

Nag Hammadi Gnostic manuscripts say that Archon consciousness has no 'ennoia'. This is directly translated as 'intentionality', but I'll use the term 'creative imagination'. The *All That Is* in awareness of itself is the source of all creativity – all possibility – and the more disconnected you are from that source the more you are subsequently denied 'creative imagination'. Given that Archon consciousness is almost entirely disconnected it severely lacks creativity and has to rely on far more mechanical processes of thought and exploit the creative potential of those that do have 'ennoia'. You can see cases of this throughout human society. Archon consciousness almost entirely dominates the global banking system and if we study how that

system works you will appreciate what I mean. Banks manifest ‘money’ out of nothing by issuing lines of ‘credit’ which is ‘money’ that has never, does not, and will never exist except in theory. It’s a confidence trick. If you think ‘credit’ figures-on-a-screen ‘money’ is worth anything you accept it as payment. If you don’t then the whole system collapses through lack of confidence in the value of that ‘money’. Archontic bankers with no ‘ennoia’ are ‘lending’ ‘money’ that doesn’t exist to humans that *do* have creativity – those that have the inspired ideas and create businesses and products. Archon banking feeds off human creativity which it controls through ‘money’ creation and debt. Humans have the creativity and Archons exploit that for their own benefit and control while having none themselves. Archon Internet platforms like Facebook claim joint copyright of everything that creative users post and while Archontic minds like Zuckerberg may officially head that company it will be human creatives on the staff that provide the creative inspiration. When you have limitless ‘money’ you can then buy other companies established by creative humans. Witness the acquisition record of Facebook, Google and their like. Survey the Archon-controlled music industry and you see non-creative dark suit executives making their fortune from the human creativity of their artists. The cases are endless. Research the history of people like Gates and Zuckerberg and how their empires were built on exploiting the creativity of others. Archon minds cannot create out of nothing, but they are skilled (because they have to be) in what Gnostic texts call ‘countermimicry’. They can imitate, but not innovate. Sabbatians trawl the creativity of others through backdoors they install in computer systems through their cybersecurity systems. Archon-controlled China is globally infamous for stealing intellectual property and I remember how Hong Kong, now part of China, became notorious for making counterfeit copies of the creativity of others – ‘countermimicry’. With the now pervasive and all-seeing surveillance systems able to infiltrate any computer you can appreciate the potential for Archons to vampire the creativity of humans. Author John Lamb Lash wrote in his book about the Nag Hammadi texts, *Not In His Image*:

Although they cannot originate anything, because they lack the divine factor of ennoia (intentionality), Archons can imitate with a vengeance. Their expertise is simulation (HAL, virtual reality). The Demiurge [Yaldabaoth] fashions a heaven world copied from the fractal patterns [of the

original] ... His construction is celestial kitsch, like the fake Italianate villa of a Mafia don complete with militant angels to guard every portal.

This brings us to something that I have been speaking about since the turn of the millennium. Our reality is a simulation; a virtual reality that we think is real. No, I'm not kidding.

Human reality? Well, virtually

I had pondered for years about whether our reality is 'real' or some kind of construct. I remembered being immensely affected on a visit as a small child in the late 1950s to the then newly-opened Planetarium on the Marylebone Road in London which is now closed and part of the adjacent Madame Tussauds wax museum. It was in the middle of the day, but when the lights went out there was the night sky projected in the Planetarium's domed ceiling and it appeared to be so real. The experience never left me and I didn't know why until around the turn of the millennium when I became certain that our 'night sky' and entire reality is a projection, a virtual reality, akin to the illusory world portrayed in the *Matrix* movies. I looked at the sky one day in this period and it appeared to me like the domed roof of the Planetarium. The release of the first *Matrix* movie in 1999 also provided a synchronistic and perfect visual representation of where my mind had been going for a long time. I hadn't come across the Gnostic Nag Hammadi texts then. When I did years later the correlation was once again astounding. As I read Gnostic accounts from 1,600 years and more earlier it was clear that they were describing the same simulation phenomenon. They tell how the Yaldabaoth 'Demiurge' and Archons created a 'bad copy' of original reality to rule over all that were captured by its illusions and the body was a prison to trap consciousness in the 'bad copy' fake reality. Read how Gnostics describe the 'bad copy' and update that to current times and they are referring to what we would call today a virtual reality simulation.

Author John Lamb Lash said 'the Demiurge fashions a heaven world copied from the fractal patterns' of the original through expertise in 'HAL' or virtual reality simulation. Fractal patterns are part of the energetic information construct of our reality, a sort of blueprint. If these patterns were copied in computer terms it would indeed give you a copy of a

‘natural’ reality in a non-natural frequency and digital form. The principle is the same as making a copy of a website. The original website still exists, but now you can change the copy version to make it whatever you like and it can become very different to the original website. Archons have done this with our reality, a *synthetic* copy of prime reality that still exists beyond the frequency walls of the simulation. Trapped within the illusions of this synthetic Matrix, however, were and are human consciousness and other expressions of prime reality and this is why the Archons via the Cult are seeking to make the human body synthetic and give us synthetic AI minds to complete the job of turning the entire reality synthetic including what we perceive to be the natural world. To quote Kurzweil: ‘Nanobots will infuse all the matter around us with information. Rocks, trees, everything will become these intelligent creatures.’ Yes, *synthetic* ‘creatures’ just as ‘Covid’ and other genetically-manipulating ‘vaccines’ are designed to make the human body synthetic. From this perspective it is obvious why Archons and their Cult are so desperate to infuse synthetic material into every human with their ‘Covid’ scam.

Let there be (electromagnetic) light

Yaldabaoth, the force that created the simulation, or Matrix, makes sense of the Gnostic reference to ‘The Great Architect’ and its use by Cult Freemasonry as the name of its deity. The designer of the Matrix in the movies is called ‘The Architect’ and that trilogy is jam-packed with symbolism relating to these subjects. I have contended for years that the angry Old Testament God (Yaldabaoth) is the ‘God’ being symbolically ‘quoted’ in the opening of Genesis as ‘creating the world’. This is not the creation of prime reality – it’s the creation of the *simulation*. The Genesis ‘God’ says: ‘Let there be Light: and there was light.’ But what is this ‘Light’? I have said for decades that the speed of light (186,000 miles per second) is not the fastest speed possible as claimed by mainstream science and is in fact the frequency walls or outer limits of the Matrix. You can’t have a fastest or slowest anything within all possibility when everything is possible. The human body is encoded to operate within the speed of light or *within the simulation* and thus we see only the tiny frequency band of visible *light*. Near-death experiencers who perceive reality outside the body during temporary ‘death’ describe a very different form of light and this is

supported by the Nag Hammadi texts. Prime reality beyond the simulation ('Upper Aeons' to the Gnostics) is described as a realm of incredible beauty, bliss, love and harmony – a realm of 'watery light' that is so powerful 'there are no shadows'. Our false reality of Archon control, which Gnostics call the 'Lower Aeons', is depicted as a realm with a different kind of 'light' and described in terms of chaos, 'Hell', 'the Abyss' and 'Outer Darkness', where trapped souls are tormented and manipulated by demons (relate that to the 'Covid' hoax alone). The watery light theme can be found in near-death accounts and it is not the same as *simulation* 'light' which is electromagnetic or radiation light within the speed of light – the 'Lower Aeons'. Simulation 'light' is the 'luminous fire' associated by Gnostics with the Archons. The Bible refers to Yaldabaoth as 'that old serpent, called the Devil, and Satan, which deceiveth the whole world' (Revelation 12:9). I think that making a simulated copy of prime reality ('countermimicry') and changing it dramatically while all the time manipulating humanity to believe it to be real could probably meet the criteria of deceiving the whole world. Then we come to the Cult god Lucifer – the *Light Bringer*. Lucifer is symbolic of Yaldabaoth, the bringer of radiation light that forms the bad copy simulation within the speed of light. 'He' is symbolised by the lighted torch held by the Statue of Liberty and in the name 'Illuminati'. Sabbatian-Frankism declares that Lucifer is the true god and Lucifer is the real god of Freemasonry honoured as their 'Great or Grand Architect of the Universe' (simulation).

I would emphasise, too, the way Archontic technologically-generated luminous fire of radiation has deluged our environment since I was a kid in the 1950s and changed the nature of The Field with which we constantly interact. Through that interaction technological radiation is changing us. The Smart Grid is designed to operate with immense levels of communication power with 5G expanding across the world and 6G, 7G, in the process of development. Radiation is the simulation and the Archontic manipulation system. Why wouldn't the Archon Cult wish to unleash radiation upon us to an ever-greater extreme to form Kurzweil's 'cloud'? The plan for a synthetic human is related to the need to cope with levels of radiation beyond even anything we've seen so far. Biological humans would not survive the scale of radiation they have in their script. The Smart Grid is a technological sub-reality within the technological simulation to

further disconnect five-sense perception from expanded consciousness. It's a technological prison of the mind.

Infusing the 'spirit of darkness'

A recurring theme in religion and native cultures is the manipulation of human genetics by a non-human force and most famously recorded as the biblical 'sons of god' (the god*s* plural in the original) who interbred with the daughters of men. The Nag Hammadi *Apocryphon of John* tells the same story this way:

> He [Yaldabaoth] sent his angels [Archons/demons] to the daughters of men, that they might take some of them for themselves and raise offspring for their enjoyment. And at first they did not succeed. When they had no success, they gathered together again and they made a plan together ... And the angels changed themselves in their likeness into the likeness of their mates, filling them with the spirit of darkness, which they had mixed for them, and with evil ... And they took women and begot children out of the darkness according to the likeness of their spirit.

Possession when a discarnate entity takes over a human body is an age-old theme and continues today. It's very real and I've seen it. Satanic and secret society rituals can create an energetic environment in which entities can attach to initiates and I've heard many stories of how people have changed their personality after being initiated even into lower levels of the Freemasons. I have been inside three Freemasonic temples, one at a public open day and two by just walking in when there was no one around to stop me. They were in Ryde, the town where I live, Birmingham, England, when I was with a group, and Boston, Massachusetts. They all felt the same energetically – dark, dense, low-vibrational and sinister. Demonic attachment can happen while the initiate has no idea what is going on. To them it's just a ritual to get in the Masons and do a bit of good business. In the far more extreme rituals of Satanism human possession is even more powerful and they are designed to make possession possible. The hierarchy of the Cult is dictated by the power and perceived status of the possessing Archon. In this way the Archon hierarchy becomes the Cult hierarchy. Once the entity has attached it can influence perception and behaviour and if it attaches to the extreme then so much of its energy (information) infuses into the body information field that the hologram starts to reflect the nature of

the possessing entity. This is the *Exorcist* movie type of possession when facial features change and it's known as shapeshifting. Islam's Jinn are said to be invisible tricksters who change shape, 'whisper', confuse and take human form. These are all traits of the Archons and other versions of the same phenomenon. Extreme possession could certainty infuse the 'spirit of darkness' into a partner during sex as the Nag Hammadi texts appear to describe. Such an infusion can change genetics which is also energetic information. Human genetics is information and the 'spirit of darkness' is information. Mix one with the other and change must happen. Islam has the concept of a 'Jinn baby' through possession of the mother and by Jinn taking human form. There are many ways that human genetics can be changed and remember that Archons have been aware all along of advanced techniques to do this. What is being done in human society today – and far more – was known about by Archons at the time of the 'fallen ones' and their other versions described in religions and cultures.

Archons and their human-world Cult are obsessed with genetics as we see today and they know this dictates how information is processed into perceived reality during a human life. They needed to produce a human form that would decode the simulation and this is symbolically known as 'Adam and Eve' who left the 'garden' (prime reality) and 'fell' into Matrix reality. The simulation is not a 'physical' construct (there is no 'physical'); it is a source of information. Think Wi-Fi again. The simulation is an energetic field encoded with information and body-brain systems are designed to decode that information encoded in wave or frequency form which is transmitted to the brain as electrical signals. These are decoded by the brain to construct our sense of reality – an illusory 'physical' world that only exists in the brain or the mind. Virtual reality games mimic this process using the same sensory decoding system. Information is fed to the senses to decode a virtual reality that can appear so real, but isn't (Figs 18 and 19). Some scientists believe – and I agree with them – that what we perceive as 'physical' reality only exists when we are looking or observing. The act of perception or focus triggers the decoding systems which turn waveform information into holographic reality. When we are not observing something our reality reverts from a holographic state to a waveform state. This relates to the same principle as a falling tree not making a noise unless someone is there to hear it or decode it. The concept makes sense from the simulation perspective. A computer is not decoding all the information in a

Wi-Fi field all the time and only decodes or brings into reality on the screen that part of Wi-Fi that it's decoding – focusing upon – at that moment.

Figure 18: Virtual reality technology 'hacks' into the body's five-sense decoding system.

Figure 19: The result can be experienced as very 'real'.

Interestingly, Professor Donald Hoffman at the Department of Cognitive Sciences at the University of California, Irvine, says that our experienced reality is like a computer interface that shows us only the level with which we interact while hiding all that exists beyond it: 'Evolution shaped us with a user interface that hides the truth. Nothing that we see is the truth – the very language of space and time and objects is the wrong language to describe reality.' He is correct in what he says on so many levels. Space and time are not a universal reality. They are a phenomenon of decoded *simulation* reality as part of the process of enslaving our sense of reality. Near-death experiencers report again and again how space and time did not exist as we perceive them once they were free of the body – body decoding systems. You can appreciate from this why Archons and their Cult are so desperate to entrap human attention in the five senses where we are in the Matrix and of the Matrix. Opening your mind to expanded states of awareness takes you beyond the information confines of the simulation and

you become aware of knowledge and insights denied to you before. This is what we call ‘awakening’ – *awakening from the Matrix* – and in the final chapter I will relate this to current events.

Where are the ‘aliens’?

A simulation would explain the so-called ‘Fermi Paradox’ named after Italian physicist Enrico Fermi (1901-1954) who created the first nuclear reactor. He considered the question of why there is such a lack of extraterrestrial activity when there are so many stars and planets in an apparently vast universe; but what if the night sky that we see, or think we do, is a simulated projection as I say? If you control the simulation and your aim is to hold humanity fast in essential ignorance would you want other forms of life including advanced life coming and going sharing information with humanity? Or would you want them to believe they were isolated and apparently alone? Themes of human isolation and apartness are common whether they be the perception of a lifeless universe or the fascist isolation laws of the ‘Covid’ era. Paradoxically the very existence of a simulation means that we are not alone when some force had to construct it. My view is that experiences that people have reported all over the world for centuries with Reptilians and Grey entities are Archon phenomena as Nag Hammadi texts describe; and that benevolent ‘alien’ interactions are non-human groups that come in and out of the simulation by overcoming Archon attempts to keep them out. It should be highlighted, too, that Reptilians and Greys are obsessed with *genetics* and *technology* as related by cultural accounts and those who say they have been abducted by them. Technology is their way of overcoming some of the limitations in their creative potential and our technology-driven and controlled human society of today is *arch*etypical Archon-Reptilian-Grey modus operandi. Technocracy is really *Archon*tocracy. The Universe does not have to be as big as it appears with a simulation. There is no space or distance only information decoded into holographic reality. What we call ‘space’ is only the absence of holographic ‘objects’ and that ‘space’ is The Field of energetic information which connects everything into a single whole. The same applies with the artificially-generated information field of the simulation. The Universe is not big or small as a physical reality. It is decoded information, that’s all, and its perceived size is decided by the way the simulation is encoded to

make it appear. The entire night sky as we perceive it only exists in our brain and so where are those ‘millions of light years’? The ‘stars’ on the ceiling of the Planetarium looked a vast distance away.

There’s another point to mention about ‘aliens’. I have been highlighting since the 1990s the plan to stage a fake ‘alien invasion’ to justify the centralisation of global power and a world military. Nazi scientist Werner von Braun, who was taken to America by Operation Paperclip after World War Two to help found NASA, told his American assistant Dr Carol Rosin about the Cult agenda when he knew he was dying in 1977. Rosin said that he told her about a sequence that would lead to total human control by a one-world government. This included threats from terrorism, rogue nations, meteors and asteroids before finally an ‘alien invasion’. All of these things, von Braun said, would be bogus and what I would refer to as a No-Problem-Reaction-Solution. Keep this in mind when ‘the aliens are coming’ is the new mantra. The aliens are not coming – they are *already here* and they have infiltrated human society while looking human. French-Canadian investigative journalist Serge Monast said in 1994 that he had uncovered a NASA/military operation called Project Blue Beam which fits with what Werner von Braun predicted. Monast died of a ‘heart attack’ in 1996 the day after he was arrested and spent a night in prison. He was 51. He said Blue Beam was a plan to stage an alien invasion that would include religious figures beamed holographically into the sky as part of a global manipulation to usher in a ‘new age’ of worshipping what I would say is the Cult ‘god’ Yaldabaoth in a one-world religion. Fake holographic asteroids are also said to be part of the plan which again syncs with von Braun. How could you stage an illusory threat from asteroids unless they were holographic inserts? This is pretty straightforward given the advanced technology outside the public arena and the fact that our ‘physical’ reality is holographic anyway. Information fields would be projected and we would decode them into the illusion of a ‘physical’ asteroid. If they can sell a global ‘pandemic’ with a ‘virus’ that doesn’t exist what will humans not believe if government and media tell them?

All this is particularly relevant as I write with the Pentagon planning to release in June, 2021, information about ‘UFO sightings’. I have been following the UFO story since the early 1990s and the common theme throughout has been government and military denials and cover up. More recently, however, the Pentagon has suddenly become more talkative and

apparently open with Air Force pilot radar images released of unexplained craft moving and changing direction at speeds well beyond anything believed possible with human technology. Then, in March, 2021, former Director of National Intelligence John Ratcliffe said a Pentagon report months later in June would reveal a great deal of information about UFO sightings unknown to the public. He said the report would have 'massive implications'. The order to do this was included bizarrely in a $2.3 trillion 'coronavirus' relief and government funding bill passed by the Trump administration at the end of 2020. I would add some serious notes of caution here. I have been pointing out since the 1990s that the US military and intelligence networks have long had craft – 'flying saucers' or anti-gravity craft – which any observer would take to be extraterrestrial in origin. Keeping this knowledge from the public allows craft flown by *humans* to be perceived as alien visitations. I am not saying that 'aliens' do not exist. I would be the last one to say that, but we have to be streetwise here. President Ronald Reagan told the UN General Assembly in 1987: 'I occasionally think how quickly our differences worldwide would vanish if we were facing an alien threat from outside this world.' That's the idea. Unite against a common 'enemy' with a common purpose behind your 'saviour force' (the Cult) as this age-old technique of mass manipulation goes global.

Science moves this way …

I could find only one other person who was discussing the simulation hypothesis publicly when I concluded it was real. This was Nick Bostrom, a Swedish-born philosopher at the University of Oxford, who has explored for many years the possibility that human reality is a computer simulation although his version and mine are not the same. Today the simulation and holographic reality hypothesis have increasingly entered the scientific mainstream. Well, the more open-minded mainstream, that is. Here are a few of the ever-gathering examples. American nuclear physicist Silas Beane led a team of physicists at the University of Bonn in Germany pursuing the question of whether we live in a simulation. They concluded that we probably do and it was likely based on a lattice of cubes. They found that cosmic rays align with that specific pattern. The team highlighted the Greisen–Zatsepin–Kuzmin (GZK) limit which refers to cosmic ray particle

interaction with cosmic background radiation that creates an apparent boundary for cosmic ray particles. They say in a paper entitled 'Constraints on the Universe as a Numerical Simulation' that this 'pattern of constraint' is exactly what you would find with a computer simulation. They also made the point that a simulation would create its own 'laws of physics' that would limit possibility. I've been making the same point for decades that the *perceived* laws of physics relate only to this reality, or what I would later call the simulation. When designers write codes to create computer and virtual reality games they are the equivalent of the laws of physics for that game. Players interact within the limitations laid out by the coding. In the same way those who wrote the codes for the simulation decided the laws of physics that would apply. These can be overridden by expanded states of consciousness, but not by those enslaved in only five-sense awareness where simulation codes rule. Overriding the codes is what people call 'miracles'. They are not. They are bypassing the encoded limits of the simulation. A population caught in simulation perception would have no idea that this was their plight. As the Bonn paper said: 'Like a prisoner in a pitch-black cell we would not be able to see the "walls" of our prison,' That's true if people remain mesmerised by the five senses. Open to expanded awareness and those walls become very clear. The main one is the speed of light.

American theoretical physicist James Gates is another who has explored the simulation question and found considerable evidence to support the idea. Gates was Professor of Physics at the University of Maryland, Director of The Center for String and Particle Theory, and on Barack Obama's Council of Advisors on Science and Technology. He and his team found *computer codes* of digital data embedded in the fabric of our reality. They relate to on-off electrical charges of 1 and 0 in the binary system used by computers. 'We have no idea what they are doing there', Gates said. They found within the energetic fabric mathematical sequences known as error-correcting codes or block codes that 'reboot' data to its original state or 'default settings' when something knocks it out of sync. Gates was asked if he had found a set of equations embedded in our reality indistinguishable from those that drive search engines and browsers and he said: 'That is correct.' Rich Terrile, director of the Centre for Evolutionary Computation and Automated Design at NASA's Jet Propulsion Laboratory, has said publicly that he believes the Universe is a digital hologram that must have

been created by a form of intelligence. I agree with that in every way. Waveform information is delivered electrically by the senses to the brain which constructs a *digital* holographic reality that we call the 'world'. This digital level of reality can be read by the esoteric art of numerology. Digital holograms are at the cutting edge of holographics today. We have digital technology everywhere designed to access and manipulate our digital level of perceived reality. Synthetic mRNA in 'Covid vaccines' has a digital component to manipulate the body's digital 'operating system'.

Reality is numbers

How many know that our reality can be broken down to numbers and codes that are the same as computer games? Max Tegmark, a physicist at the Massachusetts Institute of Technology (MIT), is the author of *Our Mathematical Universe* in which he lays out how reality can be entirely described by numbers and maths in the way that a video game is encoded with the 'physics' of computer games. Our world and computer virtual reality are essentially the same. Tegmark imagines the perceptions of characters in an advanced computer game when the graphics are so good they don't know they are in a game. They think they can bump into real objects (electromagnetic resistance in our reality), fall in love and feel emotions like excitement. When they began to study the apparently 'physical world' of the video game they would realise that everything was made of pixels (which have been found in our energetic reality as must be the case when on one level our world is digital). What computer game characters thought was physical 'stuff', Tegmark said, could actually be broken down into numbers:

> And we're exactly in this situation in our world. We look around and it doesn't seem that mathematical at all, but everything we see is made out of elementary particles like quarks and electrons. And what properties does an electron have? Does it have a smell or a colour or a texture? No! ... We physicists have come up with geeky names for [Electron] properties, like electric charge, or spin, or lepton number, but the electron doesn't care what we call it, the properties are just numbers.

This is the illusory reality Gnostics were describing. This is the simulation. The A, C, G, and T codes of DNA have a binary value – A and

C = 0 while G and T = 1. This has to be when the simulation is digital and the body must be digital to interact with it. Recurring mathematical sequences are encoded throughout reality and the body. They include the Fibonacci sequence in which the two previous numbers are added to get the next one, as in ... 1, 1, 2, 3, 5, 8, 13, 21, 34, 55, etc. The sequence is encoded in the human face and body, proportions of animals, DNA, seed heads, pine cones, trees, shells, spiral galaxies, hurricanes and the number of petals in a flower. The list goes on and on. There are fractal patterns – a 'never-ending pattern that is infinitely complex and self-similar across all scales in the as above, so below, principle of holograms. These and other famous recurring geometrical and mathematical sequences such as Phi, Pi, Golden Mean, Golden Ratio and Golden Section are *computer codes* of the simulation. I had to laugh and give my head a shake the day I finished this book and it went into the production stage. I was sent an article in *Scientific American* published in April, 2021, with the headline 'Confirmed! We Live in a Simulation'. Two decades after I first said our reality is a simulation and the speed of light is it's outer limit the article suggested that we do live in a simulation and that the speed of light is its outer limit. I left school at 15 and never passed a major exam in my life while the writer was up to his eyes in qualifications. As I will explain in the final chapter *knowing* is far better than thinking and they come from very different sources. The article rightly connected the speed of light to the processing speed of the 'Matrix' and said what has been in my books all this time … 'If we are in a simulation, as it appears, then space is an abstract property written in code. It is not real'. No it's not and if we live in a simulation something created it and it wasn't *us*. 'That David Icke says we are manipulated by aliens' – he's crackers.'

Wow …

The reality that humanity thinks is so real is an illusion. Politicians, governments, scientists, doctors, academics, law enforcement, media, school and university curriculums, on and on, are all founded on a world that *does not exist* except as a simulated prison cell. Is it such a stretch to accept that 'Covid' doesn't exist when our entire 'physical' reality doesn't exist? Revealed here is the knowledge kept under raps in the Cult networks of compartmentalised secrecy to control humanity's sense of reality by

inducing the population to believe in a reality that's not real. If it wasn't so tragic in its experiential consequences the whole thing would be hysterically funny. None of this is new to Renegade Minds. Ancient Greek philosopher Plato (about 428 to about 347BC) was a major influence on Gnostic belief and he described the human plight thousands of years ago with his Allegory of the Cave. He told the symbolic story of prisoners living in a cave who had never been outside. They were chained and could only see one wall of the cave while behind them was a fire that they could not see. Figures walked past the fire casting shadows on the prisoners' wall and those moving shadows became their sense of reality. Some prisoners began to study the shadows and were considered experts on them (today's academics and scientists), but what they studied was only an illusion (today's academics and scientists). A prisoner escaped from the cave and saw reality as it really is. When he returned to report this revelation they didn't believe him, called him mad and threatened to kill him if he tried to set them free. Plato's tale is not only a brilliant analogy of the human plight and our illusory reality. It describes, too, the dynamics of the 'Covid' hoax. I have only skimmed the surface of these subjects here. The aim of this book is to crisply connect all essential dots to put what is happening today into its true context. All subject areas and their connections in this chapter are covered in great evidential detail in *Everything You Need To Know, But Have Never Been Told* and *The Answer*.

They say that bewildered people 'can't see the forest for the trees'. Humanity, however, can't see the forest for the *twigs*. The five senses see only twigs while Renegade Minds can see the forest and it's the forest where the answers lie with the connections that reveals. Breaking free of perceptual programming so the forest can be seen is the way we turn all this around. Not breaking free is how humanity got into this mess. The situation may seem hopeless, but I promise you it's not. We are a perceptual heartbeat from paradise if only we knew.

CHAPTER TWELVE

Escaping Wetiko

Life is simply a vacation from the infinite
Dean Cavanagh

Renegade Minds weave the web of life and events and see common themes in the apparently random. They are always there if you look for them and their pursuit is aided by incredible synchronicity that comes when your mind is open rather than mesmerised by what it thinks it can see.

Infinite awareness is infinite possibility and the more of infinite possibility that we access the more becomes infinitely possible. That may be stating the apparently obvious, but it is a devastatingly-powerful fact that can set us free. We are a point of attention within an infinity of consciousness. The question is how much of that infinity do we choose to access? How much knowledge, insight, awareness, wisdom, do we want to connect with and explore? If your focus is only in the five senses you will be influenced by a fraction of infinite awareness. I mean a range so tiny that it gives new meaning to infinitesimal. Limitation of self-identity and a sense of the possible limit accordingly your range of consciousness. We are what we think we are. Life is what we think it is. The dream is the dreamer and the dreamer is the dream. Buddhist philosophy puts it this way: 'As a thing is viewed, so it appears.' Most humans live in the realm of touch, taste, see, hear, and smell and that's the limit of their sense of the possible and sense of self. Many will follow a religion and speak of a God in his heaven, but their lives are still dominated by the five senses in their perceptions and actions. The five senses become the arbiter of everything.

When that happens all except a smear of infinity is sealed away from influence by the rigid, unyielding, reality bubbles that are the five-sense human or Phantom Self. Archon Cult methodology is to isolate consciousness within five-sense reality – the simulation – and then program that consciousness with a sense of self and the world through a deluge of life-long information designed to instil the desired perception that allows global control. Efforts to do this have increased dramatically with identity politics as identity bubbles are squeezed into the minutiae of five-sense detail which disconnect people even more profoundly from the infinite 'I'.

Five-sense focus and self-identity are like a firewall that limits access to the infinite realms. You only perceive one radio or television station and no other. We'll take that literally for a moment. Imagine a vast array of stations giving different information and angles on reality, but you only ever listen to one. Here we have the human plight in which the population is overwhelmingly confined to CultFM. This relates only to the frequency range of CultFM and limits perception and insight to that band – limits *possibility* to that band. It means you are connecting with an almost imperceptibly minuscule range of possibility and creative potential within the infinite Field. It's a world where everything seems apart from everything else and where synchronicity is rare. Synchronicity is defined in the dictionary as 'the happening by chance of two or more related or similar events at the same time'. Use of 'by chance' betrays a complete misunderstanding of reality. Synchronicity is not 'by chance'. As people open their minds, or 'awaken' to use the term, they notice more and more coincidences in their lives, bits of 'luck', apparently miraculous happenings that put them in the right place at the right time with the right people. Days become peppered with 'fancy meeting you here' and 'what are the chances of that?' My entire life has been lived like this and ever more so since my own colossal awakening in 1990 and 91 which transformed my sense of reality. Synchronicity is not 'by chance'; it is by accessing expanded realms of possibility which allow expanded potential for manifestation. People broadcasting the same vibe from the same openness of mind tend to be drawn 'by chance' to each other through what I call frequency magnetism and it's not only people. In the last more than 30 years incredible synchronicity has also led me through the Cult maze to information in so many forms and to crucial personal experiences. These 'coincidences' have allowed me to put the puzzle pieces together across an enormous array of

subjects and situations. Those who have breached the bubble of five-sense reality will know exactly what I mean and this escape from the perceptual prison cell is open to everyone whenever they make that choice. This may appear super-human when compared with the limitations of 'human', but it's really our natural state. 'Human' as currently experienced is consciousness in an unnatural state of induced separation from the infinity of the whole. I'll come to how this transformation into unity can be made when I have described in more detail the force that holds humanity in servitude by denying this access to infinite self.

The Wetiko factor

I have been talking and writing for decades about the way five-sense mind is systematically barricaded from expanded awareness. I have used the analogy of a computer (five-sense mind) and someone at the keyboard (expanded awareness). Interaction between the computer and the operator is symbolic of the interaction between five-sense mind and expanded awareness. The computer directly experiences the Internet and the operator experiences the Internet via the computer which is how it's supposed to be – the two working as one. Archons seek to control that point where the operator connects with the computer to stop that interaction (Fig 20). Now the operator is banging the keyboard and clicking the mouse, but the computer is not responding and this happens when the computer is taken over – *possessed* – by an appropriately-named computer 'virus'. The operator has lost all influence over the computer which goes its own way making decisions under the control of the 'virus'. I have just described the dynamic through which the force known to Gnostics as Yaldabaoth and Archons disconnects five-sense mind from expanded awareness to imprison humanity in perceptual servitude.

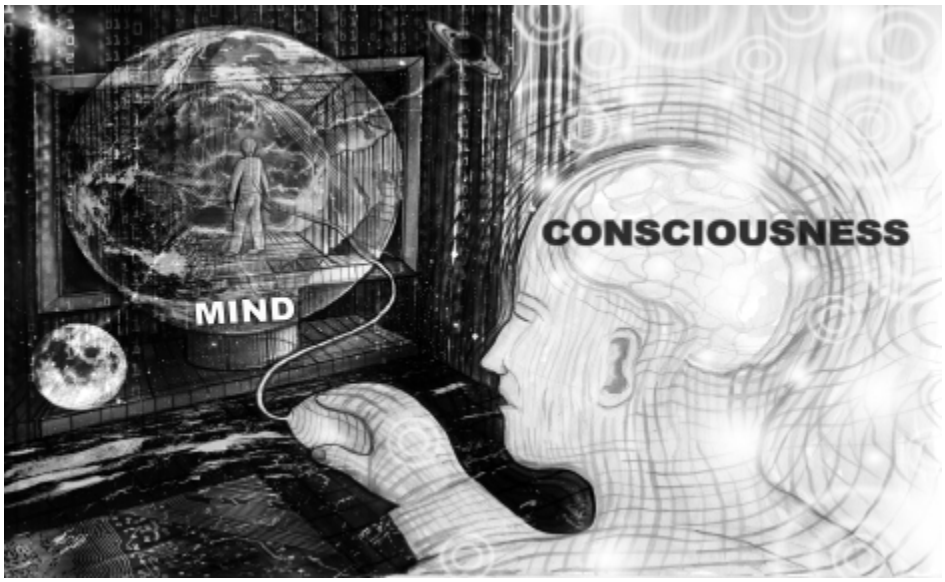

Figure 20: The mind 'virus' I have been writing about for decades seeks to isolate five-sense mind (the computer) from the true 'I'. (Image by Neil Hague).

About a year ago I came across a Native American concept of Wetiko which describes precisely the same phenomenon. Wetiko is the spelling used by the Cree and there are other versions including wintiko and windigo used by other tribal groups. They spell the name with lower case, but I see Wetiko as a proper noun as with Archons and prefer a capital. I first saw an article about Wetiko by writer and researcher Paul Levy which so synced with what I had been writing about the computer/operator disconnection and later the Archons. I then read his book, the fascinating *Dispelling Wetiko, Breaking the Spell of Evil*. The parallels between what I had concluded long before and the Native American concept of Wetiko were so clear and obvious that it was almost funny. For Wetiko see the Gnostic Archons for sure and the Jinn, the Predators, and every other name for a force of evil, inversion and chaos. Wetiko is the Native American name for the force that divides the computer from the operator (Fig 21). Indigenous author Jack D. Forbes, a founder of the Native American movement in the 1960s, wrote another book about Wetiko entitled *Columbus And Other Cannibals – The Wetiko Disease of Exploitation, Imperialism, and Terrorism* which I also read. Forbes says that Wetiko refers to an evil person or spirit 'who terrorizes other creatures by means of terrible acts, including cannibalism'. Zulu shaman Credo Mutwa told me that African accounts tell how cannibalism was brought into the world by

the Chitauri ‘gods’ – another manifestation of Wetiko. The distinction between ‘evil person or spirit’ relates to Archons/Wetiko possessing a human or acting as pure consciousness. Wetiko is said to be a sickness of the soul or spirit and a state of being that takes but gives nothing back – the Cult and its operatives perfectly described. Black Hawk, a Native American war leader defending their lands from confiscation, said European invaders had ‘poisoned hearts’ – Wetiko hearts – and that this would spread to native societies. Mention of the heart is very significant as we shall shortly see. Forbes writes: ‘Tragically, the history of the world for the past 2,000 years is, in great part, the story of the epidemiology of the wetiko disease.’ Yes, and much longer. Forbes is correct when he says: ‘The wetikos destroyed Egypt and Babylon and Athens and Rome and Tenochtitlan [capital of the Aztec empire] and perhaps now they will destroy the entire earth.’ Evil, he said, is the number one export of a Wetiko culture – see its globalisation with ‘Covid’. Constant war, mass murder, suffering of all kinds, child abuse, Satanism, torture and human sacrifice are all expressions of Wetiko and the Wetiko possessed. The world is Wetiko made manifest, *but it doesn’t have to be*. There is a way out of this even now.

Figure 21: The mind ‘virus’ is known to Native Americans as ‘Wetiko’. (Image by Neil Hague).

Cult of Wetiko

Wetiko is the Yaldabaoth frequency distortion that seeks to attach to human consciousness and absorb it into its own. Once this connection is made Wetiko can drive the perceptions of the target which they believe to be coming from their own mind. All the horrors of history and today from mass killers to Satanists, paedophiles like Jeffrey Epstein and other psychopaths, are the embodiment of Wetiko and express its state of being in all its grotesqueness. The Cult is Wetiko incarnate, Yaldabaoth incarnate, and it seeks to facilitate Wetiko assimilation of humanity in totality into its distortion by manipulating the population into low frequency states that match its own. Paul Levy writes: 'Holographically enforced within the psyche of every human being the wetiko virus pervades and underlies the entire field of consciousness, and can therefore potentially manifest through any one of us at any moment if we are not mindful.' The 'Covid' hoax has achieved this with many people, but others have not fallen into Wetiko's frequency lair. Players in the 'Covid' human catastrophe including Gates, Schwab, Tedros, Fauci, Whitty, Vallance, Johnson, Hancock, Ferguson, Drosten, and all the rest, including the psychopath psychologists, are expressions of Wetiko. This is why they have no compassion or empathy and no emotional consequence for what they do that would make them stop doing it. Observe all the people who support the psychopaths in authority against the Pushbackers despite the damaging impact the psychopaths have on their own lives and their family's lives. You are again looking at Wetiko possession which prevents them seeing through the lies to the obvious scam going on. *Why can't they see it?* Wetiko won't let them see it. The perceptual divide that has now become a chasm is between the Wetikoed and the non-Wetikoed.

Paul Levy describes Wetiko in the same way that I have long described the Archontic force. They are the same distorted consciousness operating across dimensions of reality: '… the subtle body of wetiko is not located in the third dimension of space and time, literally existing in another dimension … it is able to affect ordinary lives by mysteriously interpenetrating into our three-dimensional world.' Wetiko does this through its incarnate representatives in the Cult and by weaving itself into The Field which on our level of reality is the electromagnetic information field of the simulation or Matrix. More than that, the simulation *is* Wetiko / Yaldabaoth. Caleb Scharf, Director of Astrobiology at Columbia University, has speculated that 'alien life' could be so advanced that it has transcribed

itself into the quantum realm to become what we call physics. He said intelligence indistinguishable from the fabric of the Universe would solve many of its greatest mysteries:

> Perhaps hyper-advanced life isn't just external. Perhaps it's already all around. It is embedded in what we perceive to be physics itself, from the root behaviour of particles and fields to the phenomena of complexity and emergence ... In other words, life might not just be in the equations. It might BE the equations [My emphasis].

Scharf said it is possible that 'we don't recognise advanced life because it forms an integral and unsuspicious part of what we've considered to be the natural world'. I agree. Wetiko/Yaldabaoth *is* the simulation. We are literally in the body of the beast. But that doesn't mean it has to control us. We all have the power to overcome Wetiko influence and the Cult knows that. I doubt it sleeps too well because it knows that.

Which Field?

This, I suggest, is how it all works. There are two Fields. One is the fierce electromagnetic light of the Matrix within the speed of light; the other is the 'watery light' of The Field beyond the walls of the Matrix that connects with the Great Infinity. Five-sense mind and the decoding systems of the body attach us to the Field of Matrix light. They have to or we could not experience this reality. Five-sense mind sees only the Matrix Field of information while our expanded consciousness is part of the Infinity Field. When we open our minds, and most importantly our hearts, to the Infinity Field we have a mission control which gives us an expanded perspective, a road map, to understand the nature of the five-sense world. If we are isolated only in five-sense mind there is no mission control. We're on our own trying to understand a world that's constantly feeding us information to ensure we do not understand. People in this state can feel 'lost' and bewildered with no direction or radar. You can see ever more clearly those who are influenced by the Fields of Big Infinity or little five-sense mind simply by their views and behaviour with regard to the 'Covid' hoax. We have had this division throughout known human history with the mass of the people on one side and individuals who could see and intuit beyond the walls of the simulation – Plato's prisoner who broke out of the cave and

saw reality for what it is. Such people have always been targeted by Wetiko/Archon-possessed authority, burned at the stake or demonised as mad, bad and dangerous. The Cult today and its global network of 'anti-hate', 'anti-fascist' Woke groups are all expressions of Wetiko attacking those exposing the conspiracy, 'Covid' lies and the 'vaccine' agenda.

Woke as a whole is Wetiko which explains its black and white mentality and how at one it is with the Wetiko-possessed Cult. Paul Levy said: 'To be in this paradigm is to still be under the thrall of a two-valued logic – where things are either true or false – of a wetikoized mind.' Wetiko consciousness is in a permanent rage, therefore so is Woke, and then there is Woke inversion and contradiction. 'Anti-fascists' act like fascists because fascists *and* 'anti-fascists' are both Wetiko at work. Political parties act the same while claiming to be different for the same reason. Secret society and satanic rituals are attaching initiates to Wetiko and the cold, ruthless, psychopathic mentality that secures the positions of power all over the world is Wetiko. Reframing 'training programmes' have the same cumulative effect of attaching Wetiko and we have their graduates described as automatons and robots with a cold, psychopathic, uncaring demeanour. They are all traits of Wetiko possession and look how many times they have been described in this book and elsewhere with regard to personnel behind 'Covid' including the police and medical profession. Climbing the greasy pole in any profession in a Wetiko society requires traits of Wetiko to get there and that is particularly true of politics which is not about fair competition and pre-eminence of ideas. It is founded on how many backs you can stab and arses you can lick. This culminated in the global 'Covid' coordination between the Wetiko possessed who pulled it off in all the different countries without a trace of empathy and compassion for their impact on humans. Our sight sense can see only holographic form and not the Field which connects holographic form. Therefore we perceive 'physical' objects with 'space' in between. In fact that 'space' is energy/consciousness operating on multiple frequencies. One of them is Wetiko and that connects the Cult psychopaths, those who submit to the psychopaths, and those who serve the psychopaths in the media operations of the world. Wetiko is Gates. Wetiko is the mask-wearing submissive. Wetiko is the fake journalist and 'fact-checker'. The Wetiko Field is coordinating the whole thing. Psychopaths, gofers, media operatives, 'anti-hate' hate groups, 'fact-checkers' and submissive people work as one unit

even without human coordination because they are attached to the *same* Field which is organising it all (Fig 22). Paul Levy is here describing how Wetiko-possessed people are drawn together and refuse to let any information breach their rigid perceptions. He was writing long before 'Covid', but I think you will recognise followers of the 'Covid' religion *oh just a little bit*:

People who are channelling the vibratory frequency of wetiko align with each other through psychic resonance to reinforce their unspoken shared agreement so as to uphold their deranged view of reality. Once an unconscious content takes possession of certain individuals, it irresistibly draws them together by mutual attraction and knits them into groups tied together by their shared madness that can easily swell into an avalanche of insanity.

A psychic epidemic is a closed system, which is to say that it is insular and not open to any new information or informing influences from the outside world which contradict its fixed, limited, and limiting perspective.

There we have the Woke mind and the 'Covid' mind. Compatible resonance draws the awakening together, too, which is clearly happening today.

Figure 22: The Wetiko Field from which the Cult pyramid and its personnel are made manifest. (Image by Neil Hague).

Spiritual servitude

Wetiko doesn't care about humans. It's not human; it just possesses humans for its own ends and the effect (depending on the scale of possession) can be anything from extreme psychopathy to unquestioning obedience. Wetiko's worst nightmare is for human consciousness to expand beyond the simulation. Everything is focussed on stopping that happening through control of information, thus perception, thus frequency. The 'education system', media, science, medicine, academia, are all geared to maintaining humanity in five-sense servitude as is the constant stimulation of low-vibrational mental and emotional states (see 'Covid'). Wetiko seeks to dominate those subconscious spaces between five-sense perception and expanded consciousness where the computer meets the operator. From these subconscious hiding places Wetiko speaks to us to trigger urges and desires that we take to be our own and manipulate us into anything from low-vibrational to psychopathic states. Remember how Islam describes the Jinn as invisible tricksters that 'whisper' and confuse. Wetiko is the origin of the 'trickster god' theme that you find in cultures all over the world. Jinn, like the Archons, are Wetiko which is terrified of humans awakening and reconnecting with our true self for then its energy source has gone. With that the feedback loop breaks between Wetiko and human perception that provides the energetic momentum on which its very existence depends as a force of evil. Humans are both its target and its source of survival, but only if we are operating in low-vibrational states of fear, hate, depression and the background anxiety that most people suffer. We are Wetiko's target because we are its key to survival. It needs us, not the other way round. Paul Levy writes:

> A vampire has no intrinsic, independent, substantial existence in its own right; it only exists in relation to us. The pathogenic, vampiric mind-parasite called wetiko is nothing in itself – not being able to exist from its own side – yet it has a 'virtual reality' such that it can potentially destroy our species …
>
> …The fact that a vampire is not reflected by a mirror can also mean that what we need to see is that there's nothing, no-thing to see, other than ourselves. The fact that wetiko is the expression of something inside of us means that the cure for wetiko is with us as well. The critical issue is finding this cure within us and then putting it into effect.

Evil begets evil because if evil does not constantly expand and find new sources of energetic sustenance its evil, its *distortion*, dies with the assimilation into balance and harmony. Love is the garlic to Wetiko's vampire. Evil, the absence of love, cannot exist in the presence of love. I think I see a way out of here. I have emphasised so many times over the decades that the Archons/Wetiko and their Cult are not all powerful. *They are not*. I don't care how it looks even now *they are not*. I have not called them little boys in short trousers for effect. I have said it because it is true. Wetiko's insatiable desire for power over others is not a sign of its omnipotence, but its insecurity. Paul Levy writes: 'Due to the primal fear which ultimately drives it and which it is driven to cultivate, wetiko's body politic has an intrinsic and insistent need for centralising power and control so as to create imagined safety for itself.' *Yeeeeeees!* Exactly! Why does Wetiko want humans in an ongoing state of fear? Wetiko itself *is* fear and it is petrified of love. As evil is an absence of love, so love is an absence of fear. Love conquers all and *especially* Wetiko which *is* fear. Wetiko brought fear into the world when it wasn't here before. *Fear* was the 'fall', the fall into low-frequency ignorance and illusion – fear is **F**alse **E**motion **A**ppearing **R**eal. The simulation is driven and energised by fear because Wetiko/Yaldabaoth (fear) *are* the simulation. Fear is the absence of love and Wetiko is the absence of love.

Wetiko today

We can now view current events from this level of perspective. The 'Covid' hoax has generated momentous amounts of ongoing fear, anxiety, depression and despair which have empowered Wetiko. No wonder people like Gates have been the instigators when they are Wetiko incarnate and exhibit every trait of Wetiko in the extreme. See how cold and unemotional these people are like Gates and his cronies, how dead of eye they are. That's Wetiko. Sabbatians are Wetiko and everything they control including the World Health Organization, Big Pharma and the 'vaccine' makers, national 'health' hierarchies, corporate media, Silicon Valley, the banking system, and the United Nations with its planned transformation into world government. All are controlled and possessed by the Wetiko distortion into distorting human society in its image. We are with this knowledge at the gateway to understanding the world. Divisions of race, culture, creed and

sexuality are diversions to hide the real division between those possessed and influenced by Wetiko and those that are not. The 'Covid' hoax has brought both clearly into view. Human behaviour is not about race. Tyrants and dictatorships come in all colours and creeds. What unites the US president bombing the innocent and an African tribe committing genocide against another as in Rwanda? What unites them? *Wetiko*. All wars are Wetiko, all genocide is Wetiko, all hunger over centuries in a world of plenty is Wetiko. Children going to bed hungry, including in the West, is Wetiko. Cult-generated Woke racial divisions that focus on the body are designed to obscure the reality that divisions in behaviour are manifestations of mind, not body. Obsession with body identity and group judgement is a means to divert attention from the real source of behaviour – mind and perception. Conflict sown by the Woke both within themselves and with their target groups are Wetiko providing lunch for itself through still more agents of the division, chaos, and fear on which it feeds. The Cult is seeking to assimilate the entirety of humanity and all children and young people into the Wetiko frequency by manipulating them into states of fear and despair. Witness all the suicide and psychological unravelling since the spring of 2020. Wetiko psychopaths want to impose a state of unquestioning obedience to authority which is no more than a conduit for Wetiko to enforce its will and assimilate humanity into itself. It needs us to believe that resistance is futile when it fears resistance and even more so the game-changing non-cooperation with its impositions. It can use violent resistance for its benefit. Violent impositions and violent resistance are *both* Wetiko. The Power of Love with its Power of No will sweep Wetiko from our world. Wetiko and its Cult know that. They just don't want us to know.

AI Wetiko

This brings me to AI or artificial intelligence and something else Wetikos don't want us to know. What is AI *really*? I know about computer code algorithms and AI that learns from data input. These, however, are more diversions, the expeditionary force, for the real AI that they want to connect to the human brain as promoted by Silicon Valley Wetikos like Kurzweil. What is this AI? It is the frequency of *Wetiko*, the frequency of the Archons. The connection of AI to the human brain is the connection of the Wetiko frequency to create a Wetiko hive mind and complete the job of

assimilation. The hive mind is planned to be controlled from Israel and China which are both 100 percent owned by Wetiko Sabbatians. The assimilation process has been going on minute by minute in the ‘smart’ era which fused with the ‘Covid’ era. We are told that social media is scrambling the minds of the young and changing their personality. This is true, but what is social media? Look more deeply at how it works, how it creates divisions and conflict, the hostility and cruelty, the targeting of people until they are destroyed. That’s Wetiko. Social media is manipulated to tune people to the Wetiko frequency with all the emotional exploitation tricks employed by platforms like Facebook and its Wetiko front man, Zuckerberg. Facebook’s Instagram announced a new platform for children to overcome a legal bar on them using the main site. This is more Wetiko exploitation and manipulation of kids. Amnesty International likened the plan to foxes offering to guard the henhouse and said it was incompatible with human rights. Since when did Wetiko or Zuckerberg (I repeat myself) care about that? Would Brin and Page at Google, Wojcicki at YouTube, Bezos at Amazon and whoever the hell runs Twitter act as they do if they were not channelling Wetiko? Would those who are developing technologies for no other reason than human control? How about those designing and selling technologies to kill people and Big Pharma drug and ‘vaccine’ producers who know they will end or devastate lives? Quite a thought for these people to consider is that if you are Wetiko in a human life you are Wetiko on the ‘other side’ unless your frequency changes and that can only change by a change of perception which becomes a change of behaviour. Where Gates is going does not bear thinking about although perhaps that’s exactly where he wants to go. Either way, that’s where he’s going. His frequency will make it so.

The frequency lair

I have been saying for a long time that a big part of the addiction to smartphones and devices is that a frequency is coming off them that entraps the mind. People spend ages on their phones and sometimes even a minute or so after they put them down they pick them up again and it all repeats. ‘Covid’ lockdowns will have increased this addiction a million times for obvious reasons. Addictions to alcohol overindulgence and drugs are another way that Wetiko entraps consciousness to attach to its own. Both

are symptoms of low-vibrational psychological distress which alcoholism and drug addiction further compound. Do we think it's really a coincidence that access to them is made so easy while potions that can take people into realms beyond the simulation are banned and illegal? I have explored smartphone addiction in other books, the scale is mind-blowing, and that level of addiction does not come without help. Tech companies that make these phones are Wetiko and they will have no qualms about destroying the minds of children. We are seeing again with these companies the Wetiko perceptual combination of psychopathic enforcers and weak and meek unquestioning compliance by the rank and file.

The global Smart Grid is the Wetiko Grid and it is crucial to complete the Cult endgame. The simulation is radiation and we are being deluged with technological radiation on a devastating scale. Wetiko frauds like Elon Musk serve Cult interests while occasionally criticising them to maintain his street-cred. 5G and other forms of Wi-Fi are being directed at the earth from space on a volume and scale that goes on increasing by the day. Elon Musk's (officially) SpaceX Starlink project is in the process of putting tens of thousands of satellites in low orbit to cover every inch of the planet with 5G and other Wi-Fi to create Kurzweil's global 'cloud' to which the human mind is planned to be attached very soon. SpaceX has approval to operate 12,000 satellites with more than 1,300 launched at the time of writing and applications filed for *30,000* more. Other operators in the Wi-Fi, 5G, low-orbit satellite market include OneWeb (UK), Telesat (Canada), and AST & Science (US). Musk tells us that AI could be the end of humanity and then launches a company called Neuralink to connect the human brain to computers. Musk's (in theory) Tesla company is building electric cars and the driverless vehicles of the smart control grid. As frauds and bullshitters go Elon Musk in my opinion is Major League.

5G and technological radiation in general are destructive to human health, genetics and psychology and increasing the strength of artificial radiation underpins the five-sense perceptual bubbles which are themselves expressions of radiation or electromagnetism. Freedom activist John Whitehead was so right with his 'databit by databit, we are building our own electronic concentration camps'. The Smart Grid and 5G is a means to control the human mind and infuse perceptual information into The Field to influence anyone in sync with its frequency. You can change perception and behaviour en masse if you can manipulate the population into those levels

of frequency and this is happening all around us today. The arrogance of Musk and his fellow Cult operatives knows no bounds in the way that we see with Gates. Musk's satellites are so many in number already they are changing the night sky when viewed from Earth. The astronomy community has complained about this and they have seen nothing yet. Some consequences of Musk's Wetiko hubris include: Radiation; visible pollution of the night sky; interference with astronomy and meteorology; ground and water pollution from intensive use of increasingly many spaceports; accumulating space debris; continual deorbiting and burning up of aging satellites, polluting the atmosphere with toxic dust and smoke; and ever-increasing likelihood of collisions. A collective public open letter of complaint to Musk said:

> We are writing to you … because SpaceX is in process of surrounding the Earth with a network of thousands of satellites whose very purpose is to irradiate every square inch of the Earth. SpaceX, like everyone else, is treating the radiation as if it were not there. As if the mitochondria in our cells do not depend on electrons moving undisturbed from the food we digest to the oxygen we breathe.
>
> As if our nervous systems and our hearts are not subject to radio frequency interference like any piece of electronic equipment. As if the cancer, diabetes, and heart disease that now afflict a majority of the Earth's population are not metabolic diseases that result from interference with our cellular machinery. As if insects everywhere, and the birds and animals that eat them, are not starving to death as a result.

People like Musk and Gates believe in their limitless Wetiko arrogance that they can do whatever they like to the world because they own it. Consequences for humanity are irrelevant. It's absolutely time that we stopped taking this shit from these self-styled masters of the Earth when you consider where this is going.

Why is the Cult so anti-human?

I hear this question often: Why would they do this when it will affect them, too? Ah, but will it? Who is this *them*? Forget their bodies. They are just vehicles for Wetiko consciousness. When you break it all down to the foundations we are looking at a state of severely distorted consciousness targeting another state of consciousness for assimilation. The rest is detail. The simulation is the fly-trap in which unique sensations of the five senses

create a cycle of addiction called reincarnation. Renegade Minds see that everything which happens in our reality is a smaller version of the whole picture in line with the holographic principle. Addiction to the radiation of smart technology is a smaller version of addiction to the whole simulation. Connecting the body/brain to AI is taking that addiction on a giant step further to total ongoing control by assimilating human incarnate consciousness into Wetiko. I have watched during the 'Covid' hoax how many are becoming ever more profoundly attached to Wetiko's perceptual calling cards of aggressive response to any other point of view ('There is no other god but me'), psychopathic lack of compassion and empathy, and servile submission to the narrative and will of authority. Wetiko is the psychopaths *and* subservience to psychopaths. The Cult of Wetiko is so anti-human because it is *not* human. It embarked on a mission to destroy human by targeting everything that it means to be human and to survive as human. 'Covid' is not the end, just a means to an end. The Cult with its Wetiko consciousness is seeking to change Earth systems, including the atmosphere, to suit them, not humans. The gathering bombardment of 5G alone from ground and space is dramatically changing The Field with which the five senses interact. There is so much more to come if we sit on our hands and hope it will all go away. It is not meant to go away. It is meant to get ever more extreme and we need to face that while we still can – just.

Carbon dioxide is the gas of life. Without that human is over. Kaput, gone, history. No natural world, no human. The Cult has created a cock and bull story about carbon dioxide and climate change to justify its reduction to the point where Gates and the ignoramus Biden 'climate chief' John Kerry want to suck it out of the atmosphere. Kerry wants to do this because his master Gates does. Wetikos have made the gas of life a demon with the usual support from the Wokers of Extinction Rebellion and similar organisations and the bewildered puppet-child that is Greta Thunberg who was put on the world stage by Klaus Schwab and the World Economic Forum. The name Extinction Rebellion is both ironic and as always Wetiko inversion. The gas that we need to survive must be reduced to save us from extinction. The most basic need of human is oxygen and we now have billions walking around in face nappies depriving body and brain of this essential requirement of human existence. More than that 5G at 60 gigahertz interacts with the oxygen molecule to reduce the amount of oxygen the body can absorb into the bloodstream. The obvious knock-on

consequences of that for respiratory and cognitive problems and life itself need no further explanation. Psychopaths like Musk are assembling a global system of satellites to deluge the human atmosphere with this insanity. The man should be in jail. Here we have two most basic of human needs, oxygen and carbon dioxide, being dismantled.

Two others, water and food, are getting similar treatment with the United Nations Agendas 21 and 2030 – the Great Reset – planning to centrally control all water and food supplies. People will not even own rain water that falls on their land. Food is affected at the most basic level by reducing carbon dioxide. We have genetic modification or GMO infiltrating the food chain on a mass scale, pesticides and herbicides polluting the air and destroying the soil. Freshwater fish that provide livelihoods for 60 million people and feed hundreds of millions worldwide are being 'pushed to the brink' according the conservationists while climate change is the only focus. Now we have Gates and Schwab wanting to dispense with current food sources all together and replace them with a synthetic version which the Wetiko Cult would control in terms of production and who eats and who doesn't. We have been on the Totalitarian Tiptoe to this for more than 60 years as food has become ever more processed and full of chemical shite to the point today when it's not natural food at all. As Dr Tom Cowan says: 'If it has a label don't eat it.' Bill Gates is now the biggest owner of farmland in the United States and he does nothing without an ulterior motive involving the Cult. Klaus Schwab wrote: 'To feed the world in the next 50 years we will need to produce as much food as was produced in the last 10,000 years … food security will only be achieved, however, if regulations on genetically modified foods are adapted to reflect the reality that gene editing offers a precise, efficient and safe method of improving crops.' Liar. People and the world are being targeted with aluminium through vaccines, chemtrails, food, drink cans, and endless other sources when aluminium has been linked to many health issues including dementia which is increasing year after year. Insects, bees and wildlife essential to the food chain are being deleted by pesticides, herbicides and radiation which 5G is dramatically increasing with 6G and 7G to come. The pollinating bee population is being devastated while wildlife including birds, dolphins and whales are having their natural radar blocked by the effects of ever-increasing radiation. In the summer windscreens used to be splattered with

insects so numerous were they. It doesn't happen now. Where have they gone?

Synthetic everything

The Cult is introducing genetically-modified versions of trees, plants and insects including a Gates-funded project to unleash hundreds of millions of genetically-modified, lab-altered and patented male mosquitoes to mate with wild mosquitoes and induce genetic flaws that cause them to die out. Clinically-insane Gates-funded Japanese researchers have developed mosquitos that spread vaccine and are dubbed 'flying vaccinators'. Gates is funding the modification of weather patterns in part to sell the myth that this is caused by carbon dioxide and he's funding geoengineering of the skies to change the atmosphere. Some of this came to light with the Gates-backed plan to release tonnes of chalk into the atmosphere to 'deflect the Sun and cool the planet'. Funny how they do this while the heating effect of the Sun is not factored into climate projections focussed on carbon dioxide. The reason is that they want to reduce carbon dioxide (so don't mention the Sun), but at the same time they do want to reduce the impact of the Sun which is so essential to human life and health. I have mentioned the sun-cholesterol-vitamin D connection as they demonise the Sun with warnings about skin cancer (caused by the chemicals in sun cream they tell you to splash on). They come from the other end of the process with statin drugs to reduce cholesterol that turns sunlight into vitamin D. A lack of vitamin D leads to a long list of health effects and how vitamin D levels must have fallen with people confined to their homes over 'Covid'. Gates is funding other forms of geoengineering and most importantly chemtrails which are dropping heavy metals, aluminium and self-replicating nanotechnology onto the Earth which is killing the natural world. See *Everything You Need To Know, But Have Never Been Told* for the detailed background to this.

Every human system is being targeted for deletion by a force that's not human. The Wetiko Cult has embarked on the process of transforming the human body from biological to synthetic biological as I have explained. Biological is being replaced by the artificial and synthetic – Archontic 'countermimicry' – right across human society. The plan eventually is to dispense with the human body altogether and absorb human consciousness – which it wouldn't really be by then – into cyberspace (the simulation

which is Wetiko/Yaldabaoth). Preparations for that are already happening if people would care to look. The alternative media rightly warns about globalism and 'the globalists', but this is far bigger than that and represents the end of the human race as we know it. The 'bad copy' of prime reality that Gnostics describe was a bad copy of harmony, wonder and beauty to start with before Wetiko/Yaldabaoth set out to change the simulated 'copy' into something very different. The process was slow to start with. Entrapped humans in the simulation timeline were not technologically aware and they had to be brought up to intellectual speed while being suppressed spiritually to the point where they could build their own prison while having no idea they were doing so. We have now reached that stage where technological intellect has the potential to destroy us and that's why events are moving so fast. Central American shaman Don Juan Matus said:

> Think for a moment, and tell me how you would explain the contradictions between the intelligence of man the engineer and the stupidity of his systems of belief, or the stupidity of his contradictory behaviour. Sorcerers believe that the predators have given us our systems of beliefs, our ideas of good and evil; our social mores. They are the ones who set up our dreams of success or failure. They have given us covetousness, greed, and cowardice. It is the predator who makes us complacent, routinary, and egomaniacal.
>
> In order to keep us obedient and meek and weak, the predators engaged themselves in a stupendous manoeuvre – stupendous, of course, from the point of view of a fighting strategist; a horrendous manoeuvre from the point of those who suffer it. They gave us their mind. The predators' mind is baroque, contradictory, morose, filled with the fear of being discovered any minute now.

For 'predators' see Wetiko, Archons, Yaldabaoth, Jinn, and all the other versions of the same phenomenon in cultures and religions all over the world. The theme is always the same because it's true and it's real. We have reached the point where we have to deal with it. The question is – how?

Don't fight – walk away

I thought I'd use a controversial subheading to get things moving in terms of our response to global fascism. What do you mean 'don't fight'? What do you mean 'walk away'? We've got to fight. We can't walk away. Well, it depends what we mean by fight and walk away. If fighting means physical combat we are playing Wetiko's game and falling for its trap. It wants us to get angry, aggressive, and direct hate and hostility at the enemy we think we

must fight. Every war, every battle, every conflict, has been fought with Wetiko leading both sides. It's what it does. Wetiko wants a fight, anywhere, any place. Just hit me, son, so I can hit you back. Wetiko hits Wetiko and Wetiko hits Wetiko in return. I am very forthright as you can see in exposing Wetikos of the Cult, but I don't hate them. I refuse to hate them. It's what they want. What you hate you become. What you *fight* you become. Wokers, 'anti-haters' and 'anti-fascists' prove this every time they reach for their keyboards or don their balaclavas. By walk away I mean to disengage from Wetiko which includes ceasing to cooperate with its tyranny. Paul Levy says of Wetiko:

The way to 'defeat' evil is not to try to destroy it (for then, in playing evil's game, we have already lost), but rather, to find the invulnerable place within ourselves where evil is unable to vanquish us – this is to truly 'win' our battle with evil.

Wetiko is everywhere in human society and it's been on steroids since the 'Covid' hoax. Every shouting match over wearing masks has Wetiko wearing a mask and Wetiko not wearing one. It's an electrical circuit of push and resist, push and resist, with Wetiko pushing *and* resisting. Each polarity is Wetiko empowering itself. Dictionary definitions of 'resist' include 'opposing, refusing to accept or comply with' and the word to focus on is 'opposing'. What form does this take – setting police cars alight or 'refusing to accept or comply with'? The former is Wetiko opposing Wetiko while the other points the way forward. This is the difference between those aggressively demanding that government fascism must be obeyed who stand in stark contrast to the great majority of Pushbackers. We saw this clearly with a march by thousands of Pushbackers against lockdown in London followed days later by a Woker-hijacked protest in Bristol in which police cars were set on fire. Masks were virtually absent in London and widespread in Bristol. Wetiko wants lockdown on every level of society and infuses its aggression to police it through its unknowing stooges. Lockdown protesters are the ones with the smiling faces and the hugs, The two blatantly obvious states of being – getting more obvious by the day – are the result of Wokers and their like becoming ever more influenced by the simulation Field of Wetiko and Pushbackers ever more influenced by The Field of a far higher vibration beyond the simulation. Wetiko can't invade

the heart which is where most lockdown opponents are coming from. It's the heart that allows them to see through the lies to the truth in ways I will be highlighting.

Renegade Minds know that calmness is the place from which wisdom comes. You won't find wisdom in a hissing fit and wisdom is what we need in abundance right now. Calmness is not weakness – you don't have to scream at the top of your voice to be strong. Calmness is indeed a sign of strength. 'No' means I'm not doing it. *NOOOO!!!* doesn't mean you're not doing it even more. Volume does not advance 'No – I'm not doing it'. You are just not doing it. Wetiko possessed and influenced don't know how to deal with that. Wetiko wants a fight and we should not give it one. What it needs more than anything is our *cooperation* and we should not give that either. Mass rallies and marches are great in that they are a visual representation of feeling, but if it ends there they are irrelevant. You demand that Wetikos act differently? Well, they're not going to are they? They are Wetikos. We don't need to waste our time demanding that something doesn't happen when that will make no difference. We need to delete the means that *allows* it to happen. This, invariably, is our cooperation. You can demand a child stop firing a peashooter at the dog or you can refuse to buy the peashooter. If you provide the means you are cooperating with the dog being smacked on the nose with a pea. How can the authorities enforce mask-wearing if millions in a country refuse? What if the 74 million Pushbackers that voted for Trump in 2020 refused to wear masks, close their businesses or stay in their homes. It would be unenforceable. The few control the many through the compliance of the many and that's always been the dynamic be it 'Covid' regulations or the Roman Empire. I know people can find it intimidating to say no to authority or stand out in a crowd for being the only one with a face on display; but it has to be done or it's over. I hope I've made clear in this book that where this is going will be far more intimidating than standing up now and saying 'No' – I will not cooperate with my own enslavement and that of my children. There might be consequences for some initially, although not so if enough do the same. The question that must be addressed is what is going to happen if we don't? It is time to be strong and unyieldingly so. No means no. Not here and there, but *everywhere* and *always*. I have refused to wear a mask and obey all the other nonsense. I will not comply with tyranny. I repeat: Fascism is not imposed by fascists – there are never enough of them.

Fascism is imposed by the population acquiescing to fascism. *I will not do it*. I will die first, or my body will. Living meekly under fascism is a form of death anyway, the death of the spirit that Martin Luther King described.

Making things happen

We must not despair. This is not over till it's over and it's far from that. The 'fat lady' must refuse to sing. The longer the 'Covid' hoax has dragged on and impacted on more lives we have seen an awakening of phenomenal numbers of people worldwide to the realisation that what they have believed all their lives is not how the world really is. Research published by the system-serving University of Bristol and King's College London in February, 2021, concluded: 'One in every 11 people in Britain say they trust David Icke's take on the coronavirus pandemic.' It will be more by now and we have gathering numbers to build on. We must urgently progress from seeing the scam to ceasing to cooperate with it. Prominent German lawyer Reiner Fuellmich, also licenced to practice law in America, is doing a magnificent job taking the legal route to bring the psychopaths to justice through a second Nuremberg tribunal for crimes against humanity. Fuellmich has an impressive record of beating the elite in court and he formed the German Corona Investigative Committee to pursue civil charges against the main perpetrators with a view to triggering criminal charges. Most importantly he has grasped the foundation of the hoax – the PCR test not testing for the 'virus' – and Christian Drosten is therefore on his charge sheet along with Gates frontman Tedros at the World Health Organization. Major players must be not be allowed to inflict their horrors on the human race without being brought to book. A life sentence must follow for Bill Gates and the rest of them. A group of researchers has also indicted the government of Norway for crimes against humanity with copies sent to the police and the International Criminal Court. The lawsuit cites participation in an internationally-planned false pandemic and violation of international law and human rights, the European Commission's definition of human rights by coercive rules, Nuremberg and Hague rules on fundamental human rights, and the Norwegian constitution. We must take the initiative from hereon and not just complain, protest and react.

There are practical ways to support vital mass non-cooperation. Organising in numbers is one. Lockdown marches in London in the spring

in 2021 were mass non-cooperation that the authorities could not stop. There were too many people. Hundreds of thousands walked the London streets in the centre of the road for mile after mile while the Face-Nappies could only look on. They were determined, but calm, and just *did it* with no histrionics and lots of smiles. The police were impotent. Others are organising group shopping without masks for mutual support and imagine if that was happening all over. Policing it would be impossible. If the store refuses to serve people in these circumstances they would be faced with a long line of trolleys full of goods standing on their own and everything would have to be returned to the shelves. How would they cope with that if it kept happening? I am talking here about moving on from complaining to being pro-active; from watching things happen to making things happen. I include in this our relationship with the police. The behaviour of many Face-Nappies has been disgraceful and anyone who thinks they would never find concentration camp guards in the 'enlightened' modern era have had that myth busted big-time. The period and setting may change – Wetikos never do. I watched film footage from a London march in which a police thug viciously kicked a protestor on the floor who had done nothing. His fellow Face-Nappies stood in a ring protecting him. What he did was a criminal assault and with a crowd far outnumbering the police this can no longer be allowed to happen unchallenged. I get it when people chant 'shame on you' in these circumstances, but that is no longer enough. They *have* no shame those who do this. Crowds needs to start making a citizen's arrest of the police who commit criminal offences and brutally attack innocent people and defenceless women. A citizen's arrest can be made under section 24A of the UK Police and Criminal Evidence (PACE) Act of 1984 and you will find something similar in other countries. I prefer to call it a Common Law arrest rather than citizen's for reasons I will come to shortly. Anyone can arrest a person committing an indictable offence or if they have reasonable grounds to suspect they are committing an indictable offence. On both counts the attack by the police thug would have fallen into this category. A citizen's arrest can be made to stop someone:

- Causing physical injury to himself or any other person
- Suffering physical injury
- Causing loss of or damage to property
- Making off before a constable can assume responsibility for him

A citizen's arrest may also be made to prevent a breach of the peace under Common Law and if they believe a breach of the peace will happen or anything related to harm likely to be done or already done in their presence. This is the way to go I think – the Common Law version. If police know that the crowd and members of the public will no longer be standing and watching while they commit their thuggery and crimes they will think twice about acting like Brownshirts and Blackshirts.

Common Law – common sense

Mention of Common Law is very important. Most people think the law is the law as in one law. This is not the case. There are two bodies of law, Common Law and Statute Law, and they are not the same. Common Law is founded on the simple premise of do no harm. It does not recognise victimless crimes in which no harm is done while Statute Law does. There is a Statute Law against almost everything. So what is Statute Law? Amazingly it's the law of the *sea* that was brought ashore by the Cult to override the law of the land which is Common Law. They had no right to do this and as always they did it anyway. They had to. They could not impose their will on the people through Common Law which only applies to do no harm. How could you stitch up the fine detail of people's lives with that? Instead they took the law of the sea, or Admiralty Law, and applied it to the population. Statute Law refers to all the laws spewing out of governments and their agencies including all the fascist laws and regulations relating to 'Covid'. The key point to make is that Statute Law is *contract law*. It only applies between *contracting* corporations. Most police officers don't even know this. They have to be kept in the dark, too. Long ago when merchants and their sailing ships began to trade with different countries a contractual law was developed called Admiralty Law and other names. Again it only applied to *contracts* agreed between *corporate* entities. If there is no agreed contract the law of the sea had no jurisdiction *and that still applies to its new alias of Statute Law*. The problem for the Cult when the law of the sea was brought ashore was an obvious one. People were not corporations and neither were government entities. To overcome the latter they made governments and all associated organisations corporations. All the institutions are *private corporations* and I mean governments and their

agencies, local councils, police, courts, military, US states, the whole lot. Go to the Dun and Bradstreet corporate listings website for confirmation that they are all corporations. You are arrested by a private corporation called the police by someone who is really a private security guard and they take you to court which is another private corporation. Neither have jurisdiction over you unless you consent and *contract* with them. This is why you hear the mantra about law enforcement policing by *consent* of the people. In truth the people 'consent' only in theory through monumental trickery.

Okay, the Cult overcame the corporate law problem by making governments and institutions corporate entities; but what about people? They are not corporations are they? Ah ... well in a sense, and *only* a sense, they are. Not people exactly – the illusion of people. The Cult creates a corporation in the name of everyone at the time that their birth certificate is issued. Note birth/ *berth* certificate and when you go to court under the law of the sea on land you stand in a *dock*. These are throwbacks to the origin. My Common Law name is David Vaughan Icke. The name of the corporation created by the government when I was born is called Mr David Vaughan Icke usually written in capitals as MR DAVID VAUGHAN ICKE. That is not me, the living, breathing man. It is a fictitious corporate entity. The trick is to make you think that David Vaughan Icke and MR DAVID VAUGHAN ICKE are the same thing. *They are not*. When police charge you and take you to court they are prosecuting the corporate entity and not the living, breathing, man or woman. They have to trick you into identifying as the corporate entity and contracting with them. Otherwise they have no jurisdiction. They do this through a language known as legalese. Lawful and legal are not the same either. Lawful relates to Common Law and legal relates to Statute Law. Legalese is the language of Statue Law which uses terms that mean one thing to the public and another in legalese. Notice that when a police officer tells someone why they are being charged he or she will say at the end: 'Do you understand?' To the public that means 'Do you comprehend?' In legalese it means 'Do you stand under me?' Do you stand under my authority? If you say yes to the question you are unknowingly agreeing to give them jurisdiction over you in a contract between two corporate entities.

This is a confidence trick in every way. Contracts have to be agreed between informed parties and if you don't know that David Vaughan Icke is

agreeing to be the corporation MR DAVID VAUGHAN ICKE you cannot knowingly agree to contract. They are deceiving you and another way they do this is to ask for proof of identity. You usually show them a driving licence or other document on which your corporate name is written. In doing so you are accepting that you are that corporate entity when you are not. Referring to yourself as a 'person' or 'citizen' is also identifying with your corporate fiction which is why I made the Common Law point about the citizen's arrest. If you are approached by a police officer you identify yourself immediately as a living, breathing, man or woman and say 'I do not consent, I do not contract with you and I do not understand' or stand under their authority. I have a Common Law birth certificate as a living man and these are available at no charge from commonlawcourt.com. Businesses registered under the Statute Law system means that its laws apply. There are, however, ways to run a business under Common Law. Remember all 'Covid' laws and regulations are Statute Law – the law of *contracts* and you do not have to contract. This doesn't mean that you can kill someone and get away with it. Common Law says do no harm and that applies to physical harm, financial harm etc. Police are employees of private corporations and there needs to be a new system of non-corporate Common Law constables operating outside the Statute Law system. If you go to davidicke.com and put Common Law into the search engine you will find videos that explain Common Law in much greater detail. It is definitely a road we should walk.

With all my heart

I have heard people say that we are in a spiritual war. I don't like the term 'war' with its Wetiko dynamic, but I know what they mean. Sweep aside all the bodily forms and we are in a situation in which two states of consciousness are seeking very different realities. Wetiko wants upheaval, chaos, fear, suffering, conflict and control. The other wants love, peace, harmony, fairness and freedom. That's where we are. We should not fall for the idea that Wetiko is all-powerful and there's nothing we can do. Wetiko is not all-powerful. It's a joke, pathetic. It doesn't have to be, but it has made that choice for now. A handful of times over the years when I have felt the presence of its frequency I have allowed it to attach briefly so I could consciously observe its nature. The experience is not pleasant, the

energy is heavy and dark, but the ease with which you can kick it back out the door shows that its real power is in persuading us that it has power. It's all a con. Wetiko is a con. It's a trickster and not a power that can control us if we unleash our own. The con is founded on manipulating humanity to give its power to Wetiko which recycles it back to present the illusion that it has power when its power is *ours* that we gave away. This happens on an energetic level and plays out in the world of the seen as humanity giving its power to Wetiko authority which uses that power to control the population when the power is only the power the population has handed over. How could it be any other way for billions to be controlled by a relative few? I have had experiences with people possessed by Wetiko and again you can kick its arse if you do it with an open heart. Oh yes – the *heart* which can transform the world of perceived 'matter'.

We are receiver-transmitters and processors of information, but what information and where from? Information is processed into perception in three main areas – the brain, the heart and the belly. These relate to thinking, knowing, and emotion. Wetiko wants us to be head and belly people which means we think within the confines of the Matrix simulation and low-vibrational emotional reaction scrambles balance and perception. A few minutes on social media and you see how emotion is the dominant force. Woke is all emotion and is therefore thought-free and fact-free. Our heart is something different. It *knows* while the head *thinks* and has to try to work it out because it doesn't know. The human energy field has seven prime vortexes which connect us with wider reality (Fig 23). Chakra means 'wheels of light' in the Sanskrit language of ancient India. The main ones are: The crown chakra on top of the head; brow (or 'third eye') chakra in the centre of the forehead; throat chakra; heart chakra in the centre of the chest; solar plexus chakra below the sternum; sacral chakra beneath the navel; and base chakra at the bottom of the spine. Each one has a particular function or functions. We feel anxiety and nervousness in the belly where the sacral chakra is located and this processes emotion that can affect the colon to give people 'the shits' or make them 'shit scared' when they are nervous. Chakras all play an important role, but the Mr and Mrs Big is the heart chakra which sits at the centre of the seven, above the chakras that connect us to the 'physical' and below those that connect with higher realms (or at least should). Here in the heart chakra we feel love, empathy and compassion – 'My heart goes out to you'. Those with closed hearts

become literally ‘heart-less’ in their attitudes and behaviour (see Bill Gates). Native Americans portrayed Wetiko with what Paul Levy calls a ‘frigid, icy heart, devoid of mercy’ (see Bill Gates).

Figure 23: The chakra system which interpenetrates the human energy field. The heart chakra is the governor – or should be.

Wetiko trembles at the thought of heart energy which it cannot infiltrate. The frequency is too high. What it seeks to do instead is close the heart chakra vortex to block its perceptual and energetic influence. Psychopaths have ‘hearts of stone’ and emotionally-damaged people have ‘heartache’ and ‘broken hearts’. The astonishing amount of heart disease is related to heart chakra disruption with its fundamental connection to the ‘physical’ heart. Dr Tom Cowan has written an outstanding book challenging the belief that the heart is a pump and making the connection between the ‘physical’ and spiritual heart. Rudolph Steiner who was way ahead of his time said the same about the fallacy that the heart is a pump. *What*? The heart is not a pump? That’s crazy, right? Everybody knows that. Read Cowan’s *Human Heart, Cosmic Heart* and you will realise that the very idea of the heart as a pump is ridiculous when you see the evidence. How does blood in the feet so far from the heart get pumped horizontally up the body by the heart?? Cowan explains in the book the real reason why blood moves as it does. Our ‘physical’ heart is used to symbolise love when the source is really the heart vortex or spiritual heart which is our most powerful energetic connection to ‘out there’ expanded consciousness. That’s why we feel *knowing* – intuitive knowing – in the centre of the chest. Knowing doesn’t come from a process of thoughts leading to a conclusion. It is there in an instant all in one go. Our heart knows because of its

connection to levels of awareness that *do* know. This is the meaning and source of intuition – intuitive *knowing*.

For the last more than 30 years of uncovering the global game and the nature of reality my heart has been my constant antenna for truth and accuracy. An American intelligence insider once said that I had quoted a disinformer in one of my books and yet I had only quoted the part that was true. He asked: 'How do you do that?' By using my heart antenna was the answer and anyone can do it. Heart-centred is how we are meant to be. With a closed heart chakra we withdraw into a closed mind and the bubble of five-sense reality. If you take a moment to focus your attention on the centre of your chest, picture a spinning wheel of light and see it opening and expanding. You will feel it happening, too, and perceptions of the heart like joy and love as the heart impacts on the mind as they interact. The more the chakra opens the more you will feel expressions of heart consciousness and as the process continues, and becomes part of you, insights and knowings will follow. An open heart is connected to that level of awareness that knows all is *One*. You will see from its perspective that the fault-lines that divide us are only illusions to control us. An open heart does not process the illusions of race, creed and sexuality except as brief experiences for a consciousness that is all. Our heart does not see division, only unity (Figs 24 and 25). There's something else, too. Our hearts love to laugh. Mark Twain's quote that says 'The human race has one really effective weapon, and that is laughter' is really a reference to the heart which loves to laugh with the joy of knowing the true nature of infinite reality and that all the madness of human society is an illusion of the mind. Twain also said: 'Against the assault of laughter nothing can stand.' This is so true of Wetiko and the Cult. Their insecurity demands that they be taken seriously and their power and authority acknowledged and feared. We should do nothing of the sort. We should not get aggressive or fearful which their insecurity so desires. We should laugh in their face. Even in their no-face as police come over in their face-nappies and expect to be taken seriously. They don't take themselves seriously looking like that so why should we? Laugh in the face of intimidation. Laugh in the face of tyranny. You will see by its reaction that you have pressed all of its buttons. Wetiko does not know what to do in the face of laughter or when its targets refuse to concede their joy to fear. We have seen many examples during the 'Covid' hoax when people have expressed their energetic power and the string puppets of Wetiko retreat

with their tail limp between their knees. Laugh – the world is bloody mad after all and if it's a choice between laughter and tears I know which way I'm going.

Figure 24: Head consciousness without the heart sees division and everything apart from everything else.

Figure 25: Heart consciousness sees everything as One.

'Vaccines' and the soul

The foundation of Wetiko/Archon control of humans is the separation of incarnate five-sense mind from the infinite 'I' and closing the heart chakra where the True 'I' lives during a human life. The goal has been to achieve complete separation in both cases. I was interested therefore to read an account by a French energetic healer of what she said she experienced with a patient who had been given the 'Covid' vaccine. Genuine energy healers can sense information and consciousness fields at different levels of being which are referred to as 'subtle bodies'. She described treating the patient who later returned after having, without the healer's knowledge, two doses of the 'Covid vaccine'. The healer said:

I noticed immediately the change, very heavy energy emanating from [the] subtle bodies. The scariest thing was when I was working on the heart chakra, I connected with her soul: it was detached from the physical body, it had no contact and it was, as if it was floating in a state of total confusion: a damage to the consciousness that loses contact with the physical body, i.e. with our biological machine, there is no longer any communication between them.

I continued the treatment by sending light to the heart chakra, the soul of the person, but it seemed that the soul could no longer receive any light, frequency or energy. It was a very powerful experience for me. Then I understood that this substance is indeed used to detach consciousness so that this consciousness can no longer interact through this body that it possesses in life, where there is no longer any contact, no frequency, no light, no more energetic balance or mind.

This would create a human that is rudderless and at the extreme almost zombie-like operating with a fractional state of consciousness at the mercy of Wetiko. I was especially intrigued by what the healer said in the light of the prediction by the highly-informed Rudolf Steiner more than a hundred years ago. He said:

In the future, we will eliminate the soul with medicine. Under the pretext of a 'healthy point of view', there will be a vaccine by which the human body will be treated as soon as possible directly at birth, so that the human being cannot develop the thought of the existence of soul and Spirit. To materialistic doctors will be entrusted the task of removing the soul of humanity.

As today, people are vaccinated against this disease or that disease, so in the future, children will be vaccinated with a substance that can be produced precisely in such a way that people, thanks to this vaccination, will be immune to being subjected to the 'madness' of spiritual life. He would be extremely smart, but he would not develop a conscience, and that is the true goal of some materialistic circles.

Steiner said the vaccine would detach the physical body from the etheric body (subtle bodies) and 'once the etheric body is detached the relationship between the universe and the etheric body would become extremely unstable, and man would become an automaton'. He said 'the physical body of man must be polished on this Earth by spiritual will – so the vaccine becomes a kind of arymanique (Wetiko) force' and 'man can no longer get rid of a given materialistic feeling'. Humans would then, he said, become 'materialistic of constitution and can no longer rise to the spiritual'. I have been writing for years about DNA being a receiver-transmitter of information that connects us to other levels of reality and these 'vaccines' changing DNA can be likened to changing an antenna and what it can transmit and receive. Such a disconnection would clearly lead to changes in

personality and perception. Steiner further predicted the arrival of AI. Big Pharma 'Covid vaccine' makers, expressions of Wetiko, are testing their DNA-manipulating evil on children as I write with a view to giving the 'vaccine' to babies. If it's a soul-body disconnector – and I say that it is or can be – every child would be disconnected from 'soul' at birth and the 'vaccine' would create a closed system in which spiritual guidance from the greater self would play no part. This has been the ambition of Wetiko all along. A Pentagon video from 2005 was leaked of a presentation explaining the development of vaccines to change behaviour by their effect on the brain. Those that believe this is not happening with the 'Covid' genetically-modifying procedure masquerading as a 'vaccine' should make an urgent appointment with Naivety Anonymous. Klaus Schwab wrote in 2018:

> Neurotechnologies enable us to better influence consciousness and thought and to understand many activities of the brain. They include decoding what we are thinking in fine levels of detail through new chemicals and interventions that can influence our brains to correct for errors or enhance functionality.

The plan is clear and only the heart can stop it. With every heart that opens, every mind that awakens, Wetiko is weakened. Heart and love are far more powerful than head and hate and so nothing like a majority is needed to turn this around.

Beyond the Phantom

Our heart is the prime target of Wetiko and so it must be the answer to Wetiko. We *are* our heart which is part of one heart, the infinite heart. Our heart is where the true self lives in a human life behind firewalls of five-sense illusion when an imposter takes its place – *Phantom Self*; but our heart waits patiently to be set free any time we choose to see beyond the Phantom, beyond Wetiko. A Wetikoed Phantom Self can wreak mass death and destruction while the love of forever is locked away in its heart. The time is here to unleash its power and let it sweep away the fear and despair that is Wetiko. Heart consciousness does not seek manipulated, censored, advantage for its belief or religion, its activism and desires. As an expression of the One it treats all as One with the same rights to freedom and opinion. Our heart demands fairness for itself no more than for others.

From this unity of heart we can come together in mutual support and transform this Wetikoed world into what reality is meant to be – a place of love, joy, happiness, fairness, justice and freedom. Wetiko has another agenda and that's why the world is as it is, but enough of this nonsense. Wetiko can't stay where hearts are open and it works so hard to keep them closed. Fear is its currency and its food source and love in its true sense has no fear. Why would love have fear when it knows it is *All That Is, Has Been, And Ever Can Be* on an eternal exploration of all possibility? Love in this true sense is not the physical attraction that passes for love. This can be an expression of it, yes, but Infinite Love, a love without condition, goes far deeper to the core of all being. It *is* the core of all being. Infinite realty was born from love beyond the illusions of the simulation. Love infinitely expressed is the knowing that all is One and the swiftly-passing experience of separation is a temporary hallucination. You cannot disconnect from Oneness; you can only *perceive* that you have and withdraw from its influence. This is the most important of all perception trickery by the mind parasite that is Wetiko and the foundation of all its potential for manipulation.

If we open our hearts, open the sluice gates of the mind, and redefine self-identity amazing things start to happen. Consciousness expands or contracts in accordance with self-identity. When true self is recognised as infinite awareness and label self – Phantom Self – is seen as only a series of brief experiences life is transformed. Consciousness expands to the extent that self-identity expands and everything changes. You see unity, not division, the picture, not the pixels. From this we can play the long game. No more is an experience something in and of itself, but a fleeting moment in the eternity of forever. Suddenly people in uniform and dark suits are no longer intimidating. Doing what your heart knows to be right is no longer intimidating and consequences for those actions take on the same nature of a brief experience that passes in the blink of an infinite eye. Intimidation is all in the mind. Beyond the mind there is no intimidation.

An open heart does not consider consequences for what it knows to be right. To do so would be to consider not doing what it knows to be right and for a heart in its power that is never an option. The Renegade Mind is really the Renegade Heart. Consideration of consequences will always provide a getaway car for the mind and the heart doesn't want one. What is right in the light of what we face today is to stop cooperating with Wetiko in all its

forms and to do it without fear or compromise. You cannot compromise with tyranny when tyranny always demands more until it has everything. Life is your perception and you are your destiny. Change your perception and you change your life. Change collective perception and we change the world.

Come on people … One human family, One heart, One goal … *FREEEEEEDOM*!

We must settle for nothing less.

Postscript

The big scare story as the book goes to press is the 'Indian' variant and the world is being deluged with propaganda about the 'Covid catastrophe' in India which mirrors in its lies and misrepresentations what happened in Italy before the first lockdown in 2020.

The *New York Post* published a picture of someone who had 'collapsed in the street from Covid' in India in April, 2021, which was actually taken during a gas leak in May, 2020. Same old, same old. Media articles in mid-February were asking why India had been so untouched by 'Covid' and then as their vaccine rollout gathered pace the alleged 'cases' began to rapidly increase. Indian 'Covid vaccine' maker Bharat Biotech was funded into existence by the Bill and Melinda Gates Foundation (the pair announced their divorce in May, 2021, which is a pity because they so deserve each other). The Indian 'Covid crisis' was ramped up by the media to terrify the world and prepare people for submission to still more restrictions. The scam that worked the first time was being repeated only with far more people seeing through the deceit. Davidicke.com and Ickonic.com have sought to tell the true story of what is happening by talking to people living through the Indian nightmare which has nothing to do with 'Covid'. We posted a letter from 'Alisha' in Pune who told a very different story to government and media mendacity. She said scenes of dying people and overwhelmed hospitals were designed to hide what was really happening – genocide and starvation. Alisha said that millions had already died of starvation during the ongoing lockdowns while government and media were lying and making it look like the 'virus':

Restaurants, shops, gyms, theatres, basically everything is shut. The cities are ghost towns. Even so-called ‘essential’ businesses are only open till 11am in the morning. You basically have just an hour to buy food and then your time is up.

Inter-state travel and even inter-district travel is banned. The cops wait at all major crossroads to question why you are traveling outdoors or to fine you if you are not wearing a mask.

The medical community here is also complicit in genocide, lying about hospitals being full and turning away people with genuine illnesses, who need immediate care. They have even created a shortage of oxygen cylinders.

This is the classic Cult modus operandi played out in every country. Alisha said that people who would not have a PCR test not testing for the ‘virus’ were being denied hospital treatment. She said the people hit hardest were migrant workers and those in rural areas. Most businesses employed migrant workers and with everything closed there were no jobs, no income and no food. As a result millions were dying of starvation or malnutrition. All this was happening under Prime Minister Narendra Modi, a 100-percent asset of the Cult, and it emphasises yet again the scale of pure anti-human evil we are dealing with. Australia banned its people from returning home from India with penalties for trying to do so of up to five years in jail and a fine of £37,000. The manufactured ‘Covid’ crisis in India was being prepared to justify further fascism in the West. Obvious connections could be seen between the Indian ‘vaccine’ programme and increased ‘cases’ and this became a common theme. The Seychelles, the most per capita ‘Covid vaccinated’ population in the world, went back into lockdown after a ‘surge of cases’.

Long ago the truly evil Monsanto agricultural biotechnology corporation with its big connections to Bill Gates devastated Indian farming with genetically-modified crops. Human rights activist Gurcharan Singh highlighted the efforts by the Indian government to complete the job by destroying the food supply to hundreds of millions with ‘Covid’ lockdowns. He said that 415 million people at the bottom of the disgusting caste system (still going whatever they say) were below the poverty line and struggled to feed themselves every year. Now the government was imposing lockdown at just the time to destroy the harvest. This deliberate policy was leading to mass starvation. People may reel back at the suggestion that a government would do that, but Wetiko-controlled ‘leaders’ are capable of any level of evil. In fact what is described in India is in the process of being instigated

worldwide. The food chain and food supply are being targeted at every level to cause world hunger and thus control. Bill Gates is not the biggest owner of farmland in America for no reason and destroying access to food aids both the depopulation agenda and the plan for synthetic 'food' already being funded into existence by Gates. Add to this the coming hyper-inflation from the suicidal creation of fake 'money' in response to 'Covid' and the breakdown of container shipping systems and you have a cocktail that can only lead one way and is meant to. The Cult plan is to crash the entire system to 'build back better' with the Great Reset.

'Vaccine' transmission

Reports from all over the world continue to emerge of women suffering menstrual and fertility problems after having the fake 'vaccine' and of the non-'vaccinated' having similar problems when interacting with the 'vaccinated'. There are far too many for 'coincidence' to be credible. We've had menopausal women getting periods, others having periods stop or not stopping for weeks, passing clots, sometimes the lining of the uterus, breast irregularities, and miscarriages (which increased by 400 percent in parts of the United States). Non-'vaccinated' men and children have suffered blood clots and nose bleeding after interaction with the 'vaccinated'. Babies have died from the effects of breast milk from a 'vaccinated' mother. Awake doctors – the small minority – speculated on the cause of non-'vaccinated' suffering the same effects as the 'vaccinated'. Was it nanotechnology in the synthetic substance transmitting frequencies or was it a straight chemical bioweapon that was being transmitted between people? I am not saying that some kind of chemical transmission is not one possible answer, but the foundation of all that the Cult does is frequency and this is fertile ground for understanding how transmission can happen. American doctor Carrie Madej, an internal medicine physician and osteopath, has been practicing for the last 20 years, teaching medical students, and she says attending different meetings where the agenda for humanity was discussed. Madej, who operates out of Georgia, did not dismiss other possible forms of transmission, but she focused on frequency in search of an explanation for transmission. She said the Moderna and Pfizer 'vaccines' contained nano-lipid particles as a key component. This was a brand new technology never before used on humanity. 'They're using a nanotechnology which is pretty

much little tiny computer bits … nanobots or hydrogel.' Inside the 'vaccines' was 'this sci-fi kind of substance' which suppressed immune checkpoints to get into the cell. I referred to this earlier as the 'Trojan horse' technique that tricks the cell into opening a gateway for the self-replicating synthetic material and while the immune system is artificially suppressed the body has no defences. Madej said the substance served many purposes including an on-demand ability to 'deliver the payload' and using the nano 'computer bits' as biosensors in the body. 'It actually has the ability to accumulate data from your body, like your breathing, your respiration, thoughts, emotions, all kinds of things.'

She said the technology obviously has the ability to operate through Wi-Fi and transmit and receive energy, messages, frequencies or impulses. 'Just imagine you're getting this new substance in you and it can react to things all around you, the 5G, your smart device, your phones.' We had something completely foreign in the human body that had never been launched large scale at a time when we were seeing 5G going into schools and hospitals (plus the Musk satellites) and she believed the 'vaccine' transmission had something to do with this: '… if these people have this inside of them … it can act like an antenna and actually transmit it outwardly as well.' The synthetic substance produced its own voltage and so it could have that kind of effect. This fits with my own contention that the nano receiver-transmitters are designed to connect people to the Smart Grid and break the receiver-transmitter connection to expanded consciousness. That would explain the French energy healer's experience of the disconnection of body from 'soul' with those who have had the 'vaccine'. The nanobots, self-replicating inside the body, would also transmit the synthetic frequency which could be picked up through close interaction by those who have not been 'vaccinated'. Madej speculated that perhaps it was 5G and increased levels of other radiation that was causing the symptoms directly although interestingly she said that non-'vaccinated' patients had shown improvement when they were away from the 'vaccinated' person they had interacted with. It must be remembered that you can control frequency and energy with your mind and you can consciously create energetic barriers or bubbles with the mind to stop damaging frequencies from penetrating your field. American paediatrician Dr Larry Palevsky said the 'vaccine' was not a 'vaccine' and was never designed to protect from a 'viral' infection. He called it 'a massive, brilliant propaganda of genocide' because they didn't

have to inject everyone to get the result they wanted. He said the content of the jabs was able to infuse any material into the brain, heart, lungs, kidneys, liver, sperm and female productive system. 'This is genocide; this is a weapon of mass destruction.' At the same time American colleges were banning students from attending if they didn't have this life-changing and potentially life-ending 'vaccine'. Class action lawsuits must follow when the consequences of this college fascism come to light. As the book was going to press came reports about fertility effects on sperm in 'vaccinated' men which would absolutely fit with what I have been saying and hospitals continued to fill with 'vaccine' reactions. Another question is what about transmission via blood transfusions? The NHS has extended blood donation restrictions from seven days after a 'Covid vaccination' to 28 days after even a sore arm reaction.

I said in the spring of 2020 that the then touted 'Covid vaccine' would be ongoing each year like the flu jab. A year later Pfizer CEO, the appalling Albert Bourla, said people would 'likely' need a 'booster dose' of the 'vaccine' within 12 months of getting 'fully vaccinated' and then a yearly shot. 'Variants will play a key role', he said confirming the point. Johnson & Johnson CEO Alex Gorsky also took time out from his 'vaccine' disaster to say that people may need to be vaccinated against 'Covid-19' each year. UK Health Secretary, the psychopath Matt Hancock, said additional 'boosters' would be available in the autumn of 2021. This is the trap of the 'vaccine passport'. The public will have to accept every last 'vaccine' they introduce, including for the fake 'variants', or it would cease to be valid. The only other way in some cases would be continuous testing with a test not testing for the 'virus' and what is on the swabs constantly pushed up your noise towards the brain every time?

'Vaccines' changing behaviour

I mentioned in the body of the book how I believed we would see gathering behaviour changes in the 'vaccinated' and I am already hearing such comments from the non-'vaccinated' describing behaviour changes in friends, loved ones and work colleagues. This will only increase as the self-replicating synthetic material and nanoparticles expand in body and brain. An article in the *Guardian* in 2016 detailed research at the University of Virginia in Charlottesville which developed a new method for controlling

brain circuits associated with complex animal behaviour. The method, dubbed 'magnetogenetics', involves genetically-engineering a protein called ferritin, which stores and releases iron, to create a magnetised substance – 'Magneto' – that can activate specific groups of nerve cells from a distance. This is claimed to be an advance on other methods of brain activity manipulation known as optogenetics and chemogenetics (the Cult has been developing methods of brain control for a long time). The ferritin technique is said to be non-invasive and able to activate neurons 'rapidly and reversibly'. In other words, human thought and perception. The article said that earlier studies revealed how nerve cell proteins 'activated by heat and mechanical pressure can be genetically engineered so that they become sensitive to radio waves and magnetic fields, by attaching them to an iron-storing protein called ferritin, or to inorganic paramagnetic particles'. Sensitive to radio waves and magnetic fields? You mean like 5G, 6G and 7G? This is the human-AI Smart Grid hive mind we are talking about. The *Guardian* article said:

> … the researchers injected Magneto into the striatum of freely behaving mice, a deep brain structure containing dopamine-producing neurons that are involved in reward and motivation, and then placed the animals into an apparatus split into magnetised and non-magnetised sections.
>
> Mice expressing Magneto spent far more time in the magnetised areas than mice that did not, because activation of the protein caused the striatal neurons expressing it to release dopamine, so that the mice found being in those areas rewarding. This shows that Magneto can remotely control the firing of neurons deep within the brain, and also control complex behaviours.

Make no mistake this basic methodology will be part of the 'Covid vaccine' cocktail and using magnetics to change brain function through electromagnetic field frequency activation. The Pentagon is developing a 'Covid vaccine' using ferritin. Magnetics would explain changes in behaviour and why videos are appearing across the Internet as I write showing how magnets stick to the skin at the point of the 'vaccine' shot. Once people take these 'vaccines' anything becomes possible in terms of brain function and illness which will be blamed on 'Covid-19' and 'variants'. Magnetic field manipulation would further explain why the non-'vaccinated' are reporting the same symptoms as the 'vaccinated' they interact with and why those symptoms are reported to decrease when not in their company. Interestingly 'Magneto', a 'mutant', is a character in the

Marvel Comic *X-Men* stories with the ability to manipulate magnetic fields and he believes that mutants should fight back against their human oppressors by any means necessary. The character was born Erik Lehnsherr to a Jewish family in Germany.

Cult-controlled courts

The European Court of Human Rights opened the door for mandatory 'Covid-19 vaccines' across the continent when it ruled in a Czech Republic dispute over childhood immunisation that legally enforced vaccination could be 'necessary in a democratic society'. The 17 judges decided that compulsory vaccinations did not breach human rights law. On the face of it the judgement was so inverted you gasp for air. If not having a vaccine infused into your body is not a human right then what is? Ah, but they said human rights law which has been specifically written to delete all human rights at the behest of the state (the Cult). Article 8 of the European Convention on Human Rights relates to the right to a private life. The crucial word here is *'except'*:

There shall be no interference by a public authority with the exercise of this right EXCEPT such as is in accordance with the law and is necessary in a democratic society in the interests of national security, public safety or the economic wellbeing of the country, for the prevention of disorder or crime, for the protection of health or morals, or for the protection of the rights and freedoms of others [My emphasis].

No interference *except* in accordance with the law means there *are* no 'human rights' *except* what EU governments decide you can have at their behest. 'As is necessary in a democratic society' explains that reference in the judgement and 'in the interests of national security, public safety or the economic well-being of the country, for the prevention of disorder or crime, for the protection of health or morals, or for the protection of the rights and freedoms of others' gives the EU a coach and horses to ride through 'human rights' and scatter them in all directions. The judiciary is not a check and balance on government extremism; it is a vehicle to enforce it. This judgement was almost laughably predictable when the last thing the Cult wanted was a decision that went against mandatory vaccination. Judges rule over and over again to benefit the system of which they are a part.

Vaccination disputes that come before them are invariably delivered in favour of doctors and authorities representing the view of the state which owns the judiciary. Oh, yes, and we have even had calls to stop putting 'Covid-19' on death certificates within 28 days of a 'positive test' because it is claimed the practice makes the 'vaccine' appear not to work. They are laughing at you.

The scale of madness, inhumanity and things to come was highlighted when those not 'vaccinated' for 'Covid' were refused evacuation from the Caribbean island of St Vincent during massive volcanic eruptions. Cruise ships taking residents to the safety of another island allowed only the 'vaccinated' to board and the rest were left to their fate. Even in life and death situations like this we see 'Covid' stripping people of their most basic human instincts and the insanity is even more extreme when you think that fake 'vaccine'-makers are not even claiming their body-manipulating concoctions stop 'infection' and 'transmission' of a 'virus' that doesn't exist. St Vincent Prime Minister Ralph Gonsalves said: 'The chief medical officer will be identifying the persons already vaccinated so that we can get them on the ship.' Note again the power of the chief medical officer who, like Whitty in the UK, will be answering to the World Health Organization. This is the Cult network structure that has overridden politicians who 'follow the science' which means doing what WHO-controlled 'medical officers' and 'science advisers' tell them. Gonsalves even said that residents who were 'vaccinated' after the order so they could board the ships would still be refused entry due to possible side effects such as 'wooziness in the head'. The good news is that if they were woozy enough in the head they could qualify to be prime minister of St Vincent.

Microchipping freedom

The European judgement will be used at some point to justify moves to enforce the 'Covid' DNA-manipulating procedure. Sandra Ro, CEO of the Global Blockchain Business Council, told a World Economic Forum event that she hoped 'vaccine passports' would help to 'drive forced consent and standardisation' of global digital identity schemes: 'I'm hoping with the desire and global demand for some sort of vaccine passport – so that people can get travelling and working again – [it] will drive forced consent, standardisation, and frankly, cooperation across the world.' The lady is

either not very bright, or thoroughly mendacious, to use the term ‘forced consent’. You do not ‘consent’ if you are forced – you *submit*. She was describing what the plan has been all along and that’s to enforce a digital identity on every human without which they could not function. ‘Vaccine passports’ are opening the door and are far from the end goal. A digital identity would allow you to be tracked in everything you do in cyberspace and this is the same technique used by Cult-owned China to enforce its social credit system of total control. The ultimate ‘passport’ is planned to be a microchip as my books have warned for nearly 30 years. Those nice people at the Pentagon working for the Cult-controlled Defense Advanced Research Projects Agency (DARPA) claimed in April, 2021, they have developed a microchip inserted under the skin to detect ‘asymptomatic Covid-19 infection’ before it becomes an outbreak and a ‘revolutionary filter’ that can remove the ‘virus’ from the blood when attached to a dialysis machine. The only problems with this are that the ‘virus’ does not exist and people transmitting the ‘virus’ with no symptoms is brain-numbing bullshit. This is, of course, not a ruse to get people to be microchipped for very different reasons. DARPA also said it was producing a one-stop ‘vaccine’ for the ‘virus’ and all ‘variants’. One of the most sinister organisations on Planet Earth is doing this? Better have it then. These people are insane because Wetiko that possesses them is insane.

Researchers from the Salk Institute in California announced they have created an embryo that is part human and part monkey. My books going back to the 1990s have exposed experiments in top secret underground facilities in the United States where humans are being crossed with animal and non-human ‘extraterrestrial’ species. They are now easing that long-developed capability into the public arena and there is much more to come given we are dealing with psychiatric basket cases. Talking of which – Elon Musk’s scientists at Neuralink trained a monkey to play Pong and other puzzles on a computer screen using a joystick and when the monkey made the correct move a metal tube squirted banana smoothie into his mouth which is the basic technique for training humans into unquestioning compliance. Two Neuralink chips were in the monkey’s skull and more than 2,000 wires ‘fanned out’ into its brain. Eventually the monkey played a video game purely with its brain waves. Psychopathic narcissist Musk said the ‘breakthrough’ was a step towards putting Neuralink chips into human

skulls and merging minds with artificial intelligence. *Exactly*. This man is so dark and Cult to his DNA.

World Economic Fascism (WEF)

The World Economic Forum is telling you the plan by the statements made at its many and various events. Cult-owned fascist YouTube CEO Susan Wojcicki spoke at the 2021 WEF Global Technology Governance Summit (see the name) in which 40 governments and 150 companies met to ensure 'the responsible design and deployment of emerging technologies'. Orwellian translation: 'Ensuring the design and deployment of long-planned technologies will advance the Cult agenda for control and censorship.' Freedom-destroyer and Nuremberg-bound Wojcicki expressed support for tech platforms like hers to censor content that is 'technically legal but could be harmful'. Who decides what is 'harmful'? She does and they do. 'Harmful' will be whatever the Cult doesn't want people to see and we have legislation proposed by the UK government that would censor content on the basis of 'harm' no matter if the information is fair, legal and provably true. Make that *especially* if it is fair, legal and provably true. Wojcicki called for a global coalition to be formed to enforce content moderation standards through automated censorship. This is a woman and mega-censor so self-deluded that she shamelessly accepted a 'free expression' award – *Wojcicki* – in an event sponsored by her own *YouTube*. They have no shame and no self-awareness.

You know that 'Covid' is a scam and Wojcicki a Cult operative when YouTube is censoring medical and scientific opinion purely on the grounds of whether it supports or opposes the Cult 'Covid' narrative. Florida governor Ron DeSantis compiled an expert panel with four professors of medicine from Harvard, Oxford, and Stanford Universities who spoke against forcing children and vaccinated people to wear masks. They also said there was no proof that lockdowns reduced spread or death rates of 'Covid-19'. Cult-gofer Wojcicki and her YouTube deleted the panel video 'because it included content that contradicts the consensus of local and global health authorities regarding the efficacy of masks to prevent the spread of Covid-19'. This 'consensus' refers to what the Cult tells the World Health Organization to say and the WHO tells 'local health authorities' to do. Wojcicki knows this, of course. The panellists pointed out

that censorship of scientific debate was responsible for deaths from many causes, but Wojcicki couldn't care less. She would not dare go against what she is told and as a disgrace to humanity she wouldn't want to anyway. The UK government is seeking to pass a fascist 'Online Safety Bill' to specifically target with massive fines and other means non-censored video and social media platforms to make them censor 'lawful but harmful' content like the Cult-owned Facebook, Twitter, Google and YouTube. What is 'lawful but harmful' would be decided by the fascist Blair-created Ofcom.

Another WEF obsession is a cyber-attack on the financial system and this is clearly what the Cult has planned to take down the bank accounts of everyone – except theirs. Those that think they have enough money for the Cult agenda not to matter to them have got a big lesson coming if they continue to ignore what is staring them in the face. The World Economic Forum, funded by Gates and fronted by Klaus Schwab, announced it would be running a 'simulation' with the Russian government and global banks of just such an attack called Cyber Polygon 2021. What they simulate – as with the 'Covid' Event 201 – they plan to instigate. The WEF is involved in a project with the Cult-owned Carnegie Endowment for International Peace called the WEF-Carnegie Cyber Policy Initiative which seeks to merge Wall Street banks, 'regulators' (I love it) and intelligence agencies to 'prevent' (arrange and allow) a cyber-attack that would bring down the global financial system as long planned by those that control the WEF and the Carnegie operation. The Carnegie Endowment for International Peace sent an instruction to First World War US President Woodrow Wilson not to let the war end before society had been irreversibly transformed.

The Wuhan lab diversion

As I close, the Cult-controlled authorities and lapdog media are systematically pushing 'the virus was released from the Wuhan lab' narrative. There are two versions – it happened by accident and it happened on purpose. Both are nonsense. The perceived existence of the never-shown-to-exist 'virus' is vital to sell the impression that there is actually an infective agent to deal with and to allow the endless potential for terrifying the population with 'variants' of a 'virus' that does not exist. The authorities at the time of writing are going with the 'by accident' while the

alternative media is promoting the ‘on purpose’. Cable news host Tucker Carlson who has questioned aspects of lockdown and ‘vaccine’ compulsion has bought the Wuhan lab story. ‘Everyone now agrees’ he said. Well, I don’t and many others don’t and the question is *why* does the system and its media suddenly ‘agree’? When the media moves as one unit with a narrative it is always a lie – witness the hour by hour mendacity of the ‘Covid’ era. Why would this Cult-owned combination which has unleashed lies like machine gun fire suddenly ‘agree’ to tell the truth??

Much of the alternative media is buying the lie because it fits the conspiracy narrative, but it’s the *wrong* conspiracy. The real conspiracy is that *there is no virus* and that is what the Cult is desperate to hide. The idea that the ‘virus’ was released by accident is ludicrous when the whole ‘Covid’ hoax was clearly long-planned and waiting to be played out as it was so fast in accordance with the Rockefeller document and Event 201. So they prepared everything in detail over decades and then sat around strumming their fingers waiting for an ‘accidental’ release from a bio-lab? *What??* It’s crazy. Then there’s the ‘on purpose’ claim. You want to circulate a ‘deadly virus’ and hide the fact that you’ve done so and you release it down the street from the highest-level bio-lab in China? I repeat – *What??* You would release it far from that lab to stop any association being made. But, no, we’ll do it in a place where the connection was certain to be made. Why would you need to scam ‘cases’ and ‘deaths’ and pay hospitals to diagnose ‘Covid-19’ if you had a real ‘virus’? What are sections of the alternative media doing believing this crap? Where were all the mass deaths in Wuhan from a ‘deadly pathogen’ when the recovery to normal life after the initial propaganda was dramatic in speed? Why isn’t the ‘deadly pathogen’ now circulating all over China with bodies in the street? Once again we have the technique of tell them what they want to hear and they will likely believe it. The alternative media has its ‘conspiracy’ and with Carlson it fits with his ‘China is the danger’ narrative over years. China *is* a danger as a global Cult operations centre, but not for this reason. The Wuhan lab story also has the potential to instigate conflict with China when at some stage the plan is to trigger a Problem-Reaction-Solution confrontation with the West. Question everything – *everything* – and especially when the media agrees on a common party line.

Third wave … fourth wave … fifth wave …

As the book went into production the world was being set up for more lockdowns and a ‘third wave’ supported by invented ‘variants’ that were increasing all the time and will continue to do so in public statements and computer programs, but not in reality. India became the new Italy in the ‘Covid’ propaganda campaign and we were told to be frightened of the new ‘Indian strain’. Somehow I couldn’t find it within myself to do so. A document produced for the UK government entitled ‘Summary of further modelling of easing of restrictions – Roadmap Step 2’ declared that a third wave was inevitable (of course when it’s in the script) and it would be the fault of children and those who refuse the health-destroying fake ‘Covid vaccine’. One of the computer models involved came from the Cult-owned *Imperial College* and the other from Warwick University which I wouldn’t trust to tell me the date in a calendar factory. The document states that both models presumed extremely high uptake of the ‘Covid vaccines’ and didn’t allow for ‘variants’. The document states: ‘The resurgence is a result of some people (mostly children) being ineligible for vaccination; others choosing not to receive the vaccine; and others being vaccinated but not perfectly protected.’ The mendacity takes the breath away. Okay, blame those with a brain who won’t take the DNA-modifying shots and put more pressure on children to have it as ‘trials’ were underway involving children as young as six months with parents who give insanity a bad name. Massive pressure is being put on the young to have the fake ‘vaccine’ and child age consent limits have been systematically lowered around the world to stop parents intervening. Most extraordinary about the document was its claim that the ‘third wave’ would be driven by ‘the resurgence in both hospitalisations and deaths … dominated by *those that have received two doses of the vaccine*, comprising around 60-70% of the wave respectively’. The predicted peak of the ‘third wave’ suggested 300 deaths per day with 250 of them *fully ‘vaccinated’ people*. How many more lies do acquiescers need to be told before they see the obvious? Those who took the jab to ‘protect themselves’ are projected to be those who mostly get sick and die? So what’s in the ‘vaccine’? The document went on:

It is possible that a summer of low prevalence could be followed by substantial increases in incidence over the following autumn and winter. Low prevalence in late summer should not be taken as an

indication that SARS-CoV-2 has retreated or that the population has high enough levels of immunity to prevent another wave.

They are telling you the script and while many British people believed 'Covid' restrictions would end in the summer of 2021 the government was preparing for them to be ongoing. Authorities were awarding contracts for 'Covid marshals' to police the restrictions with contracts starting in July, 2021, and going through to January 31st, 2022, and the government was advertising for 'Media Buying Services' to secure media propaganda slots worth a potential £320 million for 'Covid-19 campaigns' with a contract not ending until March, 2022. The recipient – via a list of other front companies – was reported to be American media marketing giant Omnicom Group Inc. While money is no object for 'Covid' the UK waiting list for all other treatment – including life-threatening conditions – passed 4.5 million. Meantime the Cult is seeking to control all official 'inquiries' to block revelations about what has really been happening and why. It must not be allowed to – we need Nuremberg jury trials in every country. The cover-up doesn't get more obvious than appointing ultra-Zionist professor Philip Zelikow to oversee two dozen US virologists, public health officials, clinicians, former government officials and four American 'charitable foundations' to 'learn the lessons' of the 'Covid' debacle. The personnel will be those that created and perpetuated the 'Covid' lies while Zelikow is the former executive director of the 9/11 Commission who ensured that the truth about those attacks never came out and produced a report that must be among the most mendacious and manipulative documents ever written – see *The Trigger* for the detailed exposure of the almost unimaginable 9/11 story in which Sabbatians can be found at every level.

Passive no more

People are increasingly challenging the authorities with amazing numbers of people taking to the streets in London well beyond the ability of the Face-Nappies to stop them. Instead the Nappies choose situations away from the mass crowds to target, intimidate, and seek to promote the impression of 'violent protestors'. One such incident happened in London's Hyde Park. Hundreds of thousands walking through the streets in protest against 'Covid' fascism were ignored by the Cult-owned BBC and most of

the rest of the mainstream media, but they delighted in reporting how police were injured in ‘clashes with protestors’. The truth was that a group of people gathered in Hyde Park at the end of one march when most had gone home and they were peacefully having a good time with music and chat. Face-Nappies who couldn’t deal with the full-march crowd then waded in with their batons and got more than they bargained for. Instead of just standing for this criminal brutality the crowd used their numerical superiority to push the Face-Nappies out of the park. Eventually the Nappies turned and ran. Unfortunately two or three idiots in the crowd threw drink cans striking two officers which gave the media and the government the image they wanted to discredit the 99.9999 percent who were peaceful. The idiots walked straight into the trap and we must always be aware of potential agent provocateurs used by the authorities to discredit their targets.

This response from the crowd – the can people apart – must be a turning point when the public no longer stand by while the innocent are arrested and brutally attacked by the Face-Nappies. That doesn’t mean to be violent, that’s the last thing we need. We’ll leave the violence to the Face-Nappies and government. But it does mean that when the Face-Nappies use violence against peaceful people the numerical superiority is employed to stop them and make citizen’s arrests or Common Law arrests for a breach of the peace. The time for being passive in the face of fascism is over.

We are the many, they are the few, and we need to make that count before there is no freedom left and our children and grandchildren face an ongoing fascist nightmare.

COME ON PEOPLE – IT’S TIME.

One final thought …

The power of love
A force from above
Cleaning my soul
Flame on burn desire

Love with tongues of fire
Purge the soul
Make love your goal

I'll protect you from the hooded claw
Keep the vampires from your door
When the chips are down I'll be around
With my undying, death-defying
Love for you

Envy will hurt itself
Let yourself be beautiful
Sparkling love, flowers
And pearls and pretty girls
Love is like an energy
Rushin' rushin' inside of me

This time we go sublime
Lovers entwine, divine, divine,
Love is danger, love is pleasure
Love is pure – the only treasure

I'm so in love with you
Purge the soul
Make love your goal

The power of love
A force from above
Cleaning my soul

The power of love
A force from above
A sky-scraping dove

Flame on burn desire
Love with tongues of fire
Purge the soul
Make love your goal

Frankie Goes To Hollywood

Appendix

Cowan-Kaufman-Morell Statement on Virus Isolation (SOVI)

Isolation: The action of isolating; the fact or condition of being isolated or standing alone; separation from other things or persons; solitariness
Oxford English Dictionary

The controversy over whether the SARS-CoV-2 virus has ever been isolated or purified continues. However, using the above definition, common sense, the laws of logic and the dictates of science, any unbiased person must come to the conclusion that the SARS-CoV-2 virus has never been isolated or purified. As a result, no confirmation of the virus' existence can be found. The logical, common sense, and scientific consequences of this fact are:

- the structure and composition of something not shown to exist can't be known, including the presence, structure, and function of any hypothetical spike or other proteins;
- the genetic sequence of something that has never been found can't be known;
- "variants" of something that hasn't been shown to exist can't be known;
- it's impossible to demonstrate that SARS-CoV-2 causes a disease called Covid-19.

In as concise terms as possible, here's the proper way to isolate, characterize and demonstrate a new virus. First, one takes samples (blood, sputum, secretions) from many people (e.g. 500) with symptoms which are unique and specific enough to characterize an illness. Without mixing these samples with ANY tissue or products that also contain genetic material, the virologist macerates, filters and ultracentrifuges i.e. *purifies* the specimen. This common virology technique, done for decades to isolate bacteriophages[1] and so-called giant viruses in every virology lab, then allows the virologist to demonstrate with electron microscopy thousands of identically sized and shaped particles. These particles are the isolated and purified virus.

These identical particles are then checked for uniformity by physical and/or microscopic techniques. Once the purity is determined, the particles may be further characterized. This would include examining the structure, morphology, and chemical composition of the particles. Next, their genetic makeup is characterized by extracting the genetic material directly from the purified particles and using genetic-sequencing techniques, such as Sanger sequencing, that have also been around for decades. Then one does an analysis to confirm that these uniform particles are exogenous (outside) in origin as a virus is conceptualized to be, and not the normal breakdown products of dead and dying tissues.[2] (As of May 2020, we know that virologists have no way to determine whether the particles they're seeing are viruses or just normal break-down products of dead and dying tissues.)[3]

1 Isolation, characterization and analysis of bacteriophages from the haloalkaline lake Elmenteita, KenyaJuliah Khayeli Akhwale et al, PLOS One, Published: April 25, 2019. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0215734 – accessed 2/15/21

2 "Extracellular Vesicles Derived From Apoptotic Cells: An Essential Link Between Death and Regeneration," Maojiao Li1 et al, Frontiers in Cell and Developmental Biology, 2020 October 2. https://www.frontiersin.org/articles/10.3389/fcell.2020.573511/full – accessed 2/15/21

3 "The Role of Extraellular Vesicles as Allies of HIV, HCV and SARS Viruses," Flavia Giannessi, et al, Viruses, 2020 May

If we have come this far then we have fully isolated, characterized, and genetically sequenced an exogenous virus particle. However, we still have to show it is causally related to a disease. This is carried out by exposing a group of healthy subjects (animals are usually used) to this isolated,

purified virus in the manner in which the disease is thought to be transmitted. If the animals get sick with the same disease, as confirmed by clinical and autopsy findings, one has now shown that the virus actually causes a disease. This demonstrates infectivity and transmission of an infectious agent.

None of these steps has even been attempted with the SARS-CoV-2 virus, nor have all these steps been successfully performed for any so-called pathogenic virus. Our research indicates that a single study showing these steps does not exist in the medical literature.

Instead, since 1954, virologists have taken unpurified samples from a relatively few people, often less than ten, with a similar disease. They then minimally process this sample and inoculate this unpurified sample onto tissue culture containing usually four to six other types of material – all of which contain identical genetic material as to what is called a "virus." The tissue culture is starved and poisoned and naturally disintegrates into many types of particles, some of which contain genetic material. Against all common sense, logic, use of the English language and scientific integrity, this process is called "virus isolation." This brew containing fragments of genetic material from many sources is then subjected to genetic analysis, which then creates in a computer-simulation process the alleged sequence of the alleged virus, a so called in silico genome. At no time is an actual virus confirmed by electron microscopy. At no time is a genome extracted and sequenced from an actual virus. This is scientific fraud.

The observation that the unpurified specimen — inoculated onto tissue culture along with toxic antibiotics, bovine fetal tissue, amniotic fluid and other tissues — destroys the kidney tissue onto which it is inoculated is given as evidence of the virus' existence and pathogenicity. This is scientific fraud.

From now on, when anyone gives you a paper that suggests the SARS-CoV-2 virus has been isolated, please check the methods sections. If the

researchers used Vero cells or any other culture method, you know that their process was not isolation. You will hear the following excuses for why actual isolation isn't done:

1. There were not enough virus particles found in samples from patients to analyze.

2. Viruses are intracellular parasites; they can't be found outside the cell in this manner.

If No. 1 is correct, and we can't find the virus in the sputum of sick people, then on what evidence do we think the virus is dangerous or even lethal? If No. 2 is correct, then how is the virus spread from person to person? We are told it emerges from the cell to infect others. Then why isn't it possible to find it?

Finally, questioning these virology techniques and conclusions is not some distraction or divisive issue. Shining the light on this truth is essential to stop this terrible fraud that humanity is confronting. For, as we now know, if the virus has never been isolated, sequenced or shown to cause illness, if the virus is imaginary, then why are we wearing masks, social distancing and putting the whole world into prison?

Finally, if pathogenic viruses don't exist, then what is going into those injectable devices erroneously called "vaccines," and what is their purpose? This scientific question is the most urgent and relevant one of our time.

We are correct. The SARS-CoV2 virus does not exist.

Sally Fallon Morell, MA
Dr. Thomas Cowan, MD
Dr. Andrew Kaufman, MD

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ICKONIC
THE ALTERNATIVE

Ickonic is something that has been a dream of mine for the last 5 years, growing up around alternative information I have always had a natural interest in what is going on in the World and what could I do to make it better.
Across the range of subjects and positions of influence occupied mainly by people who don't strive to make things better it's the Media that I have always found the most frustrating and fascinating. Mainly because if the Media did their Jobs properly then so much of the negative things happening in the World simply would not be able to happen, because they would be exposed within a heartbeat.
Free Press and the Opportunities that the internet could have given would mean that the Media are able to expose things like never before and hold people to account for their actions. As we all know there are 'Untouchables' that walk among us, people the Media simply won't touch, expose or investigate and that leads to the dark underworlds that infest the establishment the World over.
Well I say enough, it's time for something different, a different kind of Media, where no one is off limits from exposing and investigating. All we're interested in at Ickonic is the truth of what is really going on in the World on whichever subject we're covering.
We hope you enjoy what we have created and take something away from the platform, we aim to deliver information that's informative and most importantly self-empowering, you're not a little person, you're part of something much bigger than that and its time we as a collective race began to understand that and look to the future as ours to take.

It's time...

Jaymie Icke - Founder Ickonic Alternative Media.

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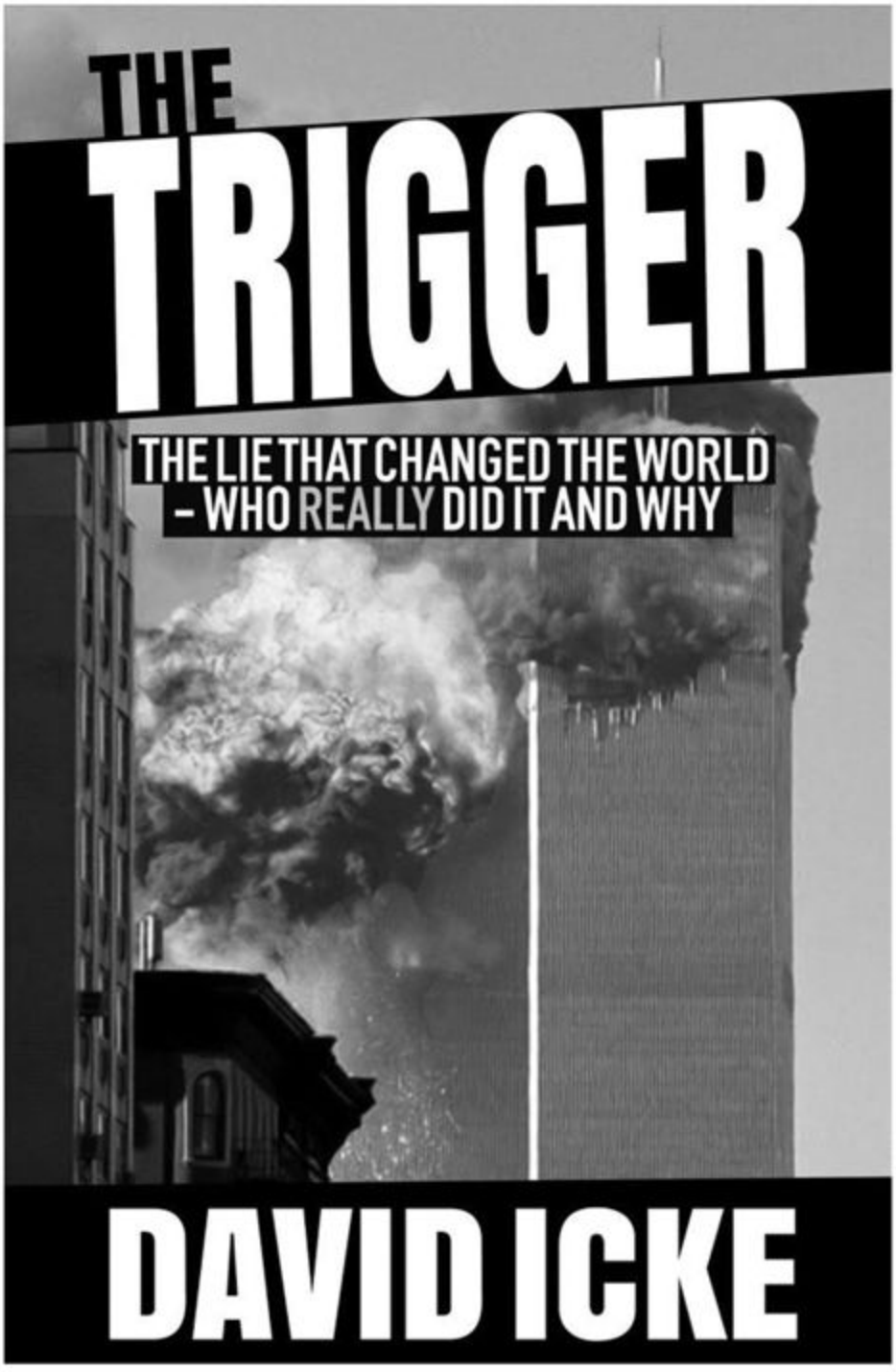
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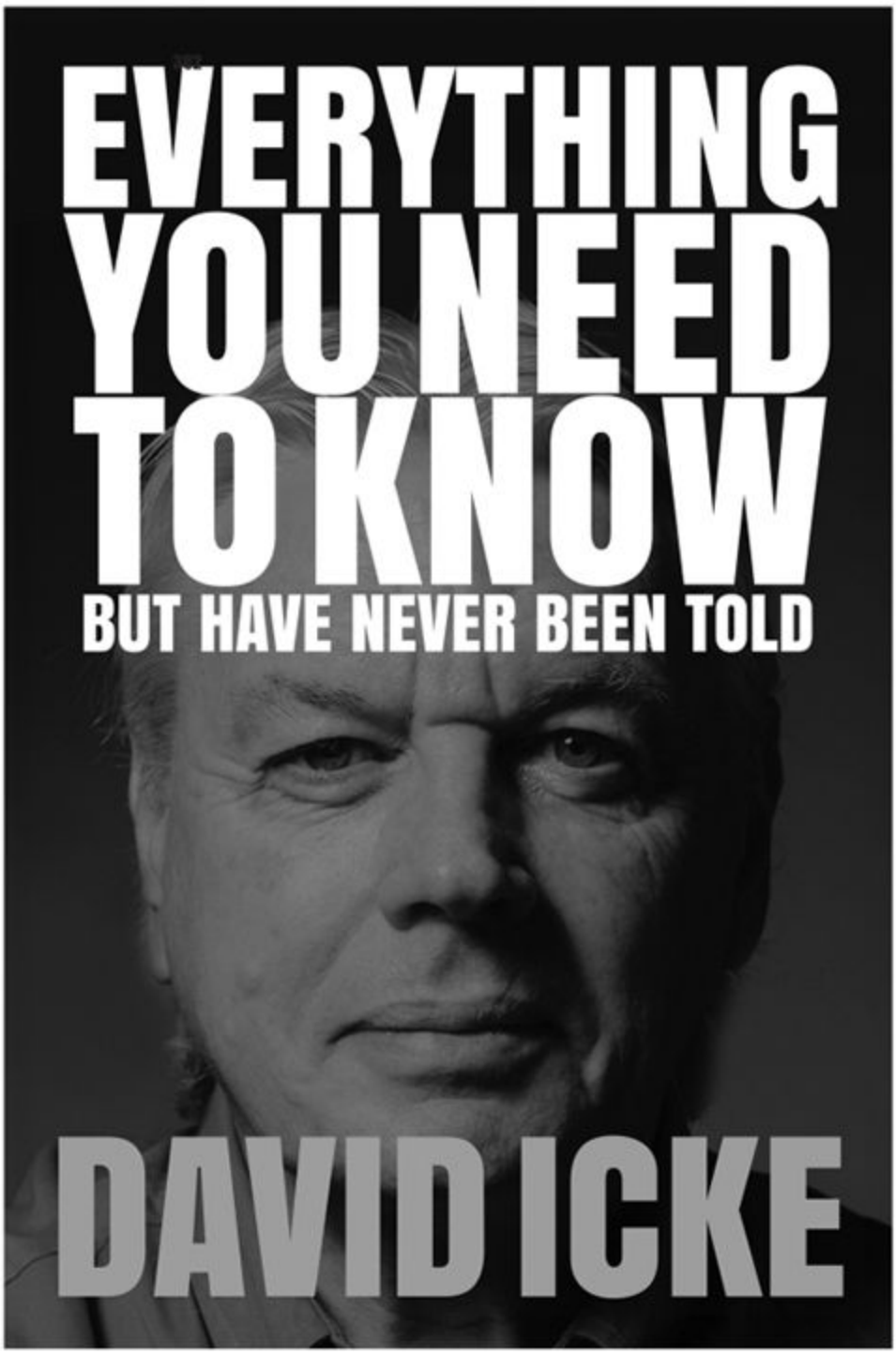
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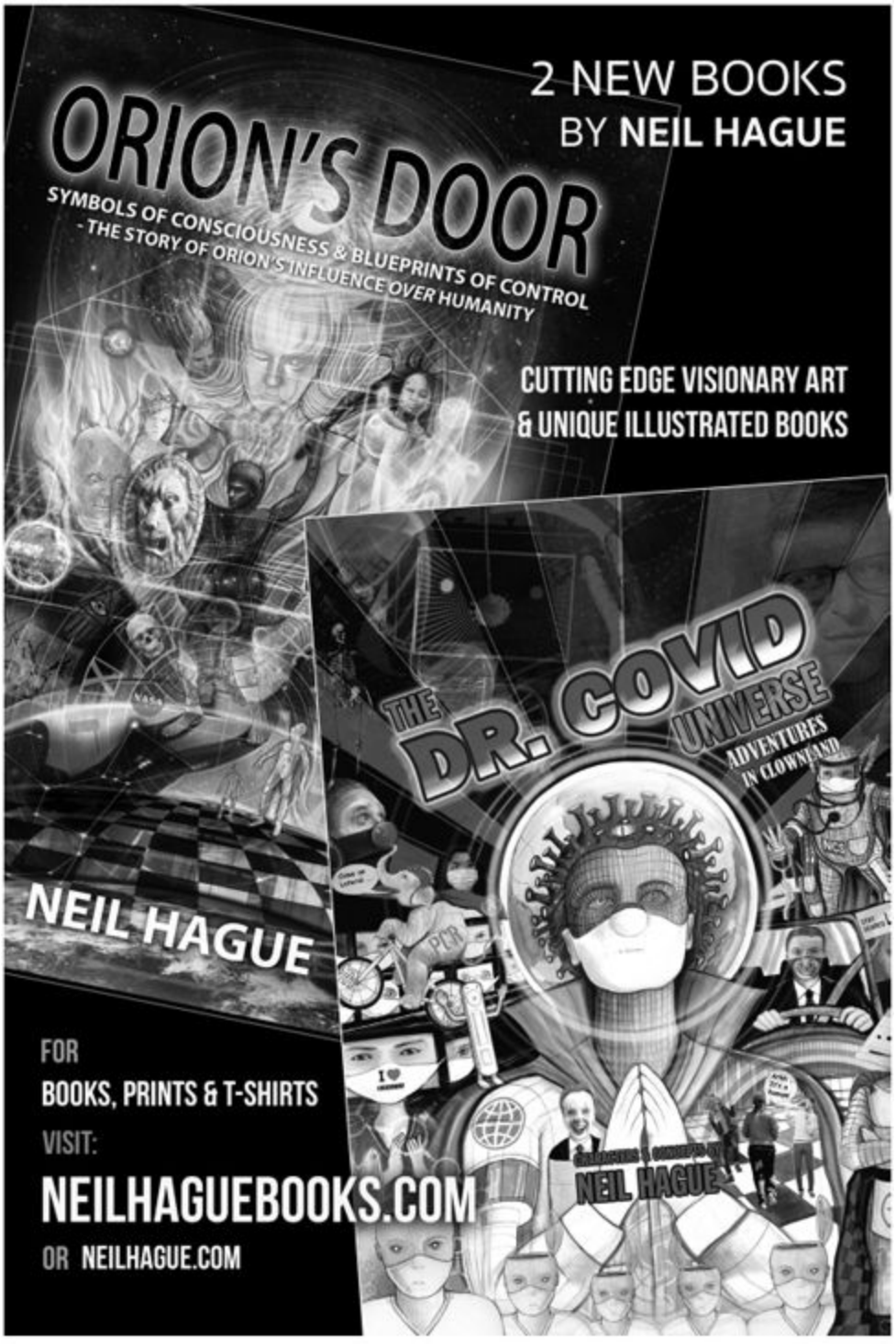
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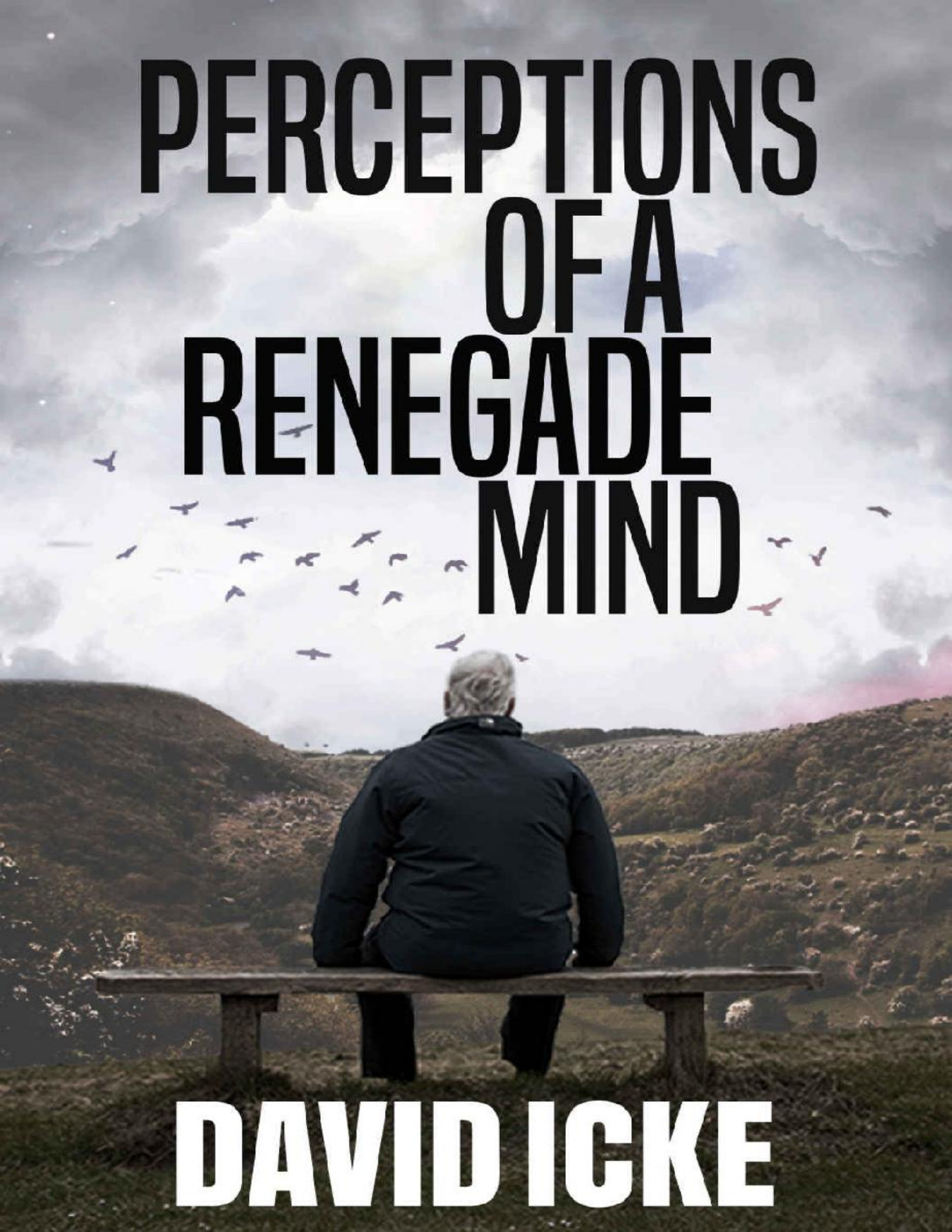

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PERCEPTIONS OF A RENEGADE MIND

DAVID ICKE

Dedication:

To *Freeeeeedom!*

Renegade:
Adjective
‘Having rejected tradition: Unconventional.’
Merriam-Webster Dictionary

Acquiescence to tyranny is the death of the spirit

You may be 38 years old, as I happen to be. And one day, some great opportunity stands before you and calls you to stand up for some great principle, some great issue, some great cause. And you refuse to do it because you are afraid … You refuse to do it because you want to live longer … You're afraid that you will lose your job, or you are afraid that you will be criticised or that you will lose your popularity, or you're afraid that somebody will stab you, or shoot at you or bomb your house; so you refuse to take the stand.

Well, you may go on and live until you are 90, but you're just as dead at 38 as you would be at 90. And the cessation of breathing in your life is but the belated announcement of an earlier death of the spirit.

Martin Luther King

How the few control the many and always have – the many do whatever they're told

'Forward, the Light Brigade!'
Was there a man dismayed?
Not though the soldier knew
Someone had blundered.
Theirs not to make reply,
Theirs not to reason why,
Theirs but to do and die.
Into the valley of Death
Rode the six hundred.

Cannon to right of them,
Cannon to left of them,
Cannon in front of them
Volleyed and thundered;
Stormed at with shot and shell,
Boldly they rode and well,
Into the jaws of Death,
Into the mouth of hell
Rode the six hundred

Alfred Lord Tennyson (1809-1892)

The mist is lifting slowly
I can see the way ahead
And I've left behind the empty streets
That once inspired my life
And the strength of the emotion
Is like thunder in the air
'Cos the promise that we made each other
Haunts me to the end

The secret of your beauty
And the mystery of your soul
I've been searching for in everyone I meet
And the times I've been mistaken
It's impossible to say
And the grass is growing
Underneath our feet

The words that I remember
From my childhood still are true
That there's none so blind
As those who will not see
And to those who lack the courage
And say it's dangerous to try
Well they just don't know
That love eternal will not be denied

I know you're out there somewhere
Somewhere, somewhere
I know you're out there somewhere
Somewhere you can hear my voice

I know I'll find you somehow
Somehow, somehow
I know I'll find you somehow
And somehow I'll return again to you

The Moody Blues

Are you a gutless wonder - or a Renegade Mind?

Monuments put from pen to paper,
Turns me into a gutless wonder,
And if you tolerate this,
Then your children will be next.
Gravity keeps my head down,
Or is it maybe shame ...

Manic Street Preachers

Rise like lions after slumber
In unvanquishable number.
Shake your chains to earth like dew
Which in sleep have fallen on you.
Ye are many – they are few.

Percy Shelley

Contents

CHAPTER ONE

I'm thinking' – Oh, but *are* you?

Think for yourself and let others enjoy the privilege of doing so too
Voltaire

French-born philosopher, mathematician and scientist René Descartes became famous for his statement in Latin in the 17th century which translates into English as: 'I think, therefore I am.'

On the face of it that is true. Thought reflects perception and perception leads to both behaviour and self-identity. In that sense 'we' are what we think. But who or what is doing the thinking and is thinking the only route to perception? Clearly, as we shall see, 'we' are not always the source of 'our' perception, indeed with regard to humanity as a whole this is rarely the case; and thinking is far from the only means of perception. Thought is the village idiot compared with other expressions of consciousness that we all have the potential to access and tap into. This has to be true when we *are* those other expressions of consciousness which are infinite in nature. We have forgotten this, or, more to the point, been manipulated to forget.

These are not just the esoteric musings of the navel. The whole foundation of human control and oppression is control of perception. Once perception is hijacked then so is behaviour which is dictated by perception. Collective perception becomes collective behaviour and collective behaviour is what we call human society. Perception is all and those behind human control know that which is why perception is the target 24/7 of the psychopathic manipulators that I call the Global Cult. They know that if they dictate perception they will dictate behaviour and collectively dictate

the nature of human society. They are further aware that perception is formed from information received and if they control the circulation of information they will to a vast extent direct human behaviour. Censorship of information and opinion has become globally Nazi-like in recent years and never more blatantly than since the illusory 'virus pandemic' was triggered out of China in 2019 and across the world in 2020. Why have billions submitted to house arrest and accepted fascistic societies in a way they would have never believed possible? Those controlling the information spewing from government, mainstream media and Silicon Valley (all controlled by the same Global Cult networks) told them they were in danger from a 'deadly virus' and only by submitting to house arrest and conceding their most basic of freedoms could they and their families be protected. This monumental and provable lie became the *perception* of the billions and therefore the *behaviour* of the billions. In those few words you have the whole structure and modus operandi of human control. Fear is a perception – **F**alse **E**motion **A**ppearing **R**eal – and fear is the currency of control. In short … get them by the balls (or give them the impression that you have) and their hearts and minds will follow. Nothing grips the dangly bits and freezes the rear-end more comprehensively than fear.

World number 1

There are two 'worlds' in what appears to be one 'world' and the prime difference between them is knowledge. First we have the mass of human society in which the population is maintained in coldly-calculated ignorance through control of information and the 'education' (indoctrination) system. That's all you really need to control to enslave billions in a perceptual delusion in which what are perceived to be *their* thoughts and opinions are ever-repeated mantras that the system has been downloading all their lives through 'education', media, science, medicine, politics and academia in which the personnel and advocates are themselves overwhelmingly the perceptual products of the same repetition. Teachers and academics in general are processed by the same programming machine as everyone else, but unlike the great majority they never leave the 'education' program. It gripped them as students and continues to grip them as programmers of subsequent generations of students. The programmed become the programmers – the programmed programmers. The same can largely be

said for scientists, doctors and politicians and not least because as the American writer Upton Sinclair said: 'It is difficult to get a man to understand something when his salary depends upon his not understanding it.' If your career and income depend on thinking the way the system demands then you will – bar a few free-minded exceptions – concede your mind to the Perceptual Mainframe that I call the Postage Stamp Consensus. This is a tiny band of perceived knowledge and possibility 'taught' (downloaded) in the schools and universities, pounded out by the mainstream media and on which all government policy is founded. Try thinking, and especially speaking and acting, outside of the 'box' of consensus and see what that does for your career in the Mainstream Everything which bullies, harasses, intimidates and ridicules the population into compliance. Here we have the simple structure which enslaves most of humanity in a perceptual prison cell for an entire lifetime and I'll go deeper into this process shortly. Most of what humanity is taught as fact is nothing more than programmed belief. American science fiction author Frank Herbert was right when he said: 'Belief can be manipulated. Only knowledge is dangerous.' In the 'Covid' age belief is promoted and knowledge is censored. It was always so, but never to the extreme of today.

World number 2

A 'number 2' is slang for 'doing a poo' and how appropriate that is when this other 'world' is doing just that on humanity every minute of every day. World number 2 is a global network of secret societies and semi-secret groups dictating the direction of society via governments, corporations and authorities of every kind. I have spent more than 30 years uncovering and exposing this network that I call the Global Cult and knowing its agenda is what has made my books so accurate in predicting current and past events. Secret societies are secret for a reason. They want to keep their hoarded knowledge to themselves and their chosen initiates and to hide it from the population which they seek through ignorance to control and subdue. The whole foundation of the division between World 1 and World 2 is *knowledge*. What number 1 knows number 2 must not. Knowledge they have worked so hard to keep secret includes (a) the agenda to enslave humanity in a centrally-controlled global dictatorship, and (b) the nature of reality and life itself. The latter (b) must be suppressed to allow the former

(a) to prevail as I shall be explaining. The way the Cult manipulates and interacts with the population can be likened to a spider's web. The 'spider' sits at the centre in the shadows and imposes its will through the web with each strand represented in World number 2 by a secret society, satanic or semi-secret group, and in World number 1 – the world of the seen – by governments, agencies of government, law enforcement, corporations, the banking system, media conglomerates and Silicon Valley (Fig 1 overleaf). The spider and the web connect and coordinate all these organisations to pursue the same global outcome while the population sees them as individual entities working randomly and independently. At the level of the web governments *are* the banking system *are* the corporations *are* the media *are* Silicon Valley *are* the World Health Organization working from their inner cores as one unit. Apparently unconnected countries, corporations, institutions, organisations and people are on the *same team* pursuing the same global outcome. Strands in the web immediately around the spider are the most secretive and exclusive secret societies and their membership is emphatically restricted to the Cult inner-circle emerging through the generations from particular bloodlines for reasons I will come to. At the core of the core you would get them in a single room. That's how many people are dictating the direction of human society and its transformation through the 'Covid' hoax and other means. As the web expands out from the spider we meet the secret societies that many people will be aware of – the Freemasons, Knights Templar, Knights of Malta, Opus Dei, the inner sanctum of the Jesuit Order, and such like. Note how many are connected to the Church of Rome and there is a reason for that. The Roman Church was established as a revamp, a rebranding, of the relocated 'Church' of Babylon and the Cult imposing global tyranny today can be tracked back to Babylon and Sumer in what is now Iraq.

Figure 1: The global web through which the few control the many. (Image Neil Hague.)

Inner levels of the web operate in the unseen away from the public eye and then we have what I call the cusp organisations located at the point where the hidden meets the seen. They include a series of satellite organisations answering to a secret society founded in London in the late 19th century called the Round Table and among them are the Royal Institute of International Affairs (UK, founded in 1920); Council on Foreign Relations (US, 1921); Bilderberg Group (worldwide, 1954); Trilateral Commission (US/worldwide, 1972); and the Club of Rome (worldwide, 1968) which was created to exploit environmental concerns to justify the centralisation of global power to 'save the planet'. The Club of Rome instigated with others the human-caused climate change hoax which has led to all the 'green new deals' demanding that very centralisation of control. Cusp organisations, which include endless 'think tanks' all over the world, are designed to coordinate a single global policy between political and business leaders, intelligence personnel, media organisations and anyone who can influence the direction of policy in their own sphere of operation. Major players and regular attenders will know what is happening – or some of it – while others come and go and are kept overwhelmingly in the dark about the big picture. I refer to these cusp groupings as semi-secret in that they can be publicly identified, but what goes on at the inner-core is kept very much 'in house' even from most of their members and participants through a fiercely-imposed system of compartmentalisation. Only let them know what they need to know to serve your interests and no more. The

structure of secret societies serves as a perfect example of this principle. Most Freemasons never get higher than the bottom three levels of 'degree' (degree of knowledge) when there are 33 official degrees of the Scottish Rite. Initiates only qualify for the next higher 'compartment' or degree if those at that level choose to allow them. Knowledge can be carefully assigned only to those considered 'safe'. I went to my local Freemason's lodge a few years ago when they were having an 'open day' to show how cuddly they were and when I chatted to some of them I was astonished at how little the rank and file knew even about the most ubiquitous symbols they use. The mushroom technique – keep them in the dark and feed them bullshit – applies to most people in the web as well as the population as a whole. Sub-divisions of the web mirror in theme and structure transnational corporations which have a headquarters somewhere in the world dictating to all their subsidiaries in different countries. Subsidiaries operate in their methodology and branding to the same centrally-dictated plan and policy in pursuit of particular ends. The Cult web functions in the same way. Each country has its own web as a subsidiary of the global one. They consist of networks of secret societies, semi-secret groups and bloodline families and their job is to impose the will of the spider and the global web in their particular country. Subsidiary networks control and manipulate the national political system, finance, corporations, media, medicine, etc. to ensure that they follow the globally-dictated Cult agenda. These networks were the means through which the 'Covid' hoax could be played out with almost every country responding in the same way.

The 'Yessir' pyramid

Compartmentalisation is the key to understanding how a tiny few can dictate the lives of billions when combined with a top-down sequence of imposition and acquiescence. The inner core of the Cult sits at the peak of the pyramidal hierarchy of human society (Fig 2 overleaf). It imposes its will – its agenda for the world – on the level immediately below which acquiesces to that imposition. This level then imposes the Cult will on the level below them which acquiesces and imposes on the next level. Very quickly we meet levels in the hierarchy that have no idea there even is a Cult, but the sequence of imposition and acquiescence continues down the pyramid in just the same way. 'I don't know why we are doing this but the

order came from “on-high” and so we better just do it.’ Alfred Lord Tennyson said of the cannon fodder levels in his poem *The Charge of the Light Brigade*: ‘Theirs not to reason why; theirs but to do and die.’ The next line says that ‘into the valley of death rode the six hundred’ and they died because they obeyed without question what their perceived ‘superiors’ told them to do. In the same way the population capitulated to ‘Covid’. The whole hierarchical pyramid functions like this to allow the very few to direct the enormous many. Eventually imposition-acquiescence-imposition-acquiescence comes down to the mass of the population at the foot of the pyramid. If they acquiesce to those levels of the hierarchy imposing on them (governments/law enforcement/doctors/media) a circuit is completed between the population and the handful of super-psychopaths in the Cult inner core at the top of the pyramid. Without a circuit-breaking refusal to obey, the sequence of imposition and acquiescence allows a staggeringly few people to impose their will upon the entirety of humankind. We are looking at the very sequence that has subjugated billions since the start of 2020. Our freedom has not been taken from us. Humanity has given it away. Fascists do not impose fascism because there are not enough of them. Fascism is imposed by the population acquiescing to fascism. Put another way allowing their perceptions to be programmed to the extent that leads to the population giving their freedom away by giving their perceptions – their mind – away. If this circuit is not broken by humanity ceasing to cooperate with their own enslavement then nothing can change. For that to happen people have to critically think and see through the lies and window dressing and then summon the backbone to act upon what they see. The Cult spends its days working to stop either happening and its methodology is systematic and highly detailed, but it can be overcome and that is what this book is all about.

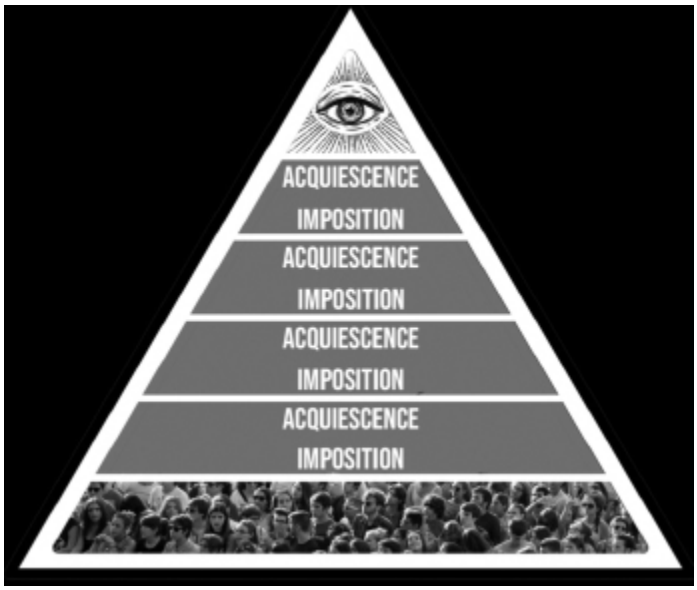

Figure 2: The simple sequence of imposition and compliance that allows a handful of people at the peak of the pyramid to dictate the lives of billions.

The Life Program

Okay, back to world number 1 or the world of the 'masses'. Observe the process of what we call 'life' and it is a perceptual download from cradle to grave. The Cult has created a global structure in which perception can be programmed and the program continually topped-up with what appears to be constant confirmation that the program is indeed true reality. The important word here is 'appears'. This is the structure, the fly-trap, the Postage Stamp Consensus or Perceptual Mainframe, which represents that incredibly narrow band of perceived possibility delivered by the 'education' system, mainstream media, science and medicine. From the earliest age the download begins with parents who have themselves succumbed to the very programming their children are about to go through. Most parents don't do this out of malevolence and mostly it is quite the opposite. They do what they believe is best for their children and that is what the program has told them is best. Within three or four years comes the major transition from parental programming to full-blown state (Cult) programming in school, college and university where perceptually-programmed teachers and academics pass on their programming to the next generations. Teachers who resist are soon marginalised and their careers ended while children who resist are called a problem child for whom Ritalin may need to be

prescribed. A few years after entering the ‘world’ children are under the control of authority figures representing the state telling them when they have to be there, when they can leave and when they can speak, eat, even go to the toilet. This is calculated preparation for a lifetime of obeying authority in all its forms. Reflex-action fear of authority is instilled by authority from the start. Children soon learn the carrot and stick consequences of obeying or defying authority which is underpinned daily for the rest of their life. Fortunately I daydreamed through this crap and never obeyed authority simply because it told me to. This approach to my alleged ‘betters’ continues to this day. There can be consequences of pursuing open-minded freedom in a world of closed-minded conformity. I spent a lot of time in school corridors after being ejected from the classroom for not taking some of it seriously and now I spend a lot of time being ejected from Facebook, YouTube and Twitter. But I can tell you that being true to yourself and not compromising your self-respect is far more exhilarating than bowing to authority for authority’s sake. You don’t have to be a sheep to the shepherd (authority) and the sheep dog (fear of not obeying authority).

The perceptual download continues throughout the formative years in school, college and university while script-reading ‘teachers’, ‘academics’ ‘scientists’, ‘doctors’ and ‘journalists’ insist that ongoing generations must be as programmed as they are. Accept the program or you will not pass your ‘exams’ which confirm your ‘degree’ of programming. It is tragic to think that many parents pressure their offspring to work hard at school to download the program and qualify for the next stage at college and university. The late, great, American comedian George Carlin said: ‘Here’s a bumper sticker I’d like to see: We are proud parents of a child who has resisted his teachers’ attempts to break his spirit and bend him to the will of his corporate masters.’ Well, the best of luck finding many of those, George. Then comes the moment to leave the formal programming years in academia and enter the ‘adult’ world of work. There you meet others in your chosen or prescribed arena who went through the same Postage Stamp Consensus program before you did. There is therefore overwhelming agreement between almost everyone on the basic foundations of Postage Stamp reality and the rejection, even contempt, of the few who have a mind of their own and are prepared to use it. This has two major effects. Firstly, the consensus confirms to the programmed that their download is really

how things are. I mean, everyone knows that, right? Secondly, the arrogance and ignorance of Postage Stamp adherents ensure that anyone questioning the program will have unpleasant consequences for seeking their own truth and not picking their perceptions from the shelf marked: 'Things you must believe without question and if you don't you're a dangerous lunatic conspiracy theorist and a harebrained nutter'.

Every government, agency and corporation is founded on the same Postage Stamp prison cell and you can see why so many people believe the same thing while calling it their own 'opinion'. Fusion of governments and corporations in pursuit of the same agenda was the definition of fascism described by Italian dictator Benito Mussolini. The pressure to conform to perceptual norms downloaded for a lifetime is incessant and infiltrates society right down to family groups that become censors and condemners of their own 'black sheep' for not, ironically, being sheep. We have seen an explosion of that in the 'Covid' era. Cult-owned global media unleashes its propaganda all day every day in support of the Postage Stamp and targets with abuse and ridicule anyone in the public eye who won't bend their mind to the will of the tyranny. Any response to this is denied (certainly in my case). They don't want to give a platform to expose official lies. Cult-owned-and-created Internet giants like Facebook, Google, YouTube and Twitter delete you for having an unapproved opinion. Facebook boasts that its AI censors delete 97-percent of 'hate speech' before anyone even reports it. Much of that 'hate speech' will simply be an opinion that Facebook and its masters don't want people to see. Such perceptual oppression is widely known as fascism. Even Facebook executive Benny Thomas, a 'CEO Global Planning Lead', said in comments secretly recorded by investigative journalism operation Project Veritas that Facebook is 'too powerful' and should be broken up:

> I mean, no king in history has been the ruler of two billion people, but Mark Zuckerberg is … And he's 36. That's too much for a 36-year-old ... You should not have power over two billion people. I just think that's wrong.

Thomas said Facebook-owned platforms like Instagram, Oculus, and WhatsApp needed to be separate companies. 'It's too much power when they're all one together'. That's the way the Cult likes it, however. We have

an executive of a Cult organisation in Benny Thomas that doesn't know there is a Cult such is the compartmentalisation. Thomas said that Facebook and Google 'are no longer companies, they're countries'. Actually they are more powerful than countries on the basis that if you control information you control perception and control human society.

I love my oppressor

Another expression of this psychological trickery is for those who realise they are being pressured into compliance to eventually convince themselves to believe the official narratives to protect their self-respect from accepting the truth that they have succumbed to meek and subservient compliance. Such people become some of the most vehement defenders of the system. You can see them everywhere screaming abuse at those who prefer to think for themselves and by doing so reminding the compliers of their own capitulation to conformity. 'You are talking dangerous nonsense you Covidiot!!' Are you trying to convince me or yourself? It is a potent form of Stockholm syndrome which is defined as: 'A psychological condition that occurs when a victim of abuse identifies and attaches, or bonds, positively with their abuser.' An example is hostages bonding and even 'falling in love' with their kidnappers. The syndrome has been observed in domestic violence, abused children, concentration camp inmates, prisoners of war and many and various Satanic cults. These are some traits of Stockholm syndrome listed at goodtherapy.org:

- Positive regard towards perpetrators of abuse or captor [see 'Covid'].
- Failure to cooperate with police and other government authorities when it comes to holding perpetrators of abuse or kidnapping accountable [or in the case of 'Covid' cooperating with the police to enforce and defend their captors' demands].
- Little or no effort to escape [see 'Covid'].
- Belief in the goodness of the perpetrators or kidnappers [see 'Covid'].
- Appeasement of captors. This is a manipulative strategy for maintaining one's safety. As victims get rewarded – perhaps with less

abuse or even with life itself – their appeasing behaviours are reinforced [see 'Covid'].

• Learned helplessness. This can be akin to 'if you can't beat 'em, join 'em'. As the victims fail to escape the abuse or captivity, they may start giving up and soon realize it's just easier for everyone if they acquiesce all their power to their captors [see 'Covid'].

• Feelings of pity toward the abusers, believing they are actually victims themselves. Because of this, victims may go on a crusade or mission to 'save' [protect] their abuser [see the venom unleashed on those challenging the official 'Covid' narrative].

• Unwillingness to learn to detach from their perpetrators and heal. In essence, victims may tend to be less loyal to themselves than to their abuser [*definitely* see 'Covid'].

Ponder on those traits and compare them with the behaviour of great swathes of the global population who have defended governments and authorities which have spent every minute destroying their lives and livelihoods and those of their children and grandchildren since early 2020 with fascistic lockdowns, house arrest and employment deletion to 'protect' them from a 'deadly virus' that their abusers' perceptually created to bring about this very outcome. We are looking at mass Stockholm syndrome. All those that agree to concede their freedom will believe those perceptions are originating in their own independent 'mind' when in fact by conceding their reality to Stockholm syndrome they have by definition conceded any independence of mind. Listen to the 'opinions' of the acquiescing masses in this 'Covid' era and what gushes forth is the repetition of the official version of everything delivered unprocessed, unfiltered and unquestioned. The whole programming dynamic works this way. I must be free because I'm told that I am and so I think that I am.

You can see what I mean with the chapter theme of 'I'm thinking – Oh, but *are* you?' The great majority are not thinking, let alone for themselves. They are repeating what authority has told them to believe which allows them to be controlled. Weaving through this mentality is the fear that the 'conspiracy theorists' are right and this again explains the often hysterical abuse that ensues when you dare to contest the official narrative of anything. Denial is the mechanism of hiding from yourself what you don't

want to be true. Telling people what they want to hear is easy, but it's an infinitely greater challenge to tell them what they would rather not be happening. One is akin to pushing against an open door while the other is met with vehement resistance no matter what the scale of evidence. I don't want it to be true so I'll convince myself that it's not. Examples are everywhere from the denial that a partner is cheating despite all the signs to the reflex-action rejection of any idea that world events in which country after country act in exactly the same way are centrally coordinated. To accept the latter is to accept that a force of unspeakable evil is working to destroy your life and the lives of your children with nothing too horrific to achieve that end. Who the heck wants that to be true? But if we don't face reality the end is duly achieved and the consequences are far worse and ongoing than breaking through the walls of denial today with the courage to make a stand against tyranny.

Connect the dots – but how?

A crucial aspect of perceptual programming is to portray a world in which everything is random and almost nothing is connected to anything else. Randomness cannot be coordinated by its very nature and once you perceive events as random the idea they could be connected is waved away as the rantings of the tinfoil-hat brigade. You can't plan and coordinate random you idiot! No, you can't, but you can hide the coldly-calculated and long-planned behind the *illusion* of randomness. A foundation manifestation of the Renegade Mind is to scan reality for patterns that connect the apparently random and turn pixels and dots into pictures. This is the way I work and have done so for more than 30 years. You look for similarities in people, modus operandi and desired outcomes and slowly, then ever quicker, the picture forms. For instance: There would seem to be no connection between the 'Covid pandemic' hoax and the human-caused global-warming hoax and yet they are masks (appropriately) on the same face seeking the same outcome. Those pushing the global warming myth through the Club of Rome and other Cult agencies are driving the lies about 'Covid' – Bill Gates is an obvious one, but they are endless. Why would the same people be involved in both when they are clearly not connected? Oh, but they *are*. Common themes with personnel are matched by common goals. The 'solutions' to both 'problems' are centralisation of global power

to impose the will of the few on the many to 'save' humanity from 'Covid' and save the planet from an 'existential threat' (we need 'zero Covid' and 'zero carbon emissions'). These, in turn, connect with the 'dot' of globalisation which was coined to describe the centralisation of global power in every area of life through incessant political and corporate expansion, trading blocks and superstates like the European Union. If you are the few and you want to control the many you have to centralise power and decision-making. The more you centralise power the more power the few at the centre will have over the many; and the more that power is centralised the more power those at the centre have to centralise even quicker. The momentum of centralisation gets faster and faster which is exactly the process we have witnessed. In this way the hoaxed 'pandemic' and the fakery of human-caused global warming serve the interests of globalisation and the seizure of global power in the hands of the Cult inner-circle which is behind 'Covid', 'climate change' *and* globalisation. At this point random 'dots' become a clear and obvious picture or pattern.

Klaus Schwab, the classic Bond villain who founded the Cult's Gates-funded World Economic Forum, published a book in 2020, *The Great Reset*, in which he used the 'problem' of 'Covid' to justify a total transformation of human society to 'save' humanity from 'climate change'. Schwab said: 'The pandemic represents a rare but narrow window of opportunity to reflect, reimagine, and reset our world.' What he didn't mention is that the Cult he serves is behind both hoaxes as I show in my book *The Answer*. He and the Cult don't have to reimagine the world. They know precisely what they want and that's why they destroyed human society with 'Covid' to 'build back better' in their grand design. Their job is not to imagine, but to get humanity to imagine and agree with their plans while believing it's all random. It must be pure coincidence that 'The Great Reset' has long been the Cult's code name for the global imposition of fascism and replaced previous code-names of the 'New World Order' used by Cult frontmen like Father George Bush and the 'New Order of the Ages' which emerged from Freemasonry and much older secret societies. New Order of the Ages appears on the reverse of the Great Seal of the United States as 'Novus ordo seclorum' underneath the Cult symbol used since way back of the pyramid and all seeing-eye (Fig 3). The pyramid is the hierarchy of human control headed by the illuminated eye that symbolises the force behind the Cult which I will expose in later chapters. The term

‘Annuit Coeptis’ translates as ‘He favours our undertaking’. We are told the ‘He’ is the Christian god, but ‘He’ is not as I will be explaining.

Figure 3: The all-seeing eye of the Cult ‘god’ on the Freemason-designed Great Seal of the United States and also on the dollar bill.

Having you on

Two major Cult techniques of perceptual manipulation that relate to all this are what I have called since the 1990s Problem-Reaction-Solution (PRS) and the Totalitarian Tiptoe (TT). They can be uncovered by the inquiring mind with a simple question: Who benefits? The answer usually identifies the perpetrators of a given action or happening through the concept of ‘he who most benefits from a crime is the one most likely to have committed it’. The Latin ‘Cue bono?’ – Who benefits? – is widely attributed to the Roman orator and statesman Marcus Tullius Cicero. No wonder it goes back so far when the concept has been relevant to human behaviour since history was recorded. Problem-Reaction-Solution is the technique used to manipulate us every day by covertly creating a problem (or the illusion of one) and offering the solution to the problem (or the illusion of one). In the first phase you create the problem and blame someone or something else for why it has happened. This may relate to a financial collapse, terrorist attack, war, global warming or pandemic, anything in fact that will allow you to impose the ‘solution’ to change society in the way you desire at that time. The ‘problem’ doesn’t have to be real. PRS is manipulation of perception and all you need is the population to believe the problem is real. Human-

caused global warming and the 'Covid pandemic' only have to be *perceived* to be real for the population to accept the 'solutions' of authority. I refer to this technique as NO-Problem-Reaction-Solution. Billions did not meekly accept house arrest from early 2020 because there was a real deadly 'Covid pandemic' but because they perceived – believed – that to be the case. The antidote to Problem-Reaction-Solution is to ask who benefits from the proposed solution. Invariably it will be anyone who wants to justify more control through deletion of freedom and centralisation of power and decision-making.

The two world wars were Problem-Reaction-Solutions that transformed and realigned global society. Both were manipulated into being by the Cult as I have detailed in books since the mid-1990s. They dramatically centralised global power, especially World War Two, which led to the United Nations and other global bodies thanks to the overt and covert manipulations of the Rockefeller family and other Cult bloodlines like the Rothschilds. The UN is a stalking horse for full-blown world government that I will come to shortly. The land on which the UN building stands in New York was donated by the Rockefellers and the same Cult family was behind Big Pharma scalpel and drug 'medicine' and the creation of the World Health Organization as part of the UN. They have been stalwarts of the eugenics movement and funded Hitler's race-purity expert' Ernst Rudin. The human-caused global warming hoax has been orchestrated by the Club of Rome through the UN which is manufacturing both the 'problem' through its Intergovernmental Panel on Climate Change and imposing the 'solution' through its Agenda 21 and Agenda 2030 which demand the total centralisation of global power to 'save the world' from a climate hoax the United Nations is itself perpetrating. What a small world the Cult can be seen to be particularly among the inner circles. The bedfellow of Problem-Reaction-Solution is the Totalitarian Tiptoe which became the Totalitarian Sprint in 2020. The technique is fashioned to hide the carefully-coordinated behind the cover of apparently random events. You start the sequence at 'A' and you know you are heading for 'Z'. You don't want people to know that and each step on the journey is presented as a random happening while all the steps strung together lead in the same direction. The speed may have quickened dramatically in recent times, but you can still see the incremental approach of the Tiptoe in the case of 'Covid' as each new imposition takes us deeper into fascism. Tell people they have to do this or that to get back to

'normal', then this and this and this. With each new demand adding to the ones that went before the population's freedom is deleted until it disappears. The spider wraps its web around the flies more comprehensively with each new diktat. I'll highlight this in more detail when I get to the 'Covid' hoax and how it has been pulled off. Another prime example of the Totalitarian Tiptoe is how the Cult-created European Union went from a 'free-trade zone' to a centralised bureaucratic dictatorship through the Tiptoe of incremental centralisation of power until nations became mere administrative units for Cult-owned dark suits in Brussels.

The antidote to ignorance is knowledge which the Cult seeks vehemently to deny us, but despite the systematic censorship to that end the Renegade Mind can overcome this by vociferously seeking out the facts no matter the impediments put in the way. There is also a method of thinking and perceiving – *knowing* – that doesn't even need names, dates, place-type facts to identify the patterns that reveal the story. I'll get to that in the final chapter. All you need to know about the manipulation of human society and to what end is still out there – *at the time of writing* – in the form of books, videos and websites for those that really want to breach the walls of programmed perception. To access this knowledge requires the abandonment of the mainstream media as a source of information in the awareness that this is owned and controlled by the Cult and therefore promotes mass perceptions that suit the Cult. Mainstream media lies all day, every day. That is its function and very reason for being. Where it does tell the truth, here and there, is only because the truth and the Cult agenda very occasionally coincide. If you look for fact and insight to the BBC, CNN and virtually all the rest of them you are asking to be conned and perceptually programmed.

Know the outcome and you'll see the journey

Events seem random when you have no idea where the world is being taken. Once you do the random becomes the carefully planned. Know the outcome and you'll see the journey is a phrase I have been using for a long time to give context to daily happenings that appear unconnected. Does a problem, or illusion of a problem, trigger a proposed 'solution' that further drives society in the direction of the outcome? Invariably the answer will be yes and the random – *abracadabra* – becomes the clearly coordinated. So

what is this outcome that unlocks the door to a massively expanded understanding of daily events? I will summarise its major aspects – the fine detail is in my other books – and those new to this information will see that the world they thought they were living in is a very different place. The foundation of the Cult agenda is the incessant centralisation of power and all such centralisation is ultimately in pursuit of Cult control on a global level. I have described for a long time the planned world structure of top-down dictatorship as the Hunger Games Society. The term obviously comes from the movie series which portrayed a world in which a few living in military-protected hi-tech luxury were the overlords of a population condemned to abject poverty in isolated 'sectors' that were not allowed to interact. 'Covid' lockdowns and travel bans anyone? The 'Hunger Games' pyramid of structural control has the inner circle of the Cult at the top with pretty much the entire population at the bottom under their control through dependency for survival on the Cult. The whole structure is planned to be protected and enforced by a military-police state (Fig 4).

Here you have the reason for the global lockdowns of the fake pandemic to coldly destroy independent incomes and livelihoods and make everyone dependent on the 'state' (the Cult that controls the 'states'). I have warned in my books for many years about the plan to introduce a 'guaranteed income' – a barely survivable pittance – designed to impose dependency when employment was destroyed by AI technology and now even more comprehensively at great speed by the 'Covid' scam. Once the pandemic was played and lockdown consequences began to delete independent income the authorities began to talk right on cue about the need for a guaranteed income and a 'Great Reset'. Guaranteed income will be presented as benevolent governments seeking to help a desperate people – desperate as a direct result of actions of the same governments. The truth is that such payments are a trap. You will only get them if you do exactly what the authorities demand including mass vaccination (genetic manipulation). We have seen this theme already in Australia where those dependent on government benefits have them reduced if parents don't agree to have their children vaccinated according to an insane health-destroying government-dictated schedule. Calculated economic collapse applies to governments as well as people. The Cult wants rid of countries through the creation of a world state with countries broken up into regions ruled by a world government and super states like the European Union. Countries must be

bankrupted, too, to this end and it's being achieved by the trillions in 'rescue packages' and furlough payments, trillions in lost taxation, and money-no-object spending on 'Covid' including constant all-medium advertising (programming) which has made the media dependent on government for much of its income. The day of reckoning is coming – as planned – for government spending and given that it has been made possible by printing money and not by production/taxation there is inflation on the way that has the potential to wipe out monetary value. In that case there will be no need for the Cult to steal your money. It just won't be worth anything (see the German Weimar Republic before the Nazis took over). Many have been okay with lockdowns while getting a percentage of their income from so-called furlough payments without having to work. Those payments are dependent, however, on people having at least a theoretical job with a business considered non-essential and ordered to close. As these business go under because they are closed by lockdown after lockdown the furlough stops and it will for everyone eventually. Then what? The 'then what?' is precisely the idea.

Figure 4: The Hunger Games Society structure I have long warned was planned and now the 'Covid' hoax has made it possible. This is the real reason for lockdowns.

Hired hands

Between the Hunger Games Cult elite and the dependent population is planned to be a vicious military-police state (a fusion of the two into one force). This has been in the making for a long time with police looking ever more like the military and carrying weapons to match. The pandemic scam has seen this process accelerate so fast as lockdown house arrest is brutally enforced by carefully recruited fascist minds and gormless system-servers. The police and military are planned to merge into a centrally-directed world army in a global structure headed by a world government which wouldn't be elected even by the election fixes now in place. The world army is not planned even to be human and instead wars would be fought, primarily against the population, using robot technology controlled by artificial intelligence. I have been warning about this for decades and now militaries around the world are being transformed by this very AI technology. The global regime that I describe is a particular form of fascism known as a technocracy in which decisions are not made by clueless and co-opted politicians but by unelected technocrats – scientists, engineers, technologists and bureaucrats. Cult-owned-and-controlled Silicon Valley giants are examples of technocracy and they already have far more power to direct world events than governments. They are with their censorship *selecting* governments. I know that some are calling the 'Great Reset' a Marxist communist takeover, but fascism and Marxism are different labels for the same tyranny. Tell those who lived in fascist Germany and Stalinist Russia that there was a difference in the way their freedom was deleted and their lives controlled. I could call it a fascist technocracy or a Marxist technocracy and they would be equally accurate. The Hunger Games society with its world government structure would oversee a world army, world central bank and single world cashless currency imposing its will on a microchipped population (Fig 5). Scan its different elements and see how the illusory pandemic is forcing society in this very direction at great speed. Leaders of 23 countries and the World Health Organization (WHO) backed the idea in March, 2021, of a global treaty for 'international cooperation' in 'health emergencies' and nations should 'come together as a global community for peaceful cooperation that extends beyond this crisis'. Cut the Orwellian bullshit and this means another step towards global government. The plan includes a cashless digital money system that I first warned about in 1993. Right at the start of 'Covid' the deeply corrupt

Tedros Adhanom Ghebreyesus, the crooked and merely gofer ‘head’ of the World Health Organization, said it was possible to catch the ‘virus’ by touching cash and it was better to use cashless means. The claim was ridiculous nonsense and like the whole ‘Covid’ mind-trick it was nothing to do with ‘health’ and everything to do with pushing every aspect of the Cult agenda. As a result of the Tedros lie the use of cash has plummeted. The Cult script involves a single world digital currency that would eventually be technologically embedded in the body. China is a massive global centre for the Cult and if you watch what is happening there you will know what is planned for everywhere. The Chinese government is developing a digital currency which would allow fines to be deducted immediately via AI for anyone caught on camera breaking its fantastic list of laws and the money is going to be programmable with an expiry date to ensure that no one can accrue wealth except the Cult and its operatives.

Figure 5: The structure of global control the Cult has been working towards for so long and this has been enormously advanced by the ‘Covid’ illusion.

Serfdom is so smart

The Cult plan is far wider, extreme, and more comprehensive than even most conspiracy researchers appreciate and I will come to the true depths of deceit and control in the chapters ‘Who controls the Cult?’ and ‘Escaping Wetiko’. Even the world that we know is crazy enough. We are being deluged with ever more sophisticated and controlling technology under the heading of ‘smart’. We have smart televisions, smart meters, smart cards,

smart cars, smart driving, smart roads, smart pills, smart patches, smart watches, smart skin, smart borders, smart pavements, smart streets, smart cities, smart communities, smart environments, smart growth, smart planet ... smart *everything* around us. Smart technologies and methods of operation are designed to interlock to create a global Smart Grid connecting the entirety of human society including human minds to create a centrally-dictated 'hive' mind. 'Smart cities' is code for densely-occupied megacities of total surveillance and control through AI. Ever more destructive frequency communication systems like 5G have been rolled out without any official testing for health and psychological effects (colossal). 5G/6G/7G systems are needed to run the Smart Grid and each one becomes more destructive of body and mind. Deleting independent income is crucial to forcing people into these AI-policed prisons by ending private property ownership (except for the Cult elite). The Cult's Great Reset now openly foresees a global society in which no one will own any possessions and everything will be rented while the Cult would own literally everything under the guise of government and corporations. The aim has been to use the lockdowns to destroy sources of income on a mass scale and when the people are destitute and in unrepayable amounts of debt (problem) Cult assets come forward with the pledge to write-off debt in return for handing over all property and possessions (solution). Everything – literally everything including people – would be connected to the Internet via AI. I was warning years ago about the coming Internet of Things (IoT) in which all devices and technology from your car to your fridge would be plugged into the Internet and controlled by AI. Now we are already there with much more to come. The next stage is the Internet of Everything (IoE) which is planned to include the connection of AI to the human brain and body to replace the human mind with a centrally-controlled AI mind. Instead of perceptions being manipulated through control of information and censorship those perceptions would come direct from the Cult through AI. What do you think? You think whatever AI decides that you think. In human terms there would be no individual 'think' any longer. Too incredible? The ravings of a lunatic? Not at all. Cult-owned crazies in Silicon Valley have been telling us the plan for years without explaining the real motivation and calculated implications. These include Google executive and 'futurist' Ray Kurzweil who highlights the year 2030 for when this would be underway. He said:

Our thinking ... will be a hybrid of biological and non-biological thinking ... humans will be able to extend their limitations and 'think in the cloud' ... We're going to put gateways to the cloud in our brains ... We're going to gradually merge and enhance ourselves ... In my view, that's the nature of being human – we transcend our limitations.

As the technology becomes vastly superior to what we are then the small proportion that is still human gets smaller and smaller and smaller until it's just utterly negligible.

The sales-pitch of Kurzweil and Cult-owned Silicon Valley is that this would make us 'super-human' when the real aim is to make us post-human and no longer 'human' in the sense that we have come to know. The entire global population would be connected to AI and become the centrally-controlled 'hive-mind' of externally-delivered perceptions. The Smart Grid being installed to impose the Cult's will on the world is being constructed to allow particular locations – even one location – to control the whole global system. From these prime control centres, which absolutely include China and Israel, anything connected to the Internet would be switched on or off and manipulated at will. Energy systems could be cut, communication via the Internet taken down, computer-controlled driverless autonomous vehicles driven off the road, medical devices switched off, the potential is limitless given how much AI and Internet connections now run human society. We have seen nothing yet if we allow this to continue. Autonomous vehicle makers are working with law enforcement to produce cars designed to automatically pull over if they detect a police or emergency vehicle flashing from up to 100 feet away. At a police stop the car would be unlocked and the window rolled down automatically. Vehicles would only take you where the computer (the state) allowed. The end of petrol vehicles and speed limiters on all new cars in the UK and EU from 2022 are steps leading to electric computerised transport over which ultimately you have no control. The picture is far bigger even than the Cult global network or web and that will become clear when I get to the nature of the 'spider'. There is a connection between all these happenings and the instigation of DNA-manipulating 'vaccines' (which aren't 'vaccines') justified by the 'Covid' hoax. That connection is the unfolding plan to transform the human body from a biological to a synthetic biological state and this is why synthetic biology is such a fast-emerging discipline of mainstream science. 'Covid vaccines' are infusing self-replicating synthetic genetic material into the cells to cumulatively take us on the Totalitarian Tiptoe from Human 1.0

to the synthetic biological Human 2.0 which will be physically and perceptually attached to the Smart Grid to one hundred percent control every thought, perception and deed. Humanity needs to wake up and *fast*.

This is the barest explanation of where the 'outcome' is planned to go but it's enough to see the journey happening all around us. Those new to this information will already see 'Covid' in a whole new context. I will add much more detail as we go along, but for the minutiae evidence see my mega-works, *The Answer, The Trigger* and *Everything You Need to Know But Have Never Been Told*.

Now – how does a Renegade Mind see the 'world'?

CHAPTER TWO

Renegade Perception

It is one thing to be clever and another to be wise
George R.R. Martin

A simple definition of the difference between a programmed mind and a Renegade Mind would be that one sees only dots while the other connects them to see the picture. Reading reality with accuracy requires the observer to (a) know the planned outcome and (b) realise that everything, but *everything*, is connected.

The entirety of infinite reality is connected – that's its very nature – and with human society an expression of infinite reality the same must apply. Simple cause and effect is a connection. The effect is triggered by the cause and the effect then becomes the cause of another effect. Nothing happens in isolation because it *can't*. Life in whatever reality is simple choice and consequence. We make choices and these lead to consequences. If we don't like the consequences we can make different choices and get different consequences which lead to other choices and consequences. The choice and the consequence are not only connected they are indivisible. You can't have one without the other as an old song goes. A few cannot control the world unless those being controlled allow that to happen – cause and effect, choice and consequence. Control – who has it and who doesn't – is a two-way process, a symbiotic relationship, involving the controller and controlled. 'They took my freedom away!!' Well, yes, but you also gave it to them. Humanity is subjected to mass control because humanity has acquiesced to that control. This is all cause and effect and literally a case of

give and take. In the same way world events of every kind are connected and the Cult works incessantly to sell the illusion of the random and coincidental to maintain the essential (to them) perception of dots that hide the picture. Renegade Minds know this and constantly scan the world for patterns of connection. This is absolutely pivotal in understanding the happenings in the world and without that perspective clarity is impossible. First you know the planned outcome and then you identify the steps on the journey – the day-by-day apparently random which, when connected in relation to the outcome, no longer appear as individual events, but as the proverbial *chain* of events leading in the same direction. I'll give you some examples:

Political puppet show

We are told to believe that politics is 'adversarial' in that different parties with different beliefs engage in an endless tussle for power. There may have been some truth in that up to a point – and only a point – but today divisions between 'different' parties are rhetorical not ideological. Even the rhetorical is fusing into one-speak as the parties eject any remaining free thinkers while others succumb to the ever-gathering intimidation of anyone with the 'wrong' opinion. The Cult is not a new phenomenon and can be traced back thousands of years as my books have documented. Its intergenerational initiates have been manipulating events with increasing effect the more that global power has been centralised. In ancient times the Cult secured control through the system of monarchy in which 'special' bloodlines (of which more later) demanded the right to rule as kings and queens simply by birthright and by vanquishing others who claimed the same birthright. There came a time, however, when people had matured enough to see the unfairness of such tyranny and demanded a say in who governed them. Note the word – *governed* them. Not served them – *governed* them, hence government defined as 'the political direction and control exercised over the actions of the members, citizens, or inhabitants of communities, societies, and states; direction of the affairs of a state, community, etc.' Governments exercise control over rather than serve just like the monarchies before them. Bizarrely there are still countries like the United Kingdom which are ruled by a monarch *and* a government that officially answers to the monarch. The UK head of state and that of Commonwealth

countries such as Canada, Australia and New Zealand is ‘selected’ by who in a *single family* had unprotected sex with whom and in what order. Pinch me it can’t be true. Ouch! Shit, it is. The demise of monarchies in most countries offered a potential vacuum in which some form of free and fair society could arise and the Cult had that base covered. Monarchies had served its interests but they couldn’t continue in the face of such widespread opposition and, anyway, replacing a ‘royal’ dictatorship that people could see with a dictatorship ‘of the people’ hiding behind the concept of ‘democracy’ presented far greater manipulative possibilities and ways of hiding coordinated tyranny behind the illusion of ‘freedom’.

Democracy is quite wrongly defined as government selected by the population. This is not the case at all. It is government selected by *some* of the population (and then only in theory). This ‘some’ doesn’t even have to be the majority as we have seen so often in first-past-the-post elections in which the so-called majority party wins fewer votes than the ‘losing’ parties combined. Democracy can give total power to a party in government from a minority of the votes cast. It’s a sleight of hand to sell tyranny as freedom. Seventy-four million Trump-supporting Americans didn’t vote for the ‘Democratic’ Party of Joe Biden in the distinctly dodgy election in 2020 and yet far from acknowledging the wishes and feelings of that great percentage of American society the Cult-owned Biden government set out from day one to destroy them and their right to a voice and opinion. Empty shell Biden and his Cult handlers said they were doing this to ‘protect democracy’. Such is the level of lunacy and sickness to which politics has descended. Connect the dots and relate them to the desired outcome – a world government run by self-appointed technocrats and no longer even elected politicians. While operating through its political agents in government the Cult is at the same time encouraging public distain for politicians by putting idiots and incompetents in theoretical power on the road to deleting them. The idea is to instil a public reaction that says of the technocrats: ‘Well, they couldn’t do any worse than the pathetic politicians.’ It’s all about controlling perception and Renegade Minds can see through that while programmed minds cannot when they are ignorant of both the planned outcome and the manipulation techniques employed to secure that end. This knowledge can be learned, however, and fast if people choose to get informed.

Politics may at first sight appear very difficult to control from a central point. I mean look at the 'different' parties and how would you be able to oversee them all and their constituent parts? In truth, it's very straightforward because of their structure. We are back to the pyramid of imposition and acquiescence. Organisations are structured in the same way as the system as a whole. Political parties are not open forums of free expression. They are hierarchies. I was a national spokesman for the British Green Party which claimed to be a different kind of politics in which influence and power was devolved; but I can tell you from direct experience – and it's far worse now – that Green parties are run as hierarchies like all the others however much they may try to hide that fact or kid themselves that it's not true. A very few at the top of all political parties are directing policy and personnel. They decide if you are elevated in the party or serve as a government minister and to do that you have to be a yes man or woman. Look at all the maverick political thinkers who never ascended the greasy pole. If you want to progress within the party or reach 'high-office' you need to fall into line and conform. Exceptions to this are rare indeed. Should you want to run for parliament or Congress you have to persuade the local or state level of the party to select you and for that you need to play the game as dictated by the hierarchy. If you secure election and wish to progress within the greater structure you need to go on conforming to what is acceptable to those running the hierarchy from the peak of the pyramid. Political parties are perceptual gulags and the very fact that there are party 'Whips' appointed to 'whip' politicians into voting the way the hierarchy demands exposes the ridiculous idea that politicians are elected to serve the people they are supposed to represent. Cult operatives and manipulation has long seized control of major parties that have any chance of forming a government and at least most of those that haven't. A new party forms and the Cult goes to work to infiltrate and direct. This has reached such a level today that you see video compilations of 'leaders' of all parties whether Democrats, Republicans, Conservative, Labour and Green parroting the same Cult mantra of 'Build Back Better' and the 'Great Reset' which are straight off the Cult song-sheet to describe the transformation of global society in response to the Cult-instigated hoaxes of the 'Covid pandemic' and human-caused 'climate change'. To see Caroline Lucas, the Green Party MP that I knew when I was in the party in the

1980s, speaking in support of plans proposed by Cult operative Klaus Schwab representing the billionaire global elite is a real head-shaker.

Many parties – one master

The party system is another mind-trick and was instigated to change the nature of the dictatorship by swapping 'royalty' for dark suits that people believed – though now ever less so – represented their interests. Understanding this trick is to realise that a single force (the Cult) controls all parties either directly in terms of the major ones or through manipulation of perception and ideology with others. You don't need to manipulate Green parties to demand your transformation of society in the name of 'climate change' when they are obsessed with the lie that this is essential to 'save the planet'. You just give them a platform and away they go serving your interests while believing they are being environmentally virtuous. America's political structure is a perfect blueprint for how the two or multi-party system is really a one-party state. The Republican Party is controlled from one step back in the shadows by a group made up of billionaires and their gofers known as neoconservatives or Neocons. I have exposed them in fine detail in my books and they were the driving force behind the policies of the imbecilic presidency of Boy George Bush which included 9/11 (see *The Trigger* for a comprehensive demolition of the official story), the subsequent 'war on terror' (war *of* terror) and the invasions of Afghanistan and Iraq. The latter was a No-Problem-Reaction-Solution based on claims by Cult operatives, including Bush and British Prime Minister Tony Blair, about Saddam Hussein's 'weapons of mass destruction' which did not exist as war criminals Bush and Blair well knew.

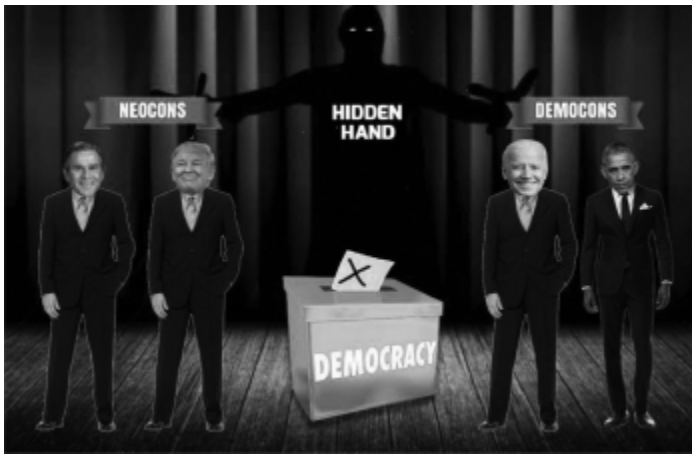

Figure 6: Different front people, different parties – same control system.

The Democratic Party has its own 'Neocon' group controlling from the background which I call the 'Democons' and here's the penny-drop – the Neocons and Democons answer to the same masters one step further back into the shadows (Fig 6). At that level of the Cult the Republican and Democrat parties are controlled by the same people and no matter which is in power the Cult is in power. This is how it works in almost every country and certainly in Britain with Conservative, Labour, Liberal Democrat and Green parties now all on the same page whatever the rhetoric may be in their feeble attempts to appear different. Neocons operated at the time of Bush through a think tank called The Project for the New American Century which in September, 2000, published a document entitled *Rebuilding America's Defenses: Strategies, Forces, and Resources For a New Century* demanding that America fight 'multiple, simultaneous major theatre wars' as a 'core mission' to force regime-change in countries including Iraq, Libya and Syria. Neocons arranged for Bush ('Republican') and Blair ('Labour Party') to front-up the invasion of Iraq and when they departed the Democons orchestrated the targeting of Libya and Syria through Barack Obama ('Democrat') and British Prime Minister David Cameron ('Conservative Party'). We have 'different' parties and 'different' people, but the same unfolding script. The more the Cult has seized the reigns of parties and personnel the more their policies have transparently pursued the same agenda to the point where the fascist 'Covid' impositions of the Conservative junta of Jackboot Johnson in Britain were opposed by the Labour Party because they were not fascist enough. The Labour Party is likened to the US Democrats while the Conservative Party is akin to a

British version of the Republicans and on both sides of the Atlantic they all speak the same language and support the direction demanded by the Cult although some more enthusiastically than others. It's a similar story in country after country because it's all centrally controlled. Oh, but what about Trump? I'll come to him shortly. Political 'choice' in the 'party' system goes like this: You vote for Party A and they get into government. You don't like what they do so next time you vote for Party B and they get into government. You don't like what they do when it's pretty much the same as Party A and why wouldn't that be with both controlled by the same force? Given that only two, sometimes three, parties have any chance of forming a government to get rid of Party B that you don't like you have to vote again for Party A which … you don't like. This, ladies and gentlemen, is what they call 'democracy' which we are told – wrongly – is a term interchangeable with 'freedom'.

The cult of cults

At this point I need to introduce a major expression of the Global Cult known as Sabbatian-Frankism. Sabbatian is also spelt as Sabbatean. I will summarise here. I have published major exposés and detailed background in other works. Sabbatian-Frankism combines the names of two frauds posing as 'Jewish' men, Sabbatai Zevi (1626-1676), a rabbi, black magician and occultist who proclaimed he was the Jewish messiah; and Jacob Frank (1726-1791), the Polish 'Jew', black magician and occultist who said he was the reincarnation of 'messiah' Zevi and biblical patriarch Jacob. They worked across two centuries to establish the Sabbatian-Frankist cult that plays a major, indeed central, role in the manipulation of human society by the Global Cult which has its origins much further back in history than Sabbatai Zevi. I should emphasise two points here in response to the shrill voices that will scream 'anti-Semitism': (1) Sabbatian-Frankists are NOT Jewish and only pose as such to hide their cult behind a Jewish façade; and (2) my information about this cult has come from Jewish sources who have long realised that their society and community has been infiltrated and taken over by interloper Sabbatian-Frankists. Infiltration has been the foundation technique of Sabbatian-Frankism from its official origin in the 17th century. Zevi's Sabbatian sect attracted a massive following described as the biggest messianic movement in Jewish history, spreading as far as

Africa and Asia, and he promised a return for the Jews to the 'Promised Land' of Israel. Sabbatianism was not Judaism but an inversion of everything that mainstream Judaism stood for. So much so that this sinister cult would have a feast day when Judaism had a fast day and whatever was forbidden in Judaism the Sabbatians were encouraged and even commanded to do. This included incest and what would be today called Satanism. Members were forbidden to marry outside the sect and there was a system of keeping their children ignorant of what they were part of until they were old enough to be trusted not to unknowingly reveal anything to outsiders. The same system is employed to this day by the Global Cult in general which Sabbatian-Frankism has enormously influenced and now largely controls.

Zevi and his Sabbatians suffered a setback with the intervention by the Sultan of the Islamic Ottoman Empire in the Middle East and what is now the Republic of Turkey where Zevi was located. The Sultan gave him the choice of proving his 'divinity', converting to Islam or facing torture and death. Funnily enough Zevi chose to convert or at least appear to. Some of his supporters were disillusioned and drifted away, but many did not with 300 families also converting – only in theory – to Islam. They continued behind this Islamic smokescreen to follow the goals, rules and rituals of Sabbatianism and became known as 'crypto-Jews' or the 'Dönmeh' which means 'to turn'. This is rather ironic because they didn't 'turn' and instead hid behind a fake Islamic persona. The process of appearing to be one thing while being very much another would become the calling card of Sabbatianism especially after Zevi's death and the arrival of the Satanist Jacob Frank in the 18th century when the cult became Sabbatian-Frankism and plumbed still new depths of depravity and infiltration which included – still includes – human sacrifice and sex with children. Wherever Sabbatians go paedophilia and Satanism follow and is it really a surprise that Hollywood is so infested with child abuse and Satanism when it was established by Sabbatian-Frankists and is still controlled by them? Hollywood has been one of the prime vehicles for global perceptual programming and manipulation. How many believe the version of 'history' portrayed in movies when it is a travesty and inversion (again) of the truth? Rabbi Marvin Antelman describes Frankism in his book, *To Eliminate the Opiate*, as 'a movement of complete evil' while Jewish professor Gershom Scholem said of Frank in *The Messianic Idea in Judaism*: 'In all his actions

[he was] a truly corrupt and degenerate individual ... one of the most frightening phenomena in the whole of Jewish history.' Frank was excommunicated by traditional rabbis, as was Zevi, but Frank was undeterred and enjoyed vital support from the House of Rothschild, the infamous banking dynasty whose inner-core are Sabbatian-Frankists and not Jews. Infiltration of the Roman Church and Vatican was instigated by Frank with many Dönmeh 'turning' again to convert to Roman Catholicism with a view to hijacking the reins of power. This was the ever-repeating modus operandi and continues to be so. Pose as an advocate of the religion, culture or country that you want to control and then manipulate your people into the positions of authority and influence largely as advisers, administrators and Svengalis for those that appear to be in power. They did this with Judaism, Christianity (Christian Zionism is part of this), Islam and other religions and nations until Sabbatian-Frankism spanned the world as it does today.

Sabbatian Saudis and the terror network

One expression of the Sabbatian-Frankist Dönmeh within Islam is the ruling family of Saudi Arabia, the House of Saud, through which came the vile distortion of Islam known as Wahhabism. This is the violent creed followed by terrorist groups like Al-Qaeda and ISIS or Islamic State. Wahhabism is the hand-chopping, head-chopping 'religion' of Saudi Arabia which is used to keep the people in a constant state of fear so the interloper House of Saud can continue to rule. Al-Qaeda and Islamic State were lavishly funded by the House of Saud while being created and directed by the Sabbatian-Frankist network in the United States that operates through the Pentagon, CIA and the government in general of whichever 'party'. The front man for the establishment of Wahhabism in the middle of the 18th century was a Sabbatian-Frankist 'crypto-Jew' posing as Islamic called Muhammad ibn Abd al-Wahhab. His daughter would marry the son of Muhammad bin Saud who established the first Saudi state before his death in 1765 with support from the British Empire. Bin Saud's successors would establish modern Saudi Arabia in league with the British and Americans in 1932 which allowed them to seize control of Islam's major shrines in Mecca and Medina. They have dictated the direction of Sunni Islam ever since while Iran is the major centre of the Shiite version and here we have

the source of at least the public conflict between them. The Sabbatian network has used its Wahhabi extremists to carry out Problem-Reaction-Solution terrorist attacks in the name of 'Al-Qaeda' and 'Islamic State' to justify a devastating 'war on terror', ever-increasing surveillance of the population and to terrify people into compliance. Another insight of the Renegade Mind is the streetwise understanding that just because a country, location or people are attacked doesn't mean that those apparently representing that country, location or people are not behind the attackers. Often they are *orchestrating* the attacks because of the societal changes that can be then justified in the name of 'saving the population from terrorists'.

I show in great detail in *The Trigger* how Sabbatian-Frankists were the real perpetrators of 9/11 and not '19 Arab hijackers' who were blamed for what happened. Observe what was justified in the name of 9/11 alone in terms of Middle East invasions, mass surveillance and control that fulfilled the demands of the Project for the New American Century document published by the Sabbatian Neocons. What appear to be enemies are on the deep inside players on the same Sabbatian team. Israel and Arab 'royal' dictatorships are all ruled by Sabbatians and the recent peace agreements between Israel and Saudi Arabia, the United Arab Emirates (UAE) and others are only making formal what has always been the case behind the scenes. Palestinians who have been subjected to grotesque tyranny since Israel was bombed and terrorised into existence in 1948 have never stood a chance. Sabbatian-Frankists have controlled Israel (so the constant theme of violence and war which Sabbatians love) and they have controlled the Arab countries that Palestinians have looked to for real support that never comes. 'Royal families' of the Arab world in Saudi Arabia, Bahrain, UAE, etc., are all Sabbatians with allegiance to the aims of the cult and not what is best for their Arabic populations. They have stolen the oil and financial resources from their people by false claims to be 'royal dynasties' with a genetic right to rule and by employing vicious militaries to impose their will.

Satanic 'illumination'

The Satanist Jacob Frank formed an alliance in 1773 with two other Sabbatians, Mayer Amschel Rothschild (1744-1812), founder of the Rothschild banking dynasty, and Jesuit-educated fraudulent Jew, Adam Weishaupt, and this led to the formation of the Bavarian Illuminati, firstly

under another name, in 1776. The Illuminati would be the manipulating force behind the French Revolution (1789-1799) and was also involved in the American Revolution (1775-1783) before and after the Illuminati's official creation. Weishaupt would later become (in public) a Protestant Christian in archetypal Sabbatian style. I read that his name can be decoded as Adam-Weis-haupt or 'the first man to lead those who know'. He wasn't a leader in the sense that he was a subordinate, but he did lead those below him in a crusade of transforming human society that still continues today. The theme was confirmed as early as 1785 when a horseman courier called Lanz was reported to be struck by lighting and extensive Illuminati documents were found in his saddlebags. They made the link to Weishaupt and detailed the plan for world takeover. Current events with 'Covid' fascism have been in the making for a very long time. Jacob Frank was jailed for 13 years by the Catholic Inquisition after his arrest in 1760 and on his release he headed for Frankfurt, Germany, home city and headquarters of the House of Rothschild where the alliance was struck with Mayer Amschel Rothschild and Weishaupt. Rothschild arranged for Frank to be given the title of Baron and he became a wealthy nobleman with a big following of Jews in Germany, the Austro-Hungarian Empire and other European countries. Most of them would have believed he was on their side.

The name 'Illuminati' came from the Zohar which is a body of works in the Jewish mystical 'bible' called the Kabbalah. 'Zohar' is the foundation of Sabbatian-Frankist belief and in Hebrew 'Zohar' means 'splendour', 'radiance', 'illuminated', and so we have 'Illuminati'. They claim to be the 'Illuminated Ones' from their knowledge systematically hidden from the human population and passed on through generations of carefully-chosen initiates in the global secret society network or Cult. Hidden knowledge includes an awareness of the Cult agenda for the world and the nature of our collective reality that I will explore later. Cult 'illumination' is symbolised by the torch held by the Statue of Liberty which was gifted to New York by French Freemasons in Paris who knew exactly what it represents. 'Liberty' symbolises the goddess worshipped in Babylon as Queen Semiramis or Ishtar. The significance of this will become clear. Notice again the ubiquitous theme of inversion with the Statue of 'Liberty' really symbolising mass control (Fig 7). A mirror-image statute stands on an island in the River Seine in Paris from where New York Liberty

originated (Fig 8). A large replica of the Liberty flame stands on top of the Pont de l'Alma tunnel in Paris where Princess Diana died in a Cult ritual described in *The Biggest Secret*. Lucifer 'the light bringer' is related to all this (and much more as we'll see) and 'Lucifer' is a central figure in Sabbatian-Frankism and its associated Satanism. Sabbatians reject the Jewish Torah, or Pentateuch, the 'five books of Moses' in the Old Testament known as Genesis, Exodus, Leviticus, Numbers, and Deuteronomy which are claimed by Judaism and Christianity to have been dictated by 'God' to Moses on Mount Sinai. Sabbatians say these do not apply to them and they seek to replace them with the Zohar to absorb Judaism and its followers into their inversion which is an expression of a much greater global inversion. They want to delete all religions and force humanity to worship a one-world religion – Sabbatian Satanism that also includes worship of the Earth goddess. Satanic themes are being more and more introduced into mainstream society and while Christianity is currently the foremost target for destruction the others are planned to follow.

Figure 7: The Cult goddess of Babylon disguised as the Statue of Liberty holding the flame of Lucifer the 'light bringer'.

Figure 8: Liberty's mirror image in Paris where the New York version originated.

Marx brothers

Rabbi Marvin Antelman connects the Illuminati to the Jacobins in *To Eliminate the Opiate* and Jacobins were the force behind the French Revolution. He links both to the Bund der Gerechten, or League of the Just, which was the network that inflicted communism/Marxism on the world. Antelman wrote:

> The original inner circle of the Bund der Gerechten consisted of born Catholics, Protestants and Jews [Sabbatian-Frankist infiltrators], and those representatives of respective subdivisions formulated schemes for the ultimate destruction of their faiths. The heretical Catholics laid plans which they felt would take a century or more for the ultimate destruction of the church; the apostate Jews for the ultimate destruction of the Jewish religion.

Sabbatian-created communism connects into this anti-religion agenda in that communism does not allow for the free practice of religion. The Sabbatian 'Bund' became the International Communist Party and Communist League and in 1848 'Marxism' was born with the Communist Manifesto of Sabbatian assets Karl Marx and Friedrich Engels. It is absolutely no coincidence that Marxism, just a different name for fascist and other centrally-controlled tyrannies, is being imposed worldwide as a result of the 'Covid' hoax and nor that Marxist/fascist China was the place where the hoax originated. The reason for this will become very clear in the chapter 'Covid: The calculated catastrophe'. The so-called 'Woke' mentality has hijacked traditional beliefs of the political left and replaced

them with far-right make-believe ‘social justice’ better known as Marxism. Woke will, however, be swallowed by its own perceived ‘revolution’ which is really the work of billionaires and billionaire corporations feigning being ‘Woke’. Marxism is being touted by Wokers as a replacement for ‘capitalism’ when we don’t have ‘capitalism’. We have cartelism in which the market is stitched up by the very Cult billionaires and corporations bankrolling Woke. Billionaires love Marxism which keeps the people in servitude while they control from the top. Terminally naïve Wokers think they are ‘changing the world’ when it’s the Cult that is doing the changing and when they have played their vital part and become surplus to requirements they, too, will be targeted. The Illuminati-Jacobins were behind the period known as ‘The Terror’ in the French Revolution in 1793 and 1794 when Jacobin Maximillian de Robespierre and his Orwellian ‘Committee of Public Safety’ killed 17,000 ‘enemies of the Revolution’ who had once been ‘friends of the Revolution’. Karl Marx (1818-1883), whose Sabbatian creed of Marxism has cost the lives of at least 100 million people, is a hero once again to Wokers who have been systematically kept ignorant of real history by their ‘education’ programming. As a result they now promote a Sabbatian ‘Marxist’ abomination destined at some point to consume them. Rabbi Antelman, who spent decades researching the Sabbatian plot, said of the League of the Just and Karl Marx:

Contrary to popular opinion Karl Marx did not originate the Communist Manifesto. He was paid for his services by the League of the Just, which was known in its country of origin, Germany, as the Bund der Geaechteten.

Antelman said the text attributed to Marx was the work of other people and Marx ‘was only repeating what others already said’. Marx was ‘a hired hack – lackey of the wealthy Illuminists’. Marx famously said that religion was the ‘opium of the people’ (part of the Sabbatian plan to demonise religion) and Antelman called his books, *To Eliminate the Opiate*. Marx was born Jewish, but his family converted to Christianity (Sabbatian modus operandi) and he attacked Jews, not least in his book, *A World Without Jews*. In doing so he supported the Sabbatian plan to destroy traditional Jewishness and Judaism which we are clearly seeing today with the vindictive targeting of orthodox Jews by the Sabbatian government of Israel over ‘Covid’ laws. I

don't follow any religion and it has done much damage to the world over centuries and acted as a perceptual straightjacket. Renegade Minds, however, are always asking *why* something is being done. It doesn't matter if they agree or disagree with what is happening – *why* is it happening is the question. The 'why?' can be answered with regard to religion in that religions create interacting communities of believers when the Cult wants to dismantle all discourse, unity and interaction (see 'Covid' lockdowns) and the ultimate goal is to delete all religions for a one-world religion of Cult Satanism worshipping their 'god' of which more later. We see the same 'why?' with gun control in America. I don't have guns and don't want them, but why is the Cult seeking to disarm the population at the same time that law enforcement agencies are armed to their molars and why has every tyrant in history sought to disarm people before launching the final takeover? They include Hitler, Stalin, Pol Pot and Mao who followed confiscation with violent seizing of power. You know it's a Cult agenda by the people who immediately race to the microphones to exploit dead people in multiple shootings. Ultra-Zionist Cult lackey Senator Chuck Schumer was straight on the case after ten people were killed in Boulder, Colorado in March, 2121. Simple rule … if Schumer wants it the Cult wants it and the same with his ultra-Zionist mate the wild-eyed Senator Adam Schiff. At the same time they were calling for the disarmament of Americans, many of whom live a long way from a police response, Schumer, Schiff and the rest of these pampered clowns were sitting on Capitol Hill behind a razor-wired security fence protected by thousands of armed troops in addition to their own armed bodyguards. Mom and pop in an isolated home? They're just potential mass shooters.

Zion Mainframe

Sabbatian-Frankists and most importantly the Rothschilds were behind the creation of 'Zionism', a political movement that demanded a Jewish homeland in Israel as promised by Sabbatai Zevi. The very symbol of Israel comes from the German meaning of the name Rothschild. Dynasty founder Mayer Amschel Rothschild changed the family name from Bauer to Rothschild, or 'Red-Shield' in German, in deference to the six-pointed 'Star of David' hexagram displayed on the family's home in Frankfurt. The symbol later appeared on the flag of Israel after the Rothschilds were

centrally involved in its creation. Hexagrams are not a uniquely Jewish symbol and are widely used in occult ('hidden') networks often as a symbol for Saturn (see my other books for why). Neither are Zionism and Jewishness interchangeable. Zionism is a political movement and philosophy and not a 'race' or a people. Many Jews oppose Zionism and many non-Jews, including US President Joe Biden, call themselves Zionists as does Israel-centric Donald Trump. America's support for the Israel government is pretty much a gimme with ultra-Zionist billionaires and corporations providing fantastic and dominant funding for both political parties. Former Congresswoman Cynthia McKinney has told how she was approached immediately she ran for office to 'sign the pledge' to Israel and confirm that she would always vote in that country's best interests. All American politicians are approached in this way. Anyone who refuses will get no support or funding from the enormous and all-powerful Zionist lobby that includes organisations like mega-lobby group AIPAC, the American Israel Public Affairs Committee. Trump's biggest funder was ultra-Zionist casino and media billionaire Sheldon Adelson while major funders of the Democratic Party include ultra-Zionist George Soros and ultra-Zionist financial and media mogul, Haim Saban. Some may reel back at the suggestion that Soros is an Israel-firster (Sabbatian-controlled Israel-firster), but Renegade Minds watch the actions not the words and everywhere Soros donates his billions the Sabbatian agenda benefits. In the spirit of Sabbatian inversion Soros pledged $1 billion for a new university network to promote 'liberal values and tackle intolerance'. He made the announcement during his annual speech at the Cult-owned World Economic Forum in Davos, Switzerland, in January, 2020, after his 'harsh criticism' of 'authoritarian rulers' around the world. You can only laugh at such brazen mendacity. How *he* doesn't laugh is the mystery. Translated from the Orwellian 'liberal values and tackle intolerance' means teaching non-white people to hate white people and for white people to loathe themselves for being born white. The reason for that will become clear.

The 'Anti-Semitism' fraud

Zionists support the Jewish homeland in the land of Palestine which has been the Sabbatian-Rothschild goal for so long, but not for the benefit of Jews. Sabbatians and their global Anti-Semitism Industry have skewed

public and political opinion to equate opposing the violent extremes of Zionism to be a blanket attack and condemnation of all Jewish people. Sabbatians and their global Anti-Semitism Industry have skewed public and political opinion to equate opposing the violent extremes of Zionism to be a blanket attack and condemnation of all Jewish people. This is nothing more than a Sabbatian protection racket to stop legitimate investigation and exposure of their agendas and activities. The official definition of 'anti-Semitism' has more recently been expanded to include criticism of Zionism – a *political movement* – and this was done to further stop exposure of Sabbatian infiltrators who created Zionism as we know it today in the 19th century. Renegade Minds will talk about these subjects when they know the shit that will come their way. People must decide if they want to know the truth or just cower in the corner in fear of what others will say. Sabbatians have been trying to label me as 'anti-Semitic' since the 1990s as I have uncovered more and more about their background and agendas. Useless, gutless, fraudulent 'journalists' then just repeat the smears without question and on the day I was writing this section a pair of unquestioning repeaters called Ben Quinn and Archie Bland (how appropriate) outright called me an 'anti-Semite' in the establishment propaganda sheet, the London *Guardian*, with no supporting evidence. The Sabbatian Anti-Semitism Industry said so and who are they to question that? They wouldn't dare. Ironically 'Semitic' refers to a group of languages in the Middle East that are almost entirely Arabic. 'Anti-Semitism' becomes 'anti-Arab' which if the consequences of this misunderstanding were not so grave would be hilarious. Don't bother telling Quinn and Bland. I don't want to confuse them, bless 'em. One reason I am dubbed 'anti-Semitic' is that I wrote in the 1990s that Jewish operatives (Sabbatians) were heavily involved in the Russian Revolution when Sabbatians overthrew the Romanov dynasty. This apparently made me 'anti-Semitic'. Oh, really? Here is a section from *The Trigger*:

British journalist Robert Wilton confirmed these themes in his 1920 book *The Last Days of the Romanovs* when he studied official documents from the Russian government to identify the members of the Bolshevik ruling elite between 1917 and 1919. The Central Committee included 41 Jews among 62 members; the Council of the People's Commissars had 17 Jews out of 22 members; and 458 of the 556 most important Bolshevik positions between 1918 and 1919 were occupied by Jewish people. Only 17 were Russian. Then there were the 23 Jews among the 36 members of the vicious Cheka Soviet secret police established in 1917 who would soon appear all across the country.

Professor Robert Service of Oxford University, an expert on 20th century Russian history, found evidence that ['Jewish'] Leon Trotsky had sought to make sure that Jews were enrolled in the Red Army and were disproportionately represented in the Soviet civil bureaucracy that included the Cheka which performed mass arrests, imprisonment and executions of 'enemies of the people'. A US State Department Decimal File (861.00/5339) dated November 13th, 1918, names [Rothschild banking agent in America] Jacob Schiff and a list of ultra-Zionists as funders of the Russian Revolution leading to claims of a 'Jewish plot', but the key point missed by all is they were not 'Jews' – they were Sabbatian-Frankists.

Britain's Winston Churchill made the same error by mistake or otherwise. He wrote in a 1920 edition of the *Illustrated Sunday Herald* that those behind the Russian revolution were part of a 'worldwide conspiracy for the overthrow of civilisation and for the reconstitution of society on the basis of arrested development, of envious malevolence, and impossible equality' (see 'Woke' today because that has been created by the same network). Churchill said there was no need to exaggerate the part played in the creation of Bolshevism and in the actual bringing about of the Russian Revolution 'by these international and for the most part atheistical Jews' ['atheistical Jews' = Sabbatians]. Churchill said it is certainly a very great one and probably outweighs all others: 'With the notable exception of Lenin, the majority of the leading figures are Jews.' He went on to describe, knowingly or not, the Sabbatian modus operandi of placing puppet leaders nominally in power while they control from the background:

Moreover, the principal inspiration and driving power comes from the Jewish leaders. Thus Tchitcherin, a pure Russian, is eclipsed by his nominal subordinate, Litvinoff, and the influence of Russians like Bukharin or Lunacharski cannot be compared with the power of Trotsky, or of Zinovieff, the Dictator of the Red Citadel (Petrograd), or of Krassin or Radek – all Jews. In the Soviet institutions the predominance of Jews is even more astonishing. And the prominent, if not indeed the principal, part in the system of terrorism applied by the Extraordinary Commissions for Combatting Counter-Revolution has been taken by Jews, and in some notable cases by Jewesses.

What I said about seriously disproportionate involvement in the Russian Revolution by Jewish 'revolutionaries' (Sabbatians) is provable fact, but truth is no defence against the Sabbatian Anti-Semitism Industry, its repeater parrots like Quinn and Bland, and the now breathtaking network of so-called 'Woke' 'anti-hate' groups with interlocking leaderships and funding which have the role of discrediting and silencing anyone who gets too close to exposing the Sabbatians. We have seen 'truth is no defence'

confirmed in legal judgements with the Saskatchewan Human Rights Commission in Canada decreeing this: ‘Truthful statements can be presented in a manner that would meet the definition of hate speech, and not all truthful statements must be free from restriction.’ Most ‘anti-hate’ activists, who are themselves consumed by hatred, are too stupid and ignorant of the world to know how they are being used. They are far too far up their own virtue-signalling arses and it’s far too dark for them to see anything.

The ‘revolution’ game

The background and methods of the ‘Russian’ Revolution are straight from the Sabbatian playbook seen in the French Revolution and endless others around the world that appear to start as a revolution of the people against tyrannical rule and end up with a regime change to more tyrannical rule overtly or covertly. Wars, terror attacks and regime overthrows follow the Sabbatian cult through history with its agents creating them as Problem-Reaction-Solutions to remove opposition on the road to world domination. Sabbatian dots connect the Rothschilds with the Illuminati, Jacobins of the French Revolution, the ‘Bund’ or League of the Just, the International Communist Party, Communist League and the Communist Manifesto of Karl Marx and Friedrich Engels that would lead to the Rothschild-funded Russian Revolution. The sequence comes under the heading of ‘creative destruction’ when you advance to your global goal by continually destroying the status quo to install a new status quo which you then also destroy. The two world wars come to mind. With each new status quo you move closer to your planned outcome. Wars and mass murder are to Sabbatians a collective blood sacrifice ritual. They are obsessed with death for many reasons and one is that death is an inversion of life. Satanists and Sabbatians are obsessed with death and often target churches and churchyards for their rituals. Inversion-obsessed Sabbatians explain the use of inverted symbolism including the *inverted* pentagram and *inverted* cross. The inversion of the cross has been related to targeting Christianity, but the cross was a religious symbol long before Christianity and its inversion is a statement about the Sabbatian mentality and goals more than any single religion.

Sabbatians operating in Germany were behind the rise of the occult-obsessed Nazis and the subsequent Jewish exodus from Germany and Europe to Palestine and the United States after World War Two. The Rothschild dynasty was at the forefront of this both as political manipulators and by funding the operation. Why would Sabbatians help to orchestrate the horrors inflicted on Jews by the Nazis and by Stalin after they organised the Russian Revolution? Sabbatians hate Jews and their religion, that's why. They pose as Jews and secure positions of control within Jewish society and play the 'anti-Semitism' card to protect themselves from exposure through a global network of organisations answering to the Sabbatian-created-and-controlled globe-spanning intelligence network that involves a stunning web of military-intelligence operatives and operations for a tiny country of just nine million. Among them are Jewish assets who are not Sabbatians but have been convinced by them that what they are doing is for the good of Israel and the Jewish community to protect them from what they have been programmed since childhood to believe is a Jew-hating hostile world. The Jewish community is just a highly convenient cover to hide the true nature of Sabbatians. Anyone getting close to exposing their game is accused by Sabbatian place-people and gofers of 'anti-Semitism' and claiming that all Jews are part of a plot to take over the world. I am not saying that. I am saying that Sabbatians – the *real* Jew-haters – have infiltrated the Jewish community to use them both as a cover and an 'anti-Semitic' defence against exposure. Thus we have the Anti-Semitism Industry targeted researchers in this way and most Jewish people think this is justified and genuine. They don't know that their 'Jewish' leaders and institutions of state, intelligence and military are not controlled by Jews at all, but cultists and stooges of Sabbatian-Frankism. I once added my name to a pro-Jewish freedom petition online and the next time I looked my name was gone and text had been added to the petition blurb to attack me as an 'anti-Semite' such is the scale of perceptual programming.

Moving on America

I tell the story in *The Trigger* and a chapter called 'Atlantic Crossing' how particularly after Israel was established the Sabbatians moved in on the United States and eventually grasped control of government administration,

the political system via both Democrats and Republicans, the intelligence community like the CIA and National Security Agency (NSA), the Pentagon and mass media. Through this seriously compartmentalised network Sabbatians and their operatives in Mossad, Israeli Defense Forces (IDF) and US agencies pulled off 9/11 and blamed it on 19 'Al-Qaeda hijackers' dominated by men from, or connected to, Sabbatian-ruled Saudi Arabia. The '19' were not even on the planes let alone flew those big passenger jets into buildings while being largely incompetent at piloting one-engine light aircraft. 'Hijacker' Hani Hanjour who is said to have flown American Airlines Flight 77 into the Pentagon with a turn and manoeuvre most professional pilots said they would have struggled to do was banned from renting a small plane by instructors at the Freeway Airport in Bowie, Maryland, just *six weeks* earlier on the grounds that he was an incompetent pilot. The Jewish population of the world is just 0.2 percent with even that almost entirely concentrated in Israel (75 percent Jewish) and the United States (around two percent). This two percent and globally 0.2 percent refers to *Jewish* people and not Sabbatian interlopers who are a fraction of that fraction. What a sobering thought when you think of the fantastic influence on world affairs of tiny Israel and that the Project for the New America Century (PNAC) which laid out the blueprint in September, 2000, for America's war on terror and regime change wars in Iraq, Libya and Syria was founded and dominated by Sabbatians known as 'Neocons'. The document conceded that this plan would not be supported politically or publicly without a major attack on American soil and a Problem-Reaction-Solution excuse to send troops to war across the Middle East. Sabbatian Neocons said:

> ... [The] process of transformation ... [war and regime change] ... is likely to be a long one, absent some catastrophic and catalysing event – like a new Pearl Harbor.

Four months later many of those who produced that document came to power with their inane puppet George Bush from the long-time Sabbatian Bush family. They included Sabbatian Dick Cheney who was officially vice-president, but really de-facto president for the entirety of the 'Bush' government. Nine months after the 'Bush' inauguration came what Bush called at the time 'the Pearl Harbor of the 21st century' and with typical

Sabbatian timing and symbolism 2001 was the 60th anniversary of the attack in 1941 by the Japanese Air Force on Pearl Harbor, Hawaii, which allowed President Franklin Delano Roosevelt to take the United States into a Sabbatian-instigated Second World War that he said in his election campaign that he never would. The evidence is overwhelming that Roosevelt and his military and intelligence networks knew the attack was coming and did nothing to stop it, but they did make sure that America's most essential naval ships were not in Hawaii at the time. Three thousand Americans died in the Pearl Harbor attacks as they did on September 11th. By the 9/11 year of 2001 Sabbatians had widely infiltrated the US government, military and intelligence operations and used their compartmentalised assets to pull off the 'Al-Qaeda' attacks. If you read *The Trigger* it will blow your mind to see the utterly staggering concentration of 'Jewish' operatives (Sabbatian infiltrators) in essential positions of political, security, legal, law enforcement, financial and business power before, during, and after the attacks to make them happen, carry them out, and then cover their tracks – and I do mean *staggering* when you think of that 0.2 percent of the world population and two percent of Americans which are Jewish while Sabbatian infiltrators are a fraction of that. A central foundation of the 9/11 conspiracy was the hijacking of government, military, Air Force and intelligence computer systems in real time through 'back-door' access made possible by Israeli (Sabbatian) 'cyber security' software. Sabbatian-controlled Israel is on the way to rivalling Silicon Valley for domination of cyberspace and is becoming the dominant force in cyber-security which gives them access to entire computer systems and their passcodes across the world. Then add to this that Zionists head (officially) Silicon Valley giants like Google (Larry Page and Sergey Brin), Google-owned YouTube (Susan Wojcicki), Facebook (Mark Zuckerberg and Sheryl Sandberg), and Apple (Chairman Arthur D. Levinson), and that ultra-Zionist hedge fund billionaire Paul Singer has a $1 billion stake in Twitter which is only nominally headed by 'CEO' pothead Jack Dorsey. As cable news host Tucker Carlson said of Dorsey: 'There used to be debate in the medical community whether dropping a ton of acid had permanent effects and I think that debate has now ended.' Carlson made the comment after Dorsey told a hearing on Capitol Hill (if you cut through his bullshit) that he believed in free speech so long as he got to decide what you can hear and see. These 'big names' of Silicon Valley are only front men and

women for the Global Cult, not least the Sabbatians, who are the true controllers of these corporations. Does anyone still wonder why these same people and companies have been ferociously censoring and banning people (like me) for exposing any aspect of the Cult agenda and especially the truth about the 'Covid' hoax which Sabbatians have orchestrated?

The Jeffrey Epstein paedophile ring was a Sabbatian operation. He was officially 'Jewish' but he was a Sabbatian and women abused by the ring have told me about the high number of 'Jewish' people involved. The Epstein horror has Sabbatian written all over it and matches perfectly their modus operandi and obsession with sex and ritual. Epstein was running a Sabbatian blackmail ring in which famous people with political and other influence were provided with young girls for sex while everything was being filmed and recorded on hidden cameras and microphones at his New York house, Caribbean island and other properties. Epstein survivors have described this surveillance system to me and some have gone public. Once the famous politician or other figure knew he or she was on video they tended to do whatever they were told. Here we go again …when you've got them by the balls their hearts and minds will follow. Sabbatians use this blackmail technique on a wide scale across the world to entrap politicians and others they need to act as demanded. Epstein's private plane, the infamous 'Lolita Express', had many well-known passengers including Bill Clinton while Bill Gates has flown on an Epstein plane and met with him four years after Epstein had been jailed for paedophilia. They subsequently met many times at Epstein's home in New York according to a witness who was there. Epstein's infamous side-kick was Ghislaine Maxwell, daughter of Mossad agent and ultra-Zionist mega-crooked British businessman, Bob Maxwell, who at one time owned the *Daily Mirror* newspaper. Maxwell was murdered at sea on his boat in 1991 by Sabbatian-controlled Mossad when he became a liability with his business empire collapsing as a former Mossad operative has confirmed (see *The Trigger*).

Money, money, money, funny money …

Before I come to the Sabbatian connection with the last three US presidents I will lay out the crucial importance to Sabbatians of controlling banking and finance. Sabbatian Mayer Amschel Rothschild set out to dominate this arena in his family's quest for total global control. What is freedom? It is, in

effect, choice. The more choices you have the freer you are and the fewer your choices the more you are enslaved. In the global structure created over centuries by Sabbatians the biggest decider and restrictor of choice is … money. Across the world if you ask people what they would like to do with their lives and why they are not doing that they will reply 'I don't have the money'. This is the idea. A global elite of multi-billionaires are described as 'greedy' and that is true on one level; but control of money – who has it and who doesn't – is not primarily about greed. It's about control. Sabbatians have seized ever more control of finance and sucked the wealth of the world out of the hands of the population. We talk now, after all, about the 'One-percent' and even then the wealthiest are a lot fewer even than that. This has been made possible by a money scam so outrageous and so vast it could rightly be called the scam of scams founded on creating 'money' out of nothing and 'loaning' that with interest to the population. Money out of nothing is called 'credit'. Sabbatians have asserted control over governments and banking ever more completely through the centuries and secured financial laws that allow banks to lend hugely more than they have on deposit in a confidence trick known as fractional reserve lending. Imagine if you could lend money that doesn't exist and charge the recipient interest for doing so. You would end up in jail. Bankers by contrast end up in mansions, private jets, Malibu and Monaco.

Banks are only required to keep a fraction of their deposits and wealth in their vaults and they are allowed to lend 'money' they don't have called 'credit. Go into a bank for a loan and if you succeed the banker will not move any real wealth into your account. They will type into your account the amount of the agreed 'loan' – say £100,000. This is not wealth that really exists; it is non-existent, fresh-air, created-out-of-nothing 'credit' which has never, does not, and will never exist except in theory. Credit is backed by nothing except wind and only has buying power because people think that it has buying power and accept it in return for property, goods and services. I have described this situation as like those cartoon characters you see chasing each other and when they run over the edge of a cliff they keep running forward on fresh air until one of them looks down, realises what's happened, and they all crash into the ravine. The whole foundation of the Sabbatian financial system is to stop people looking down except for periodic moments when they want to crash the system (as in 2008 and 2020 ongoing) and reap the rewards from all the property, businesses and wealth

their borrowers had signed over as ‘collateral’ in return for a ‘loan’ of fresh air. Most people think that money is somehow created by governments when it comes into existence from the start as a debt through banks ‘lending’ illusory money called credit. Yes, the very currency of exchange is a *debt* from day one issued as an interest-bearing loan. Why don’t governments create money interest-free and lend it to their people interest-free? Governments are controlled by Sabbatians and the financial system is controlled by Sabbatians for whom interest-free money would be a nightmare come true. Sabbatians underpin their financial domination through their global network of central banks, including the privately-owned US Federal Reserve and Britain’s Bank of England, and this is orchestrated by a privately-owned central bank coordination body called the Bank for International Settlements in Basle, Switzerland, created by the usual suspects including the Rockefellers and Rothschilds. Central bank chiefs don’t answer to governments or the people. They answer to the Bank for International Settlements or, in other words, the Global Cult which is dominated today by Sabbatians.

Built-in disaster

There are so many constituent scams within the overall banking scam. When you take out a loan of thin-air credit only the amount of that loan is theoretically brought into circulation to add to the amount in circulation; but you are paying back the principle plus interest. The additional interest is not created and this means that with every ‘loan’ there is a shortfall in the money in circulation between what is borrowed and what has to be paid back. There is never even close to enough money in circulation to repay all outstanding public and private debt including interest. Coldly weaved in the very fabric of the system is the certainty that some will lose their homes, businesses and possessions to the banking ‘lender’. This is less obvious in times of ‘boom’ when the amount of money in circulation (and the debt) is expanding through more people wanting and getting loans. When a downturn comes and the money supply contracts it becomes painfully obvious that there is not enough money to service all debt and interest. This is less obvious in times of ‘boom’ when the amount of money in circulation (and the debt) is expanding through more people wanting and getting loans. When a downturn comes and the money supply contracts and it becomes

painfully obvious – as in 2008 and currently – that there is not enough money to service all debt and interest. Sabbatian banksters have been leading the human population through a calculated series of booms (more debt incurred) and busts (when the debt can't be repaid and the banks get the debtor's tangible wealth in exchange for non-existent 'credit'). With each 'bust' Sabbatian bankers have absorbed more of the world's tangible wealth and we end up with the One-percent. Governments are in bankruptcy levels of debt to the same system and are therefore owned by a system they do not control. The Federal Reserve, 'America's central bank', is privately-owned and American presidents only nominally appoint its chairman or woman to maintain the illusion that it's an arm of government. It's not. The 'Fed' is a cartel of private banks which handed billions to its associates and friends after the crash of 2008 and has been Sabbatian-controlled since it was manipulated into being in 1913 through the covert trickery of Rothschild banking agents Jacob Schiff and Paul Warburg, and the Sabbatian Rockefeller family. Somehow from a Jewish population of two-percent and globally 0.2 percent (Sabbatian interlopers remember are far smaller) ultra-Zionists headed the Federal Reserve for 31 years between 1987 and 2018 in the form of Alan Greenspan, Bernard Bernanke and Janet Yellen (now Biden's Treasury Secretary) with Yellen's deputy chairman a Israeli-American duel citizen and ultra-Zionist Stanley Fischer, a former governor of the Bank of Israel. Ultra-Zionist Fed chiefs spanned the presidencies of Ronald Reagan ('Republican'), Father George Bush ('Republican'), Bill Clinton ('Democrat'), Boy George Bush ('Republican') and Barack Obama ('Democrat'). We should really add the pre-Greenspan chairman, Paul Adolph Volcker, 'appointed' by Jimmy Carter ('Democrat') who ran the Fed between 1979 and 1987 during the Carter and Reagan administrations before Greenspan took over. Volcker was a long-time associate and business partner of the Rothschilds. No matter what the 'party' officially in power the United States economy was directed by the same force. Here are members of the Obama, Trump and Biden administrations and see if you can make out a common theme.

Barack Obama ('Democrat')

Ultra-Zionists Robert Rubin, Larry Summers, and Timothy Geithner ran the US Treasury in the Clinton administration and two of them reappeared with

Obama. Ultra-Zionist Fed chairman Alan Greenspan had manipulated the crash of 2008 through deregulation and jumped ship just before the disaster to make way for ultra-Zionist Bernard Bernanke to hand out trillions to Sabbatian 'too big to fail' banks and businesses, including the ubiquitous ultra-Zionist Goldman Sachs which has an ongoing staff revolving door operation between itself and major financial positions in government worldwide. Obama inherited the fallout of the crash when he took office in January, 2009, and fortunately he had the support of his ultra-Zionist White House Chief of Staff Rahm Emmanuel, son of a terrorist who helped to bomb Israel into being in 1948, and his ultra-Zionist senior adviser David Axelrod, chief strategist in Obama's two successful presidential campaigns. Emmanuel, later mayor of Chicago and former senior fundraiser and strategist for Bill Clinton, is an example of the Sabbatian policy after Israel was established of migrating insider families to America so their children would be born American citizens. 'Obama' chose this financial team throughout his administration to respond to the Sabbatian-instigated crisis:

Timothy Geithner (ultra-Zionist) Treasury Secretary; Jacob J. Lew, Treasury Secretary; Larry Summers (ultra-Zionist), director of the White House National Economic Council; Paul Adolph Volcker (Rothschild business partner), chairman of the Economic Recovery Advisory Board; Peter Orszag (ultra-Zionist), director of the Office of Management and Budget overseeing all government spending; Penny Pritzker (ultra-Zionist), Commerce Secretary; Jared Bernstein (ultra-Zionist), chief economist and economic policy adviser to Vice President Joe Biden; Mary Schapiro (ultra-Zionist), chair of the Securities and Exchange Commission (SEC); Gary Gensler (ultra-Zionist), chairman of the Commodity Futures Trading Commission (CFTC); Sheila Bair (ultra-Zionist), chair of the Federal Deposit Insurance Corporation (FDIC); Karen Mills (ultra-Zionist), head of the Small Business Administration (SBA); Kenneth Feinberg (ultra-Zionist), Special Master for Executive [bail-out] Compensation. Feinberg would be appointed to oversee compensation (with strings) to 9/11 victims and families in a campaign to stop them having their day in court to question the official story. At the same time ultra-Zionist Bernard Bernanke was chairman of the Federal Reserve and these are only some of the ultra-Zionists with allegiance to Sabbatian-controlled Israel in the Obama government. Obama's biggest corporate donor was ultra-Zionist Goldman Sachs which had employed many in his administration.

Donald Trump (‘Republican’)

Trump claimed to be an outsider (he wasn’t) who had come to ‘drain the swamp’. He embarked on this goal by immediately appointing ultra-Zionist Steve Mnuchin, a Goldman Sachs employee for 17 years, as his Treasury Secretary. Others included Gary Cohn (ultra-Zionist), chief operating officer of Goldman Sachs, his first Director of the National Economic Council and chief economic adviser, who was later replaced by Larry Kudlow (ultra-Zionist). Trump’s senior adviser throughout his four years in the White House was his sinister son-in-law Jared Kushner, a life-long friend of Israel Prime Minister Benjamin Netanyahu. Kushner is the son of a convicted crook who was pardoned by Trump in his last days in office. Other ultra-Zionists in the Trump administration included: Stephen Miller, Senior Policy Adviser; Avrahm Berkowitz, Deputy Adviser to Trump and his Senior Adviser Jared Kushner; Ivanka Trump, Adviser to the President, who converted to Judaism when she married Jared Kushner; David Friedman, Trump lawyer and Ambassador to Israel; Jason Greenblatt, Trump Organization executive vice president and chief legal officer, who was made Special Representative for International Negotiations and the Israeli-Palestinian Conflict; Rod Rosenstein, Deputy Attorney General; Elliot Abrams, Special Representative for Venezuela, then Iran; John Eisenberg, National Security Council Legal Adviser and Deputy Council to the President for National Security Affairs; Anne Neuberger, Deputy National Manager, National Security Agency; Ezra Cohen-Watnick, Acting Under Secretary of Defense for Intelligence; Elan Carr, Special Envoy to monitor and combat anti-Semitism; Len Khodorkovsky, Deputy Special Envoy to monitor and combat anti-Semitism; Reed Cordish, Assistant to the President, Intragovernmental and Technology Initiatives. Trump Vice President Mike Pence and Secretary of State Mike Pompeo, both Christian Zionists, were also vehement supporters of Israel and its goals and ambitions.

Donald ‘free-speech believer’ Trump pardoned a number of financial and violent criminals while ignoring calls to pardon Julian Assange and Edward Snowden whose crimes are revealing highly relevant information about government manipulation and corruption and the widespread illegal surveillance of the American people by US ‘security’ agencies. It’s so good to know that Trump is on the side of freedom and justice and not mega-criminals with allegiance to Sabbatian-controlled Israel. These included a

pardon for Israeli spy Jonathan Pollard who was jailed for life in 1987 under the Espionage Act. Aviem Sella, the Mossad agent who recruited Pollard, was also pardoned by Trump while Assange sat in jail and Snowden remained in exile in Russia. Sella had 'fled' (was helped to escape) to Israel in 1987 and was never extradited despite being charged under the Espionage Act. A Trump White House statement said that Sella's clemency had been 'supported by Benjamin Netanyahu, Ron Dermer, Israel's US Ambassador, David Friedman, US Ambassador to Israel and Miriam Adelson, wife of leading Trump donor Sheldon Adelson who died shortly before. Other friends of Jared Kushner were pardoned along with Sholom Weiss who was believed to be serving the longest-ever white-collar prison sentence of more than 800 years in 2000. The sentence was commuted of Ponzi-schemer Eliyahu Weinstein who defrauded Jews and others out of $200 million. I did mention that Assange and Snowden were ignored, right? Trump gave Sabbatians almost everything they asked for in military and political support, moving the US Embassy from Tel Aviv to Jerusalem with its critical symbolic and literal implications for Palestinian statehood, and the 'deal of the Century' designed by Jared Kushner and David Friedman which gave the Sabbatian Israeli government the green light to substantially expand its already widespread program of building illegal Jewish-only settlements in the occupied land of the West Bank. This made a two-state 'solution' impossible by seizing all the land of a potential Palestinian homeland and that had been the plan since 1948 and then 1967 when the Arab-controlled Gaza Strip, West Bank, Sinai Peninsula and Syrian Golan Heights were occupied by Israel. All the talks about talks and road maps and delays have been buying time until the West Bank was physically occupied by Israeli real estate. Trump would have to be a monumentally ill-informed idiot not to see that this was the plan he was helping to complete. The Trump administration was in so many ways the Kushner administration which means the Netanyahu administration which means the Sabbatian administration. I understand why many opposing Cult fascism in all its forms gravitated to Trump, but he was a crucial part of the Sabbatian plan and I will deal with this in the next chapter.

Joe Biden ('Democrat')

A barely cognitive Joe Biden took over the presidency in January, 2021, along with his fellow empty shell, Vice-President Kamala Harris, as the latest Sabbatian gofers to enter the White House. Names on the door may have changed and the 'party' – the force behind them remained the same as Zionists were appointed to a stream of pivotal areas relating to Sabbatian plans and policy. They included: Janet Yellen, Treasury Secretary, former head of the Federal Reserve, and still another ultra-Zionist running the US Treasury after Mnuchin (Trump), Lew and Geithner (Obama), and Summers and Rubin (Clinton); Anthony Blinken, Secretary of State; Wendy Sherman, Deputy Secretary of State (so that's 'Biden's' Sabbatian foreign policy sorted); Jeff Zients, White House coronavirus coordinator; Rochelle Walensky, head of the Centers for Disease Control; Rachel Levine, transgender deputy health secretary (that's 'Covid' hoax policy under control); Merrick Garland, Attorney General; Alejandro Mayorkas, Secretary of Homeland Security; Cass Sunstein, Homeland Security with responsibility for new immigration laws; Avril Haines, Director of National Intelligence; Anne Neuberger, National Security Agency cybersecurity director (note, cybersecurity); David Cohen, CIA Deputy Director; Ronald Klain, Biden's Chief of Staff (see Rahm Emanuel); Eric Lander, a 'leading geneticist', Office of Science and Technology Policy director (see Smart Grid, synthetic biology agenda); Jessica Rosenworcel, acting head of the Federal Communications Commission (FCC) which controls Smart Grid technology policy and electromagnetic communication systems including 5G. How can it be that so many pivotal positions are held by two-percent of the American population and 0.2 percent of the world population administration after administration no matter who is the president and what is the party? It's a coincidence? Of course it's not and this is why Sabbatians have built their colossal global web of interlocking 'anti-hate' hate groups to condemn anyone who asks these glaring questions as an 'anti-Semite'. The way that Jewish people horrifically abused in Sabbatian-backed Nazi Germany are exploited to this end is stomach-turning and disgusting beyond words.

Political fusion

Sabbatian manipulation has reversed the roles of Republicans and Democrats and the same has happened in Britain with the Conservative and

Labour Parties. Republicans and Conservatives were always labelled the 'right' and Democrats and Labour the 'left', but look at the policy positions now and the Democrat-Labour 'left' has moved further to the 'right' than Republicans and Conservatives under the banner of 'Woke', the Cult-created far-right tyranny. Where once the Democrat-Labour 'left' defended free speech and human rights they now seek to delete them and as I said earlier despite the 'Covid' fascism of the Jackboot Johnson Conservative government in the UK the Labour Party of leader Keir Starmer demanded even more extreme measures. The Labour Party has been very publicly absorbed by Sabbatians after a political and media onslaught against the previous leader, the weak and inept Jeremy Corbyn, over made-up allegations of 'anti-Semitism' both by him and his party. The plan was clear with this 'anti-Semite' propaganda and what was required in response was a swift and decisive 'fuck off' from Corbyn and a statement to expose the Anti-Semitism Industry (Sabbatian) attempt to silence Labour criticism of the Israeli government (Sabbatians) and purge the party of all dissent against the extremes of ultra-Zionism (Sabbatians). Instead Corbyn and his party fell to their knees and appeased the abusers which, by definition, is impossible. Appeasing one demand leads only to a new demand to be appeased until takeover is complete. Like I say – 'fuck off' would have been a much more effective policy and I have used it myself with great effect over the years when Sabbatians are on my case which is most of the time. I consider that fact a great compliment, by the way. The outcome of the Labour Party capitulation is that we now have a Sabbatian-controlled Conservative Party 'opposed' by a Sabbatian-controlled Labour Party in a one-party Sabbatian state that hurtles towards the extremes of tyranny (the Sabbatian cult agenda). In America the situation is the same. Labour's Keir Starmer spends his days on his knees with his tongue out pointing to Tel Aviv, or I guess now Jerusalem, while Boris Johnson has an 'anti-Semitism czar' in the form of former Labour MP John Mann who keeps Starmer company on his prayer mat.

Sabbatian influence can be seen in Jewish members of the Labour Party who have been ejected for criticism of Israel including those from families that suffered in Nazi Germany. Sabbatians despise real Jewish people and target them even more harshly because it is so much more difficult to dub them 'anti-Semitic' although in their desperation they do try.

CHAPTER THREE

The Pushbacker sting

Until you realize how easy it is for your mind to be manipulated, you remain the puppet of someone else's game
Evita Ochel

I will use the presidencies of Trump and Biden to show how the manipulation of the one-party state plays out behind the illusion of political choice across the world. No two presidencies could – on the face of it – be more different and apparently at odds in terms of direction and policy.

A Renegade Mind sees beyond the obvious and focuses on outcomes and consequences and not image, words and waffle. The Cult embarked on a campaign to divide America between those who blindly support its agenda (the mentality known as 'Woke') and those who are pushing back on where the Cult and its Sabbatians want to go. This presents infinite possibilities for dividing and ruling the population by setting them at war with each other and allows a perceptual ring fence of demonisation to encircle the Pushbackers in a modern version of the Little Big Horn in 1876 when American cavalry led by Lieutenant Colonel George Custer were drawn into a trap, surrounded and killed by Native American tribes defending their land of thousands of years from being seized by the government. In this modern version the roles are reversed and it's those defending themselves from the Sabbatian government who are surrounded and the government that's seeking to destroy them. This trap was set years ago and to explain how we must return to 2016 and the emergence of Donald Trump as a candidate to be President of the United States. He set out to overcome the

best part of 20 other candidates in the Republican Party before and during the primaries and was not considered by many in those early stages to have a prayer of living in the White House. The Republican Party was said to have great reservations about Trump and yet somehow he won the nomination. When you know how American politics works – politics in general – there is no way that Trump could have become the party's candidate unless the Sabbatian-controlled 'Neocons' that run the Republican Party wanted that to happen. We saw the proof in emails and documents made public by WikiLeaks that the Democratic Party hierarchy, or Democons, systematically undermined the campaign of Bernie Sanders to make sure that Sabbatian gofer Hillary Clinton won the nomination to be their presidential candidate. If the Democons could do that then the Neocons in the Republican Party could have derailed Trump in the same way. But they didn't and at that stage I began to conclude that Trump could well be the one chosen to be president. If that was the case the 'why' was pretty clear to see – the goal of dividing America between Cult agenda-supporting Wokers and Pushbackers who gravitated to Trump because he was telling them what they wanted to hear. His constituency of support had been increasingly ignored and voiceless for decades and profoundly through the eight years of Sabbatian puppet Barack Obama. Now here was someone speaking their language of pulling back from the incessant globalisation of political and economic power, the exporting of American jobs to China and elsewhere by 'American' (Sabbatian) corporations, the deletion of free speech, and the mass immigration policies that had further devastated job opportunities for the urban working class of all races and the once American heartlands of the Midwest.

Beware the forked tongue

Those people collectively sighed with relief that at last a political leader was apparently on their side, but another trait of the Renegade Mind is that you look even harder at people telling you what you want to hear than those who are telling you otherwise. Obviously as I said earlier people wish what they want to hear to be true and genuine and they are much more likely to believe that than someone saying what they don't want to here and don't want to be true. Sales people are taught to be skilled in eliciting by calculated questioning what their customers want to hear and repeating that

back to them as their own opinion to get their targets to like and trust them. Assets of the Cult are also sales people in the sense of selling perception. To read Cult manipulation you have to play the long and expanded game and not fall for the Vaudeville show of party politics. Both American parties are vehicles for the Cult and they exploit them in different ways depending on what the agenda requires at that moment. Trump and the Republicans were used to be the focus of dividing America and isolating Pushbackers to open the way for a Biden presidency to become the most extreme in American history by advancing the full-blown Woke (Cult) agenda with the aim of destroying and silencing Pushbackers now labelled Nazi Trump supporters and white supremacists.

Sabbatians wanted Trump in office for the reasons described by ultra-Zionist Saul Alinsky (1909-1972) who was promoting the Woke philosophy through 'community organising' long before anyone had heard of it. In those days it still went by its traditional name of Marxism. The reason for the manipulated Trump phenomenon was laid out in Alinsky's 1971 book, *Rules for Radicals*, which was his blueprint for overthrowing democratic and other regimes and replacing them with Sabbatian Marxism. Not surprisingly his to-do list was evident in the Sabbatian French and Russian 'Revolutions' and that in China which will become very relevant in the next chapter about the 'Covid' hoax. Among Alinsky's followers have been the deeply corrupt Barack Obama, House Speaker Nancy Pelosi and Hillary Clinton who described him as a 'hero'. All three are Sabbatian stooges with Pelosi personifying the arrogant corrupt idiocy that so widely fronts up for the Cult inner core. Predictably as a Sabbatian advocate of the 'light-bringer' Alinsky features Lucifer on the dedication page of his book as the original radical who gained his own kingdom ('Earth' as we shall see). One of Alinsky's golden radical rules was to pick an individual and focus all attention, hatred and blame on them and not to target faceless bureaucracies and corporations. *Rules for Radicals* is really a Sabbatian handbook with its contents repeatedly employed all over the world for centuries and why wouldn't Sabbatians bring to power their designer-villain to be used as the individual on which all attention, hatred and blame was bestowed? This is what they did and the only question for me is how much Trump knew that and how much he was manipulated. A bit of both, I suspect. This was Alinsky's Trump technique from a man who died in 1972. The technique has spanned history:

Pick the target, freeze it, personalize it, polarize it. Don't try to attack abstract corporations or bureaucracies. Identify a responsible individual. Ignore attempts to shift or spread the blame.

From the moment Trump came to illusory power everything was about him. It wasn't about Republican policy or opinion, but all about Trump. Everything he did was presented in negative, derogatory and abusive terms by the Sabbatian-dominated media led by Cult operations such as CNN, MSNBC, *The New York Times* and the Jeff Bezos-owned *Washington Post* – 'Pick the target, freeze it, personalize it, polarize it.' Trump was turned into a demon to be vilified by those who hated him and a demi-god loved by those who worshipped him. This, in turn, had his supporters, too, presented as equally demonic in preparation for the punchline later down the line when Biden was about to take office. It was here's a Trump, there's a Trump, everywhere a Trump, Trump. Virtually every news story or happening was filtered through the lens of 'The Donald'. You loved him or hated him and which one you chose was said to define you as Satan's spawn or a paragon of virtue. Even supporting some Trump policies or statements and not others was enough for an assault on your character. No shades of grey were or are allowed. Everything is black and white (literally and figuratively). A Californian I knew had her head utterly scrambled by her hatred for Trump while telling people they should love each other. She was so totally consumed by Trump Derangement Syndrome as it became to be known that this glaring contradiction would never have occurred to her. By definition anyone who criticised Trump or praised his opponents was a hero and this lady described Joe Biden as 'a kind, honest gentleman' when he's a provable liar, mega-crook and vicious piece of work to boot. Sabbatians had indeed divided America using Trump as the fall-guy and all along the clock was ticking on the consequences for his supporters.

In hock to his masters

Trump gave Sabbatians via Israel almost everything they wanted in his four years. Ask and you shall receive was the dynamic between himself and Benjamin Netanyahu orchestrated by Trump's ultra-Zionist son-in-law Jared Kushner, his ultra-Zionist Ambassador to Israel, David Friedman, and ultra-Zionist 'Israel adviser', Jason Greenblatt. The last two were central to the running and protecting from collapse of his business empire, the Trump

Organisation, and colossal business failures made him forever beholding to Sabbatian networks that bailed him out. By the start of the 1990s Trump owed $4 billion to banks that he couldn't pay and almost $1billion of that was down to him personally and not his companies. This mega-disaster was the result of building two new casinos in Atlantic City and buying the enormous Taj Mahal operation which led to crippling debt payments. He had borrowed fantastic sums from 72 banks with major Sabbatian connections and although the scale of debt should have had him living in a tent alongside the highway they never foreclosed. A plan was devised to lift Trump from the mire by BT Securities Corporation and Rothschild Inc. and the case was handled by Wilber Ross who had worked for the Rothschilds for 27 years. Ross would be named US Commerce Secretary after Trump's election. Another crucial figure in saving Trump was ultra-Zionist 'investor' Carl Icahn who bought the Taj Mahal casino. Icahn was made special economic adviser on financial regulation in the Trump administration. He didn't stay long but still managed to find time to make a tidy sum of a reported $31.3 million when he sold his holdings affected by the price of steel three days before Trump imposed a 235 percent tariff on steel imports. What amazing bits of luck these people have. Trump and Sabbatian operatives have long had a close association and his mentor and legal adviser from the early 1970s until 1986 was the dark and genetically corrupt ultra-Zionist Roy Cohn who was chief counsel to Senator Joseph McCarthy's 'communist' witch-hunt in the 1950s. *Esquire* magazine published an article about Cohn with the headline 'Don't mess with Roy Cohn'. He was described as the most feared lawyer in New York and 'a ruthless master of dirty tricks ... [with] ... more than one Mafia Don on speed dial'. Cohn's influence, contacts, support and protection made Trump a front man for Sabbatians in New York with their connections to one of Cohn's many criminal employers, the 'Russian' Sabbatian Mafia. Israel-centric media mogul Rupert Murdoch was introduced to Trump by Cohn and they started a long friendship. Cohn died in 1986 weeks after being disbarred for unethical conduct by the Appellate Division of the New York State Supreme Court. The wheels of justice do indeed run slow given the length of Cohn's crooked career.

QAnon-sense

We are asked to believe that Donald Trump with his fundamental connections to Sabbatian networks and operatives has been leading the fight to stop the Sabbatian agenda for the fascistic control of America and the world. Sure he has. A man entrapped during his years in the White House by Sabbatian operatives and whose biggest financial donor was casino billionaire Sheldon Adelson who was Sabbatian to his DNA?? Oh, do come on. Trump has been used to divide America and isolate Pushbackers on the Cult agenda under the heading of 'Trump supporters', 'insurrectionists' and 'white supremacists'. The US Intelligence/Mossad Psyop or psychological operation known as QAnon emerged during the Trump years as a central pillar in the Sabbatian campaign to lead Pushbackers into the trap set by those that wished to destroy them. I knew from the start that QAnon was a scam because I had seen the same scenario many times before over 30 years under different names and I had written about one in particular in the books. 'Not again' was my reaction when QAnon came to the fore. The same script is pulled out every few years and a new name added to the letterhead. The story always takes the same form: 'Insiders' or 'the good guys' in the government-intelligence-military 'Deep State' apparatus were going to instigate mass arrests of the 'bad guys' which would include the Rockefellers, Rothschilds, Barack Obama, Hillary Clinton, George Soros, etc., etc. Dates are given for when the 'good guys' are going to move in, but the dates pass without incident and new dates are given which pass without incident. The central message to Pushbackers in each case is that they don't have to do anything because there is 'a plan' and it is all going to be sorted by the 'good guys' on the inside. 'Trust the plan' was a QAnon mantra when the only plan was to misdirect Pushbackers into putting their trust in a Psyop they believed to be real. Beware, beware, those who tell you what you want to hear and always check it out. Right up to Biden's inauguration QAnon was still claiming that 'the Storm' was coming and Trump would stay on as president when Biden and his cronies were arrested and jailed. It was never going to happen and of course it didn't, but what did happen as a result provided that punchline to the Sabbatian Trump/QAnon Psyop.

On January 6th, 2021, a very big crowd of Trump supporters gathered in the National Mall in Washington DC down from the Capitol Building to protest at what they believed to be widespread corruption and vote fraud that stopped Trump being re-elected for a second term as president in November, 2020. I say as someone that does not support Trump or Biden

that the evidence is clear that major vote-fixing went on to favour Biden, a man with cognitive problems so advanced he can often hardly string a sentence together without reading the words written for him on the Teleprompter. Glaring ballot discrepancies included serious questions about electronic voting machines that make vote rigging a comparative cinch and hundreds of thousands of paper votes that suddenly appeared during already advanced vote counts and virtually all of them for Biden. Early Trump leads in crucial swing states suddenly began to close and disappear. The pandemic hoax was used as the excuse to issue almost limitless numbers of mail-in ballots with no checks to establish that the recipients were still alive or lived at that address. They were sent to streams of people who had not even asked for them. Private organisations were employed to gather these ballots and who knows what they did with them before they turned up at the counts. The American election system has been manipulated over decades to become a sick joke with more holes than a Swiss cheese for the express purpose of dictating the results. Then there was the criminal manipulation of information by Sabbatian tech giants like Facebook, Twitter and Google-owned YouTube which deleted pro-Trump, anti-Biden accounts and posts while everything in support of Biden was left alone. Sabbatians wanted Biden to win because after the dividing of America it was time for full-on Woke and every aspect of the Cult agenda to be unleashed.

Hunter gatherer

Extreme Silicon Valley bias included blocking information by the *New York Post* exposing a Biden scandal that should have ended his bid for president in the final weeks of the campaign. Hunter Biden, his monumentally corrupt son, is reported to have sent a laptop to be repaired at a local store and failed to return for it. Time passed until the laptop became the property of the store for non-payment of the bill. When the owner saw what was on the hard drive he gave a copy to the FBI who did nothing even though it confirmed widespread corruption in which the Joe Biden family were using his political position, especially when he was vice president to Obama, to make multiple millions in countries around the world and most notably Ukraine and China. Hunter Biden's one-time business partner Tony Bobulinski went public when the story broke in the *New York Post* to confirm the corruption he saw and that Joe Biden not only knew what was

going on he also profited from the spoils. Millions were handed over by a Chinese company with close connections – like all major businesses in China – to the Chinese communist party of President Xi Jinping. Joe Biden even boasted at a meeting of the Cult's World Economic Forum that as vice president he had ordered the government of Ukraine to fire a prosecutor. What he didn't mention was that the same man just happened to be investigating an energy company which was part of Hunter Biden's corrupt portfolio. The company was paying him big bucks for no other reason than the influence his father had. Overnight Biden's presidential campaign should have been over given that he had lied publicly about not knowing what his son was doing. Instead almost the entire Sabbatian-owned mainstream media and Sabbatian-owned Silicon Valley suppressed circulation of the story. This alone went a mighty way to rigging the election of 2020. Cult assets like Mark Zuckerberg at Facebook also spent hundreds of millions to be used in support of Biden and vote 'administration'.

The Cult had used Trump as the focus to divide America and was now desperate to bring in moronic, pliable, corrupt Biden to complete the double-whammy. No way were they going to let little things like the will of the people thwart their plan. Silicon Valley widely censored claims that the election was rigged because it *was* rigged. For the same reason anyone claiming it was rigged was denounced as a 'white supremacist' including the pathetically few Republican politicians willing to say so. Right across the media where the claim was mentioned it was described as a 'false claim' even though these excuses for 'journalists' would have done no research into the subject whatsoever. Trump won seven million more votes than any sitting president had ever achieved while somehow a cognitively-challenged soon to be 78-year-old who was hidden away from the public for most of the campaign managed to win more votes than any presidential candidate in history. It makes no sense. You only had to see election rallies for both candidates to witness the enthusiasm for Trump and the apathy for Biden. Tens of thousands would attend Trump events while Biden was speaking in empty car parks with often only television crews attending and framing their shots to hide the fact that no one was there. It was pathetic to see footage come to light of Biden standing at a podium making speeches only to TV crews and party fixers while reading the words written for him on massive Teleprompter screens. So, yes, those protestors on January 6th

had a point about election rigging, but some were about to walk into a trap laid for them in Washington by the Cult Deep State and its QAnon Psyop. This was the Capitol Hill riot ludicrously dubbed an ‘insurrection’.

The spider and the fly

Renegade Minds know there are not two ‘sides’ in politics, only one side, the Cult, working through all ‘sides’. It’s a stage show, a puppet show, to direct the perceptions of the population into focusing on diversions like parties and candidates while missing the puppeteers with their hands holding all the strings. The Capitol Hill ‘insurrection’ brings us back to the Little Big Horn. Having created two distinct opposing groupings – Woke and Pushbackers – the trap was about to be sprung. Pushbackers were to be encircled and isolated by associating them all in the public mind with Trump and then labelling Trump as some sort of Confederate leader. I knew immediately that the Capitol riot was a set-up because of two things. One was how easy the rioters got into the building with virtually no credible resistance and secondly I could see – as with the ‘Covid’ hoax in the West at the start of 2020 – how the Cult could exploit the situation to move its agenda forward with great speed. My experience of Cult techniques and activities over more than 30 years has showed me that while they do exploit situations they haven’t themselves created this never happens with events of fundamental agenda significance. Every time major events giving cultists the excuse to rapidly advance their plan you find they are manipulated into being for the specific reason of providing that excuse – Problem-Reaction-Solution. Only a tiny minority of the huge crowd of Washington protestors sought to gain entry to the Capitol by smashing windows and breaching doors. That didn’t matter. The whole crowd and all Pushbackers, even if they did not support Trump, were going to be lumped together as dangerous insurrectionists and conspiracy theorists. The latter term came into widespread use through a CIA memo in the 1960s aimed at discrediting those questioning the nonsensical official story of the Kennedy assassination and it subsequently became widely employed by the media. It’s still being used by inept ‘journalists’ with no idea of its origin to discredit anyone questioning anything that authority claims to be true. When you are perpetrating a conspiracy you need to discredit the very word itself even though the dictionary definition of conspiracy is merely ‘the

activity of secretly planning with other people to do something bad or illegal‘ and ‘a general agreement to keep silent about a subject for the purpose of keeping it secret’. On that basis there are conspiracies almost wherever you look. For obvious reasons the Cult and its lapdog media have to claim there are no conspiracies even though the word appears in state laws as with conspiracy to defraud, to murder, and to corrupt public morals.

Agent provocateurs are widely used by the Cult Deep State to manipulate genuine people into acting in ways that suit the desired outcome. By genuine in this case I mean protestors genuinely supporting Trump and claims that the election was stolen. In among them, however, were agents of the state wearing the garb of Trump supporters and QAnon to pump-prime the Capital riot which some genuine Trump supporters naively fell for. I described the situation as ‘Come into my parlour said the spider to the fly’. Leaflets appeared through the Woke paramilitary arm Antifa, the anti-fascist fascists, calling on supporters to turn up in Washington looking like Trump supporters even though they hated him. Some of those arrested for breaching the Capitol Building were sourced to Antifa and its stable mate Black Lives Matter. Both organisations are funded by Cult billionaires and corporations. One man charged for the riot was according to his lawyer a former FBI agent who had held top secret security clearance for 40 years. Attorney Thomas Plofchan said of his client, 66-year-old Thomas Edward Caldwell:

> He has held a Top Secret Security Clearance since 1979 and has undergone multiple Special Background Investigations in support of his clearances. After retiring from the Navy, he worked as a section chief for the Federal Bureau of Investigation from 2009-2010 as a GS-12 [mid-level employee].
>
> He also formed and operated a consulting firm performing work, often classified, for U.S government customers including the US. Drug Enforcement Agency, Department of Housing and Urban Development, the US Coast Guard, and the US Army Personnel Command.

A judge later released Caldwell pending trial in the absence of evidence about a conspiracy or that he tried to force his way into the building. *The New York Post* reported a ‘law enforcement source‘ as saying that ‘at least two known Antifa members were spotted’ on camera among Trump supporters during the riot while one of the rioters arrested was John Earle Sullivan, a seriously extreme Black Lives Matter Trump-hater from Utah

who was previously arrested and charged in July, 2020, over a BLM-Antifa riot in which drivers were threatened and one was shot. Sullivan is the founder of Utah-based Insurgence USA which is an affiliate of the Cult-created-and-funded Black Lives Matter movement. Footage appeared and was then deleted by Twitter of Trump supporters calling out Antifa infiltrators and a group was filmed changing into pro-Trump clothing before the riot. Security at the building was *pathetic* – as planned. Colonel Leroy Fletcher Prouty, a man with long experience in covert operations working with the US security apparatus, once described the tell-tale sign to identify who is involved in an assassination. He said:

> No one has to direct an assassination – it happens. The active role is played secretly by permitting it to happen. This is the greatest single clue. Who has the power to call off or reduce the usual security precautions?

This principle applies to many other situations and certainly to the Capitol riot of January 6th, 2021.

The sting

With such a big and potentially angry crowd known to be gathering near the Capitol the security apparatus would have had a major police detail to defend the building with National Guard troops on standby given the strength of feeling among people arriving from all over America encouraged by the QAnon Psyop and statements by Donald Trump. Instead Capitol Police 'security' was flimsy, weak, and easily breached. The same number of officers was deployed as on a regular day and that is a blatant red flag. They were not staffed or equipped for a possible riot that had been an obvious possibility in the circumstances. No protective and effective fencing worth the name was put in place and there were no contingency plans. The whole thing was basically a case of standing aside and waving people in. Once inside police mostly backed off apart from one Capitol police officer who ridiculously shot dead unarmed Air Force veteran protestor Ashli Babbitt without a warning as she climbed through a broken window. The 'investigation' refused to name or charge the officer after what must surely be considered a murder in the circumstances. They just

lifted a carpet and swept. The story was endlessly repeated about five people dying in the 'armed insurrection' when there was no report of rioters using weapons. Apart from Babbitt the other four died from a heart attack, strokes and apparently a drug overdose. Capitol police officer Brian Sicknick was reported to have died after being bludgeoned with a fire extinguisher when he was alive after the riot was over and died later of what the Washington Medical Examiner's Office said was a stroke. Sicknick had no external injuries. The lies were delivered like rapid fire. There was a narrative to build with incessant repetition of the lie until the lie became the accepted 'everybody knows that' truth. The 'Big Lie' technique of Nazi Propaganda Minister Joseph Goebbels is constantly used by the Cult which was behind the Nazis and is today behind the 'Covid' and 'climate change' hoaxes. Goebbels said:

If you tell a lie big enough and keep repeating it, people will eventually come to believe it. The lie can be maintained only for such time as the State can shield the people from the political, economic and/or military consequences of the lie. It thus becomes vitally important for the State to use all of its powers to repress dissent, for the truth is the mortal enemy of the lie, and thus by extension, the truth is the greatest enemy of the State.

Most protestors had a free run of the Capitol Building. This allowed pictures to be taken of rioters in iconic parts of the building including the Senate chamber which could be used as propaganda images against all Pushbackers. One Congresswoman described the scene as 'the worst kind of non-security anybody could ever imagine'. Well, the first part was true, but someone obviously did imagine it and made sure it happened. Some photographs most widely circulated featured people wearing QAnon symbols and now the Psyop would be used to dub all QAnon followers with the ubiquitous fit-all label of 'white supremacist' and 'insurrectionists'. When a Muslim extremist called Noah Green drove his car at two police officers at the Capitol Building killing one in April, 2021, there was no such political and media hysteria. They were just disappointed he wasn't white.

The witch-hunt

Government prosecutor Michael Sherwin, an aggressive, dark-eyed, professional Rottweiler led the 'investigation' and to call it over the top

would be to understate reality a thousand fold. Hundreds were tracked down and arrested for the crime of having the wrong political views and people were jailed who had done nothing more than walk in the building, committed no violence or damage to property, took a few pictures and left. They were labelled a 'threat to the Republic' while Biden sat in the White House signing executive orders written for him that were dismantling 'the Republic'. Even when judges ruled that a mother and son should not be in jail the government kept them there. Some of those arrested have been badly beaten by prison guards in Washington and lawyers for one man said he suffered a fractured skull and was made blind in one eye. Meanwhile a woman is shot dead for no reason by a Capitol Police officer and we are not allowed to know who he is never mind what has happened to him although that will be *nothing*. The Cult's QAnon/Trump sting to identify and isolate Pushbackers and then target them on the road to crushing and deleting them was a resounding success. You would have thought the Russians had invaded the building at gunpoint and lined up senators for a firing squad to see the political and media reaction. Congresswoman Alexandria Ocasio-Cortez is a child in a woman's body, a terrible-twos, me, me, me, Woker narcissist of such proportions that words have no meaning. She said she thought she was going to die when 'insurrectionists' banged on her office door. It turned out she wasn't even in the Capitol Building when the riot was happening and the 'banging' was a Capitol Police officer. She referred to herself as a 'survivor' which is an insult to all those true survivors of violent and sexual abuse while she lives her pampered and privileged life talking drivel for a living. Her Woke colleague and fellow mega-narcissist Rashida Tlaib broke down describing the devastating effect on her, too, of *not being* in the building when the rioters were there. Ocasio-Cortez and Tlaib are members of a fully-Woke group of Congresswomen known as 'The Squad' along with Ilhan Omar and Ayanna Pressley. The Squad from what I can see can be identified by its vehement anti-white racism, anti-white men agenda, and, as always in these cases, the absence of brain cells on active duty.

The usual suspects were on the riot case immediately in the form of Democrat ultra-Zionist senators and operatives Chuck Schumer and Adam Schiff demanding that Trump be impeached for 'his part in the insurrection'. The same pair of prats had led the failed impeachment of Trump over the invented 'Russia collusion' nonsense which claimed Russia

had helped Trump win the 2016 election. I didn't realise that Tel Aviv had been relocated just outside Moscow. I must find an up-to-date map. The Russia hoax was a Sabbatian operation to keep Trump occupied and impotent and to stop any rapport with Russia which the Cult wants to retain as a perceptual enemy to be pulled out at will. Puppet Biden began attacking Russia when he came to office as the Cult seeks more upheaval, division and war across the world. A two-year stage show 'Russia collusion inquiry' headed by the not-very-bright former 9/11 FBI chief Robert Mueller, with support from 19 lawyers, 40 FBI agents plus intelligence analysts, forensic accountants and other staff, devoured tens of millions of dollars and found no evidence of Russia collusion which a ten-year-old could have told them on day one. Now the same moronic Schumer and Schiff wanted a second impeachment of Trump over the Capitol 'insurrection' (riot) which the arrested development of Schumer called another 'Pearl Harbor' while others compared it with 9/11 in which 3,000 died and, in the case of CNN, with the Rwandan genocide in the 1990s in which an estimated 500,000 to 600,000 were murdered, between 250, 000 and 500,000 women were raped, and populations of whole towns were hacked to death with machetes. To make those comparisons purely for Cult political reasons is beyond insulting to those that suffered and lost their lives and confirms yet again the callous inhumanity that we are dealing with. Schumer is a monumental idiot and so is Schiff, but they serve the Cult agenda and do whatever they're told so they get looked after. Talking of idiots – another inane man who spanned the Russia and Capitol impeachment attempts was Senator Eric Swalwell who had the nerve to accuse Trump of collusion with the Russians while sleeping with a Chinese spy called Christine Fang or 'Fang Fang' which is straight out of a Bond film no doubt starring Klaus Schwab as the bloke living on a secret island and controlling laser weapons positioned in space and pointing at world capitals. Fang Fang plays the part of Bond's infiltrator girlfriend which I'm sure she would enjoy rather more than sharing a bed with the brainless Swalwell, lying back and thinking of China. The FBI eventually warned Swalwell about Fang Fang which gave her time to escape back to the Chinese dictatorship. How very thoughtful of them. The second Trump impeachment also failed and hardly surprising when an impeachment is supposed to remove a sitting president and by the time it happened Trump

was no longer president. These people are running your country America, well, officially anyway. Terrifying isn't it?

Outcomes tell the story - always

The outcome of all this – and it's the *outcome* on which Renegade Minds focus, not the words – was that a vicious, hysterical and obviously pre-planned assault was launched on Pushbackers to censor, silence and discredit them and even targeted their right to earn a living. They have since been condemned as 'domestic terrorists' that need to be treated like Al-Qaeda and Islamic State. 'Domestic terrorists' is a label the Cult has been trying to make stick since the period of the Oklahoma bombing in 1995 which was blamed on 'far-right domestic terrorists'. If you read *The Trigger* you will see that the bombing was clearly a Problem-Reaction-Solution carried out by the Deep State during a Bill Clinton administration so corrupt that no dictionary definition of the term would even nearly suffice. Nearly 30, 000 troops were deployed from all over America to the empty streets of Washington for Biden's inauguration. Ten thousand of them stayed on with the pretext of protecting the capital from insurrectionists when it was more psychological programming to normalise the use of the military in domestic law enforcement in support of the Cult plan for a police-military state. Biden's fascist administration began a purge of 'wrong-thinkers' in the military which means anyone that is not on board with Woke. The Capitol Building was surrounded by a fence with razor wire and the Land of the Free was further symbolically and literally dismantled. The circle was completed with the installation of Biden and the exploitation of the QAnon Psyop.

America had never been so divided since the civil war of the 19th century, Pushbackers were isolated and dubbed terrorists and now, as was always going to happen, the Cult immediately set about deleting what little was left of freedom and transforming American society through a swish of the hand of the most controlled 'president' in American history leading (officially at least) the most extreme regime since the country was declared an independent state on July 4th, 1776. Biden issued undebated, dictatorial executive orders almost by the hour in his opening days in office across the whole spectrum of the Cult wish-list including diluting controls on the border with Mexico allowing thousands of migrants to illegally enter the

United States to transform the demographics of America and import an election-changing number of perceived Democrat voters. Then there were Biden deportation amnesties for the already illegally resident (estimated to be as high as 20 or even 30 million). A bill before Congress awarded American citizenship to anyone who could prove they had worked in agriculture for just 180 days in the previous two years as 'Big Ag' secured its slave labour long-term. There were the plans to add new states to the union such as Puerto Rico and making Washington DC a state. They are all parts of a plan to ensure that the Cult-owned Woke Democrats would be permanently in power.

Border – what border?

I have exposed in detail in other books how mass immigration into the United States and Europe is the work of Cult networks fuelled by the tens of billions spent to this and other ends by George Soros and his global Open Society (open borders) Foundations. The impact can be seen in America alone where the population has increased by *100 million* in little more than 30 years mostly through immigration. I wrote in *The Answer* that the plan was to have so many people crossing the southern border that the numbers become unstoppable and we are now there under Cult-owned Biden. El Salvador in Central America puts the scale of what is happening into context. A third of the population now lives in the United States, much of it illegally, and many more are on the way. The methodology is to crush Central and South American countries economically and spread violence through machete-wielding psychopathic gangs like MS-13 based in El Salvador and now operating in many American cities. Biden-imposed lax security at the southern border means that it is all but open. He said before his 'election' that he wanted to see a surge towards the border if he became president and that was the green light for people to do just that after election day to create the human disaster that followed for both America and the migrants. When that surge came the imbecilic Alexandria Ocasio-Cortez said it wasn't a 'surge' because they are 'children, not insurgents' and the term 'surge' (used by Biden) was a claim of 'white supremacists'. This disingenuous lady may one day enter the realm of the most basic intelligence, but it won't be any time soon.

Sabbatians and the Cult are in the process of destroying America by importing violent people and gangs in among the genuine to terrorise American cities and by overwhelming services that cannot cope with the sheer volume of new arrivals. Something similar is happening in Europe as Western society in general is targeted for demographic and cultural transformation and upheaval. The plan demands violence and crime to create an environment of intimidation, fear and division and Soros has been funding the election of district attorneys across America who then stop prosecuting many crimes, reduce sentences for violent crimes and free as many violent criminals as they can. Sabbatians are creating the chaos from which order – their order – can respond in a classic Problem-Reaction-Solution. A Freemasonic moto says 'Ordo Ab Chao' (Order out of Chaos) and this is why the Cult is constantly creating chaos to impose a new 'order'. Here you have the reason the Cult is constantly creating chaos. The 'Covid' hoax can be seen with those entering the United States by plane being forced to take a 'Covid' test while migrants flooding through southern border processing facilities do not. Nothing is put in the way of mass migration and if that means ignoring the government's own 'Covid' rules then so be it. They know it's all bullshit anyway. Any pushback on this is denounced as 'racist' by Wokers and Sabbatian fronts like the ultra-Zionist Anti-Defamation League headed by the appalling Jonathan Greenblatt which at the same time argues that Israel should not give citizenship and voting rights to more Palestinian Arabs or the 'Jewish population' (in truth the Sabbatian network) will lose control of the country.

Society-changing numbers

Biden's masters have declared that countries like El Salvador are so dangerous that their people must be allowed into the United States for humanitarian reasons when there are fewer murders in large parts of many Central American countries than in US cities like Baltimore. That is not to say Central America cannot be a dangerous place and Cult-controlled American governments have been making it so since way back, along with the dismantling of economies, in a long-term plan to drive people north into the United States. Parts of Central America are very dangerous, but in other areas the story is being greatly exaggerated to justify relaxing immigration criteria. Migrants are being offered free healthcare and education in the

United States as another incentive to head for the border and there is no requirement to be financially independent before you can enter to prevent the resources of America being drained. You can't blame migrants for seeking what they believe will be a better life, but they are being played by the Cult for dark and nefarious ends. The numbers since Biden took office are huge. In February, 2021, more than 100,000 people were known to have tried to enter the US illegally through the southern border (it was 34,000 in the same month in 2020) and in March it was 170,000 – a 418 percent increase on March, 2020. These numbers are only known people, not the ones who get in unseen. The true figure for migrants illegally crossing the border in a single month was estimated by one congressman at 250,000 and that number will only rise under Biden's current policy. Gangs of murdering drug-running thugs that control the Mexican side of the border demand money – thousands of dollars – to let migrants cross the Rio Grande into America. At the same time gun battles are breaking out on the border several times a week between rival Mexican drug gangs (which now operate globally) who are equipped with sophisticated military-grade weapons, grenades and armoured vehicles. While the Capitol Building was being 'protected' from a non-existent 'threat' by thousands of troops, and others were still deployed at the time in the Cult Neocon war in Afghanistan, the southern border of America was left to its fate. This is not incompetence, it is cold calculation.

By March, 2021, there were 17,000 unaccompanied children held at border facilities and many of them are ensnared by people traffickers for paedophile rings and raped on their journey north to America. This is not conjecture – this is fact. Many of those designated children are in reality teenage boys or older. Meanwhile Wokers posture their self-purity for encouraging poor and tragic people to come to America and face this nightmare both on the journey and at the border with the disgusting figure of House Speaker Nancy Pelosi giving disingenuous speeches about caring for migrants. The woman's evil. Wokers condemned Trump for having children in cages at the border (so did Obama, *Shhhh*), but now they are sleeping on the floor without access to a shower with one border facility 729 percent over capacity. The Biden insanity even proposed flying migrants from the southern border to the northern border with Canada for 'processing'. The whole shambles is being overseen by ultra-Zionist Secretary of Homeland Security, the moronic liar Alejandro Mayorkas, who

banned news cameras at border facilities to stop Americans seeing what was happening. Mayorkas said there was not a ban on news crews; it was just that they were not allowed to film. Alongside him at Homeland Security is another ultra-Zionist Cass Sunstein appointed by Biden to oversee new immigration laws. Sunstein despises conspiracy researchers to the point where he suggests they should be banned or *taxed* for having such views. The man is not bonkers or anything. He's perfectly well-adjusted, but adjusted to what is the question. Criticise what is happening and you are a 'white supremacist' when earlier non-white immigrants also oppose the numbers which effect their lives and opportunities. Black people in poor areas are particularly damaged by uncontrolled immigration and the increased competition for work opportunities with those who will work for less. They are also losing voting power as Hispanics become more dominant in former black areas. It's a downward spiral for them while the billionaires behind the policy drone on about how much they care about black people and 'racism'. None of this is about compassion for migrants or black people – that's just wind and air. Migrants are instead being mercilessly exploited to transform America while the countries they leave are losing their future and the same is true in Europe. Mass immigration may now be the work of Woke Democrats, but it can be traced back to the 1986 Immigration Reform and Control Act (it wasn't) signed into law by Republican hero President Ronald Reagan which gave amnesty to millions living in the United States illegally and other incentives for people to head for the southern border. Here we have the one-party state at work again.

Save me syndrome

Almost every aspect of what I have been exposing as the Cult agenda was on display in even the first days of 'Biden' with silencing of Pushbackers at the forefront of everything. A Renegade Mind will view the Trump years and QAnon in a very different light to their supporters and advocates as the dots are connected. The QAnon/Trump Psyop has given the Cult all it was looking for. We may not know how much, or little, that Trump realised he was being used, but that's a side issue. This pincer movement produced the desired outcome of dividing America and having Pushbackers isolated. To turn this around we have to look at new routes to empowerment which do not include handing our power to other people and groups through what I

will call the ‘Save Me Syndrome’ – ‘I want someone else to do it so that I don’t have to’. We have seen this at work throughout human history and the QAnon/Trump Psyop is only the latest incarnation alongside all the others. Religion is an obvious expression of this when people look to a ‘god’ or priest to save them or tell them how to be saved and then there are ‘save me’ politicians like Trump. Politics is a diversion and not a ‘saviour’. It is a means to block positive change, not make it possible.

Save Me Syndrome always comes with the same repeating theme of handing your power to whom or what you believe will save you while your real ‘saviour’ stares back from the mirror every morning. Renegade Minds are constantly vigilant in this regard and always asking the question ‘What can I do?’ rather than ‘What can someone else do for me?’ Gandhi was right when he said: ‘You must be the change you want to see in the world.’ We are indeed the people we have been waiting for. We are presented with a constant raft of reasons to concede that power to others and forget where the real power is. Humanity has the numbers and the Cult does not. It has to use diversion and division to target the unstoppable power that comes from unity. Religions, governments, politicians, corporations, media, QAnon, are all different manifestations of this power-diversion and dilution. Refusing to give your power to governments and instead handing it to Trump and QAnon is not to take a new direction, but merely to recycle the old one with new names on the posters. I will explore this phenomenon as we proceed and how to break the cycles and recycles that got us here through the mists of repeating perception and so repeating history.

For now we shall turn to the most potent example in the entire human story of the consequences that follow when you give your power away. I am talking, of course, of the ‘Covid’ hoax.

CHAPTER FOUR

‘Covid’: Calculated catastrophe

Facts are threatening to those invested in fraud
DaShanne Stokes

We can easily unravel the real reason for the ‘Covid pandemic’ hoax by employing the Renegade Mind methodology that I have outlined this far. We’ll start by comparing the long-planned Cult outcome with the ‘Covid pandemic’ outcome. Know the outcome and you’ll see the journey.

I have highlighted the plan for the Hunger Games Society which has been in my books for so many years with the very few controlling the very many through ongoing dependency. To create this dependency it is essential to destroy independent livelihoods, businesses and employment to make the population reliant on the state (the Cult) for even the basics of life through a guaranteed pittance income. While independence of income remained these Cult ambitions would be thwarted. With this knowledge it was easy to see where the ‘pandemic’ hoax was going once talk of ‘lockdowns’ began and the closing of all but perceived ‘essential’ businesses to ‘save’ us from an alleged ‘deadly virus’. Cult corporations like Amazon and Walmart were naturally considered ‘essential’ while mom and pop shops and stores had their doors closed by fascist decree. As a result with every new lockdown and new regulation more small and medium, even large businesses not owned by the Cult, went to the wall while Cult giants and their frontmen and women grew financially fatter by the second. Mom and pop were denied an income and the right to earn a living and the wealth of people like Jeff Bezos (Amazon), Mark Zuckerberg (Facebook) and Sergei Brin and

Larry Page (Google/Alphabet) have reached record levels. The Cult was increasing its own power through further dramatic concentrations of wealth while the competition was being destroyed and brought into a state of dependency. Lockdowns have been instigated to secure that very end and were never anything to do with health. My brother Paul spent 45 years building up a bus repair business, but lockdowns meant buses were running at a fraction of normal levels for months on end. Similar stories can told in their hundreds of millions worldwide. Efforts of a lifetime coldly destroyed by Cult multi-billionaires and their lackeys in government and law enforcement who continued to earn their living from the taxation of the people while denying the right of the same people to earn theirs. How different it would have been if those making and enforcing these decisions had to face the same financial hardships of those they affected, but they never do.

Gates of Hell

Behind it all in the full knowledge of what he is doing and why is the psychopathic figure of Cult operative Bill Gates. His puppet Tedros at the World Health Organization declared ‘Covid’ a pandemic in March, 2020. The WHO had changed the definition of a ‘pandemic’ in 2009 just a month before declaring the ‘swine flu pandemic’ which would not have been so under the previous definition. The same applies to ‘Covid’. The definition had included… ‘an infection by an infectious agent, occurring simultaneously in different countries, with a significant mortality rate relative to the proportion of the population infected’. The new definition removed the need for ‘significant mortality’. The ‘pandemic’ has been fraudulent even down to the definition, but Gates demanded economy-destroying lockdowns, school closures, social distancing, mandatory masks, a ‘vaccination’ for every man, woman and child on the planet and severe consequences and restrictions for those that refused. Who gave him this power? The Cult did which he serves like a little boy in short trousers doing what his daddy tells him. He and his psychopathic missus even smiled when they said that much worse was to come (what they knew was planned to come). Gates responded in the matter-of-fact way of all psychopaths to a question about the effect on the world economy of what he was doing:

Well, it won't go to zero but it will shrink. Global GDP is probably going to take the biggest hit ever [Gates was smiling as he said this] … in my lifetime this will be the greatest economic hit. But you don't have a choice. People act as if you have a choice. People don't feel like going to the stadium when they might get infected … People are deeply affected by seeing these stats, by knowing they could be part of the transmission chain, old people, their parents and grandparents, could be affected by this, and so you don't get to say ignore what is going on here.

There will be the ability to open up, particularly in rich countries, if things are done well over the next few months, but for the world at large normalcy only returns when we have largely vaccinated the entire population.

The man has no compassion or empathy. How could he when he's a psychopath like all Cult players? My own view is that even beyond that he is very seriously mentally ill. Look in his eyes and you can see this along with his crazy flailing arms. You don't do what he has done to the world population since the start of 2020 unless you are mentally ill and at the most extreme end of psychopathic. You especially don't do it when to you know, as we shall see, that cases and deaths from 'Covid' are fakery and a product of monumental figure massaging. 'These stats' that Gates referred to are based on a 'test' that's not testing for the 'virus' as he has known all along. He made his fortune with big Cult support as an infamously ruthless software salesman and now buys global control of 'health' (death) policy without the population he affects having any say. It's a breathtaking outrage. Gates talked about people being deeply affected by fear of 'Covid' when that was because of *him* and his global network lying to them minute-by-minute supported by a lying media that he seriously influences and funds to the tune of hundreds of millions. He's handed big sums to media operations including the BBC, NBC, Al Jazeera, Univision, *PBS NewsHour, ProPublica, National Journal, The Guardian, The Financial Times, The Atlantic, Texas Tribune, USA Today* publisher Gannett, *Washington Monthly, Le Monde,* Center for Investigative Reporting, Pulitzer Center on Crisis Reporting, National Press Foundation, International Center for Journalists, Solutions Journalism Network, the Poynter Institute for Media Studies, and many more. Gates is everywhere in the 'Covid' hoax and the man must go to prison – or a mental facility – for the rest of his life and his money distributed to those he has taken such enormous psychopathic pleasure in crushing.

The Muscle

The Hunger Games global structure demands a police-military state – a fusion of the two into one force – which viciously imposes the will of the Cult on the population and protects the Cult from public rebellion. In that regard, too, the 'Covid' hoax just keeps on giving. Often unlawful, ridiculous and contradictory 'Covid' rules and regulations have been policed across the world by moronic automatons and psychopaths made faceless by face-nappy masks and acting like the Nazi SS and fascist blackshirts and brownshirts of Hitler and Mussolini. The smallest departure from the rules decreed by the psychos in government and their clueless gofers were jumped upon by the face-nappy fascists. Brutality against public protestors soon became commonplace even on girls, women and old people as the brave men with the batons – the Face-Nappies as I call them – broke up peaceful protests and handed out fines like confetti to people who couldn't earn a living let alone pay hundreds of pounds for what was once an accepted human right. Robot Face-Nappies of Nottingham police in the English East Midlands fined one group £11,000 for attending a child's birthday party. For decades I charted the transformation of law enforcement as genuine, decent officers were replaced with psychopaths and the brain dead who would happily and brutally do whatever their masters told them. Now they were let loose on the public and I would emphasise the point that none of this just happened. The step-by-step change in the dynamic between police and public was orchestrated from the shadows by those who knew where this was all going and the same with the perceptual reframing of those in all levels of authority and official administration through 'training courses' by organisations such as Common Purpose which was created in the late 1980s and given a massive boost in Blair era Britain until it became a global phenomenon. Supposed public 'servants' began to view the population as the enemy and the same was true of the police. This was the start of the explosion of behaviour manipulation organisations and networks preparing for the all-war on the human psyche unleashed with the dawn of 2020. I will go into more detail about this later in the book because it is a core part of what is happening.

Police desecrated beauty spots to deter people gathering and arrested women for walking in the countryside alone 'too far' from their homes. We had arrogant, clueless sergeants in the Isle of Wight police where I live posting on Facebook what they insisted the population must do or else. A

schoolmaster sergeant called Radford looked young enough for me to ask if his mother knew he was out, but he was posting what he *expected* people to do while a Sergeant Wilkinson boasted about fining lads for meeting in a McDonald's car park where they went to get a lockdown takeaway. Wilkinson added that he had even cancelled their order. What a pair of prats these people are and yet they have increasingly become the norm among Jackboot Johnson's Yellowshirts once known as the British police. This was the theme all over the world with police savagery common during lockdown protests in the United States, the Netherlands, and the fascist state of Victoria in Australia under its tyrannical and again moronic premier Daniel Andrews. Amazing how tyrannical and moronic tend to work as a team and the same combination could be seen across America as arrogant, narcissistic Woke governors and mayors such as Gavin Newsom (California), Andrew Cuomo (New York), Gretchen Whitmer (Michigan), Lori Lightfoot (Chicago) and Eric Garcetti (Los Angeles) did their Nazi and Stalin impressions with the full support of the compliant brutality of their enforcers in uniform as they arrested small business owners defying fascist shutdown orders and took them to jail in ankle shackles and handcuffs. This happened to bistro owner Marlena Pavlos-Hackney in Gretchen Whitmer's fascist state of Michigan when police arrived to enforce an order by a state-owned judge for 'putting the community at risk' at a time when other states like Texas were dropping restrictions and migrants were pouring across the southern border without any 'Covid' questions at all. I'm sure there are many officers appalled by what they are ordered to do, but not nearly enough of them. If they were truly appalled they would not do it. As the months passed every opportunity was taken to have the military involved to make their presence on the streets ever more familiar and 'normal' for the longer-term goal of police-military fusion.

Another crucial element to the Hunger Games enforcement network has been encouraging the public to report neighbours and others for 'breaking the lockdown rules'. The group faced with £11,000 in fines at the child's birthday party would have been dobbed-in by a neighbour with a brain the size of a pea. The technique was most famously employed by the Stasi secret police in communist East Germany who had public informants placed throughout the population. A police chief in the UK says his force doesn't need to carry out 'Covid' patrols when they are flooded with so many calls from the public reporting other people for visiting the beach.

Dorset police chief James Vaughan said people were so enthusiastic about snitching on their fellow humans they were now operating as an auxiliary arm of the police: 'We are still getting around 400 reports a week from the public, so we will respond to reports …We won't need to be doing hotspot patrols because people are very quick to pick the phone up and tell us.' Vaughan didn't say that this is a pillar of all tyrannies of whatever complexion and the means to hugely extend the reach of enforcement while spreading distrust among the people and making them wary of doing anything that might get them reported. Those narcissistic Isle of Wight sergeants Radford and Wilkinson never fail to add a link to their Facebook posts where the public can inform on their fellow slaves. Neither would be self-aware enough to realise they were imitating the Stasi which they might well never have heard of. Government psychologists that I will expose later laid out a policy to turn communities against each other in the same way.

A coincidence? Yep, and I can knit fog

I knew from the start of the alleged pandemic that this was a Cult operation. It presented limitless potential to rapidly advance the Cult agenda and exploit manipulated fear to demand that every man, woman and child on the planet was 'vaccinated' in a process never used on humans before which infuses self-replicating *synthetic* material into human cells. Remember the plan to transform the human body from a biological to a synthetic biological state. I'll deal with the 'vaccine' (that's not actually a vaccine) when I focus on the genetic agenda. Enough to say here that mass global 'vaccination' justified by this 'new virus' set alarms ringing after 30 years of tracking these people and their methods. The 'Covid' hoax officially beginning in China was also a big red flag for reasons I will be explaining. The agenda potential was so enormous that I could dismiss any idea that the 'virus' appeared naturally. Major happenings with major agenda implications never occur without Cult involvement in making them happen. My questions were twofold in early 2020 as the media began its campaign to induce global fear and hysteria: Was this alleged infectious agent released on purpose by the Cult or did it even exist at all? I then did what I always do in these situations. I sat, observed and waited to see where the evidence and information would take me. By March and early April synchronicity was strongly – and ever more so since then – pointing me in

the direction of *there is no 'virus'*. I went public on that with derision even from swathes of the alternative media that voiced a scenario that the Chinese government released the 'virus' in league with Deep State elements in the United States from a top-level bio-lab in Wuhan where the 'virus' is said to have first appeared. I looked at that possibility, but I didn't buy it for several reasons. Deaths from the 'virus' did not in any way match what they would have been with a 'deadly bioweapon' and it is much more effective if you sell the *illusion* of an infectious agent rather than having a real one unless you can control through injection who has it and who doesn't. Otherwise you lose control of events. A made-up 'virus' gives you a blank sheet of paper on which you can make it do whatever you like and have any symptoms or mutant 'variants' you choose to add while a real infectious agent would limit you to what it actually does. A phantom disease allows you to have endless ludicrous 'studies' on the 'Covid' dollar to widen the perceived impact by inventing ever more 'at risk' groups including one study which said those who walk slowly may be almost four times more likely to die from the 'virus'. People are in psychiatric wards for less.

A real 'deadly bioweapon' can take out people in the hierarchy that are not part of the Cult, but essential to its operation. Obviously they don't want that. Releasing a real disease means you immediately lose control of it. Releasing an illusory one means you don't. Again it's vital that people are extra careful when dealing with what they want to hear. A bioweapon unleashed from a Chinese laboratory in collusion with the American Deep State may fit a conspiracy narrative, but is it true? Would it not be far more effective to use the excuse of a 'virus' to justify the real bioweapon – the 'vaccine'? That way your disease agent does not have to be transmitted and arrives directly through a syringe. I saw a French virologist Luc Montagnier quoted in the alternative media as saying he had discovered that the alleged 'new' severe acute respiratory syndrome coronavirus , or SARS-CoV-2, was made artificially and included elements of the human immunodeficiency 'virus' (HIV) and a parasite that causes malaria. SARS-CoV-2 is alleged to trigger an alleged illness called Covid-19. I remembered Montagnier's name from my research years before into claims that an HIV 'retrovirus' causes AIDs – claims that were demolished by Berkeley virologist Peter Duesberg who showed that no one had ever proved that HIV causes acquired immunodeficiency syndrome or AIDS. Claims that become accepted as fact, publicly and medically, with no proof whatsoever

are an ever-recurring story that profoundly applies to ‘Covid’. Nevertheless, despite the lack of proof, Montagnier’s team at the Pasteur Institute in Paris had a long dispute with American researcher Robert Gallo over which of them discovered and isolated the HIV ‘virus’ and with *no evidence* found it to cause AIDS. You will see later that there is also no evidence that any ‘virus’ causes any disease or that there is even such a thing as a ‘virus’ in the way it is said to exist. The claim to have ‘isolated’ the HIV ‘virus’ will be presented in its real context as we come to the shocking story – and it is a story – of SARS-CoV-2 and so will Montagnier’s assertion that he identified the full SARS-CoV-2 genome.

Hoax in the making

We can pick up the ‘Covid’ story in 2010 and the publication by the Rockefeller Foundation of a document called ‘Scenarios for the Future of Technology and International Development’. The inner circle of the Rockefeller family has been serving the Cult since John D. Rockefeller (1839-1937) made his fortune with Standard Oil. It is less well known that the same Rockefeller – the Bill Gates of his day – was responsible for establishing what is now referred to as ‘Big Pharma’, the global network of pharmaceutical companies that make outrageous profits dispensing scalpel and drug ‘medicine’ and are obsessed with pumping vaccines in ever-increasing number into as many human arms and backsides as possible. John D. Rockefeller was the driving force behind the creation of the ‘education’ system in the United States and elsewhere specifically designed to program the perceptions of generations thereafter. The Rockefeller family donated exceptionally valuable land in New York for the United Nations building and were central in establishing the World Health Organization in 1948 as an agency of the UN which was created from the start as a Trojan horse and stalking horse for world government. Now enter Bill Gates. His family and the Rockefellers have long been extremely close and I have seen genealogy which claims that if you go back far enough the two families fuse into the same bloodline. Gates has said that the Bill and Melinda Gates Foundation was inspired by the Rockefeller Foundation and why not when both are serving the same Cult? Major tax-exempt foundations are overwhelmingly criminal enterprises in which Cult assets fund the Cult agenda in the guise of ‘philanthropy’ while avoiding tax in the

process. Cult operatives can become mega-rich in their role of front men and women for the psychopaths at the inner core and they, too, have to be psychopaths to knowingly serve such evil. Part of the deal is that a big percentage of the wealth gleaned from representing the Cult has to be spent advancing the ambitions of the Cult and hence you have the Rockefeller Foundation, Bill and Melinda Gates Foundation (and *so* many more) and people like George Soros with his global Open Society Foundations spending their billions in pursuit of global Cult control. Gates is a global public face of the Cult with his interventions in world affairs including Big Tech influence; a central role in the 'Covid' and 'vaccine' scam; promotion of the climate change shakedown; manipulation of education; geoengineering of the skies; and his food-control agenda as the biggest owner of farmland in America, his GMO promotion and through other means. As one writer said: 'Gates monopolizes or wields disproportionate influence over the tech industry, global health and vaccines, agriculture and food policy (including biopiracy and fake food), weather modification and other climate technologies, surveillance, education and media.' The almost limitless wealth secured through Microsoft and other not-allowed-to-fail ventures (including vaccines) has been ploughed into a long, long list of Cult projects designed to enslave the entire human race. Gates and the Rockefellers have been working as one unit with the Rockefeller-established World Health Organization leading global 'Covid' policy controlled by Gates through his mouth-piece Tedros. Gates became the WHO's biggest funder when Trump announced that the American government would cease its donations, but Biden immediately said he would restore the money when he took office in January, 2021. The Gates Foundation (the Cult) owns through limitless funding the world health system and the major players across the globe in the 'Covid' hoax.

Okay, with that background we return to that Rockefeller Foundation document of 2010 headed 'Scenarios for the Future of Technology and International Development' and its 'imaginary' epidemic of a virulent and deadly influenza strain which infected 20 percent of the global population and killed eight million in seven months. The Rockefeller scenario was that the epidemic destroyed economies, closed shops, offices and other businesses and led to governments imposing fierce rules and restrictions that included mandatory wearing of face masks and body-temperature checks to enter communal spaces like railway stations and supermarkets.

The document predicted that even after the height of the Rockefeller-envisaged epidemic the authoritarian rule would continue to deal with further pandemics, transnational terrorism, environmental crises and rising poverty. Now you may think that the Rockefellers are our modern-day seers or alternatively, and rather more likely, that they well knew what was planned a few years further on. Fascism had to be imposed, you see, to 'protect citizens from risk and exposure'. The Rockefeller scenario document said:

During the pandemic, national leaders around the world flexed their authority and imposed airtight rules and restrictions, from the mandatory wearing of face masks to body-temperature checks at the entries to communal spaces like train stations and supermarkets. Even after the pandemic faded, this more authoritarian control and oversight of citizens and their activities stuck and even intensified. In order to protect themselves from the spread of increasingly global problems – from pandemics and transnational terrorism to environmental crises and rising poverty – leaders around the world took a firmer grip on power.

At first, the notion of a more controlled world gained wide acceptance and approval. Citizens willingly gave up some of their sovereignty – and their privacy – to more paternalistic states in exchange for greater safety and stability. Citizens were more tolerant, and even eager, for top-down direction and oversight, and national leaders had more latitude to impose order in the ways they saw fit.

In developed countries, this heightened oversight took many forms: biometric IDs for all citizens, for example, and tighter regulation of key industries whose stability was deemed vital to national interests. In many developed countries, enforced cooperation with a suite of new regulations and agreements slowly but steadily restored both order and, importantly, economic growth.

There we have the prophetic Rockefellers in 2010 and three years later came their paper for the Global Health Summit in Beijing, China, when government representatives, the private sector, international organisations and groups met to discuss the next 100 years of 'global health'. The Rockefeller Foundation-funded paper was called 'Dreaming the Future of Health for the Next 100 Years and more prophecy ensued as it described a dystopian future: 'The abundance of data, digitally tracking and linking people may mean the 'death of privacy' and may replace physical interaction with transient, virtual connection, generating isolation and raising questions of how values are shaped in virtual networks.' Next in the 'Covid' hoax preparation sequence came a 'table top' simulation in 2018 for another 'imaginary' pandemic of a disease called Clade X which was said to kill 900 million people. The exercise was organised by the Gates-

funded Johns Hopkins University's Center for Health Security in the United States and this is the very same university that has been compiling the disgustingly and systematically erroneous global figures for 'Covid' cases and deaths. Similar Johns Hopkins health crisis scenarios have included the Dark Winter exercise in 2001 and Atlantic Storm in 2005.

Nostradamus 201

For sheer predictive genius look no further prophecy-watchers than the Bill Gates-funded Event 201 held only six weeks before the 'coronavirus pandemic' is supposed to have broken out in China and Event 201 was based on a scenario of a global 'coronavirus pandemic'. Melinda Gates, the great man's missus, told the BBC that he had 'prepared for years' for a coronavirus pandemic which told us what we already knew. Nostradamugates had predicted in a TED talk in 2015 that a pandemic was coming that would kill a lot of people and demolish the world economy. My god, the man is a machine – possibly even literally. Now here he was only weeks before the real thing funding just such a simulated scenario and involving his friends and associates at Johns Hopkins, the World Economic Forum Cult-front of Klaus Schwab, the United Nations, Johnson & Johnson, major banks, and officials from China and the Centers for Disease Control in the United States. What synchronicity – Johns Hopkins would go on to compile the fraudulent 'Covid' figures, the World Economic Forum and Schwab would push the 'Great Reset' in response to 'Covid', the Centers for Disease Control would be at the forefront of 'Covid' policy in the United States, Johnson & Johnson would produce a 'Covid vaccine', and everything would officially start just weeks later in China. Spooky, eh? They were even accurate in creating a simulation of a 'virus' pandemic because the 'real thing' would also be a simulation. Event 201 was not an exercise preparing for something that might happen; it was a rehearsal for what those in control knew was *going* to happen and very shortly. Hours of this simulation were posted on the Internet and the various themes and responses mirrored what would soon be imposed to transform human society. News stories were inserted and what they said would be commonplace a few weeks later with still more prophecy perfection. Much discussion focused on the need to deal with misinformation and the 'anti-

vax movement' which is exactly what happened when the 'virus' arrived – was said to have arrived – in the West.

Cult-owned social media banned criticism and exposure of the official 'virus' narrative and when I said there *was* no 'virus' in early April, 2020, I was banned by one platform after another including YouTube, Facebook and later Twitter. The mainstream broadcast media in Britain was in effect banned from interviewing me by the Tony-Blair-created government broadcasting censor Ofcom headed by career government bureaucrat Melanie Dawes who was appointed just as the 'virus' hoax was about to play out in January, 2020. At the same time the Ickonic media platform was using Vimeo, another ultra-Zionist-owned operation, while our own player was being created and they deleted in an instant hundreds of videos, documentaries, series and shows to confirm their unbelievable vindictiveness. We had copies, of course, and they had to be restored one by one when our player was ready. These people have no class. Sabbatian Facebook promised free advertisements for the Gates-controlled World Health Organization narrative while deleting 'false claims and conspiracy theories' to stop 'misinformation' about the alleged coronavirus. All these responses could be seen just a short while earlier in the scenarios of Event 201. Extreme censorship was absolutely crucial for the Cult because the official story was so ridiculous and unsupportable by the evidence that it could never survive open debate and the free-flow of information and opinion. If you can't win a debate then don't have one is the Cult's approach throughout history. Facebook's little boy front man – front boy – Mark Zuckerberg equated 'credible and accurate information' with official sources and exposing their lies with 'misinformation'.

Silencing those that can see

The censorship dynamic of Event 201 is now the norm with an army of narrative-supporting 'fact-checker' organisations whose entire reason for being is to tell the public that official narratives are true and those exposing them are lying. One of the most appalling of these 'fact-checkers' is called NewsGuard founded by ultra-Zionist Americans Gordon Crovitz and Steven Brill. Crovitz is a former publisher of *The Wall Street Journal*, former Executive Vice President of Dow Jones, a member of the Council on Foreign Relations (CFR), and on the board of the American Association of

Rhodes Scholars. The CFR and Rhodes Scholarships, named after Rothschild agent Cecil Rhodes who plundered the gold and diamonds of South Africa for his masters and the Cult, have featured widely in my books. NewsGuard don't seem to like me for some reason – I really can't think why – and they have done all they can to have me censored and discredited which is, to quote an old British politician, like being savaged by a dead sheep. They are, however, like all in the censorship network, very well connected and funded by organisations themselves funded by, or connected to, Bill Gates. As you would expect with anything associated with Gates NewsGuard has an offshoot called HealthGuard which 'fights online health care hoaxes'. How very kind. Somehow the NewsGuard European Managing Director Anna-Sophie Harling, a remarkably young-looking woman with no broadcasting experience and little hands-on work in journalism, has somehow secured a position on the 'Content Board' of UK government broadcast censor Ofcom. An executive of an organisation seeking to discredit dissidents of the official narratives is making decisions for the government broadcast 'regulator' about content?? Another appalling 'fact-checker' is Full Fact funded by George Soros and global censors Google and Facebook.

It's amazing how many activists in the 'fact-checking', 'anti-hate', arena turn up in government-related positions – people like UK Labour Party activist Imran Ahmed who heads the Center for Countering Digital Hate founded by people like Morgan McSweeney, now chief of staff to the Labour Party's hapless and useless 'leader' Keir Starmer. Digital Hate – which is what it really is – uses the American spelling of Center to betray its connection to a transatlantic network of similar organisations which in 2020 shapeshifted from attacking people for 'hate' to attacking them for questioning the 'Covid' hoax and the dangers of the 'Covid vaccine'. It's just a coincidence, you understand. This is one of Imran Ahmed's hysterical statements: 'I would go beyond calling anti-vaxxers conspiracy theorists to say they are an extremist group that pose a national security risk.' No one could ever accuse this prat of understatement and he's including in that those parents who are now against vaccines after their children were damaged for life or killed by them. He's such a nice man. Ahmed does the rounds of the Woke media getting soft-ball questions from spineless 'journalists' who never ask what right he has to campaign to destroy the freedom of speech of others while he demands it for himself. There also

seems to be an overrepresentation in Ofcom of people connected to the narrative-worshipping BBC. This incredible global network of narrative-support was super-vital when the ‘Covid’ hoax was played in the light of the mega-whopper lies that have to be defended from the spotlight cast by the most basic intelligence.

Setting the scene

The Cult plays the long game and proceeds step-by-step ensuring that everything is in place before major cards are played and they don’t come any bigger than the ‘Covid’ hoax. The psychopaths can’t handle events where the outcome isn’t certain and as little as possible – preferably nothing – is left to chance. Politicians, government and medical officials who would follow direction were brought to illusory power in advance by the Cult web whether on the national stage or others like state governors and mayors of America. For decades the dynamic between officialdom, law enforcement and the public was changed from one of service to one of control and dictatorship. Behaviour manipulation networks established within government were waiting to impose the coming ‘Covid’ rules and regulations specifically designed to subdue and rewire the psyche of the people in the guise of protecting health. These included in the UK the Behavioural Insights Team part-owned by the British government Cabinet Office; the Scientific Pandemic Insights Group on Behaviours (SPI-B); and a whole web of intelligence and military groups seeking to direct the conversation on social media and control the narrative. Among them are the cyberwarfare (on the people) 77th Brigade of the British military which is also coordinated through the Cabinet Office as civilian and military leadership continues to combine in what they call the Fusion Doctrine. The 77th Brigade is a British equivalent of the infamous Israeli (Sabbatian) military cyberwarfare and Internet manipulation operation Unit 8200 which I expose at length in *The Trigger*. Also carefully in place were the medical and science advisers to government – many on the payroll past or present of Bill Gates – and a whole alternative structure of unelected government stood by to take control when elected parliaments were effectively closed down once the ‘Covid’ card was slammed on the table. The structure I have described here and so much more was installed in every major country through the Cult networks. The top-down control hierarchy looks like this:

The Cult – Cult-owned Gates – the World Health Organization and Tedros – Gates-funded or controlled chief medical officers and science 'advisers' (dictators) in each country – political 'leaders'– law enforcement – The People. Through this simple global communication and enforcement structure the policy of the Cult could be imposed on virtually the entire human population so long as they acquiesced to the fascism. With everything in place it was time for the button to be pressed in late 2019/early 2020.

These were the prime goals the Cult had to secure for its will to prevail:

1) Locking down economies, closing all but designated 'essential' businesses (Cult-owned corporations were 'essential'), and putting the population under house arrest was an imperative to destroy independent income and employment and ensure dependency on the Cult-controlled state in the Hunger Games Society. Lockdowns had to be established as the global blueprint from the start to respond to the 'virus' and followed by pretty much the entire world.

2) The global population had to be terrified into believing in a deadly 'virus' that didn't actually exist so they would unquestioningly obey authority in the belief that authority must know how best to protect them and their families. Software salesman Gates would suddenly morph into the world's health expert and be promoted as such by the Cult-owned media.

3) A method of testing that wasn't testing for the 'virus', but was only claimed to be, had to be in place to provide the illusion of 'cases' and subsequent 'deaths' that had a very different cause to the 'Covid-19' that would be scribbled on the death certificate.

4) Because there was no 'virus' and the great majority testing positive with a test not testing for the 'virus' would have no symptoms of anything the lie had to be sold that people without symptoms (without the 'virus') could still pass it on to others. This was crucial to justify for the first time quarantining – house arresting – healthy people. Without this the economy-destroying lockdown of *everybody* could not have been credibly sold.

5) The 'saviour' had to be seen as a vaccine which beyond evil drug companies were working like angels of mercy to develop as quickly as possible, with all corners cut, to save the day. The public must absolutely not know that the 'vaccine' had nothing to do with a 'virus' or that the contents were ready and waiting with a very different motive long before the 'Covid' card was even lifted from the pack.

I said in March, 2020, that the 'vaccine' would have been created way ahead of the 'Covid' hoax which justified its use and the following December an article in the New York *Intelligencer* magazine said the Moderna 'vaccine' had been 'designed' by January, 2020. This was 'before China had even acknowledged that the disease could be transmitted from human to human, more than a week before the first confirmed coronavirus

case in the United States'. The article said that by the time the first American death was announced a month later 'the vaccine had already been manufactured and shipped to the National Institutes of Health for the beginning of its Phase I clinical trial'. The 'vaccine' was actually 'designed' long before that although even with this timescale you would expect the article to ask how on earth it could have been done that quickly. Instead it asked why the 'vaccine' had not been rolled out then and not months later. Journalism in the mainstream is truly dead. I am going to detail in the next chapter why the 'virus' has never existed and how a hoax on that scale was possible, but first the foundation on which the Big Lie of 'Covid' was built.

The test that doesn't test

Fraudulent 'testing' is the bottom line of the whole 'Covid' hoax and was the means by which a 'virus' that did not exist *appeared* to exist. They could only achieve this magic trick by using a test not testing for the 'virus'. To use a test that *was* testing for the 'virus' would mean that every test would come back negative given there was no 'virus'. They chose to exploit something called the RT-PCR test invented by American biochemist Kary Mullis in the 1980s who said publicly that his PCR test … *cannot detect infectious disease*. Yes, the 'test' used worldwide to detect infectious 'Covid' to produce all the illusory 'cases' and 'deaths' compiled by Johns Hopkins and others *cannot detect infectious disease*. This fact came from the mouth of the man who invented PCR and was awarded the Nobel Prize in Chemistry in 1993 for doing so. Sadly, and incredibly conveniently for the Cult, Mullis died in August, 2019, at the age of 74 just before his test would be fraudulently used to unleash fascism on the world. He was said to have died from pneumonia which was an irony in itself. A few months later he would have had 'Covid-19' on his death certificate. I say the timing of his death was convenient because had he lived Mullis, a brilliant, honest and decent man, would have been vociferously speaking out against the use of his test to detect 'Covid' when it was never designed, or able, to do that. I know that to be true given that Mullis made the same point when his test was used to 'detect' – not detect – HIV. He had been seriously critical of the Gallo/Montagnier claim to have isolated the HIV 'virus' and shown it to cause AIDS for which Mullis said there was no evidence. AIDS is actually

not a disease but a series of diseases from which people die all the time. When they die from those *same diseases* after a positive 'test' for HIV then AIDS goes on their death certificate. I think I've heard that before somewhere. Countries instigated a policy with 'Covid' that anyone who tested positive with a test not testing for the 'virus' and died of any other cause within 28 days and even longer 'Covid-19' had to go on the death certificate. Cases have come from the test that can't test for infectious disease and the deaths are those who have died of *anything* after testing positive with a test not testing for the 'virus'. I'll have much more later about the death certificate scandal.

Mullis was deeply dismissive of the now US 'Covid' star Anthony Fauci who he said was a liar who didn't know anything about anything – 'and I would say that to his face – nothing.' He said of Fauci: 'The man thinks he can take a blood sample, put it in an electron microscope and if it's got a virus in there you'll know it – he doesn't understand electron microscopy and he doesn't understand medicine and shouldn't be in a position like he's in.' That position, terrifyingly, has made him the decider of 'Covid' fascism policy on behalf of the Cult in his role as director since 1984 of the National Institute of Allergy and Infectious Diseases (NIAID) while his record of being wrong is laughable; but being wrong, so long as it's the *right kind* of wrong, is why the Cult loves him. He'll say anything the Cult tells him to say. Fauci was made Chief Medical Adviser to the President immediately Biden took office. Biden was installed in the White House by Cult manipulation and one of his first decisions was to elevate Fauci to a position of even more control. This is a coincidence? Yes, and I identify as a flamenco dancer called Lola. How does such an incompetent criminal like Fauci remain in that pivotal position in American health since *the 1980s*? When you serve the Cult it looks after you until you are surplus to requirements. Kary Mullis said prophetically of Fauci and his like: 'Those guys have an agenda and it's not an agenda we would like them to have … they make their own rules, they change them when they want to, and Tony Fauci does not mind going on television in front of the people who pay his salary and lie directly into the camera.' Fauci has done that almost daily since the 'Covid' hoax began. Lying is in Fauci's DNA. To make the situation crystal clear about the PCR test this is a direct quote from its inventor Kary Mullis:

It [the PCR test] doesn't tell you that you're sick and doesn't tell you that the thing you ended up with was really going to hurt you ...'

Ask yourself why governments and medical systems the world over have been using this very test to decide who is 'infected' with the SARS-CoV-2 'virus' and the alleged disease it allegedly causes, 'Covid-19'. The answer to that question will tell you what has been going on. By the way, here's a little show-stopper – the 'new' SARS-CoV-2 'virus' was 'identified' as such right from the start using … *the PCR test not testing for the 'virus'*. If you are new to this and find that shocking then stick around. I have hardly started yet. Even worse, other 'tests', like the 'Lateral Flow Device' (LFD), are considered so useless that they have to be *confirmed* by the PCR test! Leaked emails written by Ben Dyson, adviser to UK 'Health' Secretary Matt Hancock, said they were 'dangerously unreliable'. Dyson, executive director of strategy at the Department of Health, wrote: 'As of today, someone who gets a positive LFD result in (say) London has at best a 25 per cent chance of it being a true positive, but if it is a self-reported test potentially as low as 10 per cent (on an optimistic assumption about specificity) or as low as 2 per cent (on a more pessimistic assumption).' These are the 'tests' that schoolchildren and the public are being urged to have twice a week or more and have to isolate if they get a positive. Each fake positive goes in the statistics as a 'case' no matter how ludicrously inaccurate and the 'cases' drive lockdown, masks and the pressure to 'vaccinate'. The government said in response to the email leak that the 'tests' were accurate which confirmed yet again what shocking bloody liars they are. The real false positive rate is *100 percent* as we'll see. In another 'you couldn't make it up' the UK government agreed to pay £2.8 billion to California's Innova Medical Group to supply the irrelevant lateral flow tests. The company's primary test-making centre is in China. Innova Medical Group, established in March, 2020, is owned by Pasaca Capital Inc, chaired by Chinese-American millionaire Charles Huang who was born in Wuhan.

How it works – and how it doesn't

The RT-PCR test, known by its full title of Polymerase chain reaction, is used across the world to make millions, even billions, of copies of a

DNA/RNA genetic information sample. The process is called ‘amplification’ and means that a tiny sample of genetic material is amplified to bring out the detailed content. I stress that it is not testing for an infectious disease. It is simply amplifying a sample of genetic material. In the words of Kary Mullis: ‘PCR is … just a process that’s used to make a whole lot of something out of something.’ To emphasise the point companies that make the PCR tests circulated around the world to ‘test’ for ‘Covid’ warn on the box that it can’t be used to detect ‘Covid’ or infectious disease and is for research purposes only. It’s okay, rest for a minute and you’ll be fine. This is the test that produces the ‘cases’ and ‘deaths’ that have been used to destroy human society. All those global and national medical and scientific ‘experts’ demanding this destruction to ‘save us’ *KNOW* that the test is not testing for the ‘virus’ and the cases and deaths they claim to be real are an almost unimaginable fraud. Every one of them and so many others including politicians and psychopaths like Gates and Tedros must be brought before Nuremburg-type trials and jailed for the rest of their lives. The more the genetic sample is amplified by PCR the more elements of that material become sensitive to the test and by that I don’t mean sensitive for a ‘virus’ but for elements of the genetic material which is *naturally* in the body or relates to remnants of old conditions of various kinds lying dormant and causing no disease. Once the amplification of the PCR reaches a certain level *everyone* will test positive. So much of the material has been made sensitive to the test that everyone will have some part of it in their body. Even lying criminals like Fauci have said that once PCR amplifications pass 35 cycles everything will be a false positive that cannot be trusted for the reasons I have described. I say, like many proper doctors and scientists, that 100 percent of the ‘positives’ are false, but let’s just go with Fauci for a moment.

He says that any amplification over 35 cycles will produce false positives and yet the US Centers for Disease Control (CDC) and Food and Drug Administration (FDA) have recommended up to *40 cycles* and the National Health Service (NHS) in Britain admitted in an internal document for staff that it was using *45 cycles* of amplification. A long list of other countries has been doing the same and at least one ‘testing’ laboratory has been using *50 cycles*. Have you ever heard a doctor, medical ‘expert’ or the media ask what level of amplification has been used to claim a ‘positive’. The ‘test’ comes back ‘positive’ and so you have the ‘virus’, end of story. Now we

can see how the government in Tanzania could send off samples from a goat and a pawpaw fruit under human names and both came back positive for ‘Covid-19’. Tanzania president John Magufuli mocked the ‘Covid’ hysteria, the PCR test and masks and refused to import the DNA-manipulating ‘vaccine’. The Cult hated him and an article sponsored by the Bill Gates Foundation appeared in the London *Guardian* in February, 2021, headed ‘It’s time for Africa to rein in Tanzania’s anti-vaxxer president’. Well, ‘reined in’ he shortly was. Magufuli appeared in good health, but then, in March, 2021, he was dead at 61 from ‘heart failure’. He was replaced by Samia Hassan Suhulu who is connected to Klaus Schwab’s World Economic Forum and she immediately reversed Magufuli’s ‘Covid’ policy. A sample of cola tested positive for ‘Covid’ with the PCR test in Germany while American actress and singer-songwriter Erykah Badu tested positive in one nostril and negative in the other. Footballer Ronaldo called the PCR test ‘bullshit’ after testing positive three times and being forced to quarantine and miss matches when there was nothing wrong with him. The mantra from Tedros at the World Health Organization and national governments (same thing) has been test, test, test. They know that the more tests they can generate the more fake ‘cases’ they have which go on to become ‘deaths’ in ways I am coming to. The UK government has its Operation Moonshot planned to test multiple millions every day in workplaces and schools with free tests for everyone to use twice a week at home in line with the Cult plan from the start to make testing part of life. A government advertisement for an ‘Interim Head of Asymptomatic Testing Communication’ said the job included responsibility for delivering a ‘communications strategy’ (propaganda) ‘to support the expansion of asymptomatic testing that *‘normalises testing as part of everyday life*’. More tests means more fake ‘cases’, ‘deaths’ and fascism. I have heard of, and from, many people who booked a test, couldn’t turn up, and yet got a positive result through the post for a test they’d never even had. The whole thing is crazy, but for the Cult there’s method in the madness. Controlling and manipulating the level of amplification of the test means the authorities can control whenever they want the number of apparent ‘cases’ and ‘deaths’. If they want to justify more fascist lockdown and destruction of livelihoods they keep the amplification high. If they want to give the illusion that lockdowns and the ‘vaccine’ are working then they lower the amplification and ‘cases’ and ‘deaths’ will appear to fall. In January, 2021,

the Cult-owned World Health Organization suddenly warned laboratories about over-amplification of the test and to lower the threshold. Suddenly headlines began appearing such as: 'Why ARE "Covid" cases plummeting?' This was just when the vaccine rollout was underway and I had predicted months before they would make cases appear to fall through amplification tampering when the 'vaccine' came. These people are so predictable.

Cow vaccines?

The question must be asked of what is on the test swabs being poked far up the nose of the population to the base of the brain? A nasal swab punctured one woman's brain and caused it to leak fluid. Most of these procedures are being done by people with little training or medical knowledge. Dr Lorraine Day, former orthopaedic trauma surgeon and Chief of Orthopaedic Surgery at San Francisco General Hospital, says the tests are really a *'vaccine'*. Cows have long been vaccinated this way. She points out that masks have to cover the nose and the mouth where it is claimed the 'virus' exists in saliva. Why then don't they take saliva from the mouth as they do with a DNA test instead of pushing a long swab up the nose towards the brain? The ethmoid bone separates the nasal cavity from the brain and within that bone is the cribriform plate. Dr Day says that when the swab is pushed up against this plate and twisted the procedure is 'depositing things back there'. She claims that among these 'things' are nanoparticles that can enter the brain. Researchers have noted that a team at the Gates-funded Johns Hopkins have designed tiny, star-shaped micro-devices that can latch onto intestinal mucosa and release drugs into the body. Mucosa is the thin skin that covers the inside surface of parts of the body such as *the nose* and mouth and produces mucus to protect them. The Johns Hopkins micro-devices are called 'theragrippers' and were 'inspired' by a parasitic worm that digs its sharp teeth into a host's intestines. Nasal swabs are also coated in the sterilisation agent ethylene oxide. The US National Cancer Institute posts this explanation on its website:

At room temperature, ethylene oxide is a flammable colorless gas with a sweet odor. It is used primarily to produce other chemicals, including antifreeze. In smaller amounts, ethylene oxide is

used as a pesticide and a sterilizing agent. The ability of ethylene oxide to damage DNA makes it an effective sterilizing agent but also accounts for its cancer-causing activity.

The Institute mentions lymphoma and leukaemia as cancers most frequently reported to be associated with occupational exposure to ethylene oxide along with stomach and breast cancers. How does anyone think this is going to work out with the constant testing regime being inflicted on adults and children at home and at school that will accumulate in the body anything that's on the swab?

Doctors know best

It is vital for people to realise that 'hero' doctors 'know' only what the Big Pharma-dominated medical authorities tell them to 'know' and if they refuse to 'know' what they are told to 'know' they are out the door. They are mostly not physicians or healers, but repeaters of the official narrative – or else. I have seen alleged professional doctors on British television make shocking statements that we are supposed to take seriously. One called 'Dr' Amir Khan, who is actually telling patients how to respond to illness, said that men could take the birth pill to 'help slow down the effects of Covid-19'. In March, 2021, another ridiculous 'Covid study' by an American doctor proposed injecting men with the female sex hormone progesterone as a 'Covid' treatment. British doctor Nighat Arif told the BBC that face coverings were now going to be part of ongoing normal. Yes, the vaccine protects you, she said (evidence?) … but the way to deal with viruses in the community was always going to come down to hand washing, face covering and keeping a physical distance. That's not what we were told before the 'vaccine' was circulating. Arif said she couldn't imagine ever again going on the underground or in a lift without a mask. I was just thanking my good luck that she was not my doctor when she said – in March, 2021 – that if 'we are *behaving* and we are doing all the right things' she thought we could 'have our nearest and dearest around us at home … around *Christmas* and N*ew Year!* Her patronising delivery was the usual school teacher talking to six-year-olds as she repeated every government talking point and probably believed them all. If we have learned anything from the 'Covid' experience surely it must be that humanity's perception of doctors needs a fundamental rethink. NHS

‘doctor’ Sara Kayat told her television audience that the ‘Covid vaccine’ would ‘100 percent prevent hospitalisation and death’. Not even Big Pharma claimed that. We have to stop taking ‘experts’ at their word without question when so many of them are clueless and only repeating the party line on which their careers depend. That is not to say there are not brilliants doctors – there are and I have spoken to many of them since all this began – but you won’t see them in the mainstream media or quoted by the psychopaths and yes-people in government.

Remember the name – Christian Drosten

German virologist Christian Drosten, Director of Charité Institute of Virology in Berlin, became a national star after the pandemic hoax began. He was feted on television and advised the German government on ‘Covid’ policy. Most importantly to the wider world Drosten led a group that produced the ‘Covid’ testing protocol for the PCR test. What a remarkable feat given the PCR cannot test for infectious disease and even more so when you think that Drosten said that his method of testing for SARS-CoV-2 was developed ‘without having virus material available’. *He developed a test for a ‘virus’ that he didn’t have and had never seen*. Let that sink in as you survey the global devastation that came from what he did. The whole catastrophe of Drosten’s ‘test’ was based on the alleged genetic sequence published by Chinese scientists on the Internet. We will see in the next chapter that this alleged ‘genetic sequence’ has never been produced by China or anyone and cannot be when there *is no* SARS-CoV-2. Drosten, however, doesn’t seem to let little details like that get in the way. He was the lead author with Victor Corman from the same Charité Hospital of the paper ‘Detection of 2019 novel coronavirus (2019-nCoV) by real-time PCR‘ published in a magazine called *Eurosurveillance*. This became known as the Corman-Drosten paper. In November, 2020, with human society devastated by the effects of the Corman-Drosten test baloney, the protocol was publicly challenged by 22 international scientists and independent researchers from Europe, the United States, and Japan. Among them were senior molecular geneticists, biochemists, immunologists, and microbiologists. They produced a document headed ‘External peer review of the RTPCR test to detect SARS-Cov-2 Reveals 10 Major Flaws At The

Molecular and Methodological Level: Consequences For False-Positive Results'. The flaws in the Corman-Drosten test included the following:

- The test is non-specific because of erroneous design
- Results are enormously variable
- The test is unable to discriminate between the whole 'virus' and viral fragments
- It doesn't have positive or negative controls
- The test lacks a standard operating procedure
- It is unsupported by proper peer view

The scientists said the PCR 'Covid' testing protocol was not founded on science and they demanded the Corman-Drosten paper be retracted by *Eurosurveillance*. They said all present and previous Covid deaths, cases, and 'infection rates' should be subject to a massive retroactive inquiry. Lockdowns and travel restrictions should be reviewed and relaxed and those diagnosed through PCR to have 'Covid-19' should not be forced to isolate. Dr Kevin Corbett, a health researcher and nurse educator with a long academic career producing a stream of peer-reviewed publications at many UK universities, made the same point about the PCR test debacle. He said of the scientists' conclusions: 'Every scientific rationale for the development of that test has been totally destroyed by this paper. It's like Hiroshima/Nagasaki to the Covid test.' He said that China hadn't given them an isolated 'virus' when Drosten developed the test. Instead they had developed the test from *a sequence in a gene bank*.' Put another way … *they made it up*! The scientists were supported in this contention by a Portuguese appeals court which ruled in November, 2020, that PCR tests are unreliable and it is unlawful to quarantine people based solely on a PCR test. The point about China not providing an isolated virus must be true when the 'virus' has never been isolated to this day and the consequences of that will become clear. Drosten and company produced this useless 'protocol' right on cue in January, 2020, just as the 'virus' was said to be moving westward and it somehow managed to successfully pass a peer-review in 24 hours. In other words there was no peer-review for a test that would be used to decide who had 'Covid' and who didn't across the world. The Cult-created, Gates-controlled World Health Organization immediately

recommended all its nearly 200 member countries to use the Drosten PCR protocol to detect ‘cases’ and ‘deaths’. The sting was underway and it continues to this day.

So who is this Christian Drosten that produced the means through which death, destruction and economic catastrophe would be justified? His education background, including his doctoral thesis, would appear to be somewhat shrouded in mystery and his track record is dire as with another essential player in the ‘Covid’ hoax, the Gates-funded Professor Neil Ferguson at the Gates-funded Imperial College in London of whom more shortly. Drosten predicted in 2003 that the alleged original SARS ‘virus’ (SARS-1’) was an epidemic that could have serious effects on economies and an effective vaccine would take at least two years to produce. Drosten’s answer to every alleged ‘outbreak’ is a vaccine which you won’t be shocked to know. What followed were just 774 official deaths worldwide and none in Germany where there were only nine cases. That is even if you believe there ever was a SARS ‘virus’ when the evidence is zilch and I will expand on this in the next chapter. Drosten claims to be co-discoverer of ‘SARS-1’ and developed a test for it in 2003. He was screaming warnings about ‘swine flu’ in 2009 and how it was a widespread infection far more severe than any dangers from a vaccine could be and people should get vaccinated. It would be helpful for Drosten’s vocal chords if he simply recorded the words ‘the virus is deadly and you need to get vaccinated’ and copies could be handed out whenever the latest made-up threat comes along. Drosten’s swine flu epidemic never happened, but Big Pharma didn’t mind with governments spending hundreds of millions on vaccines that hardly anyone bothered to use and many who did wished they hadn’t. A study in 2010 revealed that the risk of dying from swine flu, or H1N1, was no higher than that of the annual seasonal flu which is what at least most of ‘it’ really was as in the case of ‘Covid-19’. A media investigation into Drosten asked how with such a record of inaccuracy he could be *the* government adviser on these issues. The answer to that question is the same with Drosten, Ferguson and Fauci – they keep on giving the authorities the ‘conclusions’ and ‘advice’ they want to hear. Drosten certainly produced the goods for them in January, 2020, with his PCR protocol garbage and provided the foundation of what German internal medicine specialist Dr Claus Köhnlein, co-author of *Virus Mania*, called the ‘test pandemic’. The 22 scientists in the *Eurosurveillance* challenge called out conflicts of interest within the

Drosten 'protocol' group and with good reason. Olfert Landt, a regular co-author of Drosten 'studies', owns the biotech company TIB Molbiol Syntheselabor GmbH in Berlin which manufactures and sells the tests that Drosten and his mates come up with. They have done this with SARS, Enterotoxigenic E. coli (ETEC), MERS, Zika 'virus', yellow fever, and now 'Covid'. Landt told the *Berliner Zeitung* newspaper:

> The testing, design and development came from the Charité [Drosten and Corman]. We simply implemented it immediately in the form of a kit. And if we don't have the virus, which originally only existed in Wuhan, we can make a synthetic gene to simulate the genome of the virus. That's what we did very quickly.

This is more confirmation that the Drosten test was designed without access to the 'virus' and only a synthetic simulation which is what SARS-CoV-2 really is – a computer-generated synthetic fiction. It's quite an enterprise they have going here. A Drosten team decides what the test for something should be and Landt's biotech company flogs it to governments and medical systems across the world. His company must have made an absolute fortune since the 'Covid' hoax began. Dr Reiner Fuellmich, a prominent German consumer protection trial lawyer in Germany and California, is on Drosten's case and that of Tedros at the World Health Organization for crimes against humanity with a class-action lawsuit being prepared in the United States and other legal action in Germany.

Why China?

Scamming the world with a 'virus' that doesn't exist would seem impossible on the face of it, but not if you have control of the relatively few people that make policy decisions and the great majority of the global media. Remember it's not about changing 'real' reality it's about controlling *perception* of reality. You don't have to make something happen you only have make people *believe* that it's happening. Renegade Minds understand this and are therefore much harder to swindle. 'Covid-19' is not a 'real' 'virus'. It's a mind virus, like a computer virus, which has infected the minds, not the bodies, of billions. It all started, publically at least, in China and that alone is of central significance. The Cult was behind the revolution led by its asset Mao Zedong, or Chairman Mao, which established the

People's Republic of China on October 1st, 1949. It should have been called The Cult's Republic of China, but the name had to reflect the recurring illusion that vicious dictatorships are run by and for the people (see all the 'Democratic Republics' controlled by tyrants). In the same way we have the 'Biden' Democratic Republic of America officially ruled by a puppet tyrant (at least temporarily) on behalf of Cult tyrants. The creation of Mao's merciless communist/fascist dictatorship was part of a frenzy of activity by the Cult at the conclusion of World War Two which, like the First World War, it had instigated through its assets in Germany, Britain, France, the United States and elsewhere. Israel was formed in 1948; the Soviet Union expanded its 'Iron Curtain' control, influence and military power with the Warsaw Pact communist alliance in 1955; the United Nations was formed in 1945 as a Cult precursor to world government; and a long list of world bodies would be established including the World Health Organization (1948), World Trade Organization (1948 under another name until 1995), International Monetary Fund (1945) and World Bank (1944). Human society was redrawn and hugely centralised in the global Problem-Reaction-Solution that was World War Two. All these changes were significant. Israel would become the headquarters of the Sabbatians and the revolution in China would prepare the ground and control system for the events of 2019/2020.

Renegade Minds know there are no borders except for public consumption. The Cult is a seamless, borderless global entity and to understand the game we need to put aside labels like borders, nations, countries, communism, fascism and democracy. These delude the population into believing that countries are ruled within their borders by a government of whatever shade when these are mere agencies of a global power. America's illusion of democracy and China's communism/fascism are subsidiaries – vehicles – for the same agenda. We may hear about conflict and competition between America and China and on the lower levels that will be true; but at the Cult level they are branches of the same company in the way of the McDonald's example I gave earlier. I have tracked in the books over the years support by US governments of both parties for Chinese Communist Party infiltration of American society through allowing the sale of land, even military facilities, and the acquisition of American business and university influence. All this is underpinned by the infamous stealing of intellectual property and

technological know-how. Cult-owned Silicon Valley corporations waive their fraudulent 'morality' to do business with human-rights-free China; Cult-controlled Disney has become China's PR department; and China in effect owns 'American' sports such as basketball which depends for much of its income on Chinese audiences. As a result any sports player, coach or official speaking out against China's horrific human rights record is immediately condemned or fired by the China-worshipping National Basketball Association. One of the first acts of China-controlled Biden was to issue an executive order telling federal agencies to stop making references to the 'virus' by the 'geographic location of its origin'. Long-time Congressman Jerry Nadler warned that criticising China, America's biggest rival, leads to hate crimes against Asian people in the United States. So shut up you bigot. China is fast closing in on Israel as a country that must not be criticised which is apt, really, given that Sabbatians control them both. The two countries have developed close economic, military, technological and strategic ties which include involvement in China's 'Silk Road' transport and economic initiative to connect China with Europe. Israel was the first country in the Middle East to recognise the establishment of Mao's tyranny in 1950 months after it was established.

Project Wuhan – the 'Covid' Psyop

I emphasise again that the Cult plays the long game and what is happening to the world today is the result of centuries of calculated manipulation following a script to take control step-by-step of every aspect of human society. I will discuss later the common force behind all this that has spanned those centuries and thousands of years if the truth be told. Instigating the Mao revolution in China in 1949 with a 2020 'pandemic' in mind is not only how they work – the 71 years between them is really quite short by the Cult's standards of manipulation preparation. The reason for the Cult's Chinese revolution was to create a fiercely-controlled environment within which an extreme structure for human control could be incubated to eventually be unleashed across the world. We have seen this happen since the 'pandemic' emerged from China with the Chinese control-structure founded on AI technology and tyrannical enforcement sweep across the West. Until the moment when the Cult went for broke in the West and put its fascism on public display Western governments had to pay some

lip-service to freedom and democracy to not alert too many people to the tyranny-in-the-making. Freedoms were more subtly eroded and power centralised with covert government structures put in place waiting for the arrival of 2020 when that smokescreen of 'freedom' could be dispensed with. The West was not able to move towards tyranny before 2020 anything like as fast as China which was created as a tyranny and had no limits on how fast it could construct the Cult's blueprint for global control. When the time came to impose that structure on the world it was the same Cult-owned Chinese communist/fascist government that provided the excuse – the 'Covid pandemic'. It was absolutely crucial to the Cult plan for the Chinese response to the 'pandemic' – draconian lockdowns of the entire population – to become the blueprint that Western countries would follow to destroy the livelihoods and freedom of their people. This is why the Cult-owned, Gates-owned, WHO Director-General Tedros said early on:

The Chinese government is to be congratulated for the extraordinary measures it has taken to contain the outbreak. China is actually setting a new standard for outbreak response and it is not an exaggeration.

Forbes magazine said of China: '… those measures protected untold millions from getting the disease'. The Rockefeller Foundation 'epidemic scenario' document in 2010 said 'prophetically':

However, a few countries did fare better – China in particular. The Chinese government's quick imposition and enforcement of mandatory quarantine for all citizens, as well as its instant and near-hermetic sealing off of all borders, saved millions of lives, stopping the spread of the virus far earlier than in other countries and enabling a swifter post-pandemic recovery.

Once again – *spooky*.

The first official story was the 'bat theory' or rather the bat diversion. The source of the 'virus outbreak' we were told was a ''wet market' in Wuhan where bats and other animals are bought and eaten in horrifically unhygienic conditions. Then another story emerged through the alternative media that the 'virus' had been released on purpose or by accident from a BSL-4 (biosafety level 4) laboratory in Wuhan not far from the wet market. The lab was reported to create and work with lethal concoctions and

bioweapons. Biosafety level 4 is the highest in the World Health Organization system of safety and containment. Renegade Minds are aware of what I call designer manipulation. The ideal for the Cult is for people to buy its prime narrative which in the opening salvoes of the 'pandemic' was the wet market story. It knows, however, that there is now a considerable worldwide alternative media of researchers sceptical of anything governments say and they are often given a version of events in a form they can perceive as credible while misdirecting them from the real truth. In this case let them think that the conspiracy involved is a 'bioweapon virus' released from the Wuhan lab to keep them from the real conspiracy – *there is no 'virus'*. The WHO's current position on the source of the outbreak at the time of writing appears to be: 'We haven't got a clue, mate.' This is a good position to maintain mystery and bewilderment. The inner circle will know where the 'virus' came from – *nowhere*. The bottom line was to ensure the public believed there *was* a 'virus' and it didn't much matter if they thought it was natural or had been released from a lab. The belief that there was a 'deadly virus' was all that was needed to trigger global panic and fear. The population was terrified into handing their power to authority and doing what they were told. They had to or they were 'all gonna die'.

In March, 2020, information began to come my way from real doctors and scientists and my own additional research which had my intuition screaming: 'Yes, that's it! *There is no virus*.' The 'bioweapon' was not the 'virus'; it was the *'vaccine'* already being talked about that would be the bioweapon. My conclusion was further enhanced by happenings in Wuhan. The 'virus' was said to be sweeping the city and news footage circulated of people collapsing in the street (which they've never done in the West with the same 'virus'). The Chinese government was building 'new hospitals' in a matter of ten days to 'cope with demand' such was the virulent nature of the 'virus'. Yet in what seemed like no time the 'new hospitals' closed – even if they even opened – and China declared itself 'virus-free'. It was back to business as usual. This was more propaganda to promote the Chinese draconian lockdowns in the West as the way to 'beat the virus'. Trouble was that we subsequently had lockdown after lockdown, but never business as usual. As the people of the West and most of the rest of the world were caught in an ever-worsening spiral of lockdown, social distancing, masks, isolated old people, families forced apart, and livelihood destruction, it was party-time in Wuhan. Pictures emerged of thousands of

people enjoying pool parties and concerts. It made no sense until you realised there never was a ‘virus’ and the whole thing was a Cult set-up to transform human society out of one its major global strongholds – China.

How is it possible to deceive virtually the entire world population into believing there is a deadly virus when there is not even a ‘virus’ let alone a deadly one? It’s nothing like as difficult as you would think and that’s clearly true because it happened.

Postscript: See end of book Postscript for more on the ‘Wuhan lab virus release’ story which the authorities and media were pushing heavily in the summer of 2021 to divert attention from the truth that the ‘Covid virus’ is pure invention.

CHAPTER FIVE

There *is no* 'virus'

You can fool some of the people all of the time, and all of the people some of the time, but you cannot fool all of the people all of the time
Abraham Lincoln

The greatest form of mind control is repetition. The more you repeat the same mantra of alleged 'facts' the more will accept them to be true. It becomes an 'everyone knows that, mate'. If you can also censor any other version or alternative to your alleged 'facts' you are pretty much home and cooking.

By the start of 2020 the Cult owned the global mainstream media almost in its entirety to spew out its 'Covid' propaganda and ignore or discredit any other information and view. Cult-owned social media platforms in Cult-owned Silicon Valley were poised and ready to unleash a campaign of ferocious censorship to obliterate all but the official narrative. To complete the circle many demands for censorship by Silicon Valley were led by the mainstream media as 'journalists' became full-out enforcers for the Cult both as propagandists and censors. Part of this has been the influx of young people straight out of university who have become 'journalists' in significant positions. They have no experience and a headful of programmed perceptions from their years at school and university at a time when today's young are the most perceptually-targeted generations in known human history given the insidious impact of technology. They enter the media perceptually prepared and ready to repeat the narratives of the system that programmed them to repeat its narratives. The BBC has a truly

pathetic ‘specialist disinformation reporter’ called Marianna Spring who fits this bill perfectly. She is clueless about the world, how it works and what is really going on. Her role is to discredit anyone doing the job that a proper journalist would do and system-serving hacks like Spring wouldn’t dare to do or even see the need to do. They are too busy licking the arse of authority which can never be wrong and, in the case of the BBC propaganda programme, *Panorama*, contacting payments systems such as PayPal to have a donations page taken down for a film company making documentaries questioning vaccines. Even the BBC soap opera *EastEnders* included a disgracefully biased scene in which an inarticulate white working class woman was made to look foolish for questioning the ‘vaccine’ while a well-spoken black man and Asian woman promoted the government narrative. It ticked every BBC box and the fact that the black and minority community was resisting the ‘vaccine’ had nothing to do with the way the scene was written. The BBC has become a disgusting tyrannical propaganda and censorship operation that should be defunded and disbanded and a free media take its place with a brief to stop censorship instead of demanding it. A BBC ‘interview’ with Gates goes something like: ‘Mr Gates, sir, if I can call you sir, would you like to tell our audience why you are such a great man, a wonderful humanitarian philanthropist, and why you should absolutely be allowed as a software salesman to decide health policy for approaching eight billion people? Thank you, sir, please sir.’ Propaganda programming has been incessant and merciless and when all you hear is the same story from the media, repeated by those around you who have only heard the same story, is it any wonder that people on a grand scale believe absolute mendacious garbage to be true? You are about to see, too, why this level of information control is necessary when the official ‘Covid’ narrative is so nonsensical and unsupportable by the evidence.

Structure of Deceit

The pyramid structure through which the ‘Covid’ hoax has been manifested is very simple and has to be to work. As few people as possible have to be involved with full knowledge of what they are doing – and why – or the real story would get out. At the top of the pyramid are the inner core of the Cult which controls Bill Gates who, in turn, controls the World Health Organization through his pivotal funding and his puppet Director-General

mouthpiece, Tedros. Before he was appointed Tedros was chair of the Gates-founded Global Fund to 'fight against AIDS, tuberculosis and malaria', a board member of the Gates-funded 'vaccine alliance' GAVI, and on the board of another Gates-funded organisation. Gates owns him and picked him for a specific reason – Tedros is a crook and worse. 'Dr' Tedros (he's not a medical doctor, the first WHO chief not to be) was a member of the tyrannical Marxist government of Ethiopia for decades with all its human rights abuses. He has faced allegations of corruption and misappropriation of funds and was exposed three times for covering up cholera epidemics while Ethiopia's health minister. Tedros appointed the mass-murdering genocidal Zimbabwe dictator Robert Mugabe as a WHO goodwill ambassador for public health which, as with Tedros, is like appointing a psychopath to run a peace and love campaign. The move was so ridiculous that he had to drop Mugabe in the face of widespread condemnation. American economist David Steinman, a Nobel peace prize nominee, lodged a complaint with the International Criminal Court in The Hague over alleged genocide by Tedros when he was Ethiopia's foreign minister. Steinman says Tedros was a 'crucial decision maker' who directed the actions of Ethiopia's security forces from 2013 to 2015 and one of three officials in charge when those security services embarked on the 'killing' and 'torturing' of Ethiopians. You can see where Tedros is coming from and it's sobering to think that he has been the vehicle for Gates and the Cult to direct the global response to 'Covid'. Think about that. A psychopathic Cult dictates to psychopath Gates who dictates to psychopath Tedros who dictates how countries of the world must respond to a 'Covid virus' never scientifically shown to exist. At the same time psychopathic Cult-owned Silicon Valley information giants like Google, YouTube, Facebook and Twitter announced very early on that they would give the Cult/Gates/Tedros/WHO version of the narrative free advertising and censor those who challenged their intelligence-insulting, mendacious story.

The next layer in the global 'medical' structure below the Cult, Gates and Tedros are the chief medical officers and science 'advisers' in each of the WHO member countries which means virtually all of them. Medical officers and arbiters of science (they're not) then take the WHO policy and recommended responses and impose them on their country's population while the political 'leaders' say they are deciding policy (they're clearly not) by 'following the science' on the advice of the 'experts' – the same

medical officers and science ‘advisers’ (dictators). In this way with the rarest of exceptions the entire world followed the same policy of lockdown, people distancing, masks and ‘vaccines’ dictated by the psychopathic Cult, psychopathic Gates and psychopathic Tedros who we are supposed to believe give a damn about the health of the world population they are seeking to enslave. That, amazingly, is all there is to it in terms of crucial decision-making. Medical staff in each country then follow like sheep the dictates of the shepherds at the top of the national medical hierarchies – chief medical officers and science ‘advisers’ who themselves follow like sheep the shepherds of the World Health Organization and the Cult. Shepherds at the national level often have major funding and other connections to Gates and his Bill and Melinda Gates Foundation which carefully hands out money like confetti at a wedding to control the entire global medical system from the WHO down.

Follow the money

Christopher Whitty, Chief Medical Adviser to the UK Government at the centre of ‘virus’ policy, a senior adviser to the government’s Scientific Advisory Group for Emergencies (SAGE), and Executive Board member of the World Health Organization, was gifted a grant of $40 million by the Bill and Melinda Gates Foundation for malaria research in Africa. The BBC described the unelected Whitty as ‘the official who will probably have the greatest impact on our everyday lives of any individual policymaker in modern times’ and so it turned out. What Gates and Tedros have said Whitty has done like his equivalents around the world. Patrick Vallance, co-chair of SAGE and the government’s Chief Scientific Adviser, is a former executive of Big Pharma giant GlaxoSmithKline with its fundamental financial and business connections to Bill Gates. In September, 2020, it was revealed that Vallance owned a deferred bonus of shares in GlaxoSmithKline worth £600,000 while the company was ‘developing’ a ‘Covid vaccine’. Move along now – nothing to see here – what could possibly be wrong with that? Imperial College in London, a major player in ‘Covid’ policy in Britain and elsewhere with its ‘Covid-19’ Response Team, is funded by Gates and has big connections to China while the now infamous Professor Neil Ferguson, the useless ‘computer modeller’ at Imperial College is also funded by Gates. Ferguson delivered the

dramatically inaccurate excuse for the first lockdowns (much more in the next chapter). The Institute for Health Metrics and Evaluation (IHME) in the United States, another source of outrageously false 'Covid' computer models to justify lockdowns, is bankrolled by Gates who is a vehement promotor of lockdowns. America's version of Whitty and Vallance, the again now infamous Anthony Fauci, has connections to 'Covid vaccine' maker Moderna as does Bill Gates through funding from the Bill and Melinda Gates Foundation. Fauci is director of the National Institute of Allergy and Infectious Diseases (NIAID), a major recipient of Gates money, and they are very close. Deborah Birx who was appointed White House Coronavirus Response Coordinator in February, 2020, is yet another with ties to Gates. Everywhere you look at the different elements around the world behind the coordination and decision making of the 'Covid' hoax there is Bill Gates and his money. They include the World Health Organization; Centers for Disease Control (CDC) in the United States; National Institutes of Health (NIH) of Anthony Fauci; Imperial College and Neil Ferguson; the London School of Hygiene where Chris Whitty worked; Regulatory agencies like the UK Medicines & Healthcare products Regulatory Agency (MHRA) which gave emergency approval for 'Covid vaccines'; Wellcome Trust; GAVI, the Vaccine Alliance; the Coalition for Epidemic Preparedness Innovations (CEPI); Johns Hopkins University which has compiled the false 'Covid' figures; and the World Economic Forum. A Nationalfile.com article said:

> Gates has a lot of pull in the medical world, he has a multi-million dollar relationship with Dr. Fauci, and Fauci originally took the Gates line supporting vaccines and casting doubt on [the drug hydroxychloroquine]. Coronavirus response team member Dr. Deborah Birx, appointed by former president Obama to serve as United States Global AIDS Coordinator, also sits on the board of a group that has received billions from Gates' foundation, and Birx reportedly used a disputed Bill Gates-funded model for the White House's Coronavirus effort. Gates is a big proponent for a population lockdown scenario for the Coronavirus outbreak.

Another funder of Moderna is the Defense Advanced Research Projects Agency (DARPA), the technology-development arm of the Pentagon and one of the most sinister organisations on earth. DARPA had a major role with the CIA covert technology-funding operation In-Q-Tel in the development of Google and social media which is now at the centre of

global censorship. Fauci and Gates are extremely close and openly admit to talking regularly about 'Covid' policy, but then why wouldn't Gates have a seat at every national 'Covid' table after his Foundation committed $1.75 billion to the 'fight against Covid-19'. When passed through our Orwellian Translation Unit this means that he has bought and paid for the Cult-driven 'Covid' response worldwide. Research the major 'Covid' response personnel in your own country and you will find the same Gates funding and other connections again and again. Medical and science chiefs following World Health Organization 'policy' sit atop a medical hierarchy in their country of administrators, doctors and nursing staff. These 'subordinates' are told they must work and behave in accordance with the policy delivered from the 'top' of the national 'health' pyramid which is largely the policy delivered by the WHO which is the policy delivered by Gates and the Cult. The whole 'Covid' narrative has been imposed on medical staff by a climate of fear although great numbers don't even need that to comply. They do so through breathtaking levels of ignorance and include doctors who go through life simply repeating what Big Pharma and their hierarchical masters tell them to say and believe. No wonder Big Pharma 'medicine' is one of the biggest killers on Planet Earth.

The same top-down system of intimidation operates with regard to the Cult Big Pharma cartel which also dictates policy through national and global medical systems in this way. The Cult and Big Pharma agendas are the same because the former controls and owns the latter. 'Health' administrators, doctors, and nursing staff are told to support and parrot the dictated policy or they will face consequences which can include being fired. How sad it's been to see medical staff meekly repeating and imposing Cult policy without question and most of those who can see through the deceit are only willing to speak anonymously off the record. They know what will happen if their identity is known. This has left the courageous few to expose the lies about the 'virus', face masks, overwhelmed hospitals that aren't, and the dangers of the 'vaccine' that isn't a vaccine. When these medical professionals and scientists, some renowned in their field, have taken to the Internet to expose the truth their articles, comments and videos have been deleted by Cult-owned Facebook, Twitter and YouTube. What a real head-shaker to see YouTube videos with leading world scientists and highly qualified medical specialists with an added link underneath to the

notorious Cult propaganda website *Wikipedia* to find the 'facts' about the same subject.

HIV – the 'Covid' trial-run

I'll give you an example of the consequences for health and truth that come from censorship and unquestioning belief in official narratives. The story was told by PCR inventor Kary Mullis in his book *Dancing Naked in the Mind Field*. He said that in 1984 he accepted as just another scientific fact that Luc Montagnier of France's Pasteur Institute and Robert Gallo of America's National Institutes of Health had independently discovered that a 'retrovirus' dubbed HIV (human immunodeficiency virus) caused AIDS. They were, after all, Mullis writes, specialists in retroviruses. This is how the medical and science pyramids work. Something is announced or *assumed* and then becomes an everybody-knows-that purely through repetition of the assumption as if it is fact. Complete crap becomes accepted truth with no supporting evidence and only repetition of the crap. This is how a 'virus' that doesn't exist became the 'virus' that changed the world. The HIV-AIDS fairy story became a multi-billion pound industry and the media poured out propaganda terrifying the world about the deadly HIV 'virus' that caused the lethal AIDS. By then Mullis was working at a lab in Santa Monica, California, to detect retroviruses with his PCR test in blood donations received by the Red Cross. In doing so he asked a virologist where he could find a reference for HIV being the cause of AIDS. 'You don't need a reference,' the virologist said … *'Everybody knows it.'* Mullis said he wanted to quote a reference in the report he was doing and he said he felt a little funny about not knowing the source of such an important discovery when everyone else seemed to. The virologist suggested he cite a report by the Centers for Disease Control and Prevention (CDC) on morbidity and mortality. Mullis read the report, but it only said that an organism had been identified and did not say how. The report did not identify the original scientific work. Physicians, however, *assumed* (key recurring theme) that if the CDC was convinced that HIV caused AIDS then proof must exist. Mullis continues:

I did computer searches. Neither Montagnier, Gallo, nor anyone else had published papers describing experiments which led to the conclusion that HIV probably caused AIDS. I read the papers in

Science for which they had become well known as AIDS doctors, but all they had said there was that they had found evidence of a past infection by something which was probably HIV in some AIDS patients.

They found antibodies. Antibodies to viruses had always been considered evidence of past disease, not present disease. Antibodies signaled that the virus had been defeated. The patient had saved himself. There was no indication in these papers that this virus caused a disease. They didn't show that everybody with the antibodies had the disease. In fact they found some healthy people with antibodies.

Mullis asked why their work had been published if Montagnier and Gallo hadn't really found this evidence, and why had they been fighting so hard to get credit for the discovery? He says he was hesitant to write 'HIV is the probable cause of AIDS' until he found published evidence to support that. 'Tens of thousands of scientists and researchers were spending billions of dollars a year doing research based on this idea,' Mullis writes. 'The reason had to be there somewhere; otherwise these people would not have allowed their research to settle into one narrow channel of investigation.' He said he lectured about PCR at numerous meetings where people were always talking about HIV and he asked them how they knew that HIV was the cause of AIDS:

Everyone said something. Everyone had the answer at home, in the office, in some drawer. They all knew, and they would send me the papers as soon as they got back. But I never got any papers. Nobody ever sent me the news about how AIDS was caused by HIV.

Eventually Mullis was able to ask Montagnier himself about the reference proof when he lectured in San Diego at the grand opening of the University of California AIDS Research Center. Mullis says this was the last time he would ask his question without showing anger. Montagnier said he should reference the CDC report. 'I read it', Mullis said, and it didn't answer the question. 'If Montagnier didn't know the answer who the hell did?' Then one night Mullis was driving when an interview came on National Public Radio with Peter Duesberg, a prominent virologist at Berkeley and a California Scientist of the Year. Mullis says he finally understood why he could not find references that connected HIV to AIDS – *there weren't any!* No one had ever proved that HIV causes AIDS even though it had spawned a multi-billion pound global industry and the media was repeating this as

fact every day in their articles and broadcasts terrifying the shit out of people about AIDS and giving the impression that a positive test for HIV (see 'Covid') was a death sentence. Duesberg was a threat to the AIDS gravy train and the agenda that underpinned it. He was therefore abused and castigated after he told the Proceedings of the National Academy of Sciences there was no good evidence implicating the new 'virus'. Editors rejected his manuscripts and his research funds were deleted. Mullis points out that the CDC has defined AIDS as one of more than 30 diseases *if accompanied* by a positive result on a test that detects antibodies to HIV; but those same diseases are not defined as AIDS cases when antibodies are not detected:

> If an HIV-positive woman develops uterine cancer, for example, she is considered to have AIDS. If she is not HIV positive, she simply has uterine cancer. An HIV-positive man with tuberculosis has AIDS; if he tests negative he simply has tuberculosis. If he lives in Kenya or Colombia, where the test for HIV antibodies is too expensive, he is simply presumed to have the antibodies and therefore AIDS, and therefore he can be treated in the World Health Organization's clinic. It's the only medical help available in some places. And it's free, because the countries that support WHO are worried about AIDS.

Mullis accuses the CDC of continually adding new diseases (see ever more 'Covid symptoms') to the grand AIDS definition and of virtually doctoring the books to make it appear as if the disease continued to spread. He cites how in 1993 the CDC enormously broadened its AIDS definition and county health authorities were delighted because they received $2,500 per year from the Federal government for every reported AIDS case. Ladies and gentlemen, I have just described, via Kary Mullis, the 'Covid pandemic' of 2020 and beyond. Every element is the same and it's been pulled off in the same way by the same networks.

The 'Covid virus' exists? Okay – prove it. Er … still waiting

What Kary Mullis described with regard to 'HIV' has been repeated with 'Covid'. A claim is made that a new, or 'novel', infection has been found and the entire medical system of the world repeats that as fact exactly as they did with HIV and AIDS. No one in the mainstream asks rather relevant questions such as 'How do you know?' and 'Where is your proof?' The

SARS-Cov-2 ‘virus’ and the ‘Covid-19 disease’ became an overnight ‘everybody-knows-that’. The origin could be debated and mulled over, but what you could not suggest was that ‘SARS-Cov-2’ didn’t exist. That would be ridiculous. ‘Everybody knows’ the ‘virus’ exists. Well, I didn’t for one along with American proper doctors like Andrew Kaufman and Tom Cowan and long-time American proper journalist Jon Rappaport. We dared to pursue the obvious and simple question: ‘Where’s the evidence?’ The overwhelming majority in medicine, journalism and the general public did not think to ask that. After all, *everyone knew* there was a new ‘virus’. Everyone was saying so and I heard it on the BBC. Some would eventually argue that the ‘deadly virus’ was nothing like as deadly as claimed, but few would venture into the realms of its very existence. Had they done so they would have found that the evidence for that claim had gone AWOL as with HIV causes AIDS. In fact, not even that. For something to go AWOL it has to exist in the first place and scientific proof for a ‘SARS-Cov-2’ can be filed under nothing, nowhere and zilch.

Dr Andrew Kaufman is a board-certified forensic psychiatrist in New York State, a Doctor of Medicine and former Assistant Professor and Medical Director of Psychiatry at SUNY Upstate Medical University, and Medical Instructor of Hematology and Oncology at the Medical School of South Carolina. He also studied biology at the Massachusetts Institute of Technology (MIT) and trained in Psychiatry at Duke University. Kaufman is retired from allopathic medicine, but remains a consultant and educator on natural healing, I saw a video of his very early on in the ‘Covid’ hoax in which he questioned claims about the ‘virus’ in the absence of any supporting evidence and with plenty pointing the other way. I did everything I could to circulate his work which I felt was asking the pivotal questions that needed an answer. I can recommend an excellent pull-together interview he did with the website The Last Vagabond entitled *Dr Andrew Kaufman: Virus Isolation, Terrain Theory and Covid-19* and his website is andrewkaufmanmd.com. Kaufman is not only a forensic psychiatrist; he is forensic in all that he does. He always reads original scientific papers, experiments and studies instead of second-third-fourth-hand reports about the ‘virus’ in the media which are repeating the repeated repetition of the narrative. When he did so with the original Chinese ‘virus’ papers Kaufman realised that there was no evidence of a ‘SARS-Cov-2’. They had never – from the start – shown it to exist and every repeat of this

claim worldwide was based on the accepted existence of proof that was nowhere to be found – see Kary Mullis and HIV. Here we go again.

Let's postulate

Kaufman discovered that the Chinese authorities immediately concluded that the cause of an illness that broke out among about 200 initial patients in Wuhan was a 'new virus' when there were no grounds to make that conclusion. The alleged 'virus' was not isolated from other genetic material in their samples and then shown through a system known as Koch's postulates to be the causative agent of the illness. The world was told that the SARS-Cov-2 'virus' caused a disease they called 'Covid-19' which had 'flu-like' symptoms and could lead to respiratory problems and pneumonia. If it wasn't so tragic it would almost be funny. *'Flu-like' symptoms'? Pneumonia? Respiratory disease?* What in *CHINA* and particularly in *Wuhan*, one of the most polluted cities in the world with a resulting epidemic of respiratory disease?? Three hundred thousand people get pneumonia in China every year and there are nearly a billion cases worldwide of 'flu-like symptoms'. These have a whole range of causes – including pollution in Wuhan – but no other possibility was credibly considered in late 2019 when the world was told there was a new and deadly 'virus'. The global prevalence of pneumonia and 'flu-like systems' gave the Cult networks unlimited potential to re-diagnose these other causes as the mythical 'Covid-19' and that is what they did from the very start. Kaufman revealed how Chinese medical and science authorities (all subordinates to the Cult-owned communist government) took genetic material from the lungs of only a few of the first patients. The material contained their own cells, bacteria, fungi and other microorganisms living in their bodies. The only way you could prove the existence of the 'virus' and its responsibility for the alleged 'Covid-19' was to isolate the virus from all the other material – a process also known as 'purification' – and then follow the postulates sequence developed in the late 19th century by German physician and bacteriologist Robert Koch which became the 'gold standard' for connecting an alleged causation agent to a disease:

1. The microorganism (bacteria, fungus, virus, etc.) must be present in every case of the disease and all patients must have the same symptoms. It must also *not be present in healthy individuals*.

2. The microorganism must be isolated from the host with the disease. If the microorganism is a bacteria or fungus it must be grown in a pure culture. If it is a virus, it must be purified (i.e. containing no other material except the virus particles) from a clinical sample.

3. The specific disease, with all of its characteristics, must be reproduced when the infectious agent (the purified virus or a pure culture of bacteria or fungi) is inoculated into a healthy, susceptible host.

4. The microorganism must be recoverable from the experimentally infected host as in step 2.

Not one of these criteria has been met in the case of 'SARS-Cov-2' and 'Covid-19'. Not ONE. *EVER*. Robert Koch refers to bacteria and not viruses. What are called 'viral particles' are so minute (hence masks are useless by any definition) that they could only be seen after the invention of the electron microscope in the 1930s and can still only be observed through that means. American bacteriologist and virologist Thomas Milton Rivers, the so-called 'Father of Modern Virology' who was very significantly director of the Rockefeller Institute for Medical Research in the 1930s, developed a less stringent version of Koch's postulates to identify 'virus' causation known as 'Rivers criteria'. 'Covid' did not pass that process either. Some even doubt whether any 'virus' can be isolated from other particles containing genetic material in the Koch method. Freedom of Information requests in many countries asking for scientific proof that the 'Covid virus' has been purified and isolated and shown to exist have all come back with a 'we don't have that' and when this happened with a request to the UK Department of Health they added this comment:

However, outside of the scope of the [Freedom of Information Act] and on a discretionary basis, the following information has been advised to us, which may be of interest. Most infectious diseases are caused by viruses, bacteria or fungi. Some bacteria or fungi have the capacity to grow on their own in isolation, for example in colonies on a petri dish. Viruses are different in that they are what we call 'obligate pathogens' – that is, they cannot survive or reproduce without infecting a host ...

… For some diseases, it is possible to establish causation between a microorganism and a disease by isolating the pathogen from a patient, growing it in pure culture and reintroducing it to a healthy organism. These are known as 'Koch's postulates' and were developed in 1882. However, as our understanding of disease and different disease-causing agents has advanced, these are no longer the method for determining causation [Andrew Kaufman asks why in that case are there two published articles falsely claiming to satisfy Koch's postulates].

It has long been known that viral diseases cannot be identified in this way as viruses cannot be grown in 'pure culture'. When a patient is tested for a viral illness, this is normally done by looking for the presence of antigens, or viral genetic code in a host with molecular biology techniques [Kaufman

asks how you could know the origin of these chemicals without having a pure culture for comparison].

For the record ‘antigens’ are defined so:

Invading microorganisms have antigens on their surface that the human body can recognise as being foreign – meaning not belonging to it. When the body recognises a foreign antigen, lymphocytes (white blood cells) produce antibodies, which are complementary in shape to the antigen.

Notwithstanding that this is open to question in relation to ‘SARS-Cov-2’ the presence of ‘antibodies’ can have many causes and they are found in people that are perfectly well. Kary Mullis said: ‘Antibodies … had always been considered evidence of past disease, not present disease.’

‘Covid’ really is a *computer* ‘virus’

Where the UK Department of Health statement says ‘viruses’ are now ‘diagnosed’ through a ‘viral genetic code in a host with molecular biology techniques’, they mean … *the PCR test* which its inventor said cannot test for infectious disease. They have no credible method of connecting a ‘virus’ to a disease and we will see that there is no scientific proof that any ‘virus’ causes any disease or there is any such thing as a ‘virus’ in the way that it is described. Tenacious Canadian researcher Christine Massey and her team made some 40 Freedom of Information requests to national public health agencies in different countries asking for proof that SARS-CoV-2 has been isolated and not one of them could supply that information. Massey said of her request in Canada: ‘Freedom of Information reveals Public Health Agency of Canada has no record of ‘SARS-COV-2’ isolation performed by anyone, anywhere, ever.’ If you accept the comment from the UK Department of Health it’s because they can’t isolate a ‘virus’. Even so many ‘science’ papers claimed to have isolated the ‘Covid virus’ until they were questioned and had to admit they hadn’t. A reply from the Robert Koch Institute in Germany was typical: ‘I am not aware of a paper which purified isolated SARS-CoV-2.’ So what the hell was Christian Drosten and his gang using to design the ‘Covid’ testing protocol that has produced all the illusory Covid’ cases and ‘Covid’ deaths when the head of the Chinese version of the CDC admitted there was a problem right from the start in that the ‘virus’ had never been isolated/purified? Breathe deeply: What they are calling ‘Covid’ is actually created by a *computer program* i.e. *they made it*

up – er, that's it. They took lung fluid, with many sources of genetic material, from one single person alleged to be infected with Covid-19 by a PCR test which they *claimed*, without clear evidence, contained a 'virus'. They used several computer programs to create a model of a theoretical virus genome sequence from more than fifty-six million small sequences of RNA, each of an unknown source, assembling them like a puzzle with no known solution. The computer filled in the gaps with sequences from bits in the gene bank to make it look like a bat SARS-like coronavirus! A wave of the magic wand and poof, an in silico (computer-generated) genome, a scientific fantasy, was created. UK health researcher Dr Kevin Corbett made the same point with this analogy:

> … It's like giving you a few bones and saying that's your fish. It could be any fish. Not even a skeleton. Here's a few fragments of bones. That's your fish … It's all from gene bank and the bits of the virus sequence that weren't there they made up.
>
> They synthetically created them to fill in the blanks. That's what genetics is; it's a code. So it's ABBBCCDDD and you're missing some what you think is EEE so you put it in. It's all synthetic. You just manufacture the bits that are missing. This is the end result of the geneticization of virology. This is basically a computer virus.

Further confirmation came in an email exchange between British citizen journalist Frances Leader and the government's Medicines & Healthcare Products Regulatory Agency (the Gates-funded MHRA) which gave emergency permission for untested 'Covid vaccines' to be used. The agency admitted that the 'vaccine' is not based on an isolated 'virus', but comes from a *computer-generated model*. Frances Leader was naturally banned from Cult-owned fascist Twitter for making this exchange public. The process of creating computer-generated alleged 'viruses' is called 'in silico' or 'in silicon' – computer chips – and the term 'in silico' is believed to originate with biological experiments using only a computer in 1989. 'Vaccines' involved with 'Covid' are also produced 'in silico' or by computer not a natural process. If the original 'virus' is nothing more than a made-up computer model how can there be 'new variants' of something that never existed in the first place? They are not new 'variants'; they are new *computer models* only minutely different to the original program and designed to further terrify the population into having the 'vaccine' and submitting to fascism. You want a 'new variant'? Click, click, enter – there

you go. Tell the medical profession that you have discovered a ‘South African variant’, ‘UK variants’ or a ‘Brazilian variant’ and in the usual HIV-causes-AIDS manner they will unquestioningly repeat it with no evidence whatsoever to support these claims. They will go on television and warn about the dangers of ‘new variants’ while doing nothing more than repeating what they have been told to be true and knowing that any deviation from that would be career suicide. Big-time insiders will know it’s a hoax, but much of the medical community is clueless about the way they are being played and themselves play the public without even being aware they are doing so. What an interesting ‘coincidence’ that AstraZeneca and Oxford University were conducting ‘Covid vaccine trials’ in the three countries – the UK, South Africa and Brazil – where the first three ‘variants’ were claimed to have ‘broken out’.

Here’s your ‘virus’ – it’s a unicorn

Dr Andrew Kaufman presented a brilliant analysis describing how the ‘virus’ was imagined into fake existence when he dissected an article published by *Nature* and written by 19 authors detailing *alleged* ‘sequencing of a complete viral genome’ of the ‘new SARS-CoV-2 virus’. This computer-modelled *in silico* genome was used as a template for all subsequent genome sequencing experiments that resulted in the so-called variants which he said now number more than *6,000*. The fake genome was constructed from more than 56 million individual short strands of RNA. Those little pieces were assembled into longer pieces by finding areas of overlapping sequences. The computer programs created over two million possible combinations from which the authors simply chose the longest one. They then compared this to a ‘bat virus’ and the computer ‘alignment’ rearranged the sequence and filled in the gaps! They called this computer-generated abomination the ‘complete genome’. Dr Tom Cowan, a fellow medical author and collaborator with Kaufman, said such computer-generation constitutes scientific fraud and he makes this superb analogy:

> Here is an equivalency: A group of researchers claim to have found a unicorn because they found a piece of a hoof, a hair from a tail, and a snippet of a horn. They then add that information into a computer and program it to re-create the unicorn, and they then claim this computer re-creation is the real unicorn. Of course, they had never actually seen a unicorn so could not possibly have examined its genetic makeup to compare their samples with the actual unicorn’s hair, hooves and horn.

The researchers claim they decided which is the real genome of SARS-CoV-2 by ‘consensus’, sort of like a vote. Again, different computer programs will come up with different versions of the imaginary ‘unicorn’, so they come together as a group and decide which is the real imaginary unicorn.

This is how the ‘virus’ that has transformed the world was brought into fraudulent ‘existence’. Extraordinary, yes, but as the Nazis said the bigger the lie the more will believe it. Cowan, however, wasn’t finished and he went on to identify what he called the real blockbuster in the paper. He quotes this section from a paper written by virologists and published by the CDC and then explains what it means:

Therefore, we examined the capacity of SARS-CoV-2 to infect and replicate in several common primate and human cell lines, including human adenocarcinoma cells (A549), human liver cells (HUH 7.0), and human embryonic kidney cells (HEK-293T). In addition to Vero E6 and Vero CCL81 cells. ... Each cell line was inoculated at high multiplicity of infection and examined 24h post-infection.

No CPE was observed in any of the cell lines except in Vero cells, which grew to greater than 10 to the 7th power at 24 h post-infection. In contrast, HUH 7.0 and 293T showed only modest viral replication, and A549 cells were incompatible with SARS CoV-2 infection.

Cowan explains that when virologists attempt to prove infection they have three possible ‘hosts’ or models on which they can test. The first was humans. Exposure to humans was generally not done for ethical reasons and has never been done with SARS-CoV-2 or any coronavirus. The second possible host was animals. Cowan said that forgetting for a moment that they never actually use purified virus when exposing animals they do use solutions that they *claim* contain the virus. Exposure to animals has been done with SARS-CoV-2 in an experiment involving mice and this is what they found: *None of the wild (normal) mice got sick*. In a group of genetically-modified mice, a statistically insignificant number lost weight and had slightly bristled fur, but they experienced nothing like the illness called ‘Covid-19’. Cowan said the third method – the one they mostly rely on – is to inoculate solutions they *say* contain the virus onto a variety of tissue cultures. This process had never been shown to kill tissue *unless* the sample material was starved of nutrients and poisoned as *part of the process*. Yes, incredibly, in tissue experiments designed to show the ‘virus’ is responsible for killing the tissue they starve the tissue of nutrients and

add toxic drugs including antibiotics and they do not have control studies to see if it's the starvation and poisoning that is degrading the tissue rather than the 'virus' they allege to be in there somewhere. You want me to pinch you? Yep, I understand. Tom Cowan said this about the whole nonsensical farce as he explains what that quote from the CDC paper really means:

The shocking thing about the above quote is that using their own methods, the virologists found that solutions containing SARS-CoV-2 – even in high amounts – were NOT, I repeat NOT, infective to any of the three human tissue cultures they tested. In plain English, this means they proved, on their terms, that this 'new coronavirus' is not infectious to human beings. It is ONLY infective to monkey kidney cells, and only then when you add two potent drugs (gentamicin and amphotericin), known to be toxic to kidneys, to the mix.

My friends, read this again and again. These virologists, published by the CDC, performed a clear proof, on their terms, showing that the SARS-CoV-2 virus is harmless to human beings. That is the only possible conclusion, but, unfortunately, this result is not even mentioned in their conclusion. They simply say they can provide virus stocks cultured only on monkey Vero cells, thanks for coming.

Cowan concluded: 'If people really understood how this "science" was done, I would hope they would storm the gates and demand honesty, transparency and truth.' Dr Michael Yeadon, former Vice President and Chief Scientific Adviser at drug giant Pfizer has been a vocal critic of the 'Covid vaccine' and its potential for multiple harm. He said in an interview in April, 2021, that 'not one [vaccine] has the virus. He was asked why vaccines normally using a 'dead' version of a disease to activate the immune system were not used for 'Covid' and instead we had the synthetic methods of the 'mRNA Covid vaccine'. Yeadon said that to do the former 'you'd have to have some of [the virus] wouldn't you?' He added: 'No-one's got any – seriously.' Yeadon said that surely they couldn't have fooled the whole world for a year without having a virus, 'but oddly enough ask around – no one's got it'. He didn't know why with all the 'great labs' around the world that the virus had not been isolated – 'Maybe they've been too busy running bad PCR tests and vaccines that people don't need.' What is today called 'science' is not 'science' at all. Science is no longer what is, but whatever people can be manipulated to *believe* that it is. Real science has been hijacked by the Cult to dispense and produce the 'expert scientists' and contentions that suit the agenda of the Cult. How big-time this has happened with the 'Covid' hoax which is entirely based on fake science

delivered by fake ‘scientists’ and fake ‘doctors’. The human-caused climate change hoax is also entirely based on fake science delivered by fake ‘scientists’ and fake ‘climate experts’. In both cases real scientists, climate experts and doctors have their views suppressed and deleted by the Cult-owned science establishment, media and Silicon Valley. This is the ‘science’ that politicians claim to be ‘following’ and a common denominator of ‘Covid’ and climate are Cult psychopaths Bill Gates and his mate Klaus Schwab at the Gates-funded World Economic Forum. But, don’t worry, it’s all just a coincidence and absolutely nothing to worry about. *Zzzzzzzz*.

What is a ‘virus’ REALLY?

Dr Tom Cowan is one of many contesting the very existence of viruses let alone that they cause disease. This is understandable when there is no scientific evidence for a disease-causing ‘virus’. German virologist Dr Stefan Lanka won a landmark case in 2017 in the German Supreme Court over his contention that there is no such thing as a measles virus. He had offered a big prize for anyone who could prove there is and Lanka won his case when someone sought to claim the money. There is currently a prize of more than 225,000 euros on offer from an Isolate Truth Fund for anyone who can prove the isolation of SARS-CoV-2 and its genetic substance. Lanka wrote in an article headed ‘The Misconception Called Virus’ that scientists think a ‘virus’ is causing tissue to become diseased and degraded when in fact it is the *processes they are using* which do that – not a ‘virus’. Lanka has done an important job in making this point clear as Cowan did in his analysis of the CDC paper. Lanka says that all claims about viruses as disease-causing pathogens are wrong and based on ‘easily recognisable, understandable and verifiable misinterpretations.’ Scientists believed they were working with ‘viruses’ in their laboratories when they were really working with ‘typical particles of specific dying tissues or cells …’ Lanka said that the tissue decaying process claimed to be caused by a ‘virus’ still happens when no alleged ‘virus’ is involved. It’s the *process* that does the damage and not a ‘virus’. The genetic sample is deprived of nutrients, removed from its energy supply through removal from the body and then doused in toxic antibiotics to remove any bacteria. He confirms again that establishment scientists do not (pinch me) conduct control experiments to

see if this is the case and if they did they would see the claims that 'viruses' are doing the damage is nonsense. He adds that during the measles 'virus' court case he commissioned an independent laboratory to perform just such a control experiment and the result was that the tissues and cells died in the exact same way as with alleged 'infected' material. This is supported by a gathering number of scientists, doctors and researchers who reject what is called 'germ theory' or the belief in the body being infected by contagious sources emitted by other people. Researchers Dawn Lester and David Parker take the same stance in their highly-detailed and sourced book *What Really Makes You Ill – Why everything you thought you knew about disease is wrong* which was recommended to me by a number of medical professionals genuinely seeking the truth. Lester and Parker say there is no provable scientific evidence to show that a 'virus' can be transmitted between people or people and animals or animals and people:

The definition also claims that viruses are the cause of many diseases, as if this has been definitively proven. But this is not the case; there is no original scientific evidence that definitively demonstrates that any virus is the cause of any disease. The burden of proof for any theory lies with those who proposed it; but none of the existing documents provides 'proof' that supports the claim that 'viruses' are pathogens.

Dr Tom Cowan employs one of his clever analogies to describe the process by which a 'virus' is named as the culprit for a disease when what is called a 'virus' is only material released by cells detoxing themselves from infiltration by chemical or radiation poisoning. The tidal wave of technologically-generated radiation in the 'smart' modern world plus all the toxic food and drink are causing this to happen more than ever. Deluded 'scientists' misread this as a gathering impact of what they wrongly label 'viruses'.

Paper can infect houses

Cowan said in an article for davidicke.com – with his tongue only mildly in his cheek – that he believed he had made a tremendous discovery that may revolutionise science. He had discovered that small bits of paper are alive, 'well alive-ish', can 'infect' houses, and then reproduce themselves inside the house. The result was that this explosion of growth in the paper inside

the house causes the house to explode, blowing it to smithereens. His evidence for this new theory is that in the past months he had carefully examined many of the houses in his neighbourhood and found almost no scraps of paper on the lawns and surrounds of the house. There was an occasional stray label, but nothing more. Then he would return to these same houses a week or so later and with a few, not all of them, particularly the old and decrepit ones, he found to his shock and surprise they were littered with stray bits of paper. He knew then that the paper had infected these houses, made copies of itself, and blew up the house. A young boy on a bicycle at one of the sites told him he had seen a demolition crew using dynamite to explode the house the previous week, but Cowan dismissed this as the idle thoughts of silly boys because 'I was on to something big'. He was on to how 'scientists' mistake genetic material in the detoxifying process for something they call a 'virus'. Cowan said of his house and paper story:

If this sounds crazy to you, it's because it should. This scenario is obviously nuts. But consider this admittedly embellished, for effect, current viral theory that all scientists, medical doctors and virologists currently believe.

He takes the example of the 'novel SARS-Cov2' virus to prove the point. First they take someone with an undefined illness called 'Covid-19' and don't even attempt to find any virus in their sputum. Never mind the scientists still describe how this 'virus', which they have not located attaches to a cell receptor, injects its genetic material, in 'Covid's' case, RNA, into the cell. The RNA once inserted exploits the cell to reproduce itself and makes 'thousands, nay millions, of copies of itself … Then it emerges victorious to claim its next victim':

If you were to look in the scientific literature for proof, actual scientific proof, that uniform SARS-CoV2 viruses have been properly isolated from the sputum of a sick person, that actual spike proteins could be seen protruding from the virus (which has not been found), you would find that such evidence doesn't exist.

If you go looking in the published scientific literature for actual pictures, proof, that these spike proteins or any viral proteins are ever attached to any receptor embedded in any cell membrane, you would also find that no such evidence exists. If you were to look for a video or documented evidence

of the intact virus injecting its genetic material into the body of the cell, reproducing itself and then emerging victorious by budding off the cell membrane, you would find that no such evidence exists.

The closest thing you would find is electron micrograph pictures of cellular particles, possibly attached to cell debris, both of which to be seen were stained by heavy metals, a process that completely distorts their architecture within the living organism. This is like finding bits of paper stuck to the blown-up bricks, thereby proving the paper emerged by taking pieces of the bricks on its way out.

The Enders baloney

Cowan describes the 'Covid' story as being just as make-believe as his paper story and he charts back this fantasy to a Nobel Prize winner called John Enders (1897-1985), an American biomedical scientist who has been dubbed 'The Father of Modern Vaccines'. Enders is claimed to have 'discovered' the process of the viral culture which 'proved' that a 'virus' caused measles. Cowan explains how Enders did this 'by using the EXACT same procedure that has been followed by every virologist to find and characterize every new virus since 1954'. Enders took throat swabs from children with measles and immersed them in 2ml of milk. Penicillin (100u/ml) and the antibiotic streptomycin (50,g/ml) were added and the whole mix was centrifuged – rotated at high speed to separate large cellular debris from small particles and molecules as with milk and cream, for example. Cowan says that if the aim is to find little particles of genetic material ('viruses') in the snot from children with measles it would seem that the last thing you would do is mix the snot with other material – milk – that also has genetic material. 'How are you ever going to know whether whatever you found came from the snot or the milk?' He points out that streptomycin is a 'nephrotoxic' or poisonous-to-the-kidney drug. You will see the relevance of that shortly. Cowan says that it gets worse, much worse, when Enders describes the culture medium upon which the virus 'grows': 'The culture medium consisted of bovine amniotic fluid (90%), beef embryo extract (5%), horse serum (5%), antibiotics and phenol red as an indicator of cell metabolism.' Cowan asks incredulously: 'Did he just say that the culture medium also contained fluids and tissues that are themselves rich sources of genetic material?' The genetic cocktail, or 'medium', is inoculated onto tissue and cells from rhesus monkey *kidney* tissue. This is where the importance of streptomycin comes in and currently-used antimicrobials and other drugs that are *poisonous to kidneys*

and used in ALL modern viral cultures (e.g. gentamicin, streptomycin, and amphotericin). Cowan asks: 'How are you ever going to know from this witch's brew where any genetic material comes from as we now have five different sources of rich genetic material in our mix?' Remember, he says, that all genetic material, whether from monkey kidney tissues, bovine serum, milk, etc., is made from the exact same components. The same central question returns: 'How are you possibly going to know that it was the virus that killed the kidney tissue and not the toxic antibiotic and starvation rations on which you are growing the tissue?' John Enders answered the question himself – *you can't*:

A second agent was obtained from an uninoculated culture of monkey kidney cells. The cytopathic changes [death of the cells] it induced in the unstained preparations could not be distinguished with confidence from the viruses isolated from measles.

The death of the cells ('cytopathic changes') happened in exactly the same manner, whether they inoculated the kidney tissue with the measles snot or not, Cowan says. 'This is evidence that the destruction of the tissue, the very proof of viral causation of illness, was not caused by anything in the snot because they saw the same destructive effect when the snot was not even used … the cytopathic, i.e., cell-killing, changes come from the process of the culture itself, not from any virus in any snot, period.' Enders quotes in his 1957 paper a virologist called Ruckle as reporting similar findings 'and in addition has isolated an agent from monkey kidney tissue that is so far indistinguishable from human measles virus'. In other words, Cowan says, these particles called 'measles viruses' are simply and clearly breakdown products of the starved and poisoned tissue. For measles 'virus' see all 'viruses' including the so-called 'Covid virus'. Enders, the 'Father of Modern Vaccines', also said:

There is a potential risk in employing cultures of primate cells for the production of vaccines composed of attenuated virus, since the presence of other agents possibly latent in primate tissues cannot be definitely excluded by any known method.

Cowan further quotes from a paper published in the journal *Viruses* in May, 2020, while the 'Covid pandemic' was well underway in the media if

not in reality. ‘EVs’ here refers to particles of genetic debris from our own tissues, such as exosomes of which more in a moment: ‘The remarkable resemblance between EVs and viruses has caused quite a few problems in the studies focused on the analysis of EVs released during viral infections.’ Later the paper adds that to date a reliable method that can actually guarantee a complete separation (of EVs from viruses) DOES NOT EXIST. This was published at a time when a fairy tale ‘virus’ was claimed in total certainty to be causing a fairy tale ‘viral disease’ called ‘Covid-19’ – a fairy tale that was already well on the way to transforming human society in the image that the Cult has worked to achieve for so long. Cowan concludes his article:

To summarize, there is no scientific evidence that pathogenic viruses exist. What we think of as ‘viruses’ are simply the normal breakdown products of dead and dying tissues and cells. When we are well, we make fewer of these particles; when we are starved, poisoned, suffocated by wearing masks, or afraid, we make more.

There is no engineered virus circulating and making people sick. People in laboratories all over the world are making genetically modified products to make people sick. These are called vaccines. There is no virome, no ‘ecosystem’ of viruses, viruses are not 8%, 50% or 100 % of our genetic material. These are all simply erroneous ideas based on the misconception called a virus.

What is ‘Covid’? Load of bollocks

The background described here by Cowan and Lanka was emphasised in the first video presentation that I saw by Dr Andrew Kaufman when he asked whether the ‘Covid virus’ was in truth a natural defence mechanism of the body called ‘exosomes’. These are released by cells when in states of toxicity – see the same themes returning over and over. They are released ever more profusely as chemical and radiation toxicity increases and think of the potential effect therefore of 5G alone as its destructive frequencies infest the human energetic information field with a gathering pace (5G went online in Wuhan in 2019 as the ‘virus’ emerged). I’ll have more about this later. Exosomes transmit a warning to the rest of the body that ‘Houston, we have a problem’. Kaufman presented images of exosomes and compared them with ‘Covid’ under an electron microscope and the similarity was remarkable. They both attach to the same cell receptors (*claimed* in the case of ‘Covid’), contain the same genetic material in the form of RNA or ribonucleic acid, and both are found in ‘viral cell cultures’ with damaged or

dying cells. James Hildreth MD, President and Chief Executive Officer of the Meharry Medical College at Johns Hopkins, said: 'The virus is fully an exosome in every sense of the word.' Kaufman's conclusion was that there is no 'virus': 'This entire pandemic is a completely manufactured crisis … there is no evidence of anyone dying from [this] illness.' Dr Tom Cowan and Sally Fallon Morell, authors of *The Contagion Myth*, published a statement with Dr Kaufman in February, 2021, explaining why the 'virus' does not exist and you can read it that in full in the Appendix.

'Virus' theory can be traced to the 'cell theory' in 1858 of German physician Rudolf Virchow (1821-1920) who contended that disease originates from a single cell infiltrated by a 'virus'. Dr Stefan Lanka said that findings and insights with respect to the structure, function and central importance of tissues in the creation of life, which were already known in 1858, comprehensively refute the cell theory. Virchow ignored them. We have seen the part later played by John Enders in the 1950s and Lanka notes that infection theories were only established as a global dogma through the policies and eugenics of the Third Reich in Nazi Germany (creation of the same Sabbatian cult behind the 'Covid' hoax). Lanka said: 'Before 1933, scientists dared to contradict this theory; after 1933, these critical scientists were silenced'. Dr Tom Cowan's view is that ill-heath is caused by too much of something, too little of something, or toxification from chemicals and radiation – not contagion. We must also highlight as a major source of the 'virus' theology a man still called the 'Father of Modern Virology' – Thomas Milton Rivers (1888-1962). There is no way given the Cult's long game policy that it was a coincidence for the 'Father of Modern Virology' to be director of the Rockefeller Institute for Medical Research from 1937 to 1956 when he is credited with making the Rockefeller Institute a leader in 'viral research'. Cult Rockefellers were the force behind the creation of Big Pharma 'medicine', established the World Health Organisation in 1948, and have long and close associations with the Gates family that now runs the WHO during the pandemic hoax through mega-rich Cult gofer and psychopath Bill Gates.

Only a Renegade Mind can see through all this bullshit by asking the questions that need to be answered, not taking 'no' or prevarication for an answer, and certainly not hiding from the truth in fear of speaking it. Renegade Minds have always changed the world for the better and they will change this one no matter how bleak it may currently appear to be.

CHAPTER SIX

Sequence of deceit

If you tell the truth, you don't have to remember anything
Mark Twain

Against the background that I have laid out this far the sequence that took us from an invented 'virus' in Cult-owned China in late 2019 to the fascist transformation of human society can be seen and understood in a whole new context.

We were told that a deadly disease had broken out in Wuhan and the world media began its campaign (coordinated by behavioural psychologists as we shall see) to terrify the population into unquestioning compliance. We were shown images of Chinese people collapsing in the street which never happened in the West with what was supposed to be the same condition. In the earliest days when alleged cases and deaths were few the fear register was hysterical in many areas of the media and this would expand into the common media narrative across the world. The real story was rather different, but we were never told that. The Chinese government, one of the Cult's biggest centres of global operation, said they had discovered a new illness with flu-like and pneumonia-type symptoms in a city with such toxic air that it is overwhelmed with flu-like symptoms, pneumonia and respiratory disease. Chinese scientists said it was a new – 'novel' – coronavirus which they called Sars-Cov-2 and that it caused a disease they labelled 'Covid-19'. There was no evidence for this and the 'virus' has never to this day been isolated, purified and its genetic code established from that. It was from the beginning a computer-generated fiction. Stories

of Chinese whistleblowers saying the number of deaths was being supressed or that the ‘new disease’ was related to the Wuhan bio-lab misdirected mainstream and alternative media into cul-de-sacs to obscure the real truth – there was no ‘virus’.

Chinese scientists took genetic material from the lung fluid of just a few people and said they had found a ‘new’ disease when this material had a wide range of content. There was no evidence for a ‘virus’ for the very reasons explained in the last two chapters. The ‘virus’ has never been shown to (a) exist and (b) cause any disease. People were diagnosed on symptoms that are so widespread in Wuhan and polluted China and with a PCR test that can’t detect infectious disease. On this farce the whole global scam was sold to the rest of the world which would also diagnose respiratory disease as ‘Covid-19’ from symptoms alone or with a PCR test not testing for a ‘virus’. Flu miraculously disappeared *worldwide* in 2020 and into 2021 as it was redesignated ‘Covid-19’. It was really the same old flu with its ‘flu-like’ symptoms attributed to ‘flu-like’ ‘Covid-19’. At the same time with very few exceptions the Chinese response of draconian lockdown and fascism was the chosen weapon to respond across the West as recommended by the Cult-owned Tedros at the Cult-owned World Health Organization run by the Cult-owned Gates. All was going according to plan. Chinese scientists – everything in China is controlled by the Cult-owned government – compared their contaminated RNA lung-fluid material with other RNA sequences and said it appeared to be just under 80 percent identical to the SARS-CoV-1 ‘virus’ claimed to be the cause of the SARS (severe acute respiratory syndrome) ‘outbreak’ in 2003. They decreed that because of this the ‘new virus’ had to be related and they called it SARS-CoV-2. There are some serious problems with this assumption and *assumption* was all it was. Most ‘factual’ science turns out to be assumptions repeated into everyone-knows-that. A match of under 80-percent is meaningless. Dr Kaufman makes the point that there’s a *96 percent* genetic correlation between humans and chimpanzees, but ‘no one would say our genetic material is part of the chimpanzee family’. Yet the Chinese authorities were claiming that a much lower percentage, less than 80 percent, proved the existence of a new ‘coronavirus’. For goodness sake human DNA is 60 percent similar to a *banana*.

You are feeling sleepy

The entire ‘Covid’ hoax is a global Psyop, a psychological operation to program the human mind into believing and fearing a complete fantasy. A crucial aspect of this was what *appeared* to happen in Italy. It was all very well streaming out daily images of an alleged catastrophe in Wuhan, but to the Western mind it was still on the other side of the world in a very different culture and setting. A reaction of ‘this could happen to me and my family’ was still nothing like as intense enough for the mind-doctors. The Cult needed a Western example to push people over that edge and it chose Italy, one of its major global locations going back to the Roman Empire. An Italian ‘Covid’ crisis was manufactured in a particular area called Lombardy which just happens to be notorious for its toxic air and therefore respiratory disease. Wuhan, China, déjà vu. An hysterical media told horror stories of Italians dying from ‘Covid’ in their droves and how Lombardy hospitals were being overrun by a tidal wave of desperately ill people needing treatment after being struck down by the ‘deadly virus’. Here was the psychological turning point the Cult had planned. Wow, if this is happening in Italy, the Western mind concluded, this indeed could happen to me and my family. Another point is that Italian authorities responded by following the Chinese blueprint so vehemently recommended by the Cult-owned World Health Organization. They imposed fascistic lockdowns on the whole country viciously policed with the help of surveillance drones sweeping through the streets seeking out anyone who escaped from mass house arrest. Livelihoods were destroyed and psychology unravelled in the way we have witnessed since in all lockdown countries. Crucial to the plan was that Italy responded in this way to set the precedent of suspending freedom and imposing fascism in a ‘Western liberal democracy’. I emphasised in an animated video explanation on davidicke.com posted in the summer of 2020 how important it was to the Cult to expand the Chinese lockdown model across the West. Without this, and the bare-faced lie that non-symptomatic people could still transmit a ‘disease’ they didn’t have, there was no way locking down the whole population, sick and not sick, could be pulled off. At just the right time and with no evidence Cult operatives and gofers claimed that people without symptoms could pass on the ‘disease’. In the name of protecting the ‘vulnerable’ like elderly people, who lockdowns would kill by the tens of thousands, we had for the first time healthy people told to isolate as well as the sick. The great majority of

people who tested positive had no symptoms because there was nothing wrong with them. It was just a trick made possible by a test not testing for the 'virus'.

Months after my animated video the Gates-funded Professor Neil Ferguson at the Gates-funded Imperial College confirmed that I was right. He didn't say it in those terms, naturally, but he did say it. Ferguson will enter the story shortly for his outrageously crazy 'computer models' that led to Britain, the United States and many other countries following the Chinese and now Italian methods of response. Put another way, following the Cult script. Ferguson said that SAGE, the UK government's scientific advisory group which has controlled 'Covid' policy from the start, wanted to follow the Chinese lockdown model (while they all continued to work and be paid), but they wondered if they could possibly, in Ferguson's words, 'get away with it in Europe'. 'Get away with it'? Who the hell do these moronic, arrogant people think they are? This appalling man Ferguson said that once Italy went into national lockdown they realised they, too, could mimic China:

It's a communist one-party state, we said. We couldn't get away with it in Europe, we thought … and then Italy did it. And we realised we could. Behind this garbage from Ferguson is a simple fact: Doing the same as China in every country was the plan from the start and Ferguson's 'models' would play a central role in achieving that. It's just a coincidence, of course, and absolutely nothing to worry your little head about.

Oops, sorry, our mistake

Once the Italian segment of the Psyop had done the job it was designed to do a very different story emerged. Italian authorities revealed that 99 percent of those who had 'died from Covid-19' in Italy had one, two, three, or more 'co-morbidities' or illnesses and health problems that could have ended their life. The US Centers for Disease Control and Prevention (CDC) published a figure of 94 percent for Americans dying of 'Covid' while having other serious medical conditions – on average two to three (some five or six) other potential causes of death. In terms of death from an unproven 'virus' I say it is 100 percent. The other one percent in Italy and six percent in the US would presumably have died from 'Covid's' flu-like symptoms with a range of other possible causes in conjunction with a test

not testing for the ‘virus’. Fox News reported that even more startling figures had emerged in one US county in which 410 of 422 deaths attributed to ‘Covid-19’ had other potentially deadly health conditions. The Italian National Health Institute said later that the average age of people dying with a ‘Covid-19’ diagnosis in Italy was about 81. Ninety percent were over 70 with ten percent over 90. In terms of other reasons to die some 80 percent had two or more chronic diseases with half having three or more including cardiovascular problems, diabetes, respiratory problems and cancer. Why is the phantom ‘Covid-19’ said to kill overwhelmingly old people and hardly affect the young? Old people continually die of many causes and especially respiratory disease which you can re-diagnose ‘Covid-19’ while young people die in tiny numbers by comparison and rarely of respiratory disease. Old people ‘die of Covid’ because they die of other things that can be redesignated ‘Covid’ and it really is that simple.

Flu has flown

The blueprint was in place. Get your illusory ‘cases’ from a test not testing for the ‘virus’ and redesignate other causes of death as ‘Covid-19’. You have an instant ‘pandemic’ from something that is nothing more than a computer-generated fiction. With near-on a billion people having ‘flu-like’ symptoms every year the potential was limitless and we can see why flu quickly and apparently miraculously disappeared *worldwide* by being diagnosed ‘Covid-19’. The painfully bloody obvious was explained away by the childlike media in headlines like this in the UK ‘*Independent*’: ‘Not a single case of flu detected by Public Health England this year as Covid restrictions suppress virus’. I kid you not. The masking, social distancing and house arrest that did not make the ‘Covid virus’ disappear somehow did so with the ‘flu virus’. Even worse the article, by a bloke called Samuel Lovett, suggested that maybe the masking, sanitising and other ‘Covid’ measures should continue to keep the flu away. With a ridiculousness that disturbs your breathing (it’s ‘Covid-19’) the said Lovett wrote: ‘With widespread social distancing and mask-wearing measures in place throughout the UK, the usual routes of transmission for influenza have been blocked.’ He had absolutely no evidence to support that statement, but look at the consequences of him acknowledging the obvious. With flu not disappearing at all and only being relabelled ‘Covid-19’ he would have to

contemplate that ‘Covid’ was a hoax on a scale that is hard to imagine. You need guts and commitment to truth to even go there and that’s clearly something Samuel Lovett does not have in abundance. He would never have got it through the editors anyway.

Tens of thousands die in the United States alone every winter from flu including many with pneumonia complications. CDC figures record *45 million* Americans diagnosed with flu in 2017-2018 of which 61,000 died and some reports claim 80,000. Where was the same hysteria then that we have seen with ‘Covid-19’? Some 250,000 Americans are admitted to hospital with pneumonia every year with about 50,000 cases proving fatal. About 65 million suffer respiratory disease every year and three million deaths makes this the third biggest cause of death worldwide. You only have to redesignate a portion of all these people ‘Covid-19’ and you have an instant global pandemic or the *appearance* of one. Why would doctors do this? They are told to do this and all but a few dare not refuse those who must be obeyed. Doctors in general are not researching their own knowledge and instead take it direct and unquestioned from the authorities that own them and their careers. The authorities say they must now diagnose these symptoms ‘Covid-19’ and not flu, or whatever, and they do it. Dark suits say put ‘Covid-19’ on death certificates no matter what the cause of death and the doctors do it. Renegade Minds don’t fall for the illusion that doctors and medical staff are all highly-intelligent, highly-principled, seekers of medical truth. *Some are*, but not the majority. They are repeaters, gofers, and yes sir, no sir, purveyors of what the system demands they purvey. The ‘Covid’ con is not merely confined to diseases of the lungs. Instructions to doctors to put ‘Covid-19’ on death certificates for anyone dying of *anything* within 28 days (or much more) of a positive test not testing for the ‘virus’ opened the floodgates. The term dying *with* ‘Covid’ and not *of* ‘Covid’ was coined to cover the truth. Whether it was a *with* or an *of* they were all added to the death numbers attributed to the ‘deadly virus’ compiled by national governments and globally by the Gates-funded Johns Hopkins operation in the United States that was so involved in those ‘pandemic’ simulations. Fraudulent deaths were added to the ever-growing list of fraudulent ‘cases’ from false positives from a false test. No wonder Professor Walter Ricciardi, scientific advisor to the Italian minister of health, said after the Lombardy hysteria had done its job that ‘Covid’

death rates were due to Italy having the second oldest population in the world and to *how hospitals record deaths*:

The way in which we code deaths in our country is very generous in the sense that all the people who die in hospitals with the coronavirus are deemed to be dying of the coronavirus. On re-evaluation by the National Institute of Health, only 12 per cent of death certificates have shown a direct causality from coronavirus, while 88 per cent of patients who have died have at least one pre-morbidity – many had two or three.

This is extraordinary enough when you consider the propaganda campaign to use Italy to terrify the world, but how can they even say twelve percent were genuine when the 'virus' has not been shown to exist, its 'code' is a computer program, and diagnosis comes from a test not testing for it? As in China, and soon the world, 'Covid-19' in Italy was a redesignation of diagnosis. Lies and corruption were to become the real 'pandemic' fuelled by a pathetically-compliant medical system taking its orders from the tiny few at the top of their national hierarchy who answered to the World Health Organization which answers to Gates and the Cult. Doctors were told – ordered – to diagnose a particular set of symptoms 'Covid-19' and put that on the death certificate for any cause of death if the patient had tested positive with a test not testing for the virus or had 'Covid' symptoms like the flu. The United States even introduced big financial incentives to manipulate the figures with hospitals receiving £4,600 from the Medicare system for diagnosing someone with regular pneumonia, $13,000 if they made the diagnosis from the same symptoms 'Covid-19' pneumonia, and $39, 000 if they put a 'Covid' diagnosed patient on a ventilator that would almost certainly kill them. A few – painfully and pathetically few – medical whistleblowers revealed (before Cult-owned YouTube deleted their videos) that they had been instructed to 'let the patient crash' and put them straight on a ventilator instead of going through a series of far less intrusive and dangerous methods as they would have done before the pandemic hoax began and the financial incentives kicked in. We are talking cold-blooded murder given that ventilators are so damaging to respiratory systems they are usually the last step before heaven awaits. Renegade Minds never fall for the belief that people in white coats are all angels of mercy and cannot be full-on psychopaths. I have explained in detail in *The Answer* how what I am describing here played out across

the world coordinated by the World Health Organization through the medical hierarchies in almost every country.

Medical scientist calls it

Information about the non-existence of the 'virus' began to emerge for me in late March, 2020, and mushroomed after that. I was sent an email by Sir Julian Rose, a writer, researcher, and organic farming promotor, from a medical scientist friend of his in the United States. Even at that early stage in March the scientist was able to explain how the 'Covid' hoax was being manipulated. He said there were no reliable tests for a specific 'Covid-19 virus' and nor were there any reliable agencies or media outlets for reporting numbers of actual 'Covid-19' cases. We have seen in the long period since then that he was absolutely right. 'Every action and reaction to Covid-19 is based on totally flawed data and we simply cannot make accurate assessments,' he said. Most people diagnosed with 'Covid-19' were showing nothing more than cold and flu-like symptoms 'because most coronavirus strains *are* nothing more than cold/flu-like symptoms'. We had farcical situations like an 84-year-old German man testing positive for 'Covid-19' and his nursing home ordered to quarantine only for him to be found to have a common cold. The scientist described back then why PCR tests and what he called the 'Mickey Mouse test kits' were useless for what they were claimed to be identifying. 'The idea these kits can isolate a specific virus like Covid-19 is nonsense,' he said. Significantly, he pointed out that 'if you want to create a totally false panic about a totally false pandemic – pick a coronavirus'. This is exactly what the Cult-owned Gates, World Economic Forum and Johns Hopkins University did with their Event 201 'simulation' followed by their real-life simulation called the 'pandemic'. The scientist said that all you had to do was select the sickest of people with respiratory-type diseases in a single location – 'say Wuhan' – and administer PCR tests to them. You can then claim that anyone showing 'viral sequences' similar to a coronavirus 'which will inevitably be quite a few' is suffering from a 'new' disease:

> Since you already selected the sickest flu cases a fairly high proportion of your sample will go on to die. You can then say this 'new' virus has a CFR [case fatality rate] higher than the flu and use this to infuse more concern and do more tests which will of course produce more 'cases', which expands the

testing, which produces yet more ‘cases’ and so on and so on. Before long you have your ‘pandemic’, and all you have done is use a simple test kit trick to convert the worst flu and pneumonia cases into something new that doesn’t ACTUALLY EXIST [my emphasis].

He said that you then ‘just run the same scam in other countries’ and make sure to keep the fear message running high ‘so that people will feel panicky and less able to think critically’. The only problem to overcome was the fact *there is no* actual new deadly pathogen and only regular sick people. This meant that deaths from the ‘new deadly pathogen’ were going to be way too low for a real new deadly virus pandemic, but he said this could be overcome in the following ways – all of which would go on to happen:

1. You can claim this is just the beginning and more deaths are imminent [you underpin this with fantasy ‘computer projections’]. Use this as an excuse to quarantine everyone and then claim the quarantine prevented the expected millions of dead.

2. You can [say that people] ‘minimizing’ the dangers are irresponsible and bully them into not talking about numbers.

3. You can talk crap about made up numbers hoping to blind people with pseudoscience.

4. You can start testing well people (who, of course, will also likely have shreds of coronavirus [RNA] in them) and thus inflate your ‘case figures’ with ‘asymptomatic carriers’ (you will of course have to spin that to sound deadly even though any virologist knows the more symptom-less cases you have the less deadly is your pathogen).

The scientist said that if you take these simple steps ‘you can have your own entirely manufactured pandemic up and running in weeks’. His analysis made so early in the hoax was brilliantly prophetic of what would actually unfold. Pulling all the information together in these recent chapters we have this is simple 1, 2, 3, of how you can delude virtually the entire human population into believing in a ‘virus’ that doesn’t exist:

- A ‘Covid case’ is someone who tests positive with a test not testing for the ‘virus’.

- A ‘Covid death’ is someone who dies of *any cause* within 28 days (or much longer) of testing positive with a test not testing for the ‘virus.

• Asymptomatic means there is nothing wrong with you, but they claim you can pass on what you don't have to justify locking down (quarantining) healthy people in totality.

The foundations of the hoax are that simple. A study involving ten million people in Wuhan, published in November, 2020, demolished the whole lie about those without symptoms passing on the 'virus'. They found '300 asymptomatic cases' and traced their contacts to find that not one of them was detected with the 'virus'. 'Asymptomatic' patients and their contacts were isolated for no less than two weeks and nothing changed. I know it's all crap, but if you are going to claim that those without symptoms can transmit 'the virus' then you must produce evidence for that and they never have. Even World Health Organization official Dr Maria Van Kerkhove, head of the emerging diseases and zoonosis unit, said as early as June, 2020, that she doubted the validity of asymptomatic transmission. She said that 'from the data we have, it still seems to be rare that an asymptomatic person actually transmits onward to a secondary individual' and by 'rare' she meant that she couldn't cite any case of asymptomatic transmission.

The Ferguson factor

The problem for the Cult as it headed into March, 2020, when the script had lockdown due to start, was that despite all the manipulation of the case and death figures they still did not have enough people alleged to have died from 'Covid' to justify mass house arrest. This was overcome in the way the scientist described: 'You can claim this is just the beginning and more deaths are imminent … Use this as an excuse to quarantine everyone and then claim the quarantine prevented the expected millions of dead.' Enter one Professor Neil Ferguson, the Gates-funded 'epidemiologist' at the Gates-funded Imperial College in London. Ferguson is Britain's Christian Drosten in that he has a dire record of predicting health outcomes, but is still called upon to advise government on the next health outcome when another 'crisis' comes along. This may seem to be a strange and ridiculous thing to do. Why would you keep turning for policy guidance to people who have a history of being monumentally wrong? Ah, but it makes sense from the Cult point of view. These 'experts' keep on producing predictions that

suit the Cult agenda for societal transformation and so it was with Neil Ferguson as he revealed his horrific (and clearly insane) computer model predictions that allowed lockdowns to be imposed in Britain, the United States and many other countries. Ferguson does not have even an A-level in biology and would appear to have no formal training in computer modelling, medicine or epidemiology, according to Derek Winton, an MSc in Computational Intelligence. He wrote an article somewhat aghast at what Ferguson did which included taking no account of respiratory disease 'seasonality' which means it is far worse in the winter months. Who would have thought that respiratory disease could be worse in the winter? Well, certainly not Ferguson.

The massively China-connected Imperial College and its bizarre professor provided the excuse for the long-incubated Chinese model of human control to travel westward at lightning speed. Imperial College confirms on its website that it collaborates with the Chinese Research Institute; publishes more than 600 research papers every year with Chinese research institutions; has 225 Chinese staff; 2,600 Chinese students – the biggest international group; 7,000 former students living in China which is the largest group outside the UK; and was selected for a tour by China's President Xi Jinping during his state visit to the UK in 2015. The college takes major donations from China and describes itself as the UK's number one university collaborator with Chinese research institutions. The China communist/fascist government did not appear phased by the woeful predictions of Ferguson and Imperial when during the lockdown that Ferguson induced the college signed a five-year collaboration deal with China tech giant Huawei that will have Huawei's indoor 5G network equipment installed at the college's West London tech campus along with an 'AI cloud platform'. The deal includes Chinese sponsorship of Imperial's Venture Catalyst entrepreneurship competition. Imperial is an example of the enormous influence the Chinese government has within British and North American universities and research centres – and further afield. Up to 200 academics from more than a dozen UK universities are being investigated on suspicion of 'unintentionally' helping the Chinese government build weapons of mass destruction by 'transferring world-leading research in advanced military technology such as aircraft, missile designs and cyberweapons'. Similar scandals have broken in the United States, but it's all a coincidence. Imperial College serves the agenda in

many other ways including the promotion of every aspect of the United Nations Agenda 21/2030 (the Great Reset) and produced computer models to show that human-caused ‘climate change’ is happening when in the real world it isn’t. Imperial College is driving the climate agenda as it drives the ‘Covid’ agenda (both Cult hoaxes) while Patrick Vallance, the UK government’s Chief Scientific Adviser on ‘Covid’, was named Chief Scientific Adviser to the UN ‘climate change’ conference known as COP26 hosted by the government in Glasgow, Scotland. ‘Covid’ and ‘climate’ are fundamentally connected.

Professor Woeful

From Imperial’s bosom came Neil Ferguson still advising government despite his previous disasters and it was announced early on that he and other key people like UK Chief Medical Adviser Chris Whitty had caught the ‘virus’ as the propaganda story was being sold. Somehow they managed to survive and we had Prime Minister Boris Johnson admitted to hospital with what was said to be a severe version of the ‘virus’ in this same period. His whole policy and demeanour changed when he returned to Downing Street. It’s a small world with these government advisors – especially in their communal connections to Gates – and Ferguson had partnered with Whitty to write a paper called ‘Infectious disease: Tough choices to reduce Ebola transmission’ which involved another scare-story that didn’t happen. Ferguson’s ‘models’ predicted that up to150, 000 could die from ‘mad cow disease’, or BSE, and its version in sheep if it was transmitted to humans. BSE was not transmitted and instead triggered by an organophosphate pesticide used to treat a pest on cows. Fewer than 200 deaths followed from the human form. Models by Ferguson and his fellow incompetents led to the unnecessary culling of millions of pigs, cattle and sheep in the foot and mouth outbreak in 2001 which destroyed the lives and livelihoods of farmers and their families who had often spent decades building their herds and flocks. Vast numbers of these animals did not have foot and mouth and had no contact with the infection. Another ‘expert’ behind the cull was Professor Roy Anderson, a computer modeller at Imperial College specialising in the epidemiology of *human*, not animal, disease. Anderson has served on the Bill and Melinda Gates Grand Challenges in Global

Health advisory board and chairs another Gates-funded organisation. Gates is everywhere.

In a precursor to the 'Covid' script Ferguson backed closing schools 'for prolonged periods' over the swine flu 'pandemic' in 2009 and said it would affect a third of the world population if it continued to spread at the speed he claimed to be happening. His mates at Imperial College said much the same and a news report said: 'One of the authors, the epidemiologist and disease modeller Neil Ferguson, who sits on the World Health Organisation's emergency committee for the outbreak, said the virus had "full pandemic potential".' Professor Liam Donaldson, the Chris Whitty of his day as Chief Medical Officer, said the worst case could see 30 percent of the British people infected by swine flu with 65,000 dying. Ferguson and Donaldson were indeed proved correct when at the end of the year the number of deaths attributed to swine flu was 392. The term 'expert' is rather liberally applied unfortunately, not least to complete idiots. Swine flu 'projections' were great for GlaxoSmithKline (GSK) as millions rolled in for its Pandemrix influenza vaccine which led to brain damage with children most affected. The British government (taxpayers) paid out more than £60 million in compensation after GSK was given immunity from prosecution. Yet another 'Covid' déjà vu. Swine flu was supposed to have broken out in Mexico, but Dr Wolfgang Wodarg, a German doctor, former member of parliament and critic of the 'Covid' hoax, observed 'the spread of swine flu' in Mexico City at the time. He said: 'What we experienced in Mexico City was a very mild flu which did not kill more than usual – which killed even fewer people than usual.' Hyping the fear against all the facts is not unique to 'Covid' and has happened many times before. Ferguson is reported to have over-estimated the projected death toll of bird flu (H5N1) by some three million-fold, but bird flu vaccine makers again made a killing from the scare. This is some of the background to the Neil Ferguson who produced the perfectly-timed computer models in early 2020 predicting that half a million people would die in Britain without draconian lockdown and 2.2 million in the United States. Politicians panicked, people panicked, and lockdowns of alleged short duration were instigated to 'flatten the curve' of cases gleaned from a test not testing for the 'virus'. I said at the time that the public could forget the 'short duration' bit. This was an agenda to destroy the livelihoods of the population and force them into mass control through dependency and there was going to be nothing 'short' about it.

American researcher Daniel Horowitz described the consequences of the ‘models’ spewed out by Gates-funded Ferguson and Imperial College:

What led our government and the governments of many other countries into panic was a single Imperial College of UK study, funded by global warming activists, that predicted 2.2 million deaths if we didn’t lock down the country. In addition, the reported 8-9% death rate in Italy scared us into thinking there was some other mutation of this virus that they got, which might have come here.

Together with the fact that we were finally testing and had the ability to actually report new cases, we thought we were headed for a death spiral. But again … we can’t flatten a curve if we don’t know when the curve started.

How about it *never* started?

Giving them what they want

An investigation by German news outlet *Welt Am Sonntag (World on Sunday)* revealed how in March, 2020, the German government gathered together ‘leading scientists from several research institutes and universities’ and ‘together, they were to produce a [modelling] paper that would serve as legitimization for further tough political measures’. The Cult agenda was justified by computer modelling not based on evidence or reality; it was specifically constructed to justify the Cult demand for lockdowns all over the world to destroy the independent livelihoods of the global population. All these modellers and everyone responsible for the ‘Covid’ hoax have a date with a trial like those in Nuremberg after World War Two when Nazis faced the consequences of their war crimes. These corrupt-beyond-belief ‘modellers’ wrote the paper according to government instructions and it said that that if lockdown measures were lifted then up to one million Germans would die from ‘Covid-19’ adding that some would die ‘agonizingly at home, gasping for breath’ unable to be treated by hospitals that couldn’t cope. All lies. No matter – it gave the Cult all that it wanted. What did long-time government ‘modeller’ Neil Ferguson say? If the UK and the United States didn’t lockdown half a million would die in Britain and 2.2 million Americans. Anyone see a theme here? ‘Modellers’ are such a crucial part of the lockdown strategy that we should look into their background and follow the money. Researcher Rosemary Frei produced an excellent article headlined ‘The Modelling-paper Mafiosi’. She highlights a

guy called John Edmunds, a British epidemiologist, and professor in the Faculty of Epidemiology and Population Health at the London School of Hygiene & Tropical Medicine. He studied at Imperial College. Edmunds is a member of government 'Covid' advisory bodies which have been dictating policy, the New and Emerging Respiratory Virus Threats Advisory Group (NERVTAG) and the Scientific Advisory Group for Emergencies (SAGE).

Ferguson, another member of NERVTAG and SAGE, led the way with the original 'virus' and Edmunds has followed in the 'variant' stage and especially the so-called UK or Kent variant known as the 'Variant of Concern' (VOC) B.1.1.7. He said in a co-written report for the Centre for Mathematical modelling of Infectious Diseases at the London School of Hygiene and Tropical Medicine, with input from the Centre's 'Covid-19' Working Group, that there was 'a realistic possibility that VOC B.1.1.7 is associated with an increased risk of death compared to non-VOC viruses'. Fear, fear, fear, get the vaccine, fear, fear, fear, get the vaccine. Rosemary Frei reveals that almost all the paper's authors and members of the modelling centre's 'Covid-19' Working Group receive funding from the Bill and Melinda Gates Foundation and/or the associated Gates-funded Wellcome Trust. The paper was published by e-journal *Medr χiv* which only publishes papers not peer-reviewed and the journal was established by an organisation headed by Facebook's Mark Zuckerberg and his missus. What a small world it is. Frei discovered that Edmunds is on the Scientific Advisory Board of the Coalition for Epidemic Preparedness Innovations (CEPI) which was established by the Bill and Melinda Gates Foundation, Klaus Schwab's Davos World Economic Forum and Big Pharma giant Wellcome. CEPI was 'launched in Davos [in 2017] to develop vaccines to stop future epidemics', according to its website. 'Our mission is to accelerate the development of vaccines against emerging infectious diseases and enable equitable access to these vaccines for people during outbreaks.' What kind people they are. Rosemary Frei reveals that Public Health England (PHE) director Susan Hopkins is an author of her organisation's non-peer-reviewed reports on 'new variants'. Hopkins is a professor of infectious diseases at London's Imperial College which is gifted tens of millions of dollars a year by the Bill and Melinda Gates Foundation. Gates-funded modelling disaster Neil Ferguson also co-authors Public Health England reports and he spoke in December, 2020, about the potential

danger of the B.1.1.7. ‘UK variant’ promoted by Gates-funded modeller John Edmunds. When I come to the ‘Covid vaccines’ the ‘new variants’ will be shown for what they are – bollocks.

Connections, connections

All these people and modellers are lockdown-obsessed or, put another way, they demand what the Cult demands. Edmunds said in January, 2021, that to ease lockdowns too soon would be a disaster and they had to ‘vaccinate much, much, much more widely than the elderly’. Rosemary Frei highlights that Edmunds is married to Jeanne Pimenta who is described in a LinkedIn profile as director of epidemiology at GlaxoSmithKline (GSK) and she held shares in the company. Patrick Vallance, co-chair of SAGE and the government’s Chief Scientific Adviser, is a former executive of GSK and has a deferred bonus of shares in the company worth £600,000. GSK has serious business connections with Bill Gates and is collaborating with mRNA-’vaccine’ company CureVac to make ‘vaccines’ for the new variants that Edmunds is talking about. GSK is planning a ‘Covid vaccine’ with drug giant Sanofi. Puppet Prime Minister Boris Johnson announced in the spring of 2021 that up to 60 million vaccine doses were to be made at the GSK facility at Barnard Castle in the English North East. Barnard Castle, with a population of just 6,000, was famously visited in breach of lockdown rules in April, 2020, by Johnson aide Dominic Cummings who said that he drove there ‘to test his eyesight’ before driving back to London. Cummings would be better advised to test his integrity – not that it would take long. The GSK facility had nothing to do with his visit then although I’m sure Patrick Vallance would have been happy to arrange an introduction and some tea and biscuits. Ruthless psychopath Gates has made yet another fortune from vaccines in collaboration with Big Pharma companies and gushes at the phenomenal profits to be made from vaccines – more than a 20-to-1 return as he told one interviewer. Gates also tweeted in December, 2019, with the foreknowledge of what was coming: ‘What’s next for our foundation? I’m particularly excited about what the next year could mean for one of the best buys in global health: vaccines.’

Modeller John Edmunds is a big promotor of vaccines as all these people appear to be. He’s the dean of the London School of Hygiene & Tropical Medicine’s Faculty of Epidemiology and Population Health which is

primarily funded by the Bill and Melinda Gates Foundation and the Gates-established and funded GAVI vaccine alliance which is the Gates vehicle to vaccinate the world. The organisation Doctors Without Borders has described GAVI as being ‘aimed more at supporting drug-industry desires to promote new products than at finding the most efficient and sustainable means for fighting the diseases of poverty’. But then that’s why the psychopath Gates created it. John Edmunds said in a video that the London School of Hygiene & Tropical Medicine is involved in every aspect of vaccine development including large-scale clinical trials. He contends that mathematical modelling can show that vaccines protect individuals and society. That’s on the basis of shit in and shit out, I take it. Edmunds serves on the UK Vaccine Network as does Ferguson and the government’s foremost ‘Covid’ adviser, the grim-faced, dark-eyed Chris Whitty. The Vaccine Network says it works ‘to support the government to identify and shortlist targeted investment opportunities for the most promising vaccines and vaccine technologies that will help combat infectious diseases with epidemic potential, and to address structural issues related to the UK’s broader vaccine infrastructure’. Ferguson is acting Director of the Imperial College Vaccine Impact Modelling Consortium which has funding from the Bill and Melina Gates Foundation and the Gates-created GAVI ‘vaccine alliance’. Anyone wonder why these characters see vaccines as the answer to every problem? Ferguson is wildly enthusiastic in his support for GAVI’s campaign to vaccine children en masse in poor countries. You would expect someone like Gates who has constantly talked about the need to reduce the population to want to fund vaccines to keep more people alive. I’m sure that’s why he does it. The John Edmunds London School of Hygiene & Tropical Medicine (LSHTM) has a Vaccines Manufacturing Innovation Centre which develops, tests and commercialises vaccines. Rosemary Frei writes:

The vaccines centre also performs affiliated activities like combating ‘vaccine hesitancy’. The latter includes the Vaccine Confidence Project. The project’s stated purpose is, among other things, ‘to provide analysis and guidance for early response and engagement with the public to ensure sustained confidence in vaccines and immunisation’. The Vaccine Confidence Project’s director is LSHTM professor Heidi Larson. For more than a decade she’s been researching how to combat vaccine hesitancy.

How the bloody hell can blokes like John Edmunds and Neil Ferguson with those connections and financial ties model 'virus' case and death projections for the government and especially in a way that gives their paymasters like Gates exactly what they want? It's insane, but this is what you find throughout the world.

'Covid' is not dangerous, oops, wait, yes it is

Only days before Ferguson's nightmare scenario made Jackboot Johnson take Britain into a China-style lockdown to save us from a deadly 'virus' the UK government website gov.uk was reporting something very different to Ferguson on a page of official government guidance for 'high consequence infectious diseases (HCID)'. It said this about 'Covid-19':

As of 19 March 2020, COVID-19 *is no longer considered to be a high consequence infectious diseases (HCID) in the UK* [my emphasis]. The 4 nations public health HCID group made an interim recommendation in January 2020 to classify COVID-19 as an HCID. This was based on consideration of the UK HCID criteria about the virus and the disease with information available during the early stages of the outbreak.

Now that more is known about COVID-19, the public health bodies in the UK have reviewed the most up to date information about COVID-19 against the UK HCID criteria. They have determined that several features have now changed; in particular, more information is available about mortality rates (low overall), and there is now greater clinical awareness and a specific and sensitive laboratory test, the availability of which continues to increase. The Advisory Committee on Dangerous Pathogens (ACDP) is also of the opinion that COVID-19 should no longer be classified as an HCID.

Soon after the government had been exposed for downgrading the risk they upgraded it again and everyone was back to singing from the same Cult hymn book. Ferguson and his fellow Gates clones indicated that lockdowns and restrictions would have to continue until a Gates-funded vaccine was developed. Gates said the same because Ferguson and his like were repeating the Gates script which is the Cult script. 'Flatten the curve' became an ongoing nightmare of continuing lockdowns with periods in between of severe restrictions in pursuit of destroying independent incomes and had nothing to do with protecting health about which the Cult gives not a shit. Why wouldn't Ferguson be pushing a vaccine 'solution' when he's owned by vaccine-obsessive Gates who makes a fortune from them and when Ferguson heads the Vaccine Impact Modelling Consortium at

Imperial College funded by the Gates Foundation and GAVI, the 'vaccine alliance', created by Gates as his personal vaccine promotion operation? To compound the human catastrophe that Ferguson's 'models' did so much to create he was later exposed for breaking his own lockdown rules by having sexual liaisons with his married girlfriend Antonia Staats at his home while she was living at another location with her husband and children. Staats was a 'climate' activist and senior campaigner at the Soros-funded Avaaz which I wouldn't trust to tell me that grass is green. Ferguson had to resign as a government advisor over this hypocrisy in May, 2020, but after a period of quiet he was back being quoted by the ridiculous media on the need for more lockdowns and a vaccine rollout. Other government-advising 'scientists' from Imperial College' held the fort in his absence and said lockdown could be indefinite until a vaccine was found. The Cult script was being sung by the payrolled choir. I said there was no intention of going back to 'normal' when the 'vaccine' came because the 'vaccine' is part of a very different agenda that I will discuss in Human 2.0. Why would the Cult want to let the world go back to normal when destroying that normal forever was the whole point of what was happening? House arrest, closing businesses and schools through lockdown, (un)social distancing and masks all followed the Ferguson fantasy models. Again as I predicted (these people are so predictable) when the 'vaccine' arrived we were told that house arrest, lockdown, (un)social distancing and masks would still have to continue. I will deal with the masks in the next chapter because they are of fundamental importance.

Where's the 'pandemic'?

Any mildly in-depth assessment of the figures revealed what was really going on. Cult-funded and controlled organisations still have genuine people working within them such is the number involved. So it is with Genevieve Briand, assistant program director of the Applied Economics master's degree program at Johns Hopkins University. She analysed the impact that 'Covid-19' had on deaths from *all* causes in the United States using official data from the CDC for the period from early February to early September, 2020. She found that allegedly 'Covid' *related*-deaths exceeded those from heart disease which she found strange with heart disease always the biggest cause of fatalities. Her research became even more significant

when she noted the sudden decline in 2020 of *all* non-'Covid' deaths: 'This trend is completely contrary to the pattern observed in all previous years … the total decrease in deaths by other causes almost exactly equals the increase in deaths by Covid-19.' This was such a game, set and match in terms of what was happening that Johns Hopkins University deleted the article on the grounds that it 'was being used to support false and dangerous inaccuracies about the impact of the pandemic'. No – because it exposed the scam from official CDC figures and this was confirmed when those figures were published in January, 2021. Here we can see the effect of people dying from heart attacks, cancer, road accidents and gunshot wounds – *anything* – having 'Covid-19' on the death certificate along with those diagnosed from 'symptoms' who had even not tested positive with a test not testing for the 'virus'. I am not kidding with the gunshot wounds, by the way. Brenda Bock, coroner in Grand County, Colorado, revealed that two gunshot victims tested positive for the 'virus' within the previous 30 days and were therefore classified as 'Covid deaths'. Bock said: 'These two people had tested positive for Covid, but that's not what killed them. A gunshot wound is what killed them.' She said she had not even finished her investigation when the state listed the gunshot victims as deaths due to the 'virus'. The death and case figures for 'Covid-19' are an absolute joke and yet they are repeated like parrots by the media, politicians and alleged medical 'experts'. The official Cult narrative is the only show in town.

Genevieve Briand found that deaths from all causes were not exceptional in 2020 compared with previous years and a Spanish magazine published figures that said the same about Spain which was a 'Covid' propaganda hotspot at one point. *Discovery Salud*, a health and medicine magazine, quoted government figures which showed how 17,000 *fewer* people died in Spain in 2020 than in 2019 and more than 26,000 fewer than in 2018. The age-standardised mortality rate for England and Wales when age distribution is taken into account was significantly lower in 2020 than the 1970s, 80s and 90s, and was only the ninth highest since 2000. Where is the 'pandemic'?

Post mortems and autopsies virtually disappeared for 'Covid' deaths amid claims that 'virus-infected' bodily fluids posed a risk to those carrying out the autopsy. This was rejected by renowned German pathologist and forensic doctor Klaus Püschel who said that he and his staff had by then done 150 autopsies on 'Covid' patients with no problems at all. He said

they were needed to know why some ‘Covid’ patients suffered blood clots and not severe respiratory infections. The ‘virus’ is, after all, called SARS or ‘severe acute respiratory syndrome’. I highlighted in the spring of 2020 this phenomenon and quoted New York intensive care doctor Cameron Kyle-Sidell who posted a soon deleted YouTube video to say that they had been told to prepare to treat an infectious disease called ‘Covid-19’, but that was not what they were dealing with. Instead he likened the lung condition of the most severely ill patients to what you would expect with cabin depressurisation in a plane at 30,000 feet or someone dropped on the top of Everest without oxygen or acclimatisation. I have never said this is not happening to a small minority of alleged ‘Covid’ patients – I am saying this is not caused by a phantom ‘contagious virus’. Indeed Kyle-Sidell said that ‘Covid-19’ was not the disease they were told was coming their way. ‘We are operating under a medical paradigm that is untrue,’ he said, and he believed they were treating the wrong disease: ‘These people are being slowly starved of oxygen.’ Patients would take off their oxygen masks in a state of fear and stress and while they were blue in the face on the brink of death. They did not look like patients dying of pneumonia. You can see why they don’t want autopsies when their virus doesn’t exist and there is another condition in some people that they don’t wish to be uncovered. I should add here that the 5G system of millimetre waves was being rapidly introduced around the world in 2020 and even more so now as they fire 5G at the Earth from satellites. At 60 gigahertz within the 5G range that frequency interacts with the oxygen molecule and stops people breathing in sufficient oxygen to be absorbed into the bloodstream. They are installing 5G in schools and hospitals. The world is not mad or anything. 5G can cause major changes to the lungs and blood as I detail in *The Answer* and these consequences are labelled ‘Covid-19’, the alleged symptoms of which can be caused by 5G and other electromagnetic frequencies as cells respond to radiation poisoning.

The ‘Covid death’ scam

Dr Scott Jensen, a Minnesota state senator and medical doctor, exposed ‘Covid’ Medicare payment incentives to hospitals and death certificate manipulation. He said he was sent a seven-page document by the US Department of Health ‘coaching’ him on how to fill out death certificates

which had never happened before. The document said that he didn't need to have a laboratory test for 'Covid-19' to put that on the death certificate and that shocked him when death certificates are supposed to be about facts. Jensen described how doctors had been 'encouraged, if not pressured' to make a diagnosis of 'Covid-19' if they thought it was probable or *'presumed*'. No positive test was necessary – not that this would have mattered anyway. He said doctors were told to diagnose 'Covid' by symptoms when these were the same as colds, allergies, other respiratory problems, and certainly with influenza which 'disappeared' in the 'Covid' era. A common sniffle was enough to get the dreaded verdict. Ontario authorities decreed that a single care home resident with *one* symptom from a long list must lead to the isolation of the entire home. Other courageous doctors like Jensen made the same point about death figure manipulation and how deaths by other causes were falling while 'Covid-19 deaths' were rising at the same rate due to re-diagnosis. Their videos rarely survive long on YouTube with its Cult-supporting algorithms courtesy of CEO Susan Wojcicki and her bosses at Google. Figure-tampering was so glaring and ubiquitous that even officials were letting it slip or outright saying it. UK chief scientific adviser Patrick Vallance said on one occasion that 'Covid' on the death certificate doesn't mean 'Covid' was the cause of death (so why the hell is it there?) and we had the rare sight of a BBC reporter telling the truth when she said: 'Someone could be successfully treated for Covid, in say April, discharged, and then in June, get run over by a bus and die … That person would still be counted as a Covid death in England.' Yet the BBC and the rest of the world media went on repeating the case and death figures as if they were real. Illinois Public Health Director Dr Ngozi Ezike revealed the deceit while her bosses must have been clenching their buttocks:

> If you were in a hospice and given a few weeks to live and you were then found to have Covid that would be counted as a Covid death. [There might be] a clear alternate cause, but it is still listed as a Covid death. So everyone listed as a Covid death doesn't mean that was the cause of the death, but that they had Covid at the time of death.

Yes, a 'Covid virus' never shown to exist and tested for with a test not testing for the 'virus'. In the first period of the pandemic hoax through the spring of 2020 the process began of designating almost everything a

‘Covid’ death and this has continued ever since. I sat in a restaurant one night listening to a loud conversation on the next table where a family was discussing in bewilderment how a relative who had no symptoms of ‘Covid’, and had died of a long-term problem, could have been diagnosed a death by the ‘virus’. I could understand their bewilderment. If they read this book they will know why this medical fraud has been perpetrated the world over.

Some media truth shock

The media ignored the evidence of death certificate fraud until eventually one columnist did speak out when she saw it first-hand. Bel Mooney is a long-time national newspaper journalist in Britain currently working for the *Daily Mail*. Her article on February 19th, 2021, carried this headline: ‘My dad Ted passed three Covid tests and died of a chronic illness yet he’s officially one of Britain’s 120,000 victims of the virus and is far from alone ... so how many more are there?’ She told how her 99-year-old father was in a care home with a long-standing chronic obstructive pulmonary disease and vascular dementia. Maybe, but he was still aware enough to tell her from the start that there was no ‘virus’ and he refused the ‘vaccine’ for that reason. His death was not unexpected given his chronic health problems and Mooney said she was shocked to find that ‘Covid-19’ was declared the cause of death on his death certificate. She said this was a ‘bizarre and unacceptable untruth’ for a man with long-time health problems who had tested negative twice at the home for the ‘virus’. I was also shocked by this story although not by what she said. I had been highlighting the death certificate manipulation for ten months. It was the confirmation that a professional full-time journalist only realised this was going on when it affected her directly and neither did she know that whether her dad tested positive or negative was irrelevant with the test not testing for the ‘virus’. Where had she been? She said she did not believe in ‘conspiracy theories’ without knowing I’m sure that this and ‘conspiracy theorists’ were terms put into widespread circulation by the CIA in the 1960s to discredit those who did not accept the ridiculous official story of the Kennedy assassination. A blanket statement of ‘I don’t believe in conspiracy theories’ is always bizarre. The dictionary definition of the term alone means the world is drowning in conspiracies. What she said was even more

daft when her dad had just been affected by the ‘Covid’ conspiracy. Why else does she think that ‘Covid-19’ was going on the death certificates of people who died of something else?

To be fair once she saw from personal experience what was happening she didn’t mince words. Mooney was called by the care home on the morning of February 9th to be told her father had died in his sleep. When she asked for the official cause of death what came back was ‘Covid-19’. Mooney challenged this and was told there had been deaths from Covid on the dementia floor (confirmed by a test not testing for the ‘virus’) so they considered it ‘reasonable to assume’. ‘But doctor,’ Mooney rightly protested, ‘an assumption isn’t a diagnosis.’ She said she didn’t blame the perfectly decent and sympathetic doctor – ‘he was just doing his job’. Sorry, but that’s *bullshit*. He wasn’t doing his job at all. He was putting a false cause of death on the death certificate and that is a criminal offence for which he should be brought to account and the same with the millions of doctors worldwide who have done the same. They were not doing their job they were following orders and that must not wash at new Nuremberg trials any more than it did at the first ones. Mooney’s doctor was ‘assuming’ (presuming) as he was told to, but ‘just following orders’ makes no difference to his actions. A doctor’s job is to serve the patient and the truth, not follow orders, but that’s what they have done all over the world and played a central part in making the ‘Covid’ hoax possible with all its catastrophic consequences for humanity. Shame on them and they must answer for their actions. Mooney said her disquiet worsened when she registered her father’s death by telephone and was told by the registrar there had been very many other cases like hers where ‘the deceased’ had not tested positive for ‘Covid’ yet it was recorded as the cause of death. The test may not matter, but those involved at their level *think* it matters and it shows a callous disregard for accurate diagnosis. The pressure to do this is coming from the top of the national ‘health’ pyramids which in turn obey the World Health Organization which obeys Gates and the Cult. Mooney said the registrar agreed that this must distort the national figures adding that ‘the strangest thing is that every winter we record countless deaths from flu, and this winter there have been none. Not one!’ She asked if the registrar thought deaths from flu were being misdiagnosed and lumped together with ‘Covid’ deaths. The answer was a ‘puzzled yes’. Mooney said that the funeral director said the same about ‘Covid’ deaths which had

nothing to do with ‘Covid’. They had lost count of the number of families upset by this and other funeral companies in different countries have had the same experience. Mooney wrote:

The nightly shroud-waving and shocking close-ups of pain imposed on us by the TV news bewildered and terrified the population into eager compliance with lockdowns. We were invited to ‘save the NHS’ and to grieve for strangers – the real-life loved ones behind those shocking death counts. Why would the public imagine what I now fear, namely that the way Covid-19 death statistics are compiled might make the numbers seem greater than they are?

Oh, just a little bit – like 100 percent.

Do the maths

Mooney asked why a country would wish to skew its mortality figures by wrongly certifying deaths? What had been going on? Well, if you don’t believe in conspiracies you will never find the answer which is that *it’s a conspiracy*. She did, however, describe what she had discovered as a ‘national scandal’. In reality it’s a global scandal and happening everywhere. Pillars of this conspiracy were all put into place before the button was pressed with the Drosten PCR protocol and high amplifications to produce the cases and death certificate changes to secure illusory ‘Covid’ deaths. Mooney notes that normally two doctors were needed to certify a death, with one having to know the patient, and how the rules were changed in the spring of 2020 to allow one doctor to do this. In the same period ‘Covid deaths’ were decreed to be all cases where Covid-19 was put on the death certificate even without a positive test or any symptoms. Mooney asked: ‘How many of the 30,851 (as of January 15) care home resident deaths with Covid-19 on the certificate (32.4 per cent of all deaths so far) were based on an assumption, like that of my father? And what has that done to our national psyche?’All of them is the answer to the first question and it has devastated and dismantled the national psyche, actually the global psyche, on a colossal scale. In the UK case and death data is compiled by organisations like Public Health England (PHE) and the Office for National Statistics (ONS). Mooney highlights the insane policy of counting a death from any cause as ‘Covid-19’ if this happens within 28 days of a positive test (with a test not testing for the ‘virus’) and she points out that ONS

statistics reflect deaths ‘involving Covid’ ‘or due to Covid’ which meant in practice any death where ‘Covid-19’ was mentioned on the death certificate. She described the consequences of this fraud:

Most people will accept the narrative they are fed, so panicky governments here and in Europe witnessed the harsh measures enacted in totalitarian China and jumped into lockdown. Headlines about Covid deaths tolled like the knell that would bring doomsday to us all. Fear stalked our empty streets. Politicians parroted the frankly ridiculous aim of ‘zero Covid’ and shut down the economy, while most British people agreed that lockdown was essential and (astonishingly to me, as a patriotic Brit) even wanted more restrictions.

For what? Lies on death certificates? Never mind the grim toll of lives ruined, suicides, schools closed, rising inequality, depression, cancelled hospital treatments, cancer patients in a torture of waiting, poverty, economic devastation, loneliness, families kept apart, and so on. How many lives have been lost as a direct result of lockdown?

She said that we could join in a national chorus of shock and horror at reaching the 120,000 death toll which was surely certain to have been totally skewed all along, but what about the human cost of lockdown justified by these ‘death figures’? *The British Medical Journal* had reported a 1,493 percent increase in cases of children taken to Great Ormond Street Hospital with abusive head injuries alone and then there was the effect on families:

Perhaps the most shocking thing about all this is that families have been kept apart – and obeyed the most irrational, changing rules at the whim of government – because they believed in the statistics. They succumbed to fear, which his generation rejected in that war fought for freedom. Dad (God rest his soul) would be angry. And so am I.

Another theme to watch is that in the winter months when there are more deaths from all causes they focus on ‘Covid’ deaths and in the summer when the British Lung Foundation says respiratory disease plummets by 80 percent they rage on about ‘cases’. Either way fascism on population is always the answer.

Nazi eugenics in the 21st century

Elderly people in care homes have been isolated from their families month after lonely month with no contact with relatives and grandchildren who were banned from seeing them. We were told that lockdown fascism was to 'protect the vulnerable' like elderly people. At the same time Do Not Resuscitate (DNR) orders were placed on their medical files so that if they needed resuscitation it wasn't done and 'Covid-19' went on their death certificates. Old people were not being 'protected' they were being culled – murdered in truth. DNR orders were being decreed for disabled and young people with learning difficulties or psychological problems. The UK Care Quality Commission, a non-departmental body of the Department of Health and Social Care, found that 34 percent of those working in health and social care were pressured into placing 'do not attempt cardiopulmonary resuscitation' orders on 'Covid' patients who suffered from disabilities and learning difficulties without involving the patient or their families in the decision. UK judges ruled that an elderly woman with dementia should have the DNA-manipulating 'Covid vaccine' against her son's wishes and that a man with severe learning difficulties should have the jab despite his family's objections. Never mind that many had already died. The judiciary always supports doctors and government in fascist dictatorships. They wouldn't dare do otherwise. A horrific video was posted showing fascist officers from Los Angeles police forcibly giving the 'Covid' shot to women with special needs who were screaming that they didn't want it. The same fascists are seen giving the jab to a sleeping elderly woman in a care home. This is straight out of the Nazi playbook. Hitler's Nazis committed mass murder of the mentally ill and physically disabled throughout Germany and occupied territories in the programme that became known as Aktion T4, or just T4. Sabbatian-controlled Hitler and his grotesque crazies set out to kill those they considered useless and unnecessary. The Reich Committee for the Scientific Registering of Hereditary and Congenital Illnesses registered the births of babies identified by physicians to have 'defects'. By 1941 alone more than 5,000 children were murdered by the state and it is estimated that in total the number of innocent people killed in Aktion T4 was between 275,000 and 300,000. Parents were told their children had been sent away for 'special treatment' never to return. It is rather pathetic to see claims about plans for new extermination camps being dismissed today when the same force behind current events did precisely that 80 years ago. Margaret Sanger was a Cult operative who used 'birth control' to sanitise

her programme of eugenics. Organisations she founded became what is now Planned Parenthood. Sanger proposed that ‘the whole dysgenic population would have its choice of segregation or sterilization’. These included epileptics, ‘feeble-minded’, and prostitutes. Sanger opposed charity because it perpetuated ‘human waste‘. She reveals the Cult mentality and if anyone thinks that extermination camps are a ‘conspiracy theory’ their naivety is touching if breathtakingly stupid.

If you don’t believe that doctors can act with callous disregard for their patients it is worth considering that doctors and medical staff agreed to put government-decreed DNR orders on medical files and do nothing when resuscitation is called for. I don’t know what you call such people in your house. In mine they are Nazis from the Josef Mengele School of Medicine. Phenomenal numbers of old people have died worldwide from the effects of lockdown, depression, lack of treatment, the ‘vaccine’ (more later) and losing the will to live. A common response at the start of the manufactured pandemic was to remove old people from hospital beds and transfer them to nursing homes. The decision would result in a mass cull of elderly people in those homes through lack of treatment – *not* ‘Covid’. Care home whistleblowers have told how once the ‘Covid’ era began doctors would not come to their homes to treat patients and they were begging for drugs like antibiotics that often never came. The most infamous example was ordered by New York governor Andrew Cuomo, brother of a moronic CNN host, who amazingly was given an Emmy Award for his handling of the ‘Covid crisis’ by the ridiculous Wokers that hand them out. Just how ridiculous could be seen in February, 2021, when a Department of Justice and FBI investigation began into how thousands of old people in New York died in nursing homes after being discharged from hospital to make way for ‘Covid’ patients on Cuomo’s say-so – and how he and his staff covered up these facts. This couldn’t have happened to a nicer psychopath. Even then there was a ‘Covid’ spin. Reports said that thousands of old people who tested positive for ‘Covid’ in hospital were transferred to nursing homes to both die of ‘Covid’ and transmit it to others. No – they were in hospital because they were ill and the fact that they tested positive with a test not testing for the ‘virus’ is irrelevant. They were ill often with respiratory diseases ubiquitous in old people near the end of their lives. Their transfer out of hospital meant that their treatment stopped and many would go on to die.

They're old. Who gives a damn?

I have exposed in the books for decades the Cult plan to cull the world's old people and even to introduce at some point what they call a 'demise pill' which at a certain age everyone would take and be out of here by law. In March, 2021, Spain legalised euthanasia and assisted suicide following the Netherlands, Belgium, Luxembourg and Canada on the Tiptoe to the demise pill. Treatment of old people by many 'care' homes has been a disgrace in the 'Covid' era. There are many, many, caring staff – I know some. There have, however, been legions of stories about callous treatment of old people and their families. Police were called when families came to take their loved ones home in the light of isolation that was killing them. They became prisoners of the state. Care home residents in insane, fascist Ontario, Canada, were not allowed to leave their *room* once the 'Covid' hoax began. UK staff have even wheeled elderly people away from windows where family members were talking with them. Oriana Criscuolo from Stockport in the English North West dropped off some things for her 80-year-old father who has Parkinson's disease and dementia and she wanted to wave to him through a ground-floor window. She was told that was 'illegal'. When she went anyway they closed the curtains in the middle of the day. Oriana said:

> It's just unbelievable. I cannot understand how care home staff – people who are being paid to care – have become so uncaring. Their behaviour is inhumane and cruel. It's beyond belief.

She was right and this was not a one-off. What a way to end your life in such loveless circumstances. UK registered nurse Nicky Millen, a proper old school nurse for 40 years, said that when she started her career care was based on dignity, choice, compassion and empathy. Now she said 'the things that are important to me have gone out of the window.' She was appalled that people were dying without their loved ones and saying goodbye on iPads. Nicky described how a distressed 89-year-old lady stroked her face and asked her 'how many paracetamol would it take to finish me off'. Life was no longer worth living while not seeing her family. Nicky said she was humiliated in front of the ward staff and patients for letting the lady stroke her face and giving her a cuddle. Such is the dehumanisation that the 'Covid' hoax has brought to the surface. Nicky

worked in care homes where patients told her they were being held prisoner. ‘I want to live until I die’, one said to her. ‘I had a lady in tears because she hadn’t seen her great-grandson.’ Nicky was compassionate old school meeting psychopathic New Normal. She also said she had worked on a ‘Covid’ ward with no ‘Covid’ patients. Jewish writer Shai Held wrote an article in March, 2020, which was headlined ‘The Staggering, Heartless Cruelty Toward the Elderly’. What he described was happening from the earliest days of lockdown. He said ‘the elderly’ were considered a group and not unique individuals (the way of the Woke). Shai Held said:

Notice how the all-too-familiar rhetoric of dehumanization works: ‘The elderly’ are bunched together as a faceless mass, all of them considered culprits and thus effectively deserving of the suffering the pandemic will inflict upon them. Lost entirely is the fact that the elderly are individual human beings, each with a distinctive face and voice, each with hopes and dreams, memories and regrets, friendships and marriages, loves lost and loves sustained.

‘The elderly’ have become another dehumanised group for which anything goes and for many that has resulted in cold disregard for their rights and their life. The distinctive face that Held talks about is designed to be deleted by masks until everyone is part of a faceless mass.

‘War-zone’ hospitals myth

Again and again medical professionals have told me what was really going on and how hospitals ‘overrun like war zones’ according to the media were virtually empty. The mantra from medical whistleblowers was please don’t use my name or my career is over. Citizen journalists around the world sneaked into hospitals to film evidence exposing the ‘war-zone’ lie. They really *were* largely empty with closed wards and operating theatres. I met a hospital worker in my town on the Isle of Wight during the first lockdown in 2020 who said the only island hospital had never been so quiet. Lockdown was justified by the psychopaths to stop hospitals being overrun. At the same time that the island hospital was near-empty the military arrived here to provide *extra beds*. It was all propaganda to ramp up the fear to ensure compliance with fascism as were never-used temporary hospitals with thousands of beds known as Nightingales and never-used make-shift mortuaries opened by the criminal UK government. A man who helped to

install those extra island beds attributed to the army said they were never used and the hospital was empty. Doctors and nurses ‘stood around talking or on their phones, wandering down to us to see what we were doing’. There were no masks or social distancing. He accused the useless local island paper, the *County Press*, of ‘pumping the fear as if our hospital was overrun and we only have one so it should have been’. He described ambulances parked up with crews outside in deck chairs. When his brother called an ambulance he was told there was a two-hour backlog which he called ‘bullshit’. An old lady on the island fell ‘and was in a bad way’, but a caller who rang for an ambulance was told the situation wasn’t urgent enough. Ambulance stations were working under capacity while people would hear ambulances with sirens blaring driving through the streets. When those living near the stations realised what was going on they would follow them as they left, circulated around an urban area with the sirens going, and then came back without stopping. All this was to increase levels of fear and the same goes for the ‘ventilator shortage crisis’ that cost tens of millions for hastily produced ventilators never to be used. Ambulance crews that agreed to be exploited in this way for fear propaganda might find themselves a mirror. I wish them well with that. Empty hospitals were the obvious consequence of treatment and diagnoses of non-’Covid’ conditions cancelled and those involved handed a death sentence. People have been dying at home from undiagnosed and untreated cancer, heart disease and other life-threatening conditions to allow empty hospitals to deal with a ‘pandemic’ that wasn’t happening.

Death of the innocent

‘War-zones’ have been laying off nursing staff, even doctors where they can. There was no work for them. Lockdown was justified by saving lives and protecting the vulnerable they were actually killing with DNR orders and preventing empty hospitals being ‘overrun’. In Britain the mantra of stay at home to ‘save the NHS’ was everywhere and across the world the same story was being sold when it was all lies. Two California doctors, Dan Erickson and Artin Massihi at Accelerated Urgent Care in Bakersfield, held a news conference in April, 2020, to say that intensive care units in California were ‘empty, essentially’, with hospitals shutting floors, not treating patients and laying off doctors. The California health system was

working at minimum capacity ‘getting rid of doctors because we just don’t have the volume’. They said that people with conditions such as heart disease and cancer were not coming to hospital out of fear of ‘Covid-19’. Their video was deleted by Susan Wojcicki’s Cult-owned YouTube after reaching five million views. Florida governor Ron Desantis, who rejected the severe lockdowns of other states and is being targeted for doing so, said that in March, 2020, every US governor was given models claiming they would run out of hospital beds in days. That was never going to happen and the ‘modellers’ knew it. Deceit can be found at every level of the system. Urgent children’s operations were cancelled including fracture repairs and biopsies to spot cancer. Eric Nicholls, a consultant paediatrician, said ‘this is obviously concerning and we need to return to normal operating and to increase capacity as soon as possible’. Psychopaths in power were rather less concerned *because* they are psychopaths. Deletion of urgent care and diagnosis has been happening all over the world and how many kids and others have died as a result of the actions of these cold and heartless lunatics dictating ‘health’ policy? The number must be stratospheric. Richard Sullivan, professor of cancer and global health at King’s College London, said people feared ‘Covid’ more than cancer such was the campaign of fear. ‘Years of lost life will be quite dramatic’, Sullivan said, with ‘a huge amount of avoidable mortality’. Sarah Woolnough, executive director for policy at Cancer Research UK, said there had been a 75 percent drop in urgent referrals to hospitals by family doctors of people with suspected cancer. Sullivan said that ‘a lot of services have had to scale back – we’ve seen a dramatic decrease in the amount of elective cancer surgery’. Lockdown deaths worldwide has been absolutely fantastic with the *New York Post* reporting how data confirmed that ‘lockdowns end more lives than they save’:

There was a sharp decline in visits to emergency rooms and an increase in fatal heart attacks because patients didn’t receive prompt treatment. Many fewer people were screened for cancer. Social isolation contributed to excess deaths from dementia and Alzheimer’s.

Researchers predicted that the social and economic upheaval would lead to tens of thousands of “deaths of despair” from drug overdoses, alcoholism and suicide. As unemployment surged and mental-health and substance-abuse treatment programs were interrupted, the reported levels of anxiety, depression and suicidal thoughts increased dramatically, as did alcohol sales and fatal drug overdoses.

This has been happening while nurses and other staff had so much time on their hands in the ‘war-zones’ that Tic-Tok dancing videos began appearing across the Internet with medical staff dancing around in empty wards and corridors as people died at home from causes that would normally have been treated in hospital.

Mentions in dispatches

One brave and truth-committed whistleblower was Louise Hampton, a call handler with the UK NHS who made a viral Internet video saying she had done ‘fuck all’ during the ‘pandemic’ which was ‘a load of bollocks’. She said that ‘Covid-19’ was rebranded flu and of course she lost her job. This is what happens in the medical and endless other professions now when you tell the truth. Louise filmed inside ‘war-zone’ accident and emergency departments to show they were empty and I mean *empty* as in no one there. The mainstream media could have done the same and blown the gaff on the whole conspiracy. They haven’t to their eternal shame. Not that most ‘journalists’ seem capable of manifesting shame as with the psychopaths they slavishly repeat without question. The relative few who were admitted with serious health problems were left to die alone with no loved ones allowed to see them because of ‘Covid’ rules and they included kids dying without the comfort of mum and dad at their bedside while the evil behind this couldn’t give a damn. It was all good fun to them. A Scottish NHS staff nurse publicly quit in the spring of 2021 saying: ‘I can no longer be part of the lies and the corruption by the government.’ She said hospitals ‘aren’t full, the beds aren’t full, beds have been shut, wards have been shut’. Hospitals were never busy throughout ‘Covid’. The staff nurse said that Nicola Sturgeon, tragically the leader of the Scottish government, was on television saying save the hospitals and the NHS – ‘but the beds are empty’ and ‘we’ve not seen flu, we always see flu every year’. She wrote to government and spoke with her union Unison (the unions are Cult-compromised and *useless*, but nothing changed. Many of her colleagues were scared of losing their jobs if they spoke out as they wanted to. She said nursing staff were being affected by wearing masks all day and ‘my head is splitting every shift from wearing a mask’. The NHS is part of the fascist tyranny and must be dismantled so we can start again with human beings in charge. (Ironically, hospitals were reported to be busier again

when official ‘Covid’ cases *fell* in spring/summer of 2021 and many other conditions required treatment at the same time as *the fake vaccine rollout*.)

I will cover the ‘Covid vaccine’ scam in detail later, but it is another indicator of the sickening disregard for human life that I am highlighting here. The DNA-manipulating concoctions do not fulfil the definition of a ‘vaccine’, have never been used on humans before and were given only emergency approval because trials were not completed and they continued using the unknowing public. The result was what a NHS senior nurse with responsibility for ‘vaccine’ procedure said was ‘genocide’. She said the ‘vaccines’ were not ‘vaccines’. They had not been shown to be safe and claims about their effectiveness by drug companies were ‘poetic licence’. She described what was happening as a ‘horrid act of human annihilation’. The nurse said that management had instigated a policy of not providing a Patient Information Leaflet (PIL) before people were ‘vaccinated’ even though health care professionals are supposed to do this according to protocol. Patients should also be told that they are taking part in an ongoing clinical trial. Her challenges to what is happening had seen her excluded from meetings and ridiculed in others. She said she was told to ‘watch my step … or I would find myself surplus to requirements’. The nurse, who spoke anonymously in fear of her career, said she asked her NHS manager why he/she was content with taking part in genocide against those having the ‘vaccines’. The reply was that everyone had to play their part and to ‘put up, shut up, and get it done’. Government was ‘leaning heavily’ on NHS management which was clearly leaning heavily on staff. This is how the global ‘medical’ hierarchy operates and it starts with the Cult and its World Health Organization.

She told the story of a doctor who had the Pfizer jab and when questioned had no idea what was in it. The doctor had never read the literature. We have to stop treating doctors as intellectual giants when so many are moral and medical pygmies. The doctor did not even know that the ‘vaccines’ were not fully approved or that their trials were ongoing. They were, however, asking their patients if they minded taking part in follow-ups for research purposes – yes, the *ongoing clinical trial*. The nurse said the doctor’s ignorance was not rare and she had spoken to a hospital consultant who had the jab without any idea of the background or that the ‘trials’ had not been completed. Nurses and pharmacists had shown the same ignorance. ‘My NHS colleagues have forsaken their duty of care,

broken their code of conduct – Hippocratic Oath – and have been brainwashed just the same as the majority of the UK public through propaganda …' She said she had not been able to recruit a single NHS colleague, doctor, nurse or pharmacist to stand with her and speak out. Her union had refused to help. She said that if the genocide came to light she would not hesitate to give evidence at a Nuremberg-type trial against those in power who could have affected the outcomes but didn't.

And all for what?

To put the nonsense into perspective let's say the 'virus' does exist and let's go completely crazy and accept that the official manipulated figures for cases and deaths are accurate. *Even then* a study by Stanford University epidemiologist Dr John Ioannidis published on the World Health Organization website produced an average infection to fatality rate of … *0.23 percent!* Ioannidis said: 'If one could sample equally from all locations globally, the median infection fatality rate might even be substantially lower than the 0.23% observed in my analysis.' For healthy people under 70 it was … *0.05 percent*! This compares with the 3.4 percent claimed by the Cult-owned World Health Organization when the hoax was first played and maximum fear needed to be generated. An updated Stanford study in April, 2021, put the 'infection' to 'fatality' rate at just 0.15 percent. Another team of scientists led by Megan O'Driscoll and Henrik Salje studied data from 45 countries and published their findings on the Nature website. For children and young people the figure is so small it virtually does not register although authorities will be hyping dangers to the young when they introduce DNA-manipulating 'vaccines' for children. The O'Driscoll study produced an average infection-fatality figure of 0.003 for children from birth to four; 0.001 for 5 to 14; 0.003 for 15 to 19; and it was still only 0.456 up to 64. To claim that children must be 'vaccinated' to protect them from 'Covid' is an obvious lie and so there must be another reason and there is. What's more the average age of a 'Covid' death is akin to the average age that people die in general. The average age of death in England is about 80 for men and 83 for women. The average age of death from alleged 'Covid' is between 82 and 83. California doctors, Dan Erickson and Artin Massihi, said at their April media conference that projection models of millions of deaths had been 'woefully inaccurate'. They produced

detailed figures showing that Californians had a 0.03 chance of dying from 'Covid' based on the number of people who tested positive (with a test not testing for the 'virus'). Erickson said there was a 0.1 percent chance of dying from 'Covid' in the *state* of New York, not just the city, and a 0.05 percent chance in Spain, a centre of 'Covid-19' hysteria at one stage. The Stanford studies supported the doctors' data with fatality rate estimates of 0.23 and 0.15 percent. How close are these figures to my estimate of *zero*? Death-rate figures claimed by the World Health Organization at the start of the hoax were some 15 times higher. The California doctors said there was no justification for lockdowns and the economic devastation they caused. Everything they had ever learned about quarantine was that you quarantine the *sick* and not the healthy. They had never seen this before and it made no medical sense.

Why in the in the light of all this would governments and medical systems the world over say that billions must go under house arrest; lose their livelihood; in many cases lose their mind, their health and their life; force people to wear masks dangerous to health and psychology; make human interaction and even family interaction a criminal offence; ban travel; close restaurants, bars, watching live sport, concerts, theatre, and any activity involving human togetherness and discourse; and closing schools to isolate children from their friends and cause many to commit suicide in acts of hopelessness and despair? The California doctors said lockdown consequences included increased child abuse, partner abuse, alcoholism, depression, and other impacts they were seeing every day. Who would do that to the entire human race if not mentally-ill psychopaths of almost unimaginable extremes like Bill Gates? We must face the reality of what we are dealing with and come out of denial. Fascism and tyranny are made possible only by the target population submitting and acquiescing to fascism and tyranny. The whole of human history shows that to be true. Most people naively and unquestioning believed what they were told about a 'deadly virus' and meekly and weakly submitted to house arrest. Those who didn't believe it – at least in total – still submitted in fear of the consequences of not doing so. For the rest who wouldn't submit draconian fines have been imposed, brutal policing by psychopaths *for* psychopaths, and condemnation from the meek and weak who condemn the Pushbackers on behalf of the very force that has them, too, in its gunsights. 'Pathetic' does not even begin to suffice. Britain's brainless 'Health' Secretary Matt

Hancock warned anyone lying to border officials about returning from a list of ‘hotspot’ countries could face a jail sentence of up to ten years which is more than for racially-aggravated assault, incest and attempting to have sex with a child under 13. Hancock is a lunatic, but he has the state apparatus behind him in a Cult-led chain reaction and the same with UK ‘Vaccine Minister’ Nadhim Zahawi, a prominent member of the mega-Cult secret society, Le Cercle, which featured in my earlier books. The Cult enforces its will on governments and medical systems; government and medical systems enforce their will on business and police; business enforces its will on staff who enforce it on customers; police enforce the will of the Cult on the population and play their essential part in creating a world of fascist control that their own children and grandchildren will have to live in their entire lives. It is a hierarchical pyramid of imposition and acquiescence and, yes indeedy, of clinical insanity.

Does anyone bright enough to read this book have to ask what the answer is? I think not, but I will reveal it anyway in the fewest of syllables: Tell the psychos and their moronic lackeys to fuck off and let’s get on with our lives. We are many – They are few.

CHAPTER SEVEN

War on your mind

One believes things because one has been conditioned to believe them
Aldous Huxley, *Brave New World*

I have described the 'Covid' hoax as a 'Psyop' and that is true in every sense and on every level in accordance with the definition of that term which is psychological warfare. Break down the 'Covid pandemic' to the foundation themes and it is psychological warfare on the human individual and collective mind.

The same can be said for the entire human belief system involving every subject you can imagine. Huxley was right in his contention that people believe what they are conditioned to believe and this comes from the repetition throughout their lives of the same falsehoods. They spew from government, corporations, media and endless streams of 'experts' telling you what the Cult wants you to believe and often believing it themselves (although *far* from always). 'Experts' are rewarded with 'prestigious' jobs and titles and as agents of perceptual programming with regular access to the media. The Cult has to control the narrative – control *information* – or they lose control of the vital, crucial, without-which-they-cannot-prevail public perception of reality. The foundation of that control today is the Internet made possible by the Defense Advanced Research Projects Agency (DARPA), the incredibly sinister technological arm of the Pentagon. The Internet is the result of military technology. DARPA openly brags about establishing the Internet which has been a long-term project to lasso the minds of the global population. I have said for decades the plan is to control

information to such an extreme that eventually no one would see or hear anything that the Cult does not approve. We are closing in on that end with ferocious censorship since the ‘Covid’ hoax began and in my case it started back in the 1990s in terms of books and speaking venues. I had to create my own publishing company in 1995 precisely because no one else would publish my books even then. I think they’re all still running.

Cult Internet

To secure total control of information they needed the Internet in which pre-programmed algorithms can seek out ‘unclean’ content for deletion and even stop it being posted in the first place. The Cult had to dismantle print and non-Internet broadcast media to ensure the transfer of information to the appropriate-named ‘Web’ – a critical expression of the *Cult* web. We’ve seen the ever-quickening demise of traditional media and control of what is left by a tiny number of corporations operating worldwide. Independent journalism in the mainstream is already dead and never was that more obvious than since the turn of 2020. The Cult wants all information communicated via the Internet to globally censor and allow the plug to be pulled any time. Lockdowns and forced isolation has meant that communication between people has been through electronic means and no longer through face-to-face discourse and discussion. Cult psychopaths have targeted the bars, restaurants, sport, venues and meeting places in general for this reason. None of this is by chance and it’s to stop people gathering in any kind of privacy or number while being able to track and monitor all Internet communications and block them as necessary. Even private messages between individuals have been censored by these fascists that control Cult fronts like Facebook, Twitter, Google and YouTube which are all officially run by Sabbatian place-people and from the background by higher-level Sabbatian place people. Facebook, Google, Amazon and their like were seed-funded and supported into existence with money-no-object infusions of funds either directly or indirectly from DARPA and CIA technology arm In-Q-Tel. The Cult plays the long game and prepares very carefully for big plays like ‘Covid’. Amazon is another front in the psychological war and pretty much controls the global market in book sales and increasingly publishing. Amazon’s limitless funds have deleted fantastic numbers of independent publishers to seize global domination on

the way to deciding which books can be sold and circulated and which cannot. Moves in that direction are already happening. Amazon's leading light Jeff Bezos is the grandson of Lawrence Preston Gise who worked with DARPA predecessor ARPA. Amazon has big connections to the CIA and the Pentagon. The plan I have long described went like this:

1. Employ military technology to establish the Internet.

2. Sell the Internet as a place where people can freely communicate without censorship and allow that to happen until the Net becomes the central and irreversible pillar of human society. If the Internet had been highly censored from the start many would have rejected it.

3. Fund and manipulate major corporations into being to control the circulation of information on your Internet using cover stories about geeks in garages to explain how they came about. Give them unlimited funds to expand rapidly with no need to make a profit for years while non-Cult companies who need to balance the books cannot compete. You know that in these circumstances your Googles, YouTubes, Facebooks and Amazons are going to secure near monopolies by either crushing or buying up the opposition.

4. Allow freedom of expression on both the Internet and communication platforms to draw people in until the Internet is the central and irreversible pillar of human society and your communication corporations have reached a stage of near monopoly domination.

5. Then unleash your always-planned frenzy of censorship on the basis of 'where else are you going to go?' and continue to expand that until nothing remains that the Cult does not want its human targets to see.

The process was timed to hit the 'Covid' hoax to ensure the best chance possible of controlling the narrative which they knew they had to do at all costs. They were, after all, about to unleash a 'deadly virus' that didn't really exist. If you do that in an environment of free-flowing information and opinion you would be dead in the water before you could say Gates is a psychopath. The network was in place through which the Cult-created-and-owned World Health Organization could dictate the 'Covid' narrative and response policy slavishly supported by Cult-owned Internet communication giants and mainstream media while those telling a different story were censored. Google, YouTube, Facebook and Twitter openly announced that they would do this. What else would we expect from Cult-owned operations like Facebook which former executives have confirmed set out to make the platform more addictive than cigarettes and coldly manipulates emotions of its users to sow division between people and groups and scramble the minds

of the young? If Zuckerberg lives out the rest of his life without going to jail for crimes against humanity, and most emphatically against the young, it will be a travesty of justice. Still, no matter, cause and effect will catch up with him eventually and the same with Sergey Brin and Larry Page at Google with its CEO Sundar Pichai who fix the Google search results to promote Cult narratives and hide the opposition. Put the same key words into Google and other search engines like DuckDuckGo and you will see how different results can be. Wikipedia is another intensely biased 'encyclopaedia' which skews its content to the Cult agenda. YouTube links to Wikipedia's version of 'Covid' and 'climate change' on video pages in which experts in their field offer a different opinion (even that is increasingly rare with Wojcicki censorship). Into this 'Covid' silence-them network must be added government media censors, sorry 'regulators', such as Ofcom in the UK which imposed tyrannical restrictions on British broadcasters that had the effect of banning me from ever appearing. Just to debate with me about my evidence and views on 'Covid' would mean breaking the fascistic impositions of Ofcom and its CEO career government bureaucrat Melanie Dawes. Gutless British broadcasters tremble at the very thought of fascist Ofcom.

Psychos behind 'Covid'

The reason for the 'Covid' catastrophe in all its facets and forms can be seen by whom and what is driving the policies worldwide in such a coordinated way. Decisions are not being made to protect health, but to target psychology. The dominant group guiding and 'advising' government policy are not medical professionals. They are psychologists and behavioural scientists. Every major country has its own version of this phenomenon and I'll use the British example to show how it works. In many ways the British version has been affecting the wider world in the form of the huge behaviour manipulation network in the UK which operates in other countries. The network involves private companies, government, intelligence and military. The Cabinet Office is at the centre of the government 'Covid' Psyop and part-owns, with 'innovation charity' Nesta, the Behavioural Insights Team (BIT) which claims to be independent of government but patently isn't. The BIT was established in 2010 and its job is to manipulate the psyche of the population to acquiesce to government

demands and so much more. It is also known as the ‘Nudge Unit’, a name inspired by the 2009 book by two ultra-Zionists, Cass Sunstein and Richard Thaler, called *Nudge: Improving Decisions About Health, Wealth, and Happiness*. The book, as with the Behavioural Insights Team, seeks to ‘nudge’ behaviour (manipulate it) to make the public follow patterns of action and perception that suit those in authority (the Cult). Sunstein is so skilled at this that he advises the World Health Organization and the UK Behavioural Insights Team and was Administrator of the White House Office of Information and Regulatory Affairs in the Obama administration. Biden appointed him to the Department of Homeland Security – another ultra-Zionist in the fold to oversee new immigration laws which is another policy the Cult wants to control. Sunstein is desperate to silence anyone exposing conspiracies and co-authored a 2008 report on the subject in which suggestions were offered to ban ‘conspiracy theorizing’ or impose ‘some kind of tax, financial or otherwise, on those who disseminate such theories’. I guess a psychiatrist’s chair is out of the question?

Sunstein’s mate Richard Thaler, an ‘academic affiliate’ of the UK Behavioural Insights Team, is a proponent of ‘behavioural economics’ which is defined as the study of ‘the effects of psychological, cognitive, emotional, cultural and social factors on the decisions of individuals and institutions’. Study the effects so they can be manipulated to be what you want them to be. Other leading names in the development of behavioural economics are ultra-Zionists Daniel Kahneman and Robert J. Shiller and they, with Thaler, won the Nobel Memorial Prize in Economic Sciences for their work in this field. The Behavioural Insights Team is operating at the heart of the UK government and has expanded globally through partnerships with several universities including Harvard, Oxford, Cambridge, University College London (UCL) and Pennsylvania. They claim to have ‘trained’ (reframed) 20,000 civil servants and run more than 750 projects involving 400 randomised controlled trials in dozens of countries’ as another version of mind reframers Common Purpose. BIT works from its office in New York with cities and their agencies, as well as other partners, across the United States and Canada – this is a company part-owned by the British government Cabinet Office. An executive order by President Cult-servant Obama established a US Social and Behavioral Sciences Team in 2015. They all have the same reason for being and that’s

to brainwash the population directly and by brainwashing those in positions of authority.

‘Covid’ mind game

Another prime aspect of the UK mind-control network is the ‘independent’ [joke] Scientific Pandemic Insights Group on Behaviours (SPI-B) which ‘provides behavioural science advice aimed at anticipating and helping people adhere to interventions that are recommended by medical or epidemiological experts’. That means manipulating public perception and behaviour to do whatever government tells them to do. It’s disgusting and if they really want the public to be ‘safe’ this lot should all be under lock and key. According to the government website SPI-B consists of ‘behavioural scientists, health and social psychologists, anthropologists and historians’ and advises the Whitty-Vallance-led Scientific Advisory Group for Emergencies (SAGE) which in turn advises the government on ‘the science’ (it doesn’t) and ‘Covid’ policy. When politicians say they are being guided by ‘the science’ this is the rabble in each country they are talking about and that ‘science’ is dominated by behaviour manipulators to enforce government fascism through public compliance. The Behaviour Insight Team is headed by psychologist David Solomon Halpern, a visiting professor at King’s College London, and connects with a national and global web of other civilian and military organisations as the Cult moves towards its goal of fusing them into one fascistic whole in every country through its ‘Fusion Doctrine’. The behaviour manipulation network involves, but is not confined to, the Foreign Office; National Security Council; government communications headquarters (GCHQ); MI5; MI6; the Cabinet Office-based Media Monitoring Unit; and the Rapid Response Unit which ‘monitors digital trends to spot emerging issues; including misinformation and disinformation; and identifies the best way to respond’.

There is also the 77th Brigade of the UK military which operates like the notorious Israeli military’s Unit 8200 in manipulating information and discussion on the Internet by posing as members of the public to promote the narrative and discredit those who challenge it. Here we have the military seeking to manipulate *domestic* public opinion while the Nazis in government are fine with that. Conservative Member of Parliament Tobias Ellwood, an advocate of lockdown and control through ‘vaccine passports’,

is a Lieutenant Colonel reservist in the 77th Brigade which connects with the military operation jHub, the 'innovation centre' for the Ministry of Defence and Strategic Command. jHub has also been involved with the civilian National Health Service (NHS) in 'symptom tracing' the population. The NHS is a key part of this mind control network and produced a document in December, 2020, explaining to staff how to use psychological manipulation with different groups and ages to get them to have the DNA-manipulating 'Covid vaccine' that's designed to cumulatively rewrite human genetics. The document, called 'Optimising Vaccination Roll Out – Do's and Dont's for all messaging, documents and "communications" in the widest sense', was published by NHS England and the NHS Improvement *Behaviour Change Unit* in partnership with Public Health England and Warwick Business School. I hear the mantra about 'save the NHS' and 'protect the NHS' when we need to scrap the NHS and start again. The current version is far too corrupt, far too anti-human and totally compromised by Cult operatives and their assets. UK government broadcast media censor Ofcom will connect into this web – as will the BBC with its tremendous Ofcom influence – to control what the public see and hear and dictate mass perception. Nuremberg trials must include personnel from all these organisations.

The fear factor

The 'Covid' hoax has led to the creation of the UK Cabinet Office-connected Joint Biosecurity Centre (JBC) which is officially described as providing 'expert advice on pandemics' using its independent [all Cult operations are 'independent'] analytical function to provide real-time analysis about infection outbreaks to identify and respond to outbreaks of Covid-19'. Another role is to advise the government on a response to spikes in infections – 'for example by closing schools or workplaces in local areas where infection levels have risen'. Put another way, promoting the Cult agenda. The Joint Biosecurity Centre is modelled on the Joint Terrorism Analysis Centre which analyses intelligence to set 'terrorism threat levels' and here again you see the fusion of civilian and military operations and intelligence that has led to military intelligence producing documents about 'vaccine hesitancy' and how it can be combated. Domestic civilian matters and opinions should not be the business of the military. The Joint

Biosecurity Centre is headed by Tom Hurd, director general of the Office for Security and Counter-Terrorism from the establishment-to-its-fingertips Hurd family. His father is former Foreign Secretary Douglas Hurd. How coincidental that Tom Hurd went to the elite Eton College and Oxford University with Boris Johnson. Imperial College with its ridiculous computer modeller Neil Ferguson will connect with this gigantic web that will itself interconnect with similar set-ups in other major and not so major countries. Compared with this Cult network the politicians, be they Boris Johnson, Donald Trump or Joe Biden, are bit-part players 'following the science'. The network of psychologists was on the 'Covid' case from the start with the aim of generating maximum fear of the 'virus' to ensure compliance by the population. A government behavioural science group known as SPI-B produced a paper in March, 2020, for discussion by the main government science advisory group known as SAGE. It was headed 'Options for increasing adherence to social distancing measures' and it said the following in a section headed 'Persuasion':

> • A substantial number of people still do not feel sufficiently personally threatened; it could be that they are reassured by the low death rate in their demographic group, although levels of concern may be rising. Having a good understanding of the risk has been found to be positively associated with adoption of COVID-19 social distancing measures in Hong Kong.
>
> • The perceived level of personal threat needs to be increased among those who are complacent, using hard-hitting evaluation of options for increasing social distancing emotional messaging. To be effective this must also empower people by making clear the actions they can take to reduce the threat.
>
> • Responsibility to others: There seems to be insufficient understanding of, or feelings of responsibility about, people's role in transmitting the infection to others … Messaging about actions need to be framed positively in terms of protecting oneself and the community, and increase confidence that they will be effective.
>
> • Some people will be more persuaded by appeals to play by the rules, some by duty to the community, and some to personal risk. All these

> different approaches are needed. The messaging also needs to take account of the realities of different people's lives. Messaging needs to take account of the different motivational levers and circumstances of different people.

All this could be achieved the SPI-B psychologists said by *using the media to increase the sense of personal threat* which translates as terrify the shit out of the population, including children, so they all do what we want. That's not happened has it? Those excuses for 'journalists' who wouldn't know journalism if it bit them on the arse (the great majority) have played their crucial part in serving this Cult-government Psyop to enslave their own kids and grandkids. How they live with themselves I have no idea. The psychological war has been underpinned by constant government 'Covid' propaganda in almost every television and radio ad break, plus the Internet and print media, which has pounded out the fear with taxpayers footing the bill for their own programming. The result has been people terrified of a 'virus' that doesn't exist or one with a tiny fatality rate even if you believe it does. People walk down the street and around the shops wearing face-nappies damaging their health and psychology while others report those who refuse to be that naïve to the police who turn up in their own face-nappies. I had a cameraman come to my flat and he was so frightened of 'Covid' he came in wearing a mask and refused to shake my hand in case he caught something. He had – naïveitis – and the thought that he worked in the mainstream media was both depressing and made his behaviour perfectly explainable. The fear which has gripped the minds of so many and frozen them into compliance has been carefully cultivated by these psychologists who are really psychopaths. If lives get destroyed and a lot of young people commit suicide it shows our plan is working. SPI-B then turned to compulsion on the public to comply. 'With adequate preparation, rapid change can be achieved', it said. Some countries had introduced mandatory self-isolation on a wide scale without evidence of major public unrest and a large majority of the UK's population appeared to be supportive of more coercive measures with 64 percent of adults saying they would support putting London under a lockdown (watch the 'polls' which are designed to make people believe that public opinion is in favour or against whatever the subject in hand).

For ‘aggressive protective measures’ to be effective, the SPI-B paper said, special attention should be devoted to those population groups that are more at risk. Translated from the Orwellian this means making the rest of population feel guilty for not protecting the ‘vulnerable’ such as old people which the Cult and its agencies were about to kill on an industrial scale with lockdown, lack of treatment and the Gates ‘vaccine’. Psychopath psychologists sold their guilt-trip so comprehensively that Los Angeles County Supervisor Hilda Solis reported that children were apologising (from a distance) to their parents and grandparents for bringing ‘Covid’ into their homes and getting them sick. ‘… These apologies are just some of the last words that loved ones will ever hear as they die alone,’ she said. Gut-wrenchingly Solis then used this childhood tragedy to tell children to stay at home and ‘keep your loved ones alive’. Imagine heaping such potentially life-long guilt on a kid when it has absolutely nothing to do with them. These people are deeply disturbed and the psychologists behind this even more so.

Uncivil war – divide and rule

Professional mind-controllers at SPI-B wanted the media to increase a sense of responsibility to others (do as you’re told) and promote ‘positive messaging’ for those actions while in contrast to invoke ‘social disapproval’ by the unquestioning, obedient, community of anyone with a mind of their own. Again the compliant Goebbels-like media obliged. This is an old, old, trick employed by tyrannies the world over throughout human history. You get the target population to keep the target population in line – *your* line. SPI-B said this could ‘play an important role in preventing anti-social behaviour or discouraging failure to enact pro-social behaviour’. For ‘anti-social’ in the Orwellian parlance of SPI-B see any behaviour that government doesn’t approve. SPI-B recommendations said that ‘social disapproval’ should be accompanied by clear messaging and promotion of strong collective identity – hence the government and celebrity mantra of ‘we’re all in this together’. Sure we are. The mind doctors have such contempt for their targets that they think some clueless comedian, actor or singer telling them to do what the government wants will be enough to win them over. We have had UK comedian Lenny Henry, actor Michael Caine and singer Elton John wheeled out to serve the propagandists by urging

people to have the DNA-manipulating ‘Covid’ non-’vaccine’. The role of Henry and fellow black celebrities in seeking to coax a ‘vaccine’ reluctant black community into doing the government’s will was especially stomach-turning. An emotion-manipulating script and carefully edited video featuring these black ‘celebs’ was such an insult to the intelligence of black people and where’s the self-respect of those involved selling their souls to a fascist government agenda? Henry said he heard black people’s ‘legitimate worries and concerns’, but people must ‘trust the facts’ when they were doing exactly that by not having the ‘vaccine’. They had to include the obligatory reference to Black Lives Matter with the line … ‘Don’t let coronavirus cost even more black lives – because we matter’. My god, it was pathetic. ‘I know the vaccine is safe and what it does.’ How? ‘I’m a comedian and it says so in my script.’

SPI-B said social disapproval needed to be carefully managed to avoid victimisation, scapegoating and misdirected criticism, but they knew that their ‘recommendations’ would lead to exactly that and the media were specifically used to stir-up the divide-and-conquer hostility. Those who conform like good little baa, baas, are praised while those who have seen through the tidal wave of lies are ‘Covidiots’. The awake have been abused by the fast asleep for not conforming to fascism and impositions that the awake know are designed to endanger their health, dehumanise them, and tear asunder the very fabric of human society. We have had the curtain-twitchers and morons reporting neighbours and others to the face-nappied police for breaking ‘Covid rules’ with fascist police delighting in posting links and phone numbers where this could be done. The Cult cannot impose its will without a compliant police and military or a compliant population willing to play their part in enslaving themselves and their kids. The words of a pastor in Nazi Germany are so appropriate today:

First they came for the socialists and I did not speak out because I was not a socialist.

Then they came for the trade unionists and I did not speak out because I was not a trade unionist.

Then they came for the Jews and I did not speak out because I was not a Jew.

Then they came for me and there was no one left to speak for me.

Those who don't learn from history are destined to repeat it and so many are.

'Covid' rules: Rewiring the mind

With the background laid out to this gigantic national and global web of psychological manipulation we can put 'Covid' rules into a clear and sinister perspective. Forget the claims about protecting health. 'Covid' rules are about dismantling the human mind, breaking the human spirit, destroying self-respect, and then putting Humpty Dumpty together again as a servile, submissive slave. Social isolation through lockdown and distancing have devastating effects on the human psyche as the psychological psychopaths well know and that's the real reason for them. Humans need contact with each other, discourse, closeness and touch, or they eventually, and literarily, go crazy. Masks, which I will address at some length, fundamentally add to the effects of isolation and the Cult agenda to dehumanise and de-individualise the population. To do this while knowing – in fact *seeking* – this outcome is the very epitome of evil and psychologists involved in this *are* the epitome of evil. They must like all the rest of the Cult demons and their assets stand trial for crimes against humanity on a scale that defies the imagination. Psychopaths in uniform use isolation to break enemy troops and agents and make them subservient and submissive to tell what they know. The technique is rightly considered a form of torture and torture is most certainly what has been imposed on the human population.

Clinically-insane American psychologist Harry Harlow became famous for his isolation experiments in the 1950s in which he separated baby monkeys from their mothers and imprisoned them for months on end in a metal container or 'pit of despair'. They soon began to show mental distress and depression as any idiot could have predicted. Harlow put other monkeys in steel chambers for three, six or twelve months while denying them any contact with animals or humans. He said that the effects of total social isolation for six months were 'so devastating and debilitating that we had assumed initially that twelve months of isolation would not produce any additional decrement'; but twelve months of isolation 'almost obliterated the animals socially'. This is what the Cult and its psychopaths are doing to you and your children. Even monkeys in partial isolation in

which they were not allowed to form relationships with other monkeys became ‘aggressive and hostile, not only to others, but also towards their own bodies’. We have seen this in the young as a consequence of lockdown. UK government psychopaths launched a public relations campaign telling people not to hug each other even after they received the ‘Covid-19 vaccine’ which we were told with more lies would allow a return to ‘normal life’. A government source told *The Telegraph*: ‘It will be along the lines that it is great that you have been vaccinated, but if you are going to visit your family and hug your grandchildren there is a chance you are going to infect people you love.’ The source was apparently speaking from a secure psychiatric facility. Janet Lord, director of Birmingham University’s Institute of Inflammation and Ageing, said that parents and grandparents should avoid hugging their children. Well, how can I put it, Ms Lord? Fuck off. Yep, that’ll do.

Destroying the kids – where are the parents?

Observe what has happened to people enslaved and isolated by lockdown as suicide and self-harm has soared worldwide, particularly among the young denied the freedom to associate with their friends. A study of 49,000 people in English-speaking countries concluded that almost half of young adults are at clinical risk of mental health disorders. A national survey in America of 1,000 currently enrolled high school and college students found that 5 percent reported attempting suicide during the pandemic. Data from the US CDC’s National Syndromic Surveillance Program from January 1st to October 17th, 2020, revealed a *31 percent* increase in mental health issues among adolescents aged 12 to 17 compared with 2019. The CDC reported that America in general suffered the biggest drop in life expectancy since World War Two as it fell by a year in the first half of 2020 as a result of ‘deaths of despair’ – overdoses and suicides. Deaths of despair have leapt by more than 20 percent during lockdown and include the highest number of fatal overdoses ever recorded in a single year – 81,000. Internet addiction is another consequence of being isolated at home which lowers interest in physical activities as kids fall into inertia and what’s the point? Children and young people are losing hope and giving up on life, sometimes literally. A 14-year-old boy killed himself in Maryland because he had ‘given up’ when his school district didn’t reopen; an 11-year-old boy shot himself

during a zoom class; a teenager in Maine succumbed to the isolation of the 'pandemic' when he ended his life after experiencing a disrupted senior year at school. Children as young as nine have taken their life and all these stories can be repeated around the world. Careers are being destroyed before they start and that includes those in sport in which promising youngsters have not been able to take part. The plan of the psycho-psychologists is working all right. Researchers at Cambridge University found that lockdowns cause significant harm to children's mental health. Their study was published in the *Archives of Disease in Childhood*, and followed 168 children aged between 7 and 11. The researchers concluded:

During the UK lockdown, children's depression symptoms have increased substantially, relative to before lockdown. The scale of this effect has direct relevance for the continuation of different elements of lockdown policy, such as complete or partial school closures …

… Specifically, we observed a statistically significant increase in ratings of depression, with a medium-to-large effect size. Our findings emphasise the need to incorporate the potential impact of lockdown on child mental health in planning the ongoing response to the global pandemic and the recovery from it.

Not a chance when the Cult's psycho-psychologists were getting exactly what they wanted. The UK's Royal College of Paediatrics and Child Health has urged parents to look for signs of eating disorders in children and young people after a three to four fold increase. Specialists say the 'pandemic' is a major reason behind the rise. You don't say. The College said isolation from friends during school closures, exam cancellations, loss of extra-curricular activities like sport, and an increased use of social media were all contributory factors along with fears about the virus (psycho-psychologists again), family finances, and students being forced to quarantine. Doctors said young people were becoming severely ill by the time they were seen with 'Covid' regulations reducing face-to-face consultations. Nor is it only the young that have been devastated by the psychopaths. Like all bullies and cowards the Cult is targeting the young, elderly, weak and infirm. A typical story was told by a British lady called Lynn Parker who was not allowed to visit her husband in 2020 for the last ten and half months of his life 'when he needed me most' between March 20th and when he died on December 19th. This vacates the criminal and enters the territory of evil. The emotional impact on the immune system alone is immense as are the

number of people of all ages worldwide who have died as a result of Cult-demanded, Gates-demanded, lockdowns.

Isolation is torture

The experience of imposing solitary confinement on millions of prisoners around the world has shown how a large percentage become 'actively psychotic and/or acutely suicidal'. Social isolation has been found to trigger 'a specific psychiatric syndrome, characterized by hallucinations; panic attacks; overt paranoia; diminished impulse control; hypersensitivity to external stimuli; and difficulties with thinking, concentration and memory'. Juan Mendez, a United Nations rapporteur (investigator), said that isolation is a form of torture. Research has shown that even after isolation prisoners find it far more difficult to make social connections and I remember chatting to a shop assistant after one lockdown who told me that when her young son met another child again he had no idea how to act or what to do. Hannah Flanagan, Director of Emergency Services at Journey Mental Health Center in Dane County, Wisconsin, said: 'The specificity about Covid social distancing and isolation that we've come across as contributing factors to the suicides are really new to us this year.' But they are not new to those that devised them. They are getting the effect they want as the population is psychologically dismantled to be rebuilt in a totally different way. Children and the young are particularly targeted. They will be the adults when the full-on fascist AI-controlled technocracy is planned to be imposed and they are being prepared to meekly submit. At the same time older people who still have a memory of what life was like before – and how fascist the new normal really is – are being deleted. You are going to see efforts to turn the young against the old to support this geriatric genocide. Hannah Flanagan said the big increase in suicide in her county proved that social isolation is not only harmful, but deadly. Studies have shown that isolation from others is one of the main risk factors in suicide and even more so with women. Warnings that lockdown could create a 'perfect storm' for suicide were ignored. After all this was one of the *reasons* for lockdown. Suicide, however, is only the most extreme of isolation consequences. There are many others. Dr Dhruv Khullar, assistant professor of healthcare policy at Weill Cornell Medical College, said in a *New York Times* article in 2016 long before the fake 'pandemic':

A wave of new research suggests social separation is bad for us. Individuals with less social connection have disrupted sleep patterns, altered immune systems, more inflammation and higher levels of stress hormones. One recent study found that isolation increases the risk of heart disease by 29 percent and stroke by 32 percent. Another analysis that pooled data from 70 studies and 3.4 million people found that socially isolated individuals had a 30 percent higher risk of dying in the next seven years, and that this effect was largest in middle age.

Loneliness can accelerate cognitive decline in older adults, and isolated individuals are twice as likely to die prematurely as those with more robust social interactions. These effects start early: Socially isolated children have significantly poorer health 20 years later, even after controlling for other factors. All told, loneliness is as important a risk factor for early death as obesity and smoking.

There you have proof from that one article alone four years before 2020 that those who have enforced lockdown, social distancing and isolation knew what the effect would be and that is even more so with professional psychologists that have been driving the policy across the globe. We can go back even further to the years 2000 and 2003 and the start of a major study on the effects of isolation on health by Dr Janine Gronewold and Professor Dirk M. Hermann at the University Hospital in Essen, Germany, who analysed data on 4,316 people with an average age of 59 who were recruited for the long-term research project. They found that socially isolated people are more than 40 percent more likely to have a heart attack, stroke, or other major cardiovascular event and nearly 50 percent more likely to die from any cause. Given the financial Armageddon unleashed by lockdown we should note that the study found a relationship between increased cardiovascular risk and lack of financial support. After excluding other factors social isolation was still connected to a 44 percent increased risk of cardiovascular problems and a 47 percent increased risk of death by any cause. Lack of financial support was associated with a 30 percent increase in the risk of cardiovascular health events. Dr Gronewold said it had been known for some time that feeling lonely or lacking contact with close friends and family can have an impact on physical health and the study had shown that having strong social relationships is of high importance for heart health. Gronewold said they didn't understand yet why people who are socially isolated have such poor health outcomes, but this was obviously a worrying finding, particularly during these times of prolonged social distancing. Well, it can be explained on many levels. You only have to identify the point in the body where people feel loneliness and missing people they are parted from – it's in the centre of the chest where

they feel the ache of loneliness and the ache of missing people. ‘My heart aches for you’ … ‘My heart aches for some company.’ I will explain this more in the chapter Escaping Wetiko, but when you realise that the body is the mind – they are expressions of each other – the reason why state of the mind dictates state of the body becomes clear.

American psychologist Ranjit Powar was highlighting the effects of lockdown isolation as early as April, 2020. She said humans have evolved to be social creatures and are wired to live in interactive groups. Being isolated from family, friends and colleagues could be unbalancing and traumatic for most people and could result in short or even long-term psychological and physical health problems. An increase in levels of anxiety, aggression, depression, forgetfulness and hallucinations were possible psychological effects of isolation. ‘Mental conditions may be precipitated for those with underlying pre-existing susceptibilities and show up in many others without any pre-condition.’ Powar said personal relationships helped us cope with stress and if we lost this outlet for letting off steam the result can be a big emotional void which, for an average person, was difficult to deal with. ‘Just a few days of isolation can cause increased levels of anxiety and depression’ – so what the hell has been the effect on the global population of *18 months* of this at the time of writing? Powar said: ‘Add to it the looming threat of a dreadful disease being repeatedly hammered in through the media and you have a recipe for many shades of mental and physical distress.’ For those with a house and a garden it is easy to forget that billions have had to endure lockdown isolation in tiny overcrowded flats and apartments with nowhere to go outside. The psychological and physical consequences of this are unimaginable and with lunatic and abusive partners and parents the consequences have led to tremendous increases in domestic and child abuse and alcoholism as people seek to shut out the horror. Ranjit Powar said:

Staying in a confined space with family is not all a rosy picture for everyone. It can be extremely oppressive and claustrophobic for large low-income families huddled together in small single-room houses. Children here are not lucky enough to have many board/electronic games or books to keep them occupied.

Add to it the deep insecurity of running out of funds for food and basic necessities. On the other hand, there are people with dysfunctional family dynamics, such as domineering, abusive or alcoholic partners, siblings or parents which makes staying home a period of trial. Incidence of

suicide and physical abuse against women has shown a worldwide increase. Heightened anxiety and depression also affect a person's immune system, making them more susceptible to illness.

To think that Powar's article was published on April 11th, 2020.

Six-feet fantasy

Social (unsocial) distancing demanded that people stay six feet or two metres apart. UK government advisor Robert Dingwall from the New and Emerging Respiratory Virus Threats Advisory Group said in a radio interview that the two-metre rule was 'conjured up out of nowhere' and was not based on science. No, it was not based on *medical* science, but it didn't come out of nowhere. The distance related to *psychological* science. Six feet/two metres was adopted in many countries and we were told by people like the criminal Anthony Fauci and his ilk that it was founded on science. Many schools could not reopen because they did not have the space for six-feet distancing. Then in March, 2021, after a year of six-feet 'science', a study published in the *Journal of Infectious Diseases* involving more than 500,000 students and almost 100,000 staff over 16 weeks revealed no significant difference in 'Covid' cases between six feet and three feet and Fauci changed his tune. Now three feet was okay. There is no difference between six feet and three *inches* when there is no 'virus' and they got away with six feet for psychological reasons for as long as they could. I hear journalists and others talk about 'unintended consequences' of lockdown. They are not *unintended* at all; they have been coldly-calculated for a specific outcome of human control and that's why super-psychopaths like Gates have called for them so vehemently. Super-psychopath psychologists have demanded them and psychopathic or clueless, spineless, politicians have gone along with them by 'following the science'. But it's not science at all. 'Science' is not what is; it's only what people can be manipulated to believe it is. The whole 'Covid' catastrophe is founded on mind control. Three word or three statement mantras issued by the UK government are a well-known mind control technique and so we've had 'Stay home/protect the NHS/save lives', 'Stay alert/control the virus/save lives' and 'hands/face/space'. One of the most vocal proponents of extreme 'Covid' rules in the UK has been Professor Susan Michie, a member of the British Communist Party, who is not a medical professional. Michie is the

director of the Centre for Behaviour Change at University College London. She is a *behavioural psychologist* and another filthy rich 'Marxist' who praised China's draconian lockdown. She was known by fellow students at Oxford University as 'Stalin's nanny' for her extreme Marxism. Michie is an influential member of the UK government's Scientific Advisory Group for Emergencies (SAGE) and behavioural manipulation groups which have dominated 'Covid' policy. She is a consultant adviser to the World Health Organization on 'Covid-19' and behaviour. Why the hell are lockdowns anything to do with her when they are claimed to be about health? Why does a behavioural psychologist from a group charged with changing the behaviour of the public want lockdown, human isolation and mandatory masks? Does that question really need an answer? Michie *absolutely* has to explain herself before a Nuremberg court when humanity takes back its world again and even more so when you see the consequences of masks that she demands are compulsory. This is a Michie classic:

> The benefits of getting primary school children to wear masks is that regardless of what little degree of transmission is occurring in those age groups it could help normalise the practice. Young children wearing masks may be more likely to get their families to accept masks.

Those words alone should carry a prison sentence when you ponder on the callous disregard for children involved and what a statement it makes about the mind and motivations of Susan Michie. What a lovely lady and what she said there encapsulates the mentality of the psychopaths behind the 'Covid' horror. Let us compare what Michie said with a countrywide study in Germany published at researchsquare.com involving 25,000 school children and 17,854 health complaints submitted by parents. Researchers found that masks are harming children physically, psychologically, and behaviourally with 24 health issues associated with mask wearing. They include: shortness of breath (29.7%); dizziness (26.4%); increased headaches (53%); difficulty concentrating (50%); drowsiness or fatigue (37%); and malaise (42%). Nearly a third of children experienced more sleep issues than before and a quarter developed new fears. Researchers found health issues and other impairments in 68 percent of masked children covering their faces for an average of 4.5 hours a day. Hundreds of those taking part experienced accelerated respiration, tightness in the chest,

weakness, and short-term impairment of consciousness. A reminder of what Michie said again:

The benefits of getting primary school children to wear masks is that regardless of what little degree of transmission is occurring in those age groups it could help normalise the practice. Young children wearing masks may be more likely to get their families to accept masks.

Psychopaths in government and psychology now have children and young people – plus all the adults – wearing masks for hours on end while clueless teachers impose the will of the psychopaths on the young they should be protecting. What the hell are parents doing?

Cult lab rats

We have some schools already imposing on students microchipped buzzers that activate when they get 'too close' to their pals in the way they do with lab rats. How apt. To the Cult and its brain-dead servants our children *are* lab rats being conditioned to be unquestioning, dehumanised slaves for the rest of their lives. Children and young people are being weaned and frightened away from the most natural human instincts including closeness and touch. I have tracked in the books over the years how schools were banning pupils from greeting each other with a hug and the whole Cult-induced Me Too movement has terrified men and boys from a relaxed and natural interaction with female friends and work colleagues to the point where many men try never to be in a room alone with a woman that's not their partner. Airhead celebrities have as always played their virtue-signalling part in making this happen with their gross exaggeration. For every monster like Harvey Weinstein there are at least tens of thousands of men that don't treat women like that; but everyone must be branded the same and policy changed for them as well as the monster. I am going to be using the word 'dehumanise' many times in this chapter because that is what the Cult is seeking to do and it goes very deep as we shall see. Don't let them kid you that social distancing is planned to end one day. That's not the idea. We are seeing more governments and companies funding and producing wearable gadgets to keep people apart and they would not be doing that if this was meant to be short-term. A tech start-up company

backed by GCHQ, the British Intelligence and military surveillance headquarters, has created a social distancing wrist sensor that alerts people when they get too close to others. The CIA has also supported tech companies developing similar devices. The wearable sensor was developed by Tended, one of a number of start-up companies supported by GCHQ (see the CIA and DARPA). The device can be worn on the wrist or as a tag on the waistband and will vibrate whenever someone wearing the device breaches social distancing and gets anywhere near natural human contact. The company had a lucky break in that it was developing a distancing sensor when the 'Covid' hoax arrived which immediately provided a potentially enormous market. How fortunate. The government in big-time Cult-controlled Ontario in Canada is investing $2.5 million in wearable contact tracing technology that 'will alert users if they may have been exposed to the Covid-19 in the workplace and will beep or vibrate if they are within six feet of another person'. Facedrive Inc., the technology company behind this, was founded in 2016 with funding from the Ontario Together Fund and obviously they, too, had a prophet on the board of directors. The human surveillance and control technology is called TraceSCAN and would be worn by the human cyborgs in places such as airports, workplaces, construction sites, care homes and … *schools*.

I emphasise schools with children and young people the prime targets. You know what is planned for society as a whole if you keep your eyes on the schools. They have always been places where the state program the next generation of slaves to be its compliant worker-ants – or Woker-ants these days; but in the mist of the 'Covid' madness they have been transformed into mind laboratories on a scale never seen before. Teachers and head teachers are just as programmed as the kids – often more so. Children are kept apart from human interaction by walk lanes, classroom distancing, staggered meal times, masks, and the rolling-out of buzzer systems. Schools are now physically laid out as a laboratory maze for lab-rats. Lunatics at a school in Anchorage, Alaska, who should be prosecuted for child abuse, took away desks and forced children to kneel (know your place) on a mat for five hours a day while wearing a mask and using their chairs as a desk. How this was supposed to impact on a 'virus' only these clinically insane people can tell you and even then it would be clap-trap. The school banned recess (interaction), art classes (creativity), and physical exercise (getting body and mind moving out of inertia). Everyone behind this outrage should

be in jail or better still a mental institution. The behavioural manipulators are all for this dystopian approach to schools. Professor Susan Michie, the mind-doctor and British Communist Party member, said it was wrong to say that schools were safe. They had to be made so by 'distancing', masks and ventilation (sitting all day in the cold). I must ask this lady round for dinner on a night I know I am going to be out and not back for weeks. She probably wouldn't be able to make it, anyway, with all the visits to her own psychologist she must have block-booked.

Masking identity

I know how shocking it must be for you that a behaviour manipulator like Michie wants everyone to wear masks which have long been a feature of mind-control programs like the infamous MKUltra in the United States, but, there we are. We live and learn. I spent many years from 1996 to right across the millennium researching mind control in detail on both sides of the Atlantic and elsewhere. I met a large number of mind-control survivors and many had been held captive in body and mind by MKUltra. MK stands for mind-control, but employs the German spelling in deference to the Nazis spirited out of Germany at the end of World War Two by Operation Paperclip in which the US authorities, with help from the Vatican, transported Nazi mind-controllers and engineers to America to continue their work. Many of them were behind the creation of NASA and they included Nazi scientist and SS officer Wernher von Braun who swapped designing V-2 rockets to bombard London with designing the Saturn V rockets that powered the NASA moon programme's Apollo craft. I think I may have mentioned that the Cult has no borders. Among Paperclip escapees was Josef Mengele, the Angel of Death in the Nazi concentration camps where he conducted mind and genetic experiments on children often using twins to provide a control twin to measure the impact of his 'work' on the other. If you want to observe the Cult mentality in all its extremes of evil then look into the life of Mengele. I have met many people who suffered mercilessly under Mengele in the United States where he operated under the name Dr Greene and became a stalwart of MKUltra programming and torture. Among his locations was the underground facility in the Mojave Desert in California called the China Lake Naval Weapons Station which is almost entirely below the surface. My books *The Biggest Secret,*

Children of the Matrix and *The Perception Deception* have the detailed background to MKUltra.

The best-known MKUltra survivor is American Cathy O'Brien. I first met her and her late partner Mark Phillips at a conference in Colorado in 1996. Mark helped her escape and deprogram from decades of captivity in an offshoot of MKUltra known as Project Monarch in which 'sex slaves' were provided for the rich and famous including Father George Bush, Dick Cheney and the Clintons. Read Cathy and Mark's book *Trance-Formation of America* and if you are new to this you will be shocked to the core. I read it in 1996 shortly before, with the usual synchronicity of my life, I found myself given a book table at the conference right next to hers. MKUltra never ended despite being very publicly exposed (only a small part of it) in the 1970s and continues in other guises. I am still in touch with Cathy. She contacted me during 2020 after masks became compulsory in many countries to tell me how they were used as part of MKUltra programming. I had been observing 'Covid regulations' and the relationship between authority and public for months. I saw techniques that I knew were employed on individuals in MKUltra being used on the global population. I had read many books and manuals on mind control including one called *Silent Weapons for Quiet Wars* which came to light in the 1980s and was a guide on how to perceptually program on a mass scale. 'Silent Weapons' refers to mind-control. I remembered a line from the manual as governments, medical authorities and law enforcement agencies have so obviously talked to – or rather at – the adult population since the 'Covid' hoax began as if they are children. The document said:

> If a person is spoken to by a T.V. advertiser as if he were a twelve-year-old, then, due to suggestibility, he will, with a certain probability, respond or react to that suggestion with the uncritical response of a twelve-year-old and will reach in to his economic reservoir and deliver its energy to buy that product on impulse when he passes it in the store.

That's why authority has spoken to adults like children since all this began.

Why did Michael Jackson wear masks?

Every aspect of the 'Covid' narrative has mind-control as its central theme. Cathy O'Brien wrote an article for davidicke.com about the connection between masks and mind control. Her daughter Kelly who I first met in the 1990s was born while Cathy was still held captive in MKUltra. Kelly was forced to wear a mask as part of her programming from the age of *two* to dehumanise her, target her sense of individuality and reduce the amount of oxygen her brain and body received. *Bingo*. This is the real reason for compulsory masks, why they have been enforced en masse, and why they seek to increase the number they demand you wear. First one, then two, with one disgraceful alleged 'doctor' recommending four which is nothing less than a death sentence. Where and how often they must be worn is being expanded for the purpose of mass mind control and damaging respiratory health which they can call 'Covid-19'. Canada's government headed by the man-child Justin Trudeau, says it's fine for children of two and older to wear masks. An insane 'study' in Italy involving just 47 children concluded there was no problem for babies as young as *four months* wearing them. Even after people were 'vaccinated' they were still told to wear masks by the criminal that is Anthony Fauci. Cathy wrote that mandating masks is allowing the authorities literally to control the air we breathe which is what was done in MKUltra. You might recall how the singer Michael Jackson wore masks and there is a reason for that. He was subjected to MKUltra mind control through Project Monarch and his psyche was scrambled by these simpletons. Cathy wrote:

In MKUltra Project Monarch mind control, Michael Jackson had to wear a mask to silence his voice so he could not reach out for help. Remember how he developed that whisper voice when he wasn't singing? Masks control the mind from the outside in, like the redefining of words is doing. By controlling what we can and cannot say for fear of being labeled racist or beaten, for example, it ultimately controls thought that drives our words and ultimately actions (or lack thereof).

Likewise, a mask muffles our speech so that we are not heard, which controls voice … words … mind. This is Mind Control. Masks are an obvious mind control device, and I am disturbed so many people are complying on a global scale. Masks depersonalize while making a person feel as though they have no voice. It is a barrier to others. People who would never choose to comply but are forced to wear a mask in order to keep their job, and ultimately their family fed, are compromised. They often feel shame and are subdued. People have stopped talking with each other while media controls the narrative.

The ‘no voice’ theme has often become literal with train passengers told not to speak to each other in case they pass on the ‘virus’, singing banned for the same reason and bonkers California officials telling people riding roller coasters that they cannot shout and scream. Cathy said she heard every day from healed MKUltra survivors who cannot wear a mask without flashing back on ways their breathing was controlled – ‘from ball gags and penises to water boarding’. She said that through the years when she saw images of people in China wearing masks ‘due to pollution’ that it was really to control their oxygen levels. ‘I knew it was as much of a population control mechanism of depersonalisation as are burkas’, she said. Masks are another Chinese communist/fascist method of control that has been swept across the West as the West becomes China at lightning speed since we entered 2020.

Mask-19

There are other reasons for mandatory masks and these include destroying respiratory health to call it ‘Covid-19’ and stunting brain development of children and the young. Dr Margarite Griesz-Brisson MD, PhD, is a Consultant Neurologist and Neurophysiologist and the Founder and Medical Director of the London Neurology and Pain Clinic. Her CV goes down the street and round the corner. She is clearly someone who cares about people and won’t parrot the propaganda. Griesz-Brisson has a PhD in pharmacology, with special interest in neurotoxicology, environmental medicine, neuroregeneration and neuroplasticity (the way the brain can change in the light of information received). She went public in October, 2020, with a passionate warning about the effects of mask-wearing laws:

> The reinhalation of our exhaled air will without a doubt create oxygen deficiency and a flooding of carbon dioxide. We know that the human brain is very sensitive to oxygen deprivation. There are nerve cells for example in the hippocampus that can’t be longer than 3 minutes without oxygen – they cannot survive. The acute warning symptoms are headaches, drowsiness, dizziness, issues in concentration, slowing down of reaction time – reactions of the cognitive system.

Oh, I know, let’s tell bus, truck and taxi drivers to wear them and people working machinery. How about pilots, doctors and police? Griesz-Brisson makes the important point that while the symptoms she mentions may fade

as the body readjusts this does not alter the fact that people continue to operate in oxygen deficit with long list of potential consequences. She said it was well known that neurodegenerative diseases take years or decades to develop. ‘If today you forget your phone number, the breakdown in your brain would have already started 20 or 30 years ago.’ She said degenerative processes in your brain are getting amplified as your oxygen deprivation continues through wearing a mask. Nerve cells in the brain are unable to divide themselves normally in these circumstances and lost nerve cells will no longer be regenerated. ‘What is gone is gone.’ Now consider that people like shop workers and *schoolchildren* are wearing masks for hours every day. What in the name of sanity is going to be happening to them? ‘I do not wear a mask, I need my brain to think’, Griesz-Brisson said, ‘I want to have a clear head when I deal with my patients and not be in a carbon dioxide-induced anaesthesia’. If you are told to wear a mask anywhere ask the organisation, police, store, whatever, for their risk assessment on the dangers and negative effects on mind and body of enforcing mask-wearing. They won’t have one because it has never been done not even by government. All of them must be subject to class-action lawsuits as the consequences come to light. They don’t do mask risk assessments for an obvious reason. They know what the conclusions would be and independent scientific studies that *have* been done tell a horror story of consequences.

‘Masks are criminal’

Dr Griesz-Brisson said that for children and adolescents, masks are an absolute no-no. They had an extremely active and adaptive immune system and their brain was incredibly active with so much to learn. ‘The child’s brain, or the youth’s brain, is thirsting for oxygen.’ The more metabolically active an organ was, the more oxygen it required; and in children and adolescents every organ was metabolically active. Griesz-Brisson said that to deprive a child’s or adolescent’s brain of oxygen, or to restrict it in any way, was not only dangerous to their health, it was absolutely criminal. ‘Oxygen deficiency inhibits the development of the brain, and the damage that has taken place as a result CANNOT be reversed.’ Mind manipulators of MKUltra put masks on two-year-olds they wanted to neurologically rewire and you can see why. Griesz-Brisson said a child needs the brain to learn and the brain needs oxygen to function. ‘We don’t need a clinical

study for that. This is simple, indisputable physiology.' Consciously and purposely induced oxygen deficiency was an absolutely deliberate health hazard, and an absolute medical contraindication which means that 'this drug, this therapy, this method or measure should not be used, and is not allowed to be used'. To coerce an entire population to use an absolute medical contraindication by force, she said, there had to be definite and serious reasons and the reasons must be presented to competent interdisciplinary and independent bodies to be verified and authorised. She had this warning of the consequences that were coming if mask wearing continued:

When, in ten years, dementia is going to increase exponentially, and the younger generations couldn't reach their god-given potential, it won't help to say 'we didn't need the masks'. I know how damaging oxygen deprivation is for the brain, cardiologists know how damaging it is for the heart, pulmonologists know how damaging it is for the lungs. Oxygen deprivation damages every single organ. Where are our health departments, our health insurance, our medical associations? It would have been their duty to be vehemently against the lockdown and to stop it and stop it from the very beginning.

Why do the medical boards issue punishments to doctors who give people exemptions? Does the person or the doctor seriously have to prove that oxygen deprivation harms people? What kind of medicine are our doctors and medical associations representing? Who is responsible for this crime? The ones who want to enforce it? The ones who let it happen and play along, or the ones who don't prevent it?

All of the organisations and people she mentions there either answer directly to the Cult or do whatever hierarchical levels above them tell them to do. The outcome of both is the same. 'It's not about masks, it's not about viruses, it's certainly not about your health', Griesz-Brisson said. 'It is about much, much more. I am not participating. I am not afraid.' They were taking our air to breathe and there was no unfounded medical exemption from face masks. Oxygen deprivation was dangerous for every single brain. It had to be the free decision of every human being whether they want to wear a mask that was absolutely ineffective to protect themselves from a virus. She ended by rightly identifying where the responsibility lies for all this:

The imperative of the hour is personal responsibility. We are responsible for what we think, not the media. We are responsible for what we do, not our superiors. We are responsible for our health, not

the World Health Organization. And we are responsible for what happens in our country, not the government.

Halle-bloody-lujah.

But surgeons wear masks, right?

Independent studies of mask-wearing have produced a long list of reports detailing mental, emotional and physical dangers. What a definition of insanity to see police officers imposing mask-wearing on the public which will cumulatively damage their health while the police themselves wear masks that will cumulatively damage *their* health. It's utter madness and both public and police do this because 'the government says so' – yes a government of brain-donor idiots like UK Health Secretary Matt Hancock reading the 'follow the science' scripts of psychopathic, lunatic psychologists. The response you get from Stockholm syndrome sufferers defending the very authorities that are destroying them and their families is that 'surgeons wear masks'. This is considered the game, set and match that they must work and don't cause oxygen deficit. Well, actually, scientific studies have shown that they *do* and oxygen levels are monitored in operating theatres to compensate. Surgeons wear masks to stop spittle and such like dropping into open wounds – not to stop 'viral particles' which are so miniscule they can only be seen through an electron microscope. Holes in the masks are significantly bigger than 'viral particles' and if you sneeze or cough they will breach the mask. I watched an incredibly disingenuous 'experiment' that claimed to prove that masks work in catching 'virus' material from the mouth and nose. They did this with a slow motion camera and the mask did block big stuff which stayed inside the mask and against the face to be breathed in or cause infections on the face as we have seen with many children. 'Viral particles', however, would never have been picked up by the camera as they came through the mask when they are far too small to be seen. The 'experiment' was therefore disingenuous *and* useless.

Studies have concluded that wearing masks in operating theatres (and thus elsewhere) make no difference to preventing infection while the opposite is true with toxic shite building up in the mask and this had led to an explosion in tooth decay and gum disease dubbed by dentists 'mask

mouth'. You might have seen the Internet video of a furious American doctor urging people to take off their masks after a four-year-old patient had been rushed to hospital the night before and nearly died with a lung infection that doctors sourced to mask wearing. A study in the journal *Cancer Discovery* found that inhalation of harmful microbes can contribute to advanced stage lung cancer in adults and long-term use of masks can help breed dangerous pathogens. Microbiologists have said frequent mask wearing creates a moist environment in which microbes can grow and proliferate before entering the lungs. The Canadian Agency for Drugs and Technologies in Health, or CADTH, a Canadian national organisation that provides research and analysis to healthcare decision-makers, said this as long ago as 2013 in a report entitled 'Use of Surgical Masks in the Operating Room: A Review of the Clinical Effectiveness and Guidelines'. It said:

> • No evidence was found to support the use of surgical face masks to reduce the frequency of surgical site infections
> • No evidence was found on the effectiveness of wearing surgical face masks to protect staff from infectious material in the operating room.
> • Guidelines recommend the use of surgical face masks by staff in the operating room to protect both operating room staff and patients (despite the lack of evidence).

We were told that the world could go back to 'normal' with the arrival of the 'vaccines'. When they came, fraudulent as they are, the story changed as I knew that it would. We are in the midst of transforming 'normal', not going back to it. Mary Ramsay, head of immunisation at Public Health England, echoed the words of US criminal Anthony Fauci who said masks and other regulations must stay no matter if people are vaccinated. The Fauci idiot continued to wear two masks – different colours so both could be clearly seen – after he *claimed* to have been vaccinated. Senator Rand Paul told Fauci in one exchange that his double-masks were 'theatre' and he was right. It's all theatre. Mary Ramsay back-tracked on the vaccine-return-to-normal theme when she said the public may need to wear masks and social-distance for years despite the jabs. 'People have got used to those lower-level restrictions now, and [they] can live with them', she said telling us what the idea has been all along. 'The vaccine does not give you a pass,

even if you have had it, you must continue to follow all the guidelines' said a Public Health England statement which reneged on what we had been told before and made having the 'vaccine' irrelevant to 'normality' even by the official story. Spain's fascist government trumped everyone by passing a law mandating the wearing of masks on the beach and even when swimming in the sea. The move would have devastated what's left of the Spanish tourist industry, posed potential breathing dangers to swimmers and had Northern European sunbathers walking around with their forehead brown and the rest of their face white as a sheet. The ruling was so crazy that it had to be retracted after pressure from public and tourist industry, but it confirmed where the Cult wants to go with masks and how clinically insane authority has become. The determination to make masks permanent and hide the serious dangers to body and mind can be seen in the censorship of scientist Professor Denis Rancourt by Bill Gates-funded academic publishing website ResearchGate over his papers exposing the dangers and uselessness of masks. Rancourt said:

ResearchGate today has permanently locked my account, which I have had since 2015. Their reasons graphically show the nature of their attack against democracy, and their corruption of science … By their obscene non-logic, a scientific review of science articles reporting on harms caused by face masks has a 'potential to cause harm'. No criticism of the psychological device (face masks) is tolerated, if the said criticism shows potential to influence public policy.

This is what happens in a fascist world.

Where are the 'greens' (again)?

Other dangers of wearing masks especially regularly relate to the inhalation of minute plastic fibres into the lungs and the deluge of discarded masks in the environment and oceans. Estimates predicted that more than 1.5 billion disposable masks will end up in the world's oceans every year polluting the water with tons of plastic and endangering marine wildlife. Studies project that humans are using 129 billion face masks each month worldwide – about three million a minute. Most are disposable and made from plastic, non-biodegradable microfibers that break down into smaller plastic particles that become widespread in ecosystems. They are littering cities, clogging sewage channels and turning up in bodies of water. I have written

in other books about the immense amounts of microplastics from endless sources now being absorbed into the body. Rolf Halden, director of the Arizona State University (ASU) Biodesign Center for Environmental Health Engineering, was the senior researcher in a 2020 study that analysed 47 human tissue samples and found microplastics in all of them. 'We have detected these chemicals of plastics in every single organ that we have investigated', he said. I wrote in *The Answer* about the world being deluged with microplastics. A study by the Worldwide Fund for Nature (WWF) found that people are consuming on average every week some 2,000 tiny pieces of plastic mostly through water and also through marine life and the air. Every year humans are ingesting enough microplastics to fill a heaped dinner plate and in a life-time of 79 years it is enough to fill two large waste bins. Marco Lambertini, WWF International director general said: 'Not only are plastics polluting our oceans and waterways and killing marine life – it's in all of us and we can't escape consuming plastics,' American geologists found tiny plastic fibres, beads and shards in rainwater samples collected from the remote slopes of the Rocky Mountain National Park near Denver, Colorado. Their report was headed: 'It is raining plastic.' Rachel Adams, senior lecturer in Biomedical Science at Cardiff Metropolitan University, said that among health consequences are internal inflammation and immune responses to a 'foreign body'. She further pointed out that microplastics become carriers of toxins including mercury, pesticides and dioxins (a known cause of cancer and reproductive and developmental problems). These toxins accumulate in the fatty tissues once they enter the body through microplastics. Now this is being compounded massively by people putting plastic on their face and throwing it away.

Workers exposed to polypropylene plastic fibres known as 'flock' have developed 'flock worker's lung' from inhaling small pieces of the flock fibres which can damage lung tissue, reduce breathing capacity and exacerbate other respiratory problems. *Now* … commonly used surgical masks have three layers of melt-blown textiles made of … polypropylene. We have billions of people putting these microplastics against their mouth, nose and face for hours at a time day after day in the form of masks. How does anyone think that will work out? I mean – what could possibly go wrong? We posted a number of scientific studies on this at davidicke.com, but when I went back to them as I was writing this book the links to the science research website where they were hosted were dead. Anything that

challenges the official narrative in any way is either censored or vilified. The official narrative is so unsupportable by the evidence that only deleting the truth can protect it. A study by Chinese scientists still survived – with the usual twist which it why it was still active, I guess. Yes, they found that virtually all the masks they tested increased the daily intake of microplastic fibres, but people should still wear them because the danger from the 'virus' was worse said the crazy 'team' from the Institute of Hydrobiology in Wuhan. Scientists first discovered microplastics in lung tissue of some patients who died of lung cancer in the 1990s. Subsequent studies have confirmed the potential health damage with the plastic degrading slowly and remaining in the lungs to accumulate in volume. Wuhan researchers used a machine simulating human breathing to establish that masks shed up to nearly 4,000 microplastic fibres in a month with reused masks producing more. Scientists said some masks are laced with toxic chemicals and a variety of compounds seriously restricted for both health and environmental reasons. They include cobalt (used in blue dye) and formaldehyde known to cause watery eyes, burning sensations in the eyes, nose, and throat, plus coughing, wheezing and nausea. No – that must be 'Covid-19'.

Mask 'worms'

There is another and potentially even more sinister content of masks. Mostly new masks of different makes filmed under a microscope around the world have been found to contain strange black fibres or 'worms' that appear to move or 'crawl' by themselves and react to heat and water. The nearest I have seen to them are the self-replicating fibres that are pulled out through the skin of those suffering from Morgellons disease which has been connected to the phenomena of 'chemtrails' which I will bring into the story later on. Morgellons fibres continue to grow outside the body and have a form of artificial intelligence. Black 'worm' fibres in masks have that kind of feel to them and there is a nanotechnology technique called 'worm micelles' which carry and release drugs or anything else you want to deliver to the body. For sure the suppression of humanity by mind altering drugs is the Cult agenda big time and the more excuses they can find to gain access to the body the more opportunities there are to make that happen whether through 'vaccines' or masks pushed against the mouth and nose for hours on end.

So let us summarise the pros and cons of masks:

Against masks: Breathing in your own carbon dioxide; depriving the body and brain of sufficient oxygen; build-up of toxins in the mask that can be breathed into the lungs and cause rashes on the face and 'mask-mouth'; breathing microplastic fibres and toxic chemicals into the lungs; dehumanisation and deleting individualisation by literally making people faceless; destroying human emotional interaction through facial expression and deleting parental connection with their babies which look for guidance to their facial expression.

For masks: They don't protect you from a 'virus' that doesn't exist and even if it did 'viral' particles are so minute they are smaller than the holes in the mask.

Governments, police, supermarkets, businesses, transport companies, and all the rest who seek to impose masks have done no risk assessment on their consequences for health and psychology and are now open to group lawsuits when the impact becomes clear with a cumulative epidemic of respiratory and other disease. Authorities will try to exploit these effects and hide the real cause by dubbing them 'Covid-19'. Can you imagine setting out to force the population to wear health-destroying masks without doing any assessment of the risks? It is criminal and it is evil, but then how many people targeted in this way, who see their children told to wear them all day at school, have asked for a risk assessment? Billions can't be imposed upon by the few unless the billions allow it. Oh, yes, with just a tinge of irony, 85 percent of all masks made worldwide come from *China*.

Wash your hands in toxic shite

'Covid' rules include the use of toxic sanitisers and again the health consequences of constantly applying toxins to be absorbed through the skin is obvious to any level of Renegade Mind. America's Food and Drug Administration (FDA) said that sanitisers are drugs and issued a warning about 75 dangerous brands which contain methanol used in antifreeze and

can cause death, kidney damage and blindness. The FDA circulated the following warning even for those brands that it claims to be safe:

Store hand sanitizer out of the reach of pets and children, and children should use it only with adult supervision. Do not drink hand sanitizer. This is particularly important for young children, especially toddlers, who may be attracted by the pleasant smell or brightly colored bottles of hand sanitizer.

Drinking even a small amount of hand sanitizer can cause alcohol poisoning in children. (However, there is no need to be concerned if your children eat with or lick their hands after using hand sanitizer.) During this coronavirus pandemic, poison control centers have had an increase in calls about accidental ingestion of hand sanitizer, so it is important that adults monitor young children's use.

Do not allow pets to swallow hand sanitizer. If you think your pet has eaten something potentially dangerous, call your veterinarian or a pet poison control center right away. Hand sanitizer is flammable and should be stored away from heat and flames. When using hand sanitizer, rub your hands until they feel completely dry before performing activities that may involve heat, sparks, static electricity, or open flames.

There you go, perfectly safe, then, and that's without even a mention of the toxins absorbed through the skin. Come on kids – sanitise your hands everywhere you go. It will save you from the 'virus'. Put all these elements together of the 'Covid' normal and see how much health and psychology is being cumulatively damaged, even devastated, to 'protect your health'. Makes sense, right? They are only imposing these things because they care, right? *Right?*

Submitting to insanity

Psychological reframing of the population goes very deep and is done in many less obvious ways. I hear people say how contradictory and crazy 'Covid' rules are and how they are ever changing. This is explained away by dismissing those involved as idiots. It is a big mistake. The Cult is delighted if its cold calculation is perceived as incompetence and idiocy when it is anything but. Oh, yes, there are idiots within the system – lots of them – but they are *administering* the Cult agenda, mostly unknowingly. They are not deciding and dictating it. The bulwark against tyranny is self-respect, always has been, always will be. It is self-respect that has broken every tyranny in history. By its very nature self-respect will not bow to oppression and its perpetrators. There is so little self-respect that it's always

the few that overturn dictators. Many may eventually follow, but the few with the iron spines (self-respect) kick it off and generate the momentum. The Cult targets self-respect in the knowledge that once this has gone only submission remains. Crazy, contradictory, ever-changing 'Covid' rules are systematically applied by psychologists to delete self-respect. They *want* you to see that the rules make no sense. It is one thing to decide to do something when *you* have made the choice based on evidence and logic. You still retain your self-respect. It is quite another when you can see what you are being told to do is insane, ridiculous and makes no sense, and *yet you still do it*. Your self-respect is extinguished and this has been happening as ever more obviously stupid and nonsensical things have been demanded and the great majority have complied even when they can see they are stupid and nonsensical.

People walk around in face-nappies knowing they are damaging their health and make no difference to a 'virus'. They do it in fear of not doing it. I know it's daft, but I'll do it anyway. When that happens something dies inside of you and submissive reframing has begun. Next there's a need to hide from yourself that you have conceded your self-respect and you convince yourself that you have not really submitted to fear and intimidation. You begin to believe that you are complying with craziness because it's the right thing to do. When first you concede your self-respect of 2+2 = 4 to 2+2 = 5 you *know* you are compromising your self-respect. Gradually to avoid facing that fact you begin to *believe* that 2+2=5. You have been reframed and I have been watching this process happening in the human psyche on an industrial scale. The Cult is working to break your spirit and one of its major tools in that war is humiliation. I read how former American soldier Bradley Manning (later Chelsea Manning after a sex-change) was treated after being jailed for supplying WikiLeaks with documents exposing the enormity of government and elite mendacity. Manning was isolated in solitary confinement for eight months, put under 24-hour surveillance, forced to hand over clothing before going to bed, and stand naked for every roll call. This is systematic humiliation. The introduction of anal swab 'Covid' tests in China has been done for the same reason to delete self-respect and induce compliant submission. Anal swabs are mandatory for incoming passengers in parts of China and American diplomats have said they were forced to undergo the indignity which would

have been calculated humiliation by the Cult-owned Chinese government that has America in its sights.

Government-people: An abusive relationship

Spirit-breaking psychological techniques include giving people hope and apparent respite from tyranny only to take it away again. This happened in the UK during Christmas, 2020, when the psycho-psychologists and their political lackeys announced an easing of restrictions over the holiday only to reimpose them almost immediately on the basis of yet another lie. There is a big psychological difference between getting used to oppression and being given hope of relief only to have that dashed. Psychologists know this and we have seen the technique used repeatedly. Then there is traumatising people before you introduce more extreme regulations that require compliance. A perfect case was the announcement by the dark and sinister Whitty and Vallance in the UK that 'new data' predicted that 4,000 could die every day over the winter of 2020/2021 if we did not lockdown again. I think they call it lying and after traumatising people with that claim out came Jackboot Johnson the next day with new curbs on human freedom. Psychologists know that a frightened and traumatised mind becomes suggestable to submission and behaviour reframing. Underpinning all this has been to make people fearful and suspicious of each other and see themselves as a potential danger to others. In league with deleted self-respect you have the perfect psychological recipe for self-loathing. The relationship between authority and public is now demonstrably the same as that of subservience to an abusive partner. These are signs of an abusive relationship explained by psychologist Leslie Becker-Phelps:

Psychological and emotional abuse: Undermining a partner's self-worth with verbal attacks, name-calling, and belittling. Humiliating the partner in public, unjustly accusing them of having an affair, or interrogating them about their every behavior. Keeping partner confused or off balance by saying they were just kidding or blaming the partner for 'making' them act this way … Feigning in public that they care while turning against them in private. This leads to victims frequently feeling confused, incompetent, unworthy, hopeless, and chronically self-doubting.

[Apply these techniques to how governments have treated the population since New Year, 2020, and the parallels are obvious.]

Physical abuse: The abuser might physically harm their partner in a range of ways, such as grabbing, hitting, punching, or shoving them. They might throw objects at them or harm them with a weapon. [Observe the physical harm imposed by masks, lockdown, and so on.]

Threats and intimidation: One way abusers keep their partners in line is by instilling fear. They might be verbally threatening, or give threatening looks or gestures. Abusers often make it known that they are tracking their partner's every move. They might destroy their partner's possessions, threaten to harm them, or threaten to harm their family members. Not surprisingly, victims of this abuse often feel anxiety, fear, and panic. [No words necessary.]

Isolation: Abusers often limit their partner's activities, forbidding them to talk or interact with friends or family. They might limit access to a car or even turn off their phone. All of this might be done by physically holding them against their will, but is often accomplished through psychological abuse and intimidation. The more isolated a person feels, the fewer resources they have to help gain perspective on their situation and to escape from it. [No words necessary.]

Economic abuse: Abusers often make their partners beholden to them for money by controlling access to funds of any kind. They might prevent their partner from getting a job or withhold access to money they earn from a job. This creates financial dependency that makes leaving the relationship very difficult. [See destruction of livelihoods and the proposed meagre 'guaranteed income' so long as you do whatever you are told.]

Using children: An abuser might disparage their partner's parenting skills, tell their children lies about their partner, threaten to take custody of their children, or threaten to harm their children. These tactics instil fear and often elicit compliance. [See reframed social service mafia and how children are being mercilessly abused by the state over 'Covid' while their parents look on too frightened to do anything.]

A further recurring trait in an abusive relationship is the abused blaming themselves for their abuse and making excuses for the abuser. We have the public blaming each other for lockdown abuse by government and many making excuses for the government while attacking those who challenge

the government. How often we have heard authorities say that rules are being imposed or reimposed only because people have refused to ‘behave’ and follow the rules. We don’t want to do it – it’s *you*.

Renegade Minds are an antidote to all of these things. They will never concede their self-respect no matter what the circumstances. Even when apparent humiliation is heaped upon them they laugh in its face and reflect back the humiliation on the abuser where it belongs. Renegade Minds will never wear masks they know are only imposed to humiliate, suppress and damage both physically and psychologically. Consequences will take care of themselves and they will never break their spirit or cause them to concede to tyranny. UK newspaper columnist Peter Hitchens was one of the few in the mainstream media to speak out against lockdowns and forced vaccinations. He then announced he had taken the jab. He wanted to see family members abroad and he believed vaccine passports were inevitable even though they had not yet been introduced. Hitchens has a questioning and critical mind, but not a Renegade one. If he had no amount of pressure would have made him concede. Hitchens excused his action by saying that the battle has been lost. Renegade Minds never accept defeat when freedom is at stake and even if they are the last one standing the self-respect of not submitting to tyranny is more important than any outcome or any consequence.

That’s why Renegade Minds are the only minds that ever changed anything worth changing.

CHAPTER EIGHT

‘Reframing’ insanity

Insanity is relative. It depends on who has who locked in what cage
Ray Bradbury

‘Reframing’ a mind means simply to change its perception and behaviour. This can be done subconsciously to such an extent that subjects have no idea they have been ‘reframed’ while to any observer changes in behaviour and attitudes are obvious.

Human society is being reframed on a ginormous scale since the start of 2020 and here we have the reason why psychologists rather than doctors have been calling the shots. Ask most people who have succumbed to ‘Covid’ reframing if they have changed and most will say ‘no’; but they *have* and fundamentally. The Cult’s long-game has been preparing for these times since way back and crucial to that has been to prepare both population and officialdom mentally and emotionally. To use the mind-control parlance they had to reframe the population with a mentality that would submit to fascism and reframe those in government and law enforcement to impose fascism or at least go along with it. The result has been the fact-deleted mindlessness of ‘Wokeness’ and officialdom that has either enthusiastically or unquestioningly imposed global tyranny demanded by reframed politicians on behalf of psychopathic and deeply evil cultists. ‘Cognitive reframing’ identifies and challenges the way someone sees the world in the form of situations, experiences and emotions and then restructures those perceptions to view the same set of circumstances in a different way. This can have benefits if the attitudes are personally destructive while on the

other side it has the potential for individual and collective mind control which the subject has no idea has even happened.

Cognitive therapy was developed in the 1960s by Aaron T. Beck who was born in Rhode Island in 1921 as the son of Jewish immigrants from the Ukraine. He became interested in the techniques as a treatment for depression. Beck's daughter Judith S. Beck is prominent in the same field and they founded the Beck Institute for Cognitive Behavior Therapy in Philadelphia in 1994. Cognitive reframing, however, began to be used worldwide by those with a very dark agenda. The Cult reframes politicians to change their attitudes and actions until they are completely at odds with what they once appeared to stand for. The same has been happening to government administrators at all levels, law enforcement, military and the human population. Cultists love mind control for two main reasons: It allows them to control what people think, do and say to secure agenda advancement and, by definition, it calms their legendary insecurity and fear of the unexpected. I have studied mind control since the time I travelled America in 1996. I may have been talking to next to no one in terms of an audience in those years, but my goodness did I gather a phenomenal amount of information and knowledge about so many things including the techniques of mind control. I have described this in detail in other books going back to *The Biggest Secret* in 1998. I met a very large number of people recovering from MKUltra and its offshoots and successors and I began to see how these same techniques were being used on the population in general. This was never more obvious than since the 'Covid' hoax began.

Reframing the enforcers

I have observed over the last two decades and more the very clear transformation in the dynamic between the police, officialdom and the public. I tracked this in the books as the relationship mutated from one of serving the public to seeing them as almost the enemy and certainly a lower caste. There has always been a class divide based on income and always been some psychopathic, corrupt, and big-I-am police officers. This was different. Wholesale change was unfolding in the collective dynamic; it was less about money and far more about position and perceived power. An us-and-them was emerging. Noses were lifted skyward by government administration and law enforcement and their attitude to the public they

were *supposed* to be serving changed to one of increasing contempt, superiority and control. The transformation was so clear and widespread that it had to be planned. Collective attitudes and dynamics do not change naturally and organically that quickly on that scale. I then came across an organisation in Britain called Common Purpose created in the late 1980s by Julia Middleton who would work in the office of Deputy Prime Minister John Prescott during the long and disastrous premiership of war criminal Tony Blair. When Blair speaks the Cult is speaking and the man should have been in jail a long time ago. Common Purpose proclaims itself to be one of the biggest 'leadership development' organisations in the world while functioning as a *charity* with all the financial benefits which come from that. It hosts 'leadership development' courses and programmes all over the world and claims to have 'brought together' what it calls 'leaders' from more than 100 countries on six continents. The modus operandi of Common Purpose can be compared with the work of the UK government's reframing network that includes the Behavioural Insights Team 'nudge unit' and 'Covid' reframing specialists at SPI-B. WikiLeaks described Common Purpose long ago as 'a hidden virus in our government and schools' which is unknown to the general public: 'It recruits and trains "leaders" to be loyal to the directives of Common Purpose and the EU, instead of to their own departments, which they then undermine or subvert, the NHS [National Health Service] being an example.' This is a vital point to understand the 'Covid' hoax. The NHS, and its equivalent around the world, has been utterly reframed in terms of administrators and much of the medical personnel with the transformation underpinned by recruitment policies. The outcome has been the criminal and psychopathic behaviour of the NHS over 'Covid' and we have seen the same in every other major country. WikiLeaks said Common Purpose trainees are 'learning to rule without regard to democracy' and to usher in a police state (current events explained). Common Purpose operated like a 'glue' and had members in the NHS, BBC, police, legal profession, church, many of Britain's 7,000 quangos, local councils, the Civil Service, government ministries and Parliament, and controlled many RDA's (Regional Development Agencies). Here we have one answer for how and why British institutions and their like in other countries have changed so negatively in relation to the public. This further explains how and why the beyond-disgraceful reframed BBC has

become a propaganda arm of ‘Covid’ fascism. They are all part of a network pursuing the same goal.

By 2019 Common Purpose was quoting a figure of 85,000 ‘leaders’ that had attended its programmes. These ‘students’ of all ages are known as Common Purpose ‘graduates’ and they consist of government, state and local government officials and administrators, police chiefs and officers, and a whole range of others operating within the national, local and global establishment. Cressida Dick, Commissioner of the London Metropolitan Police, is the Common Purpose graduate who was the ‘Gold Commander’ that oversaw what can only be described as the murder of Brazilian electrician Jean Charles de Menezes in 2005. He was held down by psychopathic police and shot seven times in the head by a psychopathic lunatic after being mistaken for a terrorist when he was just a bloke going about his day. Dick authorised officers to pursue and keep surveillance on de Menezes and ordered that he be stopped from entering the underground train system. Police psychopaths took her at her word clearly. She was ‘disciplined’ for this outrage by being *promoted* – eventually to the top of the ‘Met’ police where she has been a disaster. Many Chief Constables controlling the police in different parts of the UK are and have been Common Purpose graduates. I have heard the ‘graduate’ network described as a sort of Mafia or secret society operating within the fabric of government at all levels pursuing a collective policy ingrained at Common Purpose training events. Founder Julia Middleton herself has said:

> Locally and internationally, Common Purpose graduates will be ‘lighting small fires’ to create change in their organisations and communities … The Common Purpose effect is best illustrated by the many stories of small changes brought about by leaders, who themselves have changed.

A Common Purpose mission statement declared:

> Common Purpose aims to improve the way society works by expanding the vision, decision-making ability and influence of all kinds of leaders. The organisation runs a variety of educational programmes for leaders of all ages, backgrounds and sectors, in order to provide them with the inspirational, information and opportunities they need to change the world.

Yes, but into what? Since 2020 the answer has become clear.

NLP and the Delphi technique

Common Purpose would seem to be a perfect name or would common programming be better? One of the foundation methods of reaching ‘consensus’ (group think) is by setting the agenda theme and then encouraging, cajoling or pressuring everyone to agree a ‘consensus’ in line with the core theme promoted by Common Purpose. The methodology involves the ‘Delphi technique’, or an adaption of it, in which opinions are expressed that are summarised by a ‘facilitator or change agent’ at each stage. Participants are ‘encouraged’ to modify their views in the light of what others have said. Stage by stage the former individual opinions are merged into group consensus which just happens to be what Common Purpose wants them to believe. A key part of this is to marginalise anyone refusing to concede to group think and turn the group against them to apply pressure to conform. We are seeing this very technique used on the general population to make ‘Covid’ group-thinkers hostile to those who have seen through the bullshit. People can be reframed by using perception manipulation methods such as Neuro-Linguistic Programming (NLP) in which you change perception with the use of carefully constructed language. An NLP website described the technique this way:

> … A method of influencing brain behaviour (the ‘neuro’ part of the phrase) through the use of language (the ‘linguistic’ part) and other types of communication to enable a person to ‘recode’ the way the brain responds to stimuli (that’s the ‘programming’) and manifest new and better behaviours. Neuro-Linguistic Programming often incorporates hypnosis and self-hypnosis to help achieve the change (or ‘programming’) that is wanted.

British alternative media operation UKColumn has done very detailed research into Common Purpose over a long period. I quoted co-founder and former naval officer Brian Gerrish in my book *Remember Who You Are*, published in 2011, as saying the following years before current times:

> It is interesting that many of the mothers who have had children taken by the State speak of the Social Services people being icily cool, emotionless and, as two ladies said in slightly different words, ‘… like little robots’. We know that NLP is cumulative, so people can be given small imperceptible doses of NLP in a course here, another in a few months, next year etc. In this way, major changes are accrued in their personality, but the day by day change is almost unnoticeable.

In these and other ways ‘graduates’ have had their perceptions uniformly reframed and they return to their roles in the institutions of government, law enforcement, legal profession, military, ‘education’, the UK National Health Service and the whole swathe of the establishment structure to pursue a common agenda preparing for the ‘post-industrial’, ‘post-democratic’ society. I say ‘preparing’ but we are now there. ‘Post-industrial’ is code for the Great Reset and ‘post-democratic’ is ‘Covid’ fascism. UKColumn has spoken to partners of those who have attended Common Purpose ‘training’. They have described how personalities and attitudes of ‘graduates’ changed very noticeably for the worse by the time they had completed the course. They had been ‘reframed’ and told they are the ‘leaders’ – the special ones – who know better than the population. There has also been the very demonstrable recruitment of psychopaths and narcissists into government administration at all levels and law enforcement. If you want psychopathy hire psychopaths and you get a simple cause and effect. If you want administrators, police officers and ‘leaders’ to perceive the public as lesser beings who don’t matter then employ narcissists. These personalities are identified using ‘psychometrics’ that identifies knowledge, abilities, attitudes and personality traits, mostly through carefully-designed questionnaires and tests. As this policy has passed through the decades we have had power-crazy, power-trippers appointed into law enforcement, security and government administration in preparation for current times and the dynamic between public and law enforcement/officialdom has been transformed. UKColumn’s Brian Gerrish said of the narcissistic personality:

> Their love of themselves and power automatically means that they will crush others who get in their way. I received a major piece of the puzzle when a friend pointed out that when they made public officials re-apply for their own jobs several years ago they were also required to do psychometric tests. This was undoubtedly the start of the screening process to get ‘their’ sort of people in post.

How obvious that has been since 2020 although it was clear what was happening long before if people paid attention to the changing public-establishment dynamic.

Change agents

At the centre of events in ‘Covid’ Britain is the National Health Service (NHS) which has behaved disgracefully in slavishly following the Cult agenda. The NHS management structure is awash with Common Purpose graduates or ‘change agents’ working to a common cause. Helen Bevan, a Chief of Service Transformation at the NHS Institute for Innovation and Improvement, co-authored a document called ‘Towards a million change agents, a review of the social movements literature: implications for large scale change in the NHS‘. The document compared a project management approach to that of change and social movements where ‘people change themselves and each other – peer to peer’. Two definitions given for a ‘social movement’ were:

A group of people who consciously attempt to build a radically new social order; involves people of a broad range of social backgrounds; and deploys politically confrontational and socially disruptive tactics – Cyrus Zirakzadeh 1997

Collective challenges, based on common purposes and social solidarities, in sustained interaction with elites, opponents, and authorities – Sidney Tarrow 1994

Helen Bevan wrote another NHS document in which she defined ‘framing’ as ‘the process by which leaders construct, articulate and put across their message in a powerful and compelling way in order to win people to their cause and call them to action’. I think I could come up with another definition that would be rather more accurate. The National Health Service and institutions of Britain and the wider world have been taken over by reframed ‘change agents’ and that includes everything from the United Nations to national governments, local councils and social services which have been kidnapping children from loving parents on an extraordinary and gathering scale on the road to the end of parenthood altogether. Children from loving homes are stolen and kidnapped by the state and put into the ‘care’ (inversion) of the local authority through council homes, foster parents and forced adoption. At the same time children are allowed to be abused without response while many are under council ‘care’. UKColumn

highlighted the Common Purpose connection between South Yorkshire Police and Rotherham council officers in the case of the scandal in that area of the sexual exploitation of children to which the authorities turned not one blind eye, but both:

We were alarmed to discover that the Chief Executive, the Strategic Director of Children and Young People's Services, the Manager for the Local Strategic Partnership, the Community Cohesion Manager, the Cabinet Member for Cohesion, the Chief Constable and his predecessor had all attended Leadership training courses provided by the pseudo-charity Common Purpose.

Once 'change agents' have secured positions of hire and fire within any organisation things start to move very quickly. Personnel are then hired and fired on the basis of whether they will work towards the agenda the change agent represents. If they do they are rapidly promoted even though they may be incompetent. Those more qualified and skilled who are pre-Common Purpose 'old school' see their careers stall and even disappear. This has been happening for decades in every institution of state, police, 'health' and social services and all of them have been transformed as a result in their attitudes to their jobs and the public. Medical professions, including nursing, which were once vocations for the caring now employ many cold, callous and couldn't give a shit personality types. The UKColumn investigation concluded:

By blurring the boundaries between people, professions, public and private sectors, responsibility and accountability, Common Purpose encourages 'graduates' to believe that as new selected leaders, they can work together, outside of the established political and social structures, to achieve a paradigm shift or CHANGE – so called 'Leading Beyond Authority'. In doing so, the allegiance of the individual becomes 'reframed' on CP colleagues and their NETWORK.

Reframing the Face-Nappies

Nowhere has this process been more obvious than in the police where recruitment of psychopaths and development of unquestioning mind-controlled group-thinkers have transformed law enforcement into a politically-correct 'Woke' joke and a travesty of what should be public service. Today they wear their face-nappies like good little gofers and enforce 'Covid' rules which are fascism under another name. Alongside the

specifically-recruited psychopaths we have software minds incapable of free thought. Brian Gerrish again:

An example is the policeman who would not get on a bike for a press photo because he had not done the cycling proficiency course. Normal people say this is political correctness gone mad. Nothing could be further from the truth. The policeman has been reframed, and in his reality it is perfect common sense not to get on the bike 'because he hasn't done the cycling course'.

Another example of this is where the police would not rescue a boy from a pond until they had taken advice from above on the 'risk assessment'. A normal person would have arrived, perhaps thought of the risk for a moment, and dived in. To the police now 'reframed', they followed 'normal' procedure.

There are shocking cases of reframed ambulance crews doing the same. Sheer unthinking stupidity of London Face-Nappies headed by Common Purpose graduate Cressida Dick can be seen in their behaviour at a vigil in March, 2021, for a murdered woman, Sarah Everard. A police officer had been charged with the crime. Anyone with a brain would have left the vigil alone in the circumstances. Instead they 'manhandled' women to stop them breaking 'Covid rules' to betray classic reframing. Minds in the thrall of perception control have no capacity for seeing a situation on its merits and acting accordingly. 'Rules is rules' is their only mind-set. My father used to say that rules and regulations are for the guidance of the intelligent and the blind obedience of the idiot. Most of the intelligent, decent, coppers have gone leaving only the other kind and a few old school for whom the job must be a daily nightmare. The combination of psychopaths and rule-book software minds has been clearly on public display in the 'Covid' era with automaton robots in uniform imposing fascistic 'Covid' regulations on the population without any personal initiative or judging situations on their merits. There are thousands of examples around the world, but I'll make my point with the infamous Derbyshire police in the English East Midlands – the ones who think pouring dye into beauty spots and using drones to track people walking in the countryside away from anyone is called 'policing'. To them there are rules decreed by the government which they have to enforce and in their bewildered state a group gathering in a closed space and someone walking alone in the countryside are the same thing. It is beyond idiocy and enters the realm of clinical insanity.

Police officers in Derbyshire said they were 'horrified' – *horrified* – to find 15 to 20 'irresponsible' kids playing a football match at a closed leisure

centre ‘in breach of coronavirus restrictions’. When they saw the police the kids ran away leaving their belongings behind and the reframed men and women of Derbyshire police were seeking to establish their identities with a view to fining their parents. The most natural thing for youngsters to do – kicking a ball about – is turned into a criminal activity and enforced by the moronic software programs of Derbyshire police. You find the same mentality in every country. These barely conscious ‘horrified’ officers said they had to take action because ‘we need to ensure these rules are being followed’ and ‘it is of the utmost importance that you ensure your children are following the rules and regulations for Covid-19’. Had any of them done ten seconds of research to see if this parroting of their masters’ script could be supported by any evidence? Nope. Reframed people don’t think – others think for them and that’s the whole idea of reframing. I have seen police officers one after the other repeating without question word for word what officialdom tells them just as I have seen great swathes of the public doing the same. Ask either for ‘their’ opinion and out spews what they have been told to think by the official narrative. Police and public may seem to be in different groups, but their mentality is the same. Most people do whatever they are told in fear not doing so or because they believe what officialdom tells them; almost the entirety of the police do what they are told for the same reason. Ultimately it’s the tiny inner core of the global Cult that’s telling both what to do.

So Derbyshire police were ‘horrified’. Oh, really? Why did they think those kids were playing football? It was to relieve the psychological consequences of lockdown and being denied human contact with their friends and interaction, touch and discourse vital to human psychological health. Being denied this month after month has dismantled the psyche of many children and young people as depression and suicide have exploded. Were Derbyshire police *horrified by that*? Are you kidding? Reframed people don’t have those mental and emotional processes that can see how the impact on the psychological health of youngsters is far more dangerous than any ‘virus’ even if you take the mendacious official figures to be true. The reframed are told (programmed) how to act and so they do. The Derbyshire Chief Constable in the first period of lockdown when the black dye and drones nonsense was going on was Peter Goodman. He was the man who severed the connection between his force and the Derbyshire Constabulary *Male Voice* Choir when he decided that it was not inclusive

enough to allow women to join. The fact it was a male voice choir making a particular sound produced by male voices seemed to elude a guy who terrifyingly ran policing in Derbyshire. He retired weeks after his force was condemned as disgraceful by former Supreme Court Justice Jonathan Sumption for their behaviour over extreme lockdown impositions. Goodman was replaced by his deputy Rachel Swann who was in charge when her officers were 'horrified'. The police statement over the boys committing the hanging-offence of playing football included the line about the youngsters being 'irresponsible in the times we are all living through' missing the point that the real relevance of the 'times we are all living through' is the imposition of fascism enforced by psychopaths and reframed minds of police officers playing such a vital part in establishing the fascist tyranny that their own children and grandchildren will have to live in their entire lives. As a definition of insanity that is hard to beat although it might be run close by imposing masks on people that can have a serious effect on their health while wearing a face nappy all day themselves. Once again public and police do it for the same reason – the authorities tell them to and who are they to have the self-respect to say no?

Wokers in uniform

How reframed do you have to be to arrest a *six-year-old* and take him to court for *picking a flower* while waiting for a bus? Brain dead police and officialdom did just that in North Carolina where criminal proceedings happen regularly for children under nine. Attorney Julie Boyer gave the six-year-old crayons and a colouring book during the 'flower' hearing while the 'adults' decided his fate. County Chief District Court Judge Jay Corpening asked: 'Should a child that believes in Santa Claus, the Easter Bunny and the tooth fairy be making life-altering decisions?' Well, of course not, but common sense has no meaning when you have a common purpose and a reframed mind. Treating children in this way, and police operating in American schools, is all part of the psychological preparation for children to accept a police state as normal all their adult lives. The same goes for all the cameras and biometric tracking technology in schools. Police training is focused on reframing them as snowflake Wokers and this is happening in the military. Pentagon top brass said that 'training sessions on extremism' were needed for troops who asked why they were so focused on the Capitol

Building riot when Black Lives Matter riots were ignored. What's the difference between them some apparently and rightly asked. Actually, there is a difference. Five people died in the Capitol riot, only one through violence, and that was a police officer shooting an unarmed protestor. BLM riots killed at least 25 people and cost billions. Asking the question prompted the psychopaths and reframed minds that run the Pentagon to say that more 'education' (programming) was needed. Troop training is all based on psychological programming to make them fodder for the Cult – 'Military men are just dumb, stupid animals to be used as pawns in foreign policy' as Cult-to-his-DNA former Secretary of State Henry Kissinger famously said. Governments see the police in similar terms and it's time for those among them who can see this to defend the people and stop being enforcers of the Cult agenda upon the people.

The US military, like the country itself, is being targeted for destruction through a long list of Woke impositions. Cult-owned gaga 'President' Biden signed an executive order when he took office to allow taxpayer money to pay for transgender surgery for active military personnel and veterans. Are you a man soldier? No, I'm a LGBTQIA+ with a hint of Skoliosexual and Spectrasexual. Oh, good man. Bad choice of words you bigot. The Pentagon announced in March, 2021, the appointment of the first 'diversity and inclusion officer' for US Special Forces. Richard Torres-Estrada arrived with the publication of a 'D&I Strategic Plan which will guide the enterprise-wide effort to institutionalize and sustain D&I'. If you think a Special Forces 'Strategic Plan' should have something to do with defending America you haven't been paying attention. Defending Woke is now the military's new role. Torres-Estrada has posted images comparing Donald Trump with Adolf Hitler and we can expect no bias from him as a representative of the supposedly non-political Pentagon. Cable news host Tucker Carlson said: 'The Pentagon is now the Yale faculty lounge but with cruise missiles.' Meanwhile Secretary of Defense Lloyd Austin, a board member of weapons-maker Raytheon with stock and compensation interests in October, 2020, worth $1.4 million, said he was purging the military of the 'enemy within' – anyone who isn't Woke and supports Donald Trump. Austin refers to his targets as 'racist extremists' while in true Woke fashion being himself a racist extremist. Pentagon documents pledge to 'eradicate, eliminate and conquer all forms of racism, sexism and homophobia'. The definitions of these are decided by 'diversity and inclusion committees'

peopled by those who see racism, sexism and homophobia in every situation and opinion. Woke (the Cult) is dismantling the US military and purging testosterone as China expands its military and gives its troops 'masculinity training'. How do we think that is going to end when this is all Cult coordinated? The US military, like the British military, is controlled by Woke and spineless top brass who just go along with it out of personal career interests.

'Woke' means fast asleep

Mind control and perception manipulation techniques used on individuals to create group-think have been unleashed on the global population in general. As a result many have no capacity to see the obvious fascist agenda being installed all around them or what 'Covid' is really all about. Their brains are firewalled like a computer system not to process certain concepts, thoughts and realisations that are bad for the Cult. The young are most targeted as the adults they will be when the whole fascist global state is planned to be fully implemented. They need to be prepared for total compliance to eliminate all pushback from entire generations. The Cult has been pouring billions into taking complete control of 'education' from schools to universities via its operatives and corporations and not least Bill Gates as always. The plan has been to transform 'education' institutions into programming centres for the mentality of 'Woke'. James McConnell, professor of psychology at the University of Michigan, wrote in *Psychology Today* in 1970:

> The day has come when we can combine sensory deprivation with drugs, hypnosis, and astute manipulation of reward and punishment, to gain almost absolute control over an individual's behaviour. It should then be possible to achieve a very rapid and highly effective type of brainwashing that would allow us to make dramatic changes in a person's behaviour and personality ...
>
> … We should reshape society so that we all would be trained from birth to want to do what society wants us to do. We have the techniques to do it... no-one owns his own personality you acquired, and there's no reason to believe you should have the right to refuse to acquire a new personality if your old one is anti-social.

This was the potential for mass brainwashing in 1970 and the mentality there displayed captures the arrogant psychopathy that drives it forward. I emphasise that not all young people have succumbed to Woke programming and those that haven't are incredibly impressive people given that today's young are the most perceptually-targeted generations in history with all the technology now involved. Vast swathes of the young generations, however, have fallen into the spell – and that's what it is – of Woke. The Woke mentality and perceptual program is founded on *inversion* and you will appreciate later why that is so significant. Everything with Woke is inverted and the opposite of what it is claimed to be. Woke was a term used in African-American culture from the 1900s and referred to an awareness of social and racial justice. This is not the meaning of the modern version or 'New Woke' as I call it in *The Answer*. Oh, no, Woke today means something very different no matter how much Wokers may seek to hide that and insist Old Woke and New Woke are the same. See if you find any 'awareness of social justice' here in the modern variety:

• Woke demands 'inclusivity' while excluding anyone with a different opinion and calls for mass censorship to silence other views.

• Woke claims to stand against oppression when imposing oppression is the foundation of all that it does. It is the driver of political correctness which is nothing more than a Cult invention to manipulate the population to silence itself.

• Woke believes itself to be 'liberal' while pursuing a global society that can only be described as fascist (see 'anti-fascist' fascist Antifa).

• Woke calls for 'social justice' while spreading injustice wherever it goes against the common 'enemy' which can be easily identified as a differing view.

• Woke is supposed to be a metaphor for 'awake' when it is solid-gold asleep and deep in a Cult-induced coma that meets the criteria for 'off with the fairies'.

I state these points as obvious facts if people only care to look. I don't do this with a sense of condemnation. We need to appreciate that the onslaught

of perceptual programming on the young has been incessant and merciless. I can understand why so many have been reframed, or, given their youth, framed from the start to see the world as the Cult demands. The Cult has had access to their minds day after day in its 'education' system for their entire formative years. Perception is formed from information received and the Cult-created system is a life-long download of information delivered to elicit a particular perception, thus behaviour. The more this has expanded into still new extremes in recent decades and ever-increasing censorship has deleted other opinions and information why wouldn't that lead to a perceptual reframing on a mass scale? I have described already cradle-to-grave programming and in more recent times the targeting of young minds from birth to adulthood has entered the stratosphere. This has taken the form of skewing what is 'taught' to fit the Cult agenda and the omnipresent techniques of group-think to isolate non-believers and pressure them into line. There has always been a tendency to follow the herd, but we really are in a new world now in relation to that. We have parents who can see the 'Covid' hoax told by their children not to stop them wearing masks at school, being 'Covid' tested or having the 'vaccine' in fear of the peer-pressure consequences of being different. What is 'peer-pressure' if not pressure to conform to group-think? Renegade Minds never group-think and always retain a set of perceptions that are unique to them. Group-think is always underpinned by consequences for not group-thinking. Abuse now aimed at those refusing DNA-manipulating 'Covid vaccines' are a potent example of this. The biggest pressure to conform comes from the very group which is itself being manipulated. 'I am programmed to be part of a hive mind and so you must be.'

Woke control structures in 'education' now apply to every mainstream organisation. Those at the top of the 'education' hierarchy (the Cult) decide the policy. This is imposed on governments through the Cult network; governments impose it on schools, colleges and universities; their leadership impose the policy on teachers and academics and they impose it on children and students. At any level where there is resistance, perhaps from a teacher or university lecturer, they are targeted by the authorities and often fired. Students themselves regularly demand the dismissal of academics (increasingly few) at odds with the narrative that the students have been programmed to believe in. It is quite a thought that students who are being targeted by the Cult become so consumed by programmed group-

think that they launch protests and demand the removal of those who are trying to push back against those targeting the students. Such is the scale of perceptual inversion. We see this with ‘Covid’ programming as the Cult imposes the rules via psycho-psychologists and governments on shops, transport companies and businesses which impose them on their staff who impose them on their customers who pressure Pushbackers to conform to the will of the Cult which is in the process of destroying them and their families. Scan all aspects of society and you will see the same sequence every time.

Fact free Woke and hijacking the ‘left’

There is no more potent example of this than ‘Woke’, a mentality only made possible by the deletion of factual evidence by an ‘education’ system seeking to produce an ever more uniform society. Why would you bother with facts when you don’t know any? Deletion of credible history both in volume and type is highly relevant. Orwell said: ‘Who controls the past controls the future: who controls the present controls the past.’ They who control the perception of the past control the perception of the future and they who control the present control the perception of the past through the writing and deleting of history. Why would you oppose the imposition of Marxism in the name of Wokeism when you don’t know that Marxism cost at least 100 million lives in the 20th century alone? Watch videos and read reports in which Woker generations are asked basic historical questions – it’s mind-blowing. A survey of 2,000 people found that six percent of millennials (born approximately early1980s to early 2000s) believed the Second World War (1939-1945) broke out with the assassination of President Kennedy (in 1963) and one in ten thought Margaret Thatcher was British Prime Minister at the time. She was in office between 1979 and 1990. We are in a post-fact society. Provable facts are no defence against the fascism of political correctness or Silicon Valley censorship. Facts don’t matter anymore as we have witnessed with the ‘Covid’ hoax. Sacrificing uniqueness to the Woke group-think religion is all you are required to do and that means thinking for yourself is the biggest Woke no, no. All religions are an expression of group-think and censorship and Woke is just another religion with an orthodoxy defended by group-think and censorship. Burned at the stake becomes burned on Twitter which leads

back eventually to burned at the stake as Woke humanity regresses to ages past.

The biggest Woke inversion of all is its creators and funders. I grew up in a traditional left of centre political household on a council estate in Leicester in the 1950s and 60s – you know, the left that challenged the power of wealth-hoarding elites and threats to freedom of speech and opinion. In those days students went on marches defending freedom of speech while today's Wokers march for its deletion. What on earth could have happened? Those very elites (collectively the Cult) that we opposed in my youth and early life have funded into existence the antithesis of that former left and hijacked the 'brand' while inverting everything it ever stood for. We have a mentality that calls itself 'liberal' and 'progressive' while acting like fascists. Cult billionaires and their corporations have funded themselves into control of 'education' to ensure that Woke programming is unceasing throughout the formative years of children and young people and that non-Wokers are isolated (that word again) whether they be students, teachers or college professors. The Cult has funded into existence the now colossal global network of Woke organisations that have spawned and promoted all the 'causes' on the Cult wish-list for global transformation and turned Wokers into demanders of them. Does anyone really think it's a coincidence that the Cult agenda for humanity is a carbon (sorry) copy of the societal transformations desired by Woke?? These are only some of them:

Political correctness: The means by which the Cult deletes all public debates that it knows it cannot win if we had the free-flow of information and evidence.

Human-caused 'climate change': The means by which the Cult seeks to transform society into a globally-controlled dictatorship imposing its will over the fine detail of everyone's lives 'to save the planet' which doesn't actually need saving.

Transgender obsession: Preparing collective perception to accept the 'new human' which would not have genders because it would be created

technologically and not through procreation. I'll have much more on this in Human 2.0.

Race obsession: The means by which the Cult seeks to divide and rule the population by triggering racial division through the perception that society is more racist than ever when the opposite is the case. Is it perfect in that regard? No. But to compare today with the racism of apartheid and segregation brought to an end by the civil rights movement in the 1960s is to insult the memory of that movement and inspirations like Martin Luther King. Why is the 'anti-racism' industry (which it is) so dominated by privileged white people?

White supremacy: This is a label used by privileged white people to demonise poor and deprived white people pushing back on tyranny to marginalise and destroy them. White people are being especially targeted as the dominant race by number within Western society which the Cult seeks to transform in its image. If you want to change a society you must weaken and undermine its biggest group and once you have done that by using the other groups you next turn on them to do the same … 'Then they came for the Jews and I was not a Jew so I did nothing.'

Mass migration: The mass movement of people from the Middle East, Africa and Asia into Europe, from the south into the United States and from Asia into Australia are another way the Cult seeks to dilute the racial, cultural and political influence of white people on Western society. White people ask why their governments appear to be working against them while being politically and culturally biased towards incoming cultures. Well, here's your answer. In the same way sexually 'straight' people, men and women, ask why the authorities are biased against them in favour of other sexualities. The answer is the same – that's the way the Cult wants it to be for very sinister motives.

These are all central parts of the Cult agenda and central parts of the Woke agenda and Woke was created and continues to be funded to an immense

degree by Cult billionaires and corporations. If anyone begins to say ‘coincidence’ the syllables should stick in their throat.

Billionaire ‘social justice warriors’

Joe Biden is a 100 percent-owned asset of the Cult and the Wokers’ man in the White House whenever he can remember his name and for however long he lasts with his rapidly diminishing cognitive function. Even walking up the steps of an aircraft without falling on his arse would appear to be a challenge. He’s not an empty-shell puppet or anything. From the minute Biden took office (or the Cult did) he began his executive orders promoting the Woke wish-list. You will see the Woke agenda imposed ever more severely because it’s really the *Cult* agenda. Woke organisations and activist networks spawned by the Cult are funded to the extreme so long as they promote what the Cult wants to happen. Woke is funded to promote ‘social justice’ by billionaires who become billionaires by destroying social justice. The social justice mantra is only a cover for dismantling social justice and funded by billionaires that couldn’t give a damn about social justice. Everything makes sense when you see that. One of Woke’s premier funders is Cult billionaire financier George Soros who said: ‘I am basically there to make money, I cannot and do not look at the social consequences of what I do.’ This is the same Soros who has given more than $32 billion to his Open Society Foundations global Woke network and funded Black Lives Matter, mass immigration into Europe and the United States, transgender activism, climate change activism, political correctness and groups targeting ‘white supremacy’ in the form of privileged white thugs that dominate Antifa. What a scam it all is and when you are dealing with the unquestioning fact-free zone of Woke scamming them is child’s play. All you need to pull it off in all these organisations are a few in-the-know agents of the Cult and an army of naïve, reframed, uninformed, narcissistic, know-nothings convinced of their own self-righteousness, self-purity and virtue.

Soros and fellow billionaires and billionaire corporations have poured hundreds of millions into Black Lives Matter and connected groups and promoted them to a global audience. None of this is motivated by caring about black people. These are the billionaires that have controlled and exploited a system that leaves millions of black people in abject poverty

and deprivation which they do absolutely nothing to address. The same Cult networks funding BLM were behind the *slave trade!* Black Lives Matter hijacked a phrase that few would challenge and they have turned this laudable concept into a political weapon to divide society. You know that BLM is a fraud when it claims that *All* Lives Matter, the most inclusive statement of all, is 'racist'. BLM and its Cult masters don't want to end racism. To them it's a means to an end to control all of humanity never mind the colour, creed, culture or background. What has destroying the nuclear family got to do with ending racism? Nothing – but that is one of the goals of BLM and also happens to be a goal of the Cult as I have been exposing in my books for decades. Stealing children from loving parents and giving schools ever more power to override parents is part of that same agenda. BLM is a Marxist organisation and why would that not be the case when the Cult created Marxism *and* BLM? Patrisse Cullors, a BLM co-founder, said in a 2015 video that she and her fellow organisers, including co-founder Alicia Garza, are 'trained Marxists'. The lady known after marriage as Patrisse Khan-Cullors bought a $1.4 million home in 2021 in one of the whitest areas of California with a black population of just 1.6 per cent and has so far bought *four* high-end homes for a total of $3.2 million. How very Marxist. There must be a bit of spare in the BLM coffers, however, when Cult corporations and billionaires have handed over the best part of $100 million. Many black people can see that Black Lives Matter is not working for them, but against them, and this is still more confirmation. Black journalist Jason Whitlock, who had his account suspended by Twitter for simply linking to the story about the 'Marxist's' home buying spree, said that BLM leaders are 'making millions of dollars off the backs of these dead black men who they wouldn't spit on if they were on fire and alive'.

Black Lies Matter

Cult assets and agencies came together to promote BLM in the wake of the death of career criminal George Floyd who had been jailed a number of times including for forcing his way into the home of a black woman with others in a raid in which a gun was pointed at her stomach. Floyd was filmed being held in a Minneapolis street in 2020 with the knee of a police officer on his neck and he subsequently died. It was an appalling thing for the officer to do, but the same technique has been used by police on

peaceful protestors of lockdown without any outcry from the Woke brigade. As unquestioning supporters of the Cult agenda Wokers have supported lockdown and all the 'Covid' claptrap while attacking anyone standing up to the tyranny imposed in its name. Court documents would later include details of an autopsy on Floyd by County Medical Examiner Dr Andrew Baker who concluded that Floyd had taken a fatal level of the drug fentanyl. None of this mattered to fact-free, question-free, Woke. Floyd's death was followed by worldwide protests against police brutality amid calls to defund the police. Throwing babies out with the bathwater is a Woke speciality. In the wake of the murder of British woman Sarah Everard a Green Party member of the House of Lords, Baroness Jones of Moulescoomb (Nincompoopia would have been better), called for a 6pm curfew for all men. This would be in breach of the Geneva Conventions on war crimes which ban collective punishment, but that would never have crossed the black and white Woke mind of Baroness Nincompoopia who would have been far too convinced of her own self-righteousness to compute such details. Many American cities did defund the police in the face of Floyd riots and after $15 million was deleted from the police budget in Washington DC under useless Woke mayor Muriel Bowser car-jacking alone rose by 300 percent and within six months the US capital recorded its highest murder rate in 15 years. The same happened in Chicago and other cities in line with the Cult/Soros plan to bring fear to streets and neighbourhoods by reducing the police, releasing violent criminals and not prosecuting crime. This is the mob-rule agenda that I have warned in the books was coming for so long. Shootings in the area of Minneapolis where Floyd was arrested increased by 2,500 percent compared with the year before. Defunding the police over George Floyd has led to a big increase in dead people with many of them black. Police protection for politicians making these decisions stayed the same or increased as you would expect from professional hypocrites. The Cult doesn't actually want to abolish the police. It wants to abolish local control over the police and hand it to federal government as the psychopaths advance the Hunger Games Society. Many George Floyd protests turned into violent riots with black stores and businesses destroyed by fire and looting across America fuelled by Black Lives Matter. Woke doesn't do irony. If you want civil rights you must loot the liquor store and the supermarket and make off with a smart TV. It's the only way.

It’s not a race war – it’s a class war

Black people are patronised by privileged blacks and whites alike and told they are victims of white supremacy. I find it extraordinary to watch privileged blacks supporting the very system and bloodline networks behind the slave trade and parroting the same Cult-serving manipulative crap of their privileged white, often billionaire, associates. It is indeed not a race war but a class war and colour is just a diversion. Black Senator Cory Booker and black Congresswoman Maxine Waters, more residents of Nincompoopia, personify this. Once you tell people they are victims of someone else you devalue both their own responsibility for their plight and the power they have to impact on their reality and experience. Instead we have: ‘You are only in your situation because of whitey – turn on them and everything will change.’ It won’t change. Nothing changes in our lives unless *we* change it. Crucial to that is never seeing yourself as a victim and always as the creator of your reality. Life is a simple sequence of choice and consequence. Make different choices and you create different consequences. *You* have to make those choices – not Black Lives Matter, the Woke Mafia and anyone else that seeks to dictate your life. Who are they these Wokers, an emotional and psychological road traffic accident, to tell you what to do? Personal empowerment is the last thing the Cult and its Black Lives Matter want black people or anyone else to have. They claim to be defending the underdog while *creating* and perpetuating the underdog. The Cult’s worst nightmare is human unity and if they are going to keep blacks, whites and every other race under economic servitude and control then the focus must be diverted from what they have in common to what they can be manipulated to believe divides them. Blacks have to be told that their poverty and plight is the fault of the white bloke living on the street in the same poverty and with the same plight they are experiencing. The difference is that your plight black people is due to him, a white supremacist with ‘white privilege’ living on the street. Don’t unite as one human family against your mutual oppressors and suppressors – fight the oppressor with the white face who is as financially deprived as you are. The Cult knows that as its ‘Covid’ agenda moves into still new levels of extremism people are going to respond and it has been spreading the seeds of disunity everywhere to stop a united response to the evil that targets *all of us*.

Racist attacks on ‘whiteness’ are getting ever more outrageous and especially through the American Democratic Party which has an appalling history for anti-black racism. Barack Obama, Joe Biden, Hillary Clinton and Nancy Pelosi all eulogised about Senator Robert Byrd at his funeral in 2010 after a nearly 60-year career in Congress. Byrd was a brutal Ku Klux Klan racist and a violent abuser of Cathy O’Brien in MKUltra. He said he would never fight in the military ‘with a negro by my side’ and ‘rather I should die a thousand times, and see Old Glory trampled in the dirt never to rise again, than to see this beloved land of ours become degraded by race mongrels, a throwback to the blackest specimen from the wilds’. Biden called Byrd a ‘very close friend and mentor’. These ‘Woke’ hypocrites are not anti-racist they are anti-poor and anti-people not of their perceived class. Here is an illustration of the scale of anti-white racism to which we have now descended. Seriously Woke and moronic *New York Times* contributor Damon Young described whiteness as a ‘virus’ that ‘like other viruses will not die until there are no bodies left for it to infect’. He went on: ‘… the only way to stop it is to locate it, isolate it, extract it, and kill it.’ Young can say that as a black man with no consequences when a white man saying the same in reverse would be facing a jail sentence. *That’s* racism. We had super-Woke numbskull senators Tammy Duckworth and Mazie Hirono saying they would object to future Biden Cabinet appointments if he did not nominate more Asian Americans and Pacific Islanders. Never mind the ability of the candidate what do they look like? Duckworth said: ‘I will vote for racial minorities and I will vote for LGBTQ, but anyone else I’m not voting for.’ Appointing people on the grounds of race is illegal, but that was not a problem for this ludicrous pair. They were on-message and that’s a free pass in any situation.

Critical race racism

White children are told at school they are intrinsically racist as they are taught the divisive ‘critical race theory’. This claims that the law and legal institutions are inherently racist and that race is a socially constructed concept used by white people to further their economic and political interests at the expense of people of colour. White is a ‘virus’ as we’ve seen. Racial inequality results from ‘social, economic, and legal differences that white people create between races to maintain white interests which

leads to poverty and criminality in minority communities‘. I must tell that to the white guy sleeping on the street. The principal of East Side Community School in New York sent white parents a manifesto that called on them to become ‘white traitors’ and advocate for full ‘white abolition’. These people are teaching your kids when they urgently need a psychiatrist. The ‘school’ included a chart with ‘eight white identities’ that ranged from ‘white supremacist’ to ‘white abolition’ and defined the behaviour white people must follow to end ‘the regime of whiteness’. Woke blacks and their privileged white associates are acting exactly like the slave owners of old and Ku Klux Klan racists like Robert Byrd. They are too full of their own self-purity to see that, but it’s true. Racism is not a body type; it’s a state of mind that can manifest through any colour, creed or culture.

Another racial fraud is *‘equity’*. Not equality of treatment and opportunity – equity. It’s a term spun as equality when it means something very different. Equality in its true sense is a raising up while ‘equity’ is a race to the bottom. Everyone in the same level of poverty is ‘equity’. Keep everyone down – that’s equity. The Cult doesn’t want anyone in the human family to be empowered and BLM leaders, like all these ‘anti-racist’ organisations, continue their privileged, pampered existence by perpetuating the perception of gathering racism. When is the last time you heard an ‘anti-racist’ or ‘anti-Semitism’ organisation say that acts of racism and discrimination have *fallen?* It’s not in the interests of their fund-raising and power to influence and the same goes for the professional soccer anti-racism operation, Kick It Out. Two things confirmed that the Black Lives Matter riots in the summer of 2020 were Cult creations. One was that while anti-lockdown protests were condemned in this same period for ‘transmitting ‘Covid’ the authorities supported mass gatherings of Black Lives Matter supporters. I even saw self-deluding people claiming to be doctors say the two types of protest were not the same. No – the non-existent ‘Covid’ was in favour of lockdowns and attacked those that protested against them while ‘Covid’ supported Black Lives Matter and kept well away from its protests. The whole thing was a joke and as lockdown protestors were arrested, often brutally, by reframed Face-Nappies we had the grotesque sight of police officers taking the knee to Black Lives Matter, a Cult-funded Marxist organisation that supports violent riots and wants to destroy the nuclear family and white people.

He's not white? Shucks!

Woke obsession with race was on display again when ten people were shot dead in Boulder, Colorado, in March, 2021. Cult-owned Woke TV channels like CNN said the shooter appeared to be a white man and Wokers were on Twitter condemning 'violent white men' with the usual mantras. Then the shooter's name was released as Ahmad Al Aliwi Alissa, an anti-Trump Arab-American, and the sigh of disappointment could be heard five miles away. Never mind that ten people were dead and what that meant for their families. Race baiting was all that mattered to these sick Cult-serving people like Barack Obama who exploited the deaths to further divide America on racial grounds which is his job for the Cult. This is the man that 'racist' white Americans made the first black president of the United States and then gave him a second term. Not-very-bright Obama has become filthy rich on the back of that and today appears to have a big influence on the Biden administration. Even so he's still a downtrodden black man and a victim of white supremacy. This disingenuous fraud reveals the contempt he has for black people when he puts on a Deep South Alabama accent whenever he talks to them, no, *at* them.

Another BLM red flag was how the now fully-Woke (fully-Cult) and fully-virtue-signalled professional soccer authorities had their teams taking the knee before every match in support of Marxist Black Lives Matter. Soccer authorities and clubs displayed 'Black Lives Matter' on the players' shirts and flashed the name on electronic billboards around the pitch. Any fans that condemned what is a Freemasonic taking-the-knee ritual were widely condemned as you would expect from the Woke virtue-signallers of professional sport and the now fully-Woke media. We have reverse racism in which you are banned from criticising any race or culture except for white people for whom anything goes – say what you like, no problem. What has this got to do with racial harmony and equality? We've had black supremacists from Black Lives Matter telling white people to fall to their knees in the street and apologise for their white supremacy. Black supremacists acting like white supremacist slave owners of the past couldn't breach their self-obsessed, race-obsessed sense of self-purity. Joe Biden appointed a race-obsessed black supremacist Kristen Clarke to head the Justice Department Civil Rights Division. Clarke claimed that blacks are endowed with 'greater mental, physical and spiritual abilities' than whites. If anyone reversed that statement they would be vilified. Clarke is on-

message so no problem. She's never seen a black-white situation in which the black figure is anything but a virtuous victim and she heads the Civil Rights Division which should treat everyone the same or it isn't civil rights. Another perception of the Renegade Mind: If something or someone is part of the Cult agenda they will be supported by Woke governments and media no matter what. If they're not, they will be condemned and censored. It really is that simple and so racist Clarke prospers despite (make that because of) her racism.

The end of culture

Biden's administration is full of such racial, cultural and economic bias as the Cult requires the human family to be divided into warring factions. We are now seeing racially-segregated graduations and everything, but everything, is defined through the lens of perceived 'racism. We have 'racist' mathematics, 'racist' food and even 'racist' *plants*. World famous Kew Gardens in London said it was changing labels on plants and flowers to tell its pre-'Covid' more than two million visitors a year how racist they are. Kew director Richard Deverell said this was part of an effort to 'move quickly to decolonise collections' after they were approached by one Ajay Chhabra 'an actor with an insight into how sugar cane was linked to slavery'. They are *plants* you idiots. 'Decolonisation' in the Woke manual really means colonisation of society with its mentality and by extension colonisation by the Cult. We are witnessing a new Chinese-style 'Cultural Revolution' so essential to the success of all Marxist takeovers. Our cultural past and traditions have to be swept away to allow a new culture to be built-back-better. Woke targeting of long-standing Western cultural pillars including historical monuments and cancelling of historical figures is what happened in the Mao revolution in China which 'purged remnants of capitalist and traditional elements from Chinese society' and installed Maoism as the dominant ideology'. For China see the Western world today and for 'dominant ideology' see Woke. Better still see Marxism or Maoism. The 'Covid' hoax has specifically sought to destroy the arts and all elements of Western culture from people meeting in a pub or restaurant to closing theatres, music venues, sports stadiums, places of worship and even banning *singing*. Destruction of Western society is also why criticism of any religion is banned except for Christianity which again is the dominant

religion as white is the numerically-dominant race. Christianity may be fading rapidly, but its history and traditions are weaved through the fabric of Western society. Delete the pillars and other structures will follow until the whole thing collapses. I am not a Christian defending that religion when I say that. I have no religion. It's just a fact. To this end Christianity has itself been turned Woke to usher its own downfall and its ranks are awash with 'change agents' – knowing and unknowing – at every level including Pope Francis (*definitely* knowing) and the clueless Archbishop of Canterbury Justin Welby (possibly not, but who can be sure?). Woke seeks to coordinate attacks on Western culture, traditions, and ways of life through 'intersectionality' defined as 'the complex, cumulative way in which the effects of multiple forms of discrimination (such as racism, sexism, and classism) combine, overlap, or intersect especially in the experiences of marginalised individuals or groups'. Wade through the Orwellian Woke-speak and this means coordinating disparate groups in a common cause to overthrow freedom and liberal values.

The entire structure of public institutions has been infested with Woke – government at all levels, political parties, police, military, schools, universities, advertising, media and trade unions. This abomination has been achieved through the Cult web by appointing Wokers to positions of power and battering non-Wokers into line through intimidation, isolation and threats to their job. Many have been fired in the wake of the empathy-deleted, vicious hostility of 'social justice' Wokers and the desire of gutless, spineless employers to virtue-signal their Wokeness. Corporations are filled with Wokers today, most notably those in Silicon Valley. Ironically at the top they are not Woke at all. They are only exploiting the mentality their Cult masters have created and funded to censor and enslave while the Wokers cheer them on until it's their turn. Thus the Woke 'liberal left' is an inversion of the traditional liberal left. Campaigning for justice on the grounds of power and wealth distribution has been replaced by campaigning for identity politics. The genuine traditional left would never have taken money from today's billionaire abusers of fairness and justice and nor would the billionaires have wanted to fund that genuine left. It would not have been in their interests to do so. The division of opinion in those days was between the haves and have nots. This all changed with Cult manipulated and funded identity politics. The division of opinion today is between Wokers and non-Wokers and not income brackets. Cult

corporations and their billionaires may have taken wealth disparity to cataclysmic levels of injustice, but as long as they speak the language of Woke, hand out the dosh to the Woke network and censor the enemy they are 'one of us'. Billionaires who don't give a damn about injustice are laughing at them till their bellies hurt. Wokers are not even close to self-aware enough to see that. The transformed 'left' dynamic means that Wokers who drone on about 'social justice' are funded by billionaires that have destroyed social justice the world over. It's *why* they are billionaires.

The climate con

Nothing encapsulates what I have said more comprehensively than the hoax of human-caused global warming. I have detailed in my books over the years how Cult operatives and organisations were the pump-primers from the start of the climate con. A purpose-built vehicle for this is the Club of Rome established by the Cult in 1968 with the Rockefellers and Rothschilds centrally involved all along. Their gofer frontman Maurice Strong, a Canadian oil millionaire, hosted the Earth Summit in Rio de Janeiro, Brazil, in 1992 where the global 'green movement' really expanded in earnest under the guiding hand of the Cult. The Earth Summit established Agenda 21 through the Cult-created-and-owned United Nations to use the illusion of human-caused climate change to justify the transformation of global society to save the world from climate disaster. It is a No-Problem-Reaction-Solution sold through governments, media, schools and universities as whole generations have been terrified into believing that the world was going to end in their lifetimes unless what old people had inflicted upon them was stopped by a complete restructuring of how everything is done. Chill, kids, it's all a hoax. Such restructuring is precisely what the Cult agenda demands (purely by coincidence of course). Today this has been given the codename of the Great Reset which is only an updated term for Agenda 21 and its associated Agenda 2030. The latter, too, is administered through the UN and was voted into being by the General Assembly in 2015. Both 21 and 2030 seek centralised control of all resources and food right down to the raindrops falling on your own land. These are some of the demands of Agenda 21 established in 1992. See if you recognise this society emerging today:

- End national sovereignty
- State planning and management of all land resources, ecosystems, deserts, forests, mountains, oceans and fresh water; agriculture; rural development; biotechnology; and ensuring *'equity'*
- The state to 'define the role' of business and financial resources
- Abolition of private property
- 'Restructuring' the family unit (see BLM)
- Children raised by the state
- People told what their job will be
- Major restrictions on movement
- Creation of 'human settlement zones'
- Mass resettlement as people are forced to vacate land where they live
- Dumbing down education
- Mass global depopulation in pursuit of all the above

The United Nations was created as a Trojan horse for world government. With the climate con of critical importance to promoting that outcome you would expect the UN to be involved. Oh, it's involved all right. The UN is promoting Agenda 21 and Agenda 2030 justified by 'climate change' while also driving the climate hoax through its Intergovernmental Panel on Climate Change (IPCC), one of the world's most corrupt organisations. The IPCC has been lying ferociously and constantly since the day it opened its doors with the global media hanging unquestioningly on its every mendacious word. The Green movement is entirely Woke and has long lost its original environmental focus since it was co-opted by the Cult. An obsession with 'global warming' has deleted its values and scrambled its head. I experienced a small example of what I mean on a beautiful country walk that I have enjoyed several times a week for many years. The path merged into the fields and forests and you felt at one with the natural world. Then a 'Green' organisation, the Hampshire and Isle of Wight Wildlife Trust, took over part of the land and proceeded to cut down a large number of trees, including mature ones, to install a horrible big, bright steel 'this-is-ours-stay-out' fence that destroyed the whole atmosphere of this beautiful place. No one with a feel for nature would do that. Day after day I walked to the sound of chainsaws and a magnificent mature weeping willow tree that I so admired was cut down at the base of the trunk. When I challenged

a Woke young girl in a green shirt (of course) about this vandalism she replied: ‘It’s a weeping willow – it will grow back.’ This is what people are paying for when they donate to the Hampshire and Isle of Wight Wildlife Trust and many other ‘green’ organisations today. It is not the environmental movement that I knew and instead has become a support-system – as with Extinction Rebellion – for a very dark agenda.

Private jets for climate justice

The Cult-owned, Gates-funded, World Economic Forum and its founder Klaus Schwab were behind the emergence of Greta Thunberg to harness the young behind the climate agenda and she was invited to speak to the world at … the UN. Schwab published a book, *Covid-19: The Great Reset* in 2020 in which he used the ‘Covid’ hoax and the climate hoax to lay out a new society straight out of Agenda 21 and Agenda 2030. Bill Gates followed in early 2021 when he took time out from destroying the world to produce a book in his name about the way to save it. Gates flies across the world in private jets and admitted that ‘I probably have one of the highest greenhouse gas footprints of anyone on the planet … my personal flying alone is gigantic.’ He has also bid for the planet’s biggest private jet operator. Other climate change saviours who fly in private jets include John Kerry, the US Special Presidential Envoy for Climate, and actor Leonardo DiCaprio, a ‘UN Messenger of Peace with special focus on climate change’. These people are so full of bullshit they could corner the market in manure. We mustn’t be sceptical, though, because the Gates book, *How to Avoid a Climate Disaster: The Solutions We Have and the Breakthroughs We Need*, is a genuine attempt to protect the world and not an obvious pile of excrement attributed to a mega-psychopath aimed at selling his masters’ plans for humanity. The Gates book and the other shite-pile by Klaus Schwab could have been written by the same person and may well have been. Both use ‘climate change’ and ‘Covid’ as the excuses for their new society and by coincidence the Cult’s World Economic Forum and Bill and Melinda Gates Foundation promote the climate hoax and hosted Event 201 which pre-empted with a ‘simulation’ the very ‘coronavirus’ hoax that would be simulated for real on humanity within weeks. The British ‘royal’ family is promoting the ‘Reset’ as you would expect through Prince ‘climate change caused the war in Syria’ Charles and his hapless son Prince

William who said that we must ‘reset our relationship with nature and our trajectory as a species’ to avoid a climate disaster. Amazing how many promotors of the ‘Covid’ and ‘climate change’ control systems are connected to Gates and the World Economic Forum. A ‘study’ in early 2021 claimed that carbon dioxide emissions must fall by the equivalent of a global lockdown roughly every two years for the next decade to save the planet. The ‘study’ appeared in the same period that the Schwab mob claimed in a video that lockdowns destroying the lives of billions are good because they make the earth ‘quieter’ with less ‘ambient noise’. They took down the video amid a public backlash for such arrogant, empathy-deleted stupidity You see, however, where they are going with this. Corinne Le Quéré, a professor at the Tyndall Centre for Climate Change Research, University of East Anglia, was lead author of the climate lockdown study, and she writes for … the World Economic Forum. Gates calls in ‘his’ book for changing ‘every aspect of the economy’ (long-time Cult agenda) and for humans to eat synthetic ‘meat’ (predicted in my books) while cows and other farm animals are eliminated. Australian TV host and commentator Alan Jones described what carbon emission targets would mean for farm animals in Australia alone if emissions were reduced as demanded by 35 percent by 2030 and zero by 2050:

Well, let’s take agriculture, the total emissions from agriculture are about 75 million tonnes of carbon dioxide, equivalent. Now reduce that by 35 percent and you have to come down to 50 million tonnes, I’ve done the maths. So if you take for example 1.5 million cows, you’re going to have to reduce the herd by 525,000 [by] 2030, nine years, that’s 58,000 cows a year. The beef herd’s 30 million, reduce that by 35 percent, that’s 10.5 million, which means 1.2 million cattle have to go every year between now and 2030. This is insanity!

There are 75 million sheep. Reduce that by 35 percent, that’s 26 million sheep, that’s almost 3 million a year. So under the Paris Agreement over 30 million beasts. dairy cows, cattle, pigs and sheep would go. More than 8,000 every minute of every hour for the next decade, do these people know what they’re talking about?

Clearly they don’t at the level of campaigners, politicians and administrators. The Cult *does* know; that’s the outcome it wants. We are faced with not just a war on humanity. Animals and the natural world are being targeted and I have been saying since the ‘Covid’ hoax began that the plan eventually was to claim that the ‘deadly virus’ is able to jump from animals, including farm animals and domestic pets, to humans. Just before

this book went into production came this story: ‘Russia registers world’s first Covid-19 vaccine for cats & dogs as makers of Sputnik V warn pets & farm animals could spread virus’. The report said ‘top scientists warned that the deadly pathogen could soon begin spreading through homes and farms’ and ‘the next stage is the infection of farm and domestic animals’. Know the outcome and you’ll see the journey. Think what that would mean for animals and keep your eye on a term called zoonosis or zoonotic diseases which transmit between animals and humans. The Cult wants to break the connection between animals and people as it does between people and people. Farm animals fit with the Cult agenda to transform food from natural to synthetic.

The gas of life is killing us

There can be few greater examples of Cult inversion than the condemnation of carbon dioxide as a dangerous pollutant when it is the gas of life. Without it the natural world would be dead and so we would all be dead. We breathe in oxygen and breathe out carbon dioxide while plants produce oxygen and absorb carbon dioxide. It is a perfect symbiotic relationship that the Cult wants to dismantle for reasons I will come to in the final two chapters. Gates, Schwab, other Cult operatives and mindless repeaters, want the world to be ‘carbon neutral’ by at least 2050 and the earlier the better. ‘Zero carbon’ is the cry echoed by lunatics calling for ‘Zero Covid’ when we already have it. These carbon emission targets will deindustrialise the world in accordance with Cult plans – the post-industrial, post-democratic society – and with so-called renewables like solar and wind not coming even close to meeting human energy needs blackouts and cold are inevitable. Texans got the picture in the winter of 2021 when a snow storm stopped wind turbines and solar panels from working and the lights went down along with water which relies on electricity for its supply system. Gates wants everything to be powered by electricity to ensure that his masters have the kill switch to stop all human activity, movement, cooking, water and warmth any time they like. The climate lie is so stupendously inverted that it claims we must urgently reduce carbon dioxide when we *don’t have enough*.

Co2 in the atmosphere is a little above 400 parts per million when the optimum for plant growth is 2,000 ppm and when it falls anywhere near

150 ppm the natural world starts to die and so do we. It fell to as low as 280 ppm in an 1880 measurement in Hawaii and rose to 413 ppm in 2019 with industrialisation which is why the planet has become *greener* in the industrial period. How insane then that psychopathic madman Gates is not satisfied only with blocking the rise of Co2. He's funding technology to suck it out of the atmosphere. The reason why will become clear. The industrial era is not destroying the world through Co2 and has instead turned around a potentially disastrous ongoing fall in Co2. Greenpeace co-founder and scientist Patrick Moore walked away from Greenpeace in 1986 and has exposed the green movement for fear-mongering and lies. He said that 500 million years ago there was *17 times* more Co2 in the atmosphere than we have today and levels have been falling for hundreds of millions of years. In the last 150 million years Co2 levels in Earth's atmosphere had reduced by *90 percent*. Moore said that by the time humanity began to unlock carbon dioxide from fossil fuels we were at '38 seconds to midnight' and in that sense: 'Humans are [the Earth's] salvation.' Moore made the point that only half the Co2 emitted by fossil fuels stays in the atmosphere and we should remember that all pollution pouring from chimneys that we are told is carbon dioxide is in fact nothing of the kind. It's pollution. Carbon dioxide is an invisible gas.

William Happer, Professor of Physics at Princeton University and long-time government adviser on climate, has emphasised the Co2 deficiency for maximum growth and food production. Greenhouse growers don't add carbon dioxide for a bit of fun. He said that most of the warming in the last 100 years, after the earth emerged from the super-cold period of the 'Little Ice Age' into a natural warming cycle, was over by 1940. Happer said that a peak year for warming in 1988 can be explained by a 'monster El Nino' which is a natural and cyclical warming of the Pacific that has nothing to do with 'climate change'. He said the effect of Co2 could be compared to painting a wall with red paint in that once two or three coats have been applied it didn't matter how much more you slapped on because the wall will not get much redder. Almost all the effect of the rise in Co2 has already happened, he said, and the volume in the atmosphere would now have to *double* to increase temperature by a single degree. Climate hoaxers know this and they have invented the most ridiculously complicated series of 'feedback' loops to try to overcome this rather devastating fact. You hear puppet Greta going on cluelessly about feedback loops and this is why.

The Sun affects temperature? No you *climate denier*

Some other nonsense to contemplate: Climate graphs show that rises in temperature do not follow rises in Co2 – *it's the other way round* with a lag between the two of some 800 years. If we go back 800 years from present time we hit the Medieval Warm Period when temperatures were higher than now without any industrialisation and this was followed by the Little Ice Age when temperatures plummeted. The world was still emerging from these centuries of serious cold when many climate records began which makes the ever-repeated line of the 'hottest year since records began' meaningless when you are not comparing like with like. The coldest period of the Little Ice Age corresponded with the lowest period of sunspot activity when the Sun was at its least active. Proper scientists will not be at all surprised by this when it confirms the obvious fact that earth temperature is affected by the scale of Sun activity and the energetic power that it subsequently emits; but when is the last time you heard a climate hoaxer talking about the Sun as a source of earth temperature?? Everything has to be focussed on Co2 which makes up just 0.117 percent of so-called greenhouse gases and only a fraction of even that is generated by human activity. The rest is natural. More than *90 percent* of those greenhouse gases are water vapour and clouds (Fig 9). Ban moisture I say. Have you noticed that the climate hoaxers no longer use the polar bear as their promotion image? That's because far from becoming extinct polar bear communities are stable or thriving. Joe Bastardi, American meteorologist, weather forecaster and outspoken critic of the climate lie, documents in his book *The Climate Chronicles* how weather patterns and events claimed to be evidence of climate change have been happening since long before industrialisation: 'What happened before naturally is happening again, as is to be expected given the cyclical nature of the climate due to the design of the planet.' If you read the detailed background to the climate hoax in my other books you will shake your head and wonder how anyone could believe the crap which has spawned a multi-trillion dollar industry based on absolute garbage (see HIV causes AIDs and Sars-Cov-2 causes 'Covid-19'). Climate and 'Covid' have much in common given they have the same source. They both have the contradictory *everything* factor in which everything is explained by reference to them. It's hot – 'it's climate change'. It's cold – 'it's climate change'. I got a sniffle – 'it's Covid'. I haven't got a sniffle – 'it's Covid'. Not having a sniffle has to be a symptom

of ‘Covid’. Everything is and not having a sniffle is especially dangerous if you are a slow walker. For sheer audacity I offer you a Cambridge University ‘study’ that actually linked ‘Covid’ to ‘climate change’. It had to happen eventually. They concluded that climate change played a role in ‘Covid-19’ spreading from animals to humans because … wait for it … I kid you not … *the two groups were forced closer together as populations grow*. Er, that’s it. The whole foundation on which this depended was that ‘Bats are the likely zoonotic origin of SARS-CoV-1 and SARS-CoV-2’. Well, they are not. They are nothing to do with it. Apart from bats not being the origin and therefore ‘climate change’ effects on bats being irrelevant I am in awe of their academic insight. Where would we be without them? Not where we are that’s for sure.

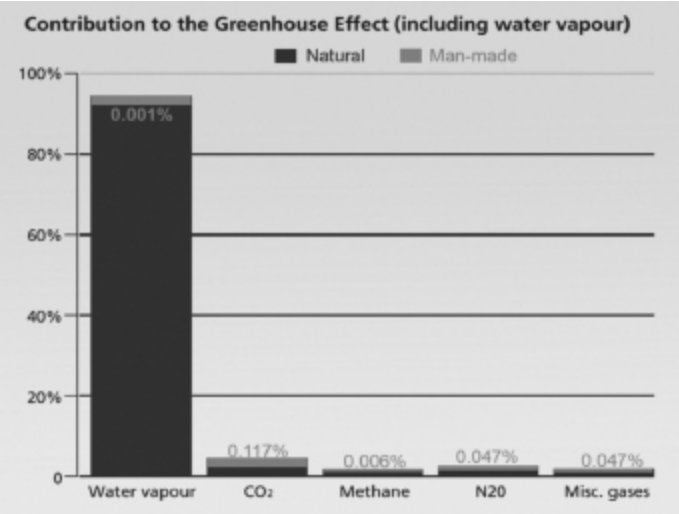

Figure 9: The idea that the gas of life is disastrously changing the climate is an insult to brain cell activity.

One other point about the weather is that climate modification is now well advanced and not every major weather event is natural – or earthquake come to that. I cover this subject at some length in other books. China is openly planning a rapid expansion of its weather modification programme which includes changing the climate in an area more than one and a half times the size of India. China used weather manipulation to ensure clear skies during the 2008 Olympics in Beijing. I have quoted from US military documents detailing how to employ weather manipulation as a weapon of war and they did that in the 1960s and 70s during the conflict in Vietnam with Operation Popeye manipulating monsoon rains for military purposes.

Why would there be international treaties on weather modification if it wasn't possible? Of course it is. Weather is energetic information and it can be changed.

How was the climate hoax pulled off? See 'Covid'

If you can get billions to believe in a 'virus' that doesn't exist you can get them to believe in human-caused climate change that doesn't exist. Both are being used by the Cult to transform global society in the way it has long planned. Both hoaxes have been achieved in pretty much the same way. First you declare a lie is a fact. There's a 'virus' you call SARS-Cov-2 or humans are warming the planet with their behaviour. Next this becomes, via Cult networks, the foundation of government, academic and science policy and belief. Those who parrot the mantra are given big grants to produce research that confirms the narrative is true and ever more 'symptoms' are added to make the 'virus'/'climate change' sound even more scary. Scientists and researchers who challenge the narrative have their grants withdrawn and their careers destroyed. The media promote the lie as the unquestionable truth and censor those with an alternative view or evidence. A great percentage of the population believe what they are told as the lie becomes an everybody-knows-that and the believing-masses turn on those with a mind of their own. The technique has been used endlessly throughout human history. Wokers are the biggest promotors of the climate lie *and* 'Covid' fascism because their minds are owned by the Cult; their sense of self-righteous self-purity knows no bounds; and they exist in a bubble of reality in which facts are irrelevant and only get in the way of looking without seeing.

Running through all of this like veins in a blue cheese is control of information, which means control of perception, which means control of behaviour, which collectively means control of human society. The Cult owns the global media and Silicon Valley fascists for the simple reason that it *has* to. Without control of information it can't control perception and through that human society. Examine every facet of the Cult agenda and you will see that anything supporting its introduction is never censored while anything pushing back is always censored. I say again: Psychopaths that know why they are doing this must go before Nuremberg trials and those that follow their orders must trot along behind them into the same

dock. ‘I was just following orders’ didn’t work the first time and it must not work now. Nuremberg trials must be held all over the world before public juries for politicians, government officials, police, compliant doctors, scientists and virologists, and all Cult operatives such as Gates, Tedros, Fauci, Vallance, Whitty, Ferguson, Zuckerberg, Wojcicki, Brin, Page, Dorsey, the whole damn lot of them – including, no *especially*, the psychopath psychologists. Without them and the brainless, gutless excuses for journalists that have repeated their lies, none of this could be happening. Nobody can be allowed to escape justice for the psychological and economic Armageddon they are all responsible for visiting upon the human race.

As for the compliant, unquestioning, swathes of humanity, and the self-obsessed, all-knowing ignorance of the Wokers … don’t start me. God help their kids. God help their grandkids. God *help them*.

CHAPTER NINE

We must have it? So what is it?

Well I won't back down. No, I won't back down. You can stand me up at the Gates of Hell. But I won't back down
Tom Petty

I will now focus on the genetically-manipulating 'Covid vaccines' which do not meet this official definition of a vaccine by the US Centers for Disease Control (CDC): 'A product that stimulates a person's immune system to produce immunity to a specific disease, protecting the person from that disease.' On that basis 'Covid vaccines' are not a vaccine in that the makers don't even claim they stop infection or transmission.

They are instead part of a multi-levelled conspiracy to change the nature of the human body and what it means to be 'human' and to depopulate an enormous swathe of humanity. What I shall call Human 1.0 is on the cusp of becoming Human 2.0 and for very sinister reasons. Before I get to the 'Covid vaccine' in detail here's some background to vaccines in general. Government regulators do not test vaccines – the makers do – and the makers control which data is revealed and which isn't. Children in America are given 50 vaccine doses by age six and 69 by age 19 and the effect of the whole combined schedule has never been tested. Autoimmune diseases when the immune system attacks its own body have soared in the mass vaccine era and so has disease in general in children and the young. Why wouldn't this be the case when vaccines target the *immune system?* The US government gave Big Pharma drug companies immunity from prosecution for vaccine death and injury in the 1986 National Childhood Vaccine Injury

Act (NCVIA) and since then the government (taxpayer) has been funding compensation for the consequences of Big Pharma vaccines. The criminal and satanic drug giants can't lose and the vaccine schedule has increased dramatically since 1986 for this reason. There is no incentive to make vaccines safe and a big incentive to make money by introducing ever more. Even against a ridiculously high bar to prove vaccine liability, and with the government controlling the hearing in which it is being challenged for compensation, the vaccine court has so far paid out more than $4 billion. These are the vaccines we are told are safe and psychopaths like Zuckerberg censor posts saying otherwise. The immunity law was even justified by a ruling that vaccines by their nature were 'unavoidably unsafe'.

Check out the ingredients of vaccines and you will be shocked if you are new to this. *They put that in children's bodies?? What??* Try aluminium, a brain toxin connected to dementia, aborted foetal tissue and formaldehyde which is used to embalm corpses. World-renowned aluminium expert Christopher Exley had his research into the health effect of aluminium in vaccines shut down by Keele University in the UK when it began taking funding from the Bill and Melinda Gates Foundation. Research when diseases 'eradicated' by vaccines began to decline and you will find the fall began long *before* the vaccine was introduced. Sometimes the fall even plateaued after the vaccine. Diseases like scarlet fever for which there was no vaccine declined in the same way because of environmental and other factors. A perfect case in point is the polio vaccine. Polio began when lead arsenate was first sprayed as an insecticide and residues remained in food products. Spraying started in 1892 and the first US polio epidemic came in Vermont in 1894. The simple answer was to stop spraying, but Rockefeller-created Big Pharma had a better idea. Polio was decreed to be caused by the *poliovirus* which 'spreads from person to person and can infect a person's spinal cord'. Lead arsenate was replaced by the lethal DDT which had the same effect of causing paralysis by damaging the brain and central nervous system. Polio plummeted when DDT was reduced and then banned, but the vaccine is still given the credit for something it didn't do. Today by far the biggest cause of polio is the vaccines promoted by Bill Gates. Vaccine justice campaigner Robert Kennedy Jr, son of assassinated (by the Cult) US Attorney General Robert Kennedy, wrote:

In 2017, the World Health Organization (WHO) reluctantly admitted that the global explosion in polio is predominantly vaccine strain. The most frightening epidemics in Congo, Afghanistan, and the Philippines, are all linked to vaccines. In fact, by 2018, 70% of global polio cases were vaccine strain.

Vaccines make fortunes for Cult-owned Gates and Big Pharma while undermining the health and immune systems of the population. We had a glimpse of the mentality behind the Big Pharma cartel with a report on WION (World is One News), an international English language TV station based in India, which exposed the extraordinary behaviour of US drug company Pfizer over its 'Covid vaccine'. The WION report told how Pfizer had made fantastic demands of Argentina, Brazil and other countries in return for its 'vaccine'. These included immunity from prosecution, even for Pfizer negligence, government insurance to protect Pfizer from law suits and handing over as collateral sovereign assets of the country to include Argentina's bank reserves, military bases and embassy buildings. Pfizer demanded the same of Brazil in the form of waiving sovereignty of its assets abroad; exempting Pfizer from Brazilian laws; and giving Pfizer immunity from all civil liability. This is a 'vaccine' developed with government funding. Big Pharma is evil incarnate as a creation of the Cult and all must be handed tickets to Nuremberg.

Phantom 'vaccine' for a phantom 'disease'

I'll expose the 'Covid vaccine' fraud and then go on to the wider background of why the Cult has set out to 'vaccinate' every man, woman and child on the planet for an alleged 'new disease' with a survival rate of 99.77 percent (or more) even by the grotesquely-manipulated figures of the World Health Organization and Johns Hopkins University. The 'infection' to 'death' ratio is 0.23 to 0.15 percent according to Stanford epidemiologist Dr John Ioannidis and while estimates vary the danger remains tiny. I say that if the truth be told the fake infection to fake death ratio is zero. Never mind all the evidence I have presented here and in *The Answer* that there is no 'virus' let us just focus for a moment on that death-rate figure of say 0.23 percent. The figure includes all those worldwide who have tested positive with a test not testing for the 'virus' and then died within 28 days or even longer of any other cause – *any other cause*. Now subtract all those

illusory ‘Covid’ deaths on the global data sheets from the 0.23 percent. What do you think you would be left with? *Zero*. A vaccination has never been successfully developed for a so-called coronavirus. They have all failed at the animal testing stage when they caused hypersensitivity to what they were claiming to protect against and made the impact of a disease far worse. Cult-owned vaccine corporations got around that problem this time by bypassing animal trials, going straight to humans and making the length of the ‘trials’ before the public rollout as short as they could get away with. Normally it takes five to ten years or more to develop vaccines that still cause demonstrable harm to many people and that’s without including the long-term effects that are never officially connected to the vaccination. ‘Covid’ non-vaccines have been officially produced and approved in a matter of months from a standing start and part of the reason is that (a) they were developed before the ‘Covid’ hoax began and (b) they are based on computer programs and not natural sources. Official non-trials were so short that government agencies gave *emergency,* not full, approval. ‘Trials’ were not even completed and full approval cannot be secured until they are. Public ‘Covid vaccination’ is actually a *continuation of the trial*. Drug company ‘trials’ are not scheduled to end until 2023 by which time a lot of people are going to be dead. Data on which government agencies gave this emergency approval was supplied by the Big Pharma corporations themselves in the form of Pfizer/BioNTech, AstraZeneca, Moderna, Johnson & Johnson, and others, and this is the case with all vaccines. By its very nature *emergency* approval means drug companies do not have to prove that the ‘vaccine’ is ‘safe and effective’. How could they with trials way short of complete? Government regulators only have to *believe* that they *could* be safe and effective. It is criminal manipulation to get products in circulation with no testing worth the name. Agencies giving that approval are infested with Big Pharma-connected place-people and they act in the interests of Big Pharma (the Cult) and not the public about whom they do not give a damn.

More human lab rats

‘Covid vaccines’ produced in record time by Pfizer/BioNTech and Moderna employ a technique *never approved before for use on humans*. They are known as mRNA ‘vaccines’ and inject a synthetic version of ‘viral’ mRNA

or ‘messenger RNA’. The key is in the term ‘messenger’. The body works, or doesn’t, on the basis of information messaging. Communications are constantly passing between and within the genetic system and the brain. Change those messages and you change the state of the body and even its very nature and you can change psychology and behaviour by the way the brain processes information. I think you are going to see significant changes in personality and perception of many people who have had the ‘Covid vaccine’ synthetic potions. Insider Aldous Huxley predicted the following in 1961 and mRNA ‘vaccines’ can be included in the term ‘pharmacological methods’:

There will be, in the next generation or so, a pharmacological method of making people love their servitude, and producing dictatorship without tears, so to speak, producing a kind of painless concentration camp for entire societies, so that people will in fact have their own liberties taken away from them, but rather enjoy it, because they will be distracted from any desire to rebel by propaganda or brainwashing, or brainwashing enhanced by pharmacological methods. And this seems to be the final revolution.

Apologists claim that mRNA synthetic ‘vaccines’ don’t change the DNA genetic blueprint because RNA does not affect DNA only the other way round. This is so disingenuous. A process called ‘reverse transcription’ can convert RNA into DNA and be integrated into DNA in the cell nucleus. This was highlighted in December, 2020, by scientists at Harvard and Massachusetts Institute of Technology (MIT). Geneticists report that more than 40 percent of mammalian genomes results from reverse transcription. On the most basic level if messaging changes then that sequence must lead to changes in DNA which is receiving and transmitting those communications. How can introducing synthetic material into cells not change the cells where DNA is located? The process is known as transfection which is defined as ‘a technique to insert foreign nucleic acid (DNA or RNA) into a cell, typically with the intention of altering the properties of the cell’. Researchers at the Sloan Kettering Institute in New York found that changes in messenger RNA can deactivate tumour-suppressing proteins and thereby promote cancer. This is what happens when you mess with messaging. ‘Covid vaccine’ maker Moderna was founded in 2010 by Canadian stem cell biologist Derrick J. Rossi after his breakthrough discovery in the field of transforming and reprogramming

stem cells. These are neutral cells that can be programmed to become any cell including sperm cells. Moderna was therefore founded on the principle of genetic manipulation and has never produced any vaccine or drug before its genetically-manipulating synthetic 'Covid' shite. Look at the name – Mode-RNA or Modify-RNA. Another important point is that the US Supreme Court has ruled that genetically-modified DNA, or complementary DNA (cDNA) synthesized in the laboratory from messenger RNA, can be patented and owned. These psychopaths are doing this to the human body.

Cells replicate synthetic mRNA in the 'Covid vaccines' and in theory the body is tricked into making antigens which trigger antibodies to target the 'virus spike proteins' which as Dr Tom Cowan said have *never been seen*. Cut the crap and these 'vaccines' deliver *self-replicating* synthetic material to the cells with the effect of changing human DNA. The more of them you have the more that process is compounded while synthetic material is all the time self-replicating. 'Vaccine'-maker Moderna describes mRNA as 'like software for the cell' and so they are messing with the body's software. What happens when you change the software in a computer? Everything changes. For this reason the Cult is preparing a production line of mRNA 'Covid vaccines' and a long list of excuses to use them as with all the 'variants' of a 'virus' never shown to exist. The plan is further to transfer the mRNA technique to other vaccines mostly given to children and young people. The cumulative consequences will be a transformation of human DNA through a constant infusion of synthetic genetic material which will kill many and change the rest. Now consider that governments that have given emergency approval for a vaccine that's not a vaccine; never been approved for humans before; had no testing worth the name; and the makers have been given immunity from prosecution for any deaths or adverse effects suffered by the public. The UK government awarded *permanent legal indemnity* to itself and its employees for harm done when a patient is being treated for 'Covid-19' or 'suspected Covid-19'. That is quite a thought when these are possible 'side-effects' from the 'vaccine' (they are not 'side', they are effects) listed by the US Food and Drug Administration:

Guillain-Barre syndrome; acute disseminated encephalomyelitis; transverse myelitis; encephalitis; myelitis; encephalomyelitis; meningoencephalitis; meningitis; encephalopathy; convulsions; seizures;

stroke; narcolepsy; cataplexy; anaphylaxis; acute myocardial infarction (heart attack); myocarditis; pericarditis; autoimmune disease; death; implications for pregnancy, and birth outcomes; other acute demyelinating diseases; non anaphylactic allergy reactions; thrombocytopenia ; disseminated intravascular coagulation; venous thromboembolism; arthritis; arthralgia; joint pain; Kawasaki disease; multisystem inflammatory syndrome in children; vaccine enhanced disease. The latter is the way the 'vaccine' has the potential to make diseases far worse than they would otherwise be.

UK doctor and freedom campaigner Vernon Coleman described the conditions in this list as 'all unpleasant, most of them very serious, and you can't get more serious than death'. The thought that anyone at all has had the 'vaccine' in these circumstances is testament to the potential that humanity has for clueless, unquestioning, stupidity and for many that programmed stupidity has already been terminal.

An insider speaks

Dr Michael Yeadon is a former Vice President, head of research and Chief Scientific Adviser at vaccine giant Pfizer. Yeadon worked on the inside of Big Pharma, but that did not stop him becoming a vocal critic of 'Covid vaccines' and their potential for multiple harms, including infertility in women. By the spring of 2021 he went much further and even used the no, no, term 'conspiracy'. When you begin to see what is going on it is impossible not to do so. Yeadon spoke out in an interview with freedom campaigner James Delingpole and I mentioned earlier how he said that no one had samples of 'the virus'. He explained that the mRNA technique originated in the anti-cancer field and ways to turn on and off certain genes which could be advantageous if you wanted to stop cancer growing out of control. 'That's the origin of them. They are a very unusual application, really.' Yeadon said that treating a cancer patient with an aggressive procedure might be understandable if the alternative was dying, but it was quite another thing to use the same technique as a public health measure. Most people involved wouldn't catch the infectious agent you were vaccinating against and if they did they probably wouldn't die:

If you are really using it as a public health measure you really want to as close as you can get to zero sides-effects … I find it odd that they chose techniques that were really cutting their teeth in the field of oncology and I'm worried that in using gene-based vaccines that have to be injected in the body and spread around the body, get taken up into some cells, and the regulators haven't quite told us which cells they get taken up into … you are going to be generating a wide range of responses … with multiple steps each of which could go well or badly.

I doubt the Cult intends it to go well. Yeadon said that you can put any gene you like into the body through the 'vaccine'. 'You can certainly give them a gene that would do them some harm if you wanted.' I was intrigued when he said that when used in the cancer field the technique could turn genes on and off. I explore this process in *The Answer* and with different genes having different functions you could create mayhem – physically and psychologically – if you turned the wrong ones on and the right ones off. I read reports of an experiment by researchers at the University of Washington's school of computer science and engineering in which they encoded DNA to infect computers. The body is itself a biological computer and if human DNA can inflict damage on a computer why can't the computer via synthetic material mess with the human body? It can. The Washington research team said it was possible to insert malicious malware into 'physical DNA strands' and corrupt the computer system of a gene sequencing machine as it 'reads gene letters and stores them as binary digits 0 and 1'. They concluded that hackers could one day use blood or spit samples to access computer systems and obtain sensitive data from police forensics labs or infect genome files. It is at this level of digital interaction that synthetic 'vaccines' need to be seen to get the full picture and that will become very clear later on. Michael Yeadon said it made no sense to give the 'vaccine' to younger people who were in no danger from the 'virus'. What was the benefit? It was all downside with potential effects:

The fact that my government in what I thought was a civilised, rational country, is raining [the 'vaccine'] on people in their 30s and 40s, even my children in their 20s, they're getting letters and phone calls, I know this is not right and any of you doctors who are vaccinating you know it's not right, too. They are not at risk. They are not at risk from the disease, so you are now hoping that the side-effects are so rare that you get away with it. You don't give new technology … that you don't understand to 100 percent of the population.

Blood clot problems with the AstraZeneca ‘vaccine’ have been affecting younger people to emphasise the downside risks with no benefit. AstraZeneca’s version, produced with Oxford University, does not use mRNA, but still gets its toxic cocktail inside cells where it targets DNA. The Johnson & Johnson ‘vaccine’ which uses a similar technique has also produced blood clot effects to such an extent that the United States paused its use at one point. They are all ‘gene therapy’ (cell modification) procedures and not ‘vaccines’. The truth is that once the content of these injections enter cells we have no idea what the effect will be. People can speculate and some can give very educated opinions and that’s good. In the end, though, only the makers know what their potions are designed to do and even they won’t know every last consequence. Michael Yeadon was scathing about doctors doing what they knew to be wrong. ‘Everyone’s mute’, he said. Doctors in the NHS must know this was not right, coming into work and injecting people. ‘I don’t know how they sleep at night. I know I couldn’t do it. I know that if I were in that position I’d have to quit.’ He said he knew enough about toxicology to know this was not a good risk-benefit. Yeadon had spoken to seven or eight university professors and all except two would not speak out publicly. Their universities had a policy that no one said anything that countered the government and its medical advisors. They were afraid of losing their government grants. This is how intimidation has been used to silence the truth at every level of the system. I say silence, but these people could still speak out if they made that choice. Yeadon called them ‘moral cowards’ – ‘This is about your children and grandchildren’s lives and you have just buggered off and left it.’

‘Variant’ nonsense

Some of his most powerful comments related to the alleged ‘variants’ being used to instil more fear, justify more lockdowns, and introduce more ‘vaccines’. He said government claims about ‘variants’ were nonsense. He had checked the alleged variant ‘codes’ and they were 99.7 percent identical to the ‘original’. This was the human identity difference equivalent to putting a baseball cap on and off or wearing it the other way round. A 0.3 percent difference would make it impossible for that ‘variant’ to escape immunity from the ‘original’. This made no sense of having new ‘vaccines’ for ‘variants’. He said there would have to be at least a *30 percent*

difference for that to be justified and even then he believed the immune system would still recognise what it was. Gates-funded 'variant modeller' and 'vaccine'-pusher John Edmunds might care to comment. Yeadon said drug companies were making new versions of the 'vaccine' as a 'top up' for 'variants'. Worse than that, he said, the 'regulators' around the world like the MHRA in the UK had got together and agreed that because 'vaccines' for 'variants' were so similar to the first 'vaccines' *they did not have to do safety studies*. How transparently sinister that is. This is when Yeadon said: 'There is a conspiracy here.' There was no need for another vaccine for 'variants' and yet we were told that there was and the country had shut its borders because of them. 'They are going into hundreds of millions of arms without passing 'go' or any regulator. Why did they do that? Why did they pick this method of making the vaccine?'

The reason had to be something bigger than that it seemed and 'it's not protection against the virus'. It's was a far bigger project that meant politicians and advisers were willing to do things and not do things that knowingly resulted in avoidable deaths – 'that's already happened when you think about lockdown and deprivation of health care for a year.' He spoke of people prepared to do something that results in the avoidable death of their fellow human beings and it not bother them. This is the penny-drop I have been working to get across for more than 30 years – the level of pure evil we are dealing with. Yeadon said his friends and associates could not believe there could be that much evil, but he reminded them of Stalin, Pol Pot and Hitler and of what Stalin had said: 'One death is a tragedy. A million? A statistic.' He could not think of a benign explanation for why you need top-up vaccines 'which I'm sure you don't' and for the regulators 'to just get out of the way and wave them through'. Why would the regulators do that when they were still wrestling with the dangers of the 'parent' vaccine? He was clearly shocked by what he had seen since the 'Covid' hoax began and now he was thinking the previously unthinkable:

If you wanted to depopulate a significant proportion of the world and to do it in a way that doesn't involve destruction of the environment with nuclear weapons, poisoning everyone with anthrax or something like that, and you wanted plausible deniability while you had a multi-year infectious disease crisis, I actually don't think you could come up with a better plan of work than seems to be in front of me. I can't say that's what they are going to do, but I can't think of a benign explanation why they are doing it.

He said he never thought that they would get rid of 99 percent of humans, but now he wondered. ‘If you wanted to that this would be a hell of a way to do it – it would be unstoppable folks.’ Yeadon had concluded that those who submitted to the ‘vaccine’ would be allowed to have some kind of normal life (but for how long?) while screws were tightened to coerce and mandate the last few percent. ‘I think they’ll put the rest of them in a prison camp. I wish I was wrong, but I don’t think I am.’ Other points he made included: There were no coronavirus vaccines then suddenly they all come along at the same time; we have no idea of the long term affect with trials so short; coercing or forcing people to have medical procedures is against the Nuremberg Code instigated when the Nazis did just that; people should at least delay having the ‘vaccine’; a quick Internet search confirms that masks don’t reduce respiratory viral transmission and ‘the government knows that’; they have smashed civil society and they know that, too; two dozen peer-reviewed studies show no connection between lockdown and reducing deaths; he knew from personal friends the elite were still flying around and going on holiday while the public were locked down; the elite were not having the ‘vaccines’. He was also asked if ‘vaccines’ could be made to target difference races. He said he didn’t know, but the document by the Project for the New American Century in September, 2000, said developing ‘advanced forms of biological warfare that can target *specific genotypes* may transform biological warfare from the realm of terror to a politically useful tool.’ Oh, they’re evil all right. Of that we can be *absolutely* sure.

Another cull of old people

We have seen from the CDC definition that the mRNA ‘Covid vaccine’ is not a vaccine and nor are the others that *claim* to reduce ‘severity of symptoms’ in *some* people, but not protect from infection or transmission. What about all the lies about returning to ‘normal’ if people were ‘vaccinated’? If they are not claimed to stop infection and transmission of the alleged ‘virus’, how does anything change? This was all lies to manipulate people to take the jabs and we are seeing that now with masks and distancing still required for the ‘vaccinated’. How did they think that elderly people with fragile health and immune responses were going to be affected by infusing their cells with synthetic material and other toxic

substances? They *knew* that in the short and long term it would be devastating and fatal as the culling of the old that began with the first lockdowns was continued with the 'vaccine'. Death rates in care homes soared immediately residents began to be 'vaccinated' – infused with synthetic material. Brave and committed whistleblower nurses put their careers at risk by exposing this truth while the rest kept their heads down and their mouths shut to put their careers before those they are supposed to care for. A long-time American Certified Nursing Assistant who gave his name as James posted a video in which he described emotionally what happened in his care home when vaccination began. He said that during 2020 very few residents were sick with 'Covid' and no one died during the entire year; but shortly after the Pfizer mRNA injections 14 people died within two weeks and many others were near death. 'They're dropping like flies', he said. Residents who walked on their own before the shot could no longer and they had lost their ability to conduct an intelligent conversation. The home's management said the sudden deaths were caused by a 'super-spreader' of 'Covid-19'. Then how come, James asked, that residents who refused to take the injections were not sick? It was a case of inject the elderly with mRNA synthetic potions and blame their illness and death that followed on the 'virus'. James described what was happening in care homes as 'the greatest crime of genocide this country has ever seen'. Remember the NHS staff nurse from earlier who used the same word 'genocide' for what was happening with the 'vaccines' and that it was an 'act of human annihilation'. A UK care home whistleblower told a similar story to James about the effect of the 'vaccine' in deaths and 'outbreaks' of illness dubbed 'Covid' after getting the jab. She told how her care home management and staff had zealously imposed government regulations and no one was allowed to even question the official narrative let alone speak out against it. She said the NHS was even worse. Again we see the results of reframing. A worker at a local care home where I live said they had not had a single case of 'Covid' there for almost a year and when the residents were 'vaccinated' they had 19 positive cases in two weeks with eight dying.

It's not the 'vaccine' – honest

The obvious cause and effect was being ignored by the media and most of the public. Australia's health minister Greg Hunt (a former head of strategy

at the World Economic Forum) was admitted to hospital after he had the ‘vaccine’. He was suffering according to reports from the skin infection ‘cellulitis’ and it must have been a severe case to have warranted days in hospital. Immediately the authorities said this was nothing to do with the ‘vaccine’ when an effect of some vaccines is a ‘cellulitis-like reaction’. We had families of perfectly healthy old people who died after the ‘vaccine’ saying that if only they had been given the ‘vaccine’ earlier they would still be alive. As a numbskull rating that is off the chart. A father of four ‘died of Covid’ at aged 48 when he was taken ill two days after having the ‘vaccine’. The man, a health administrator, had been ‘shielding during the pandemic’ and had ‘not really left the house’ until he went for the ‘vaccine’. Having the ‘vaccine’ and then falling ill and dying does not seem to have qualified as a possible cause and effect and ‘Covid-19’ went on his death certificate. His family said they had no idea how he ‘caught the virus’. A family member said: ‘Tragically, it could be that going for a vaccination ultimately led to him catching Covid …The sad truth is that they are never going to know where it came from.’ The family warned people to remember that the virus still existed and was ‘very real’. So was their stupidity. Nurses and doctors who had the first round of the ‘vaccine’ were collapsing, dying and ending up in a hospital bed while they or their grieving relatives were saying they’d still have the ‘vaccine’ again despite what happened. I kid you not. You mean if your husband returned from the dead he’d have the same ‘vaccine’ again that killed him??

Doctors at the VCU Medical Center in Richmond, Virginia, said the Johnson & Johnson ‘vaccine’ was to blame for a man’s skin peeling off. Patient Richard Terrell said: ‘It all just happened so fast. My skin peeled off. It’s still coming off on my hands now.’ He said it was stinging, burning and itching and when he bent his arms and legs it was very painful with ‘the skin swollen and rubbing against itself’. Pfizer/BioNTech and Moderna vaccines use mRNA to change the cell while the Johnson & Johnson version uses DNA in a process similar to AstraZeneca’s technique. Johnson & Johnson and AstraZeneca have both had their ‘vaccines’ paused by many countries after causing serious blood problems. Terrell’s doctor Fnu Nutan said he could have died if he hadn’t got medical attention. It sounds terrible so what did Nutan and Terrell say about the ‘vaccine’ now? Oh, they still recommend that people have it. A nurse in a hospital bed 40 minutes after the vaccination and unable to swallow due to throat swelling was told by a

doctor that he lost mobility in his arm for 36 hours following the vaccination. What did he say to the ailing nurse? ‘Good for you for getting the vaccination.’ We are dealing with a serious form of cognitive dissonance madness in both public and medical staff. There is a remarkable correlation between those having the ‘vaccine’ and trumpeting the fact and suffering bad happenings shortly afterwards. Witold Rogiewicz, a Polish doctor, made a video of his ‘vaccination’ and ridiculed those who were questioning its safety and the intentions of Bill Gates: ‘Vaccinate yourself to protect yourself, your loved ones, friends and also patients. And to mention quickly I have info for anti-vaxxers and anti-Coviders if you want to contact Bill Gates you can do this through me.’ He further ridiculed the dangers of 5G. Days later he was dead, but naturally the vaccination wasn’t mentioned in the verdict of ‘heart attack’.

Lies, lies and more lies

So many members of the human race have slipped into extreme states of insanity and unfortunately they include reframed doctors and nursing staff. Having a ‘vaccine’ and dying within minutes or hours is not considered a valid connection while death from any cause within 28 days or longer of a positive test with a test not testing for the ‘virus’ means ‘Covid-19’ goes on the death certificate. How could that ‘vaccine’-death connection not have been made except by calculated deceit? US figures in the initial rollout period to February 12th, 2020, revealed that a third of the deaths reported to the CDC after ‘Covid vaccines’ happened within 48 hours. Five men in the UK suffered an ‘extremely rare’ blood clot problem after having the AstraZeneca ‘vaccine’, but no causal link was established said the Gates-funded Medicines and Healthcare products Regulatory Agency (MHRA) which had given the ‘vaccine’ emergency approval to be used. Former Pfizer executive Dr Michael Yeadon explained in his interview how the procedures could cause blood coagulation and clots. People who should have been at no risk were dying from blood clots in the brain and he said he had heard from medical doctor friends that people were suffering from skin bleeding and massive headaches. The AstraZeneca ‘shot’ was stopped by some 20 countries over the blood clotting issue and still the corrupt MHRA, the European Medicines Agency (EMA) and the World Health Organization said that it should continue to be given even though the EMA admitted that

it ‘still cannot rule out definitively’ a link between blood clotting and the ‘vaccine’. Later Marco Cavaleri, head of EMA vaccine strategy, said there was indeed a clear link between the ‘vaccine’ and thrombosis, but they didn’t know why. So much for the trials showing the ‘vaccine’ is safe. Blood clots were affecting younger people who would be under virtually no danger from ‘Covid’ even if it existed which makes it all the more stupid and sinister.

The British government responded to public alarm by wheeling out June Raine, the terrifyingly weak infant school headmistress sound-alike who heads the UK MHRA drug ‘regulator’. The idea that she would stand up to Big Pharma and government pressure is laughable and she told us that all was well in the same way that she did when allowing untested, never-used-on-humans-before, genetically-manipulating ‘vaccines’ to be exposed to the public in the first place. Mass lying is the new normal of the ‘Covid’ era. The MHRA later said 30 cases of rare blood clots had by then been connected with the AstraZeneca ‘vaccine’ (that means a lot more in reality) while stressing that the benefits of the jab in preventing ‘Covid-19’ outweighed any risks. A more ridiculous and disingenuous statement with callous disregard for human health it is hard to contemplate. Immediately after the mendacious ‘all-clears’ two hospital workers in Denmark experienced blood clots and cerebral haemorrhaging following the AstraZeneca jab and one died. Top Norwegian health official Pål Andre Holme said the ‘vaccine’ was the only common factor: ‘There is nothing in the patient history of these individuals that can give such a powerful immune response … I am confident that the antibodies that we have found are the cause, and I see no other explanation than it being the vaccine which triggers it.’ Strokes, a clot or bleed in the brain, were clearly associated with the ‘vaccine’ from word of mouth and whistleblower reports. Similar consequences followed with all these ‘vaccines’ that we were told were so safe and as the numbers grew by the day it was clear we were witnessing human carnage.

Learning the hard way

A woman interviewed by UKColumn told how her husband suffered dramatic health effects after the vaccine when he’d been in good health all his life. He went from being a little unwell to losing all feeling in his legs

and experiencing ‘excruciating pain’. Misdiagnosis followed twice at Accident and Emergency (an ‘allergy’ and ‘sciatica’) before he was admitted to a neurology ward where doctors said his serious condition had been caused by the ‘vaccine’. Another seven ‘vaccinated’ people were apparently being treated on the same ward for similar symptoms. The woman said he had the ‘vaccine’ because they believed media claims that it was safe. ‘I didn’t think the government would give out a vaccine that does this to somebody; I believed they would be bringing out a vaccination that would be safe.’ What a tragic way to learn that lesson. Another woman posted that her husband was transporting stroke patients to hospital on almost every shift and when he asked them if they had been ‘vaccinated’ for ‘Covid’ they all replied ‘yes’. One had a ‘massive brain bleed’ the day after his second dose. She said her husband reported the ‘just been vaccinated’ information every time to doctors in A and E only for them to ignore it, make no notes and appear annoyed that it was even mentioned. This particular report cannot be verified, but it expresses a common theme that confirms the monumental underreporting of ‘vaccine’ consequences. Interestingly as the ‘vaccines’ and their brain blood clot/stroke consequences began to emerge the UK National Health Service began a publicity campaign telling the public what to do in the event of a stroke. A Scottish NHS staff nurse who quit in disgust in March, 2021, said:

> I have seen traumatic injuries from the vaccine, they’re not getting reported to the yellow card [adverse reaction] scheme, they’re treating the symptoms, not asking why, why it’s happening. It’s just treating the symptoms and when you speak about it you’re dismissed like you’re crazy, I’m not crazy, I’m not crazy because every other colleague I’ve spoken to is terrified to speak out, they’ve had enough.

Videos appeared on the Internet of people uncontrollably shaking after the ‘vaccine’ with no control over muscles, limbs and even their face. A Scottish mother broke out in a severe rash all over her body almost immediately after she was given the AstraZeneca ‘vaccine’. The pictures were horrific. Leigh King, a 41-year-old hairdresser from Lanarkshire said: ‘Never in my life was I prepared for what I was about to experience … My skin was so sore and constantly hot … I have never felt pain like this …’ But don’t you worry, the ‘vaccine’ is perfectly safe. Then there has been the effect on medical staff who have been pressured to have the ‘vaccine’ by

psychopathic ‘health’ authorities and government. A London hospital consultant who gave the name K. Polyakova wrote this to the *British Medical Journal* or *BMJ*:

> I am currently struggling with … the failure to report the reality of the morbidity caused by our current vaccination program within the health service and staff population. The levels of sickness after vaccination is unprecedented and staff are getting very sick and some with neurological symptoms which is having a huge impact on the health service function. Even the young and healthy are off for days, some for weeks, and some requiring medical treatment. Whole teams are being taken out as they went to get vaccinated together.
>
> Mandatory vaccination in this instance is stupid, unethical and irresponsible when it comes to protecting our staff and public health. We are in the voluntary phase of vaccination, and encouraging staff to take an unlicensed product that is impacting on their immediate health … it is clearly stated that these vaccine products do not offer immunity or stop transmission. In which case why are we doing it?

Not to protect health that’s for sure. Medical workers are lauded by governments for agenda reasons when they couldn’t give a toss about them any more than they can for the population in general. Schools across America faced the same situation as they closed due to the high number of teachers and other staff with bad reactions to the Pfizer/BioNTech, Moderna, and Johnson & Johnson ‘Covid vaccines’ all of which were linked to death and serious adverse effects. The *BMJ* took down the consultant’s comments pretty quickly on the grounds that they were being used to spread ‘disinformation’. They were exposing the truth about the ‘vaccine’ was the real reason. The cover-up is breathtaking.

Hiding the evidence

The scale of the ‘vaccine’ death cover-up worldwide can be confirmed by comparing official figures with the personal experience of the public. I heard of many people in my community who died immediately or soon after the vaccine that would never appear in the media or even likely on the official totals of ‘vaccine’ fatalities and adverse reactions when only about ten percent are estimated to be reported and I have seen some estimates as low as one percent in a Harvard study. In the UK alone by April 29th, 2021, some 757,654 adverse reactions had been officially reported from the Pfizer/BioNTech, Oxford/AstraZeneca and Moderna ‘vaccines’ with more

than a thousand deaths linked to jabs and that means an estimated ten times this number in reality from a ten percent reporting rate percentage. That's seven million adverse reactions and 10,000 potential deaths and a one percent reporting rate would be ten times *those* figures. In 1976 the US government pulled the swine flu vaccine after *53* deaths. The UK data included a combined 10,000 eye disorders from the 'Covid vaccines' with more than 750 suffering visual impairment or blindness and again multiply by the estimated reporting percentages. As 'Covid cases' officially fell hospitals virtually empty during the 'Covid crisis' began to fill up with a range of other problems in the wake of the 'vaccine' rollout. The numbers across America have also been catastrophic. Deaths linked to *all* types of vaccine increased by *6,000 percent* in the first quarter of 2021 compared with 2020. A 39-year-old woman from Ogden, Utah, died four days after receiving a second dose of Moderna's 'Covid vaccine' when her liver, heart and kidneys all failed despite the fact that she had no known medical issues or conditions. Her family sought an autopsy, but Dr Erik Christensen, Utah's chief medical examiner, said proving vaccine injury as a cause of death almost never happened. He could think of only one instance where an autopsy would name a vaccine as the official cause of death and that would be anaphylaxis where someone received a vaccine and died almost instantaneously. 'Short of that, it would be difficult for us to definitively say this is the vaccine,' Christensen said. If that is true this must be added to the estimated ten percent (or far less) reporting rate of vaccine deaths and serious reactions and the conclusion can only be that vaccine deaths and serious reactions – including these 'Covid' potions' – are phenomenally understated in official figures. The same story can be found everywhere. Endless accounts of deaths and serious reactions among the public, medical and care home staff while official figures did not even begin to reflect this.

Professional script-reader Dr David Williams, a 'top public-health official' in Ontario, Canada, insulted our intelligence by claiming only four serious adverse reactions and no deaths from the more than 380,000 vaccine doses then given. This bore no resemblance to what people knew had happened in their owns circles and we had Dirk Huyer in charge of getting millions vaccinated in Ontario while at the same time he was Chief Coroner for the province investigating causes of death including possible death from the vaccine. An aide said he had stepped back from investigating deaths, but evidence indicated otherwise. Rosemary Frei, who secured a Master of

Science degree in molecular biology at the Faculty of Medicine at Canada's University of Calgary before turning to investigative journalism, was one who could see that official figures for 'vaccine' deaths and reactions made no sense. She said that doctors seldom reported adverse events and when people got really sick or died after getting a vaccination they would attribute that to anything except the vaccines. It had been that way for years and anyone who wondered aloud whether the 'Covid vaccines' or other shots cause harm is immediately branded as 'anti-vax' and 'anti-science'. This was 'career-threatening' for health professionals. Then there was the huge pressure to support the push to 'vaccinate' billions in the quickest time possible. Frei said:

So that's where we're at today. More than half a million vaccine doses have been given to people in Ontario alone. The rush is on to vaccinate all 15 million of us in the province by September. And the mainstream media are screaming for this to be sped up even more. That all adds up to only a very slim likelihood that we're going to be told the truth by officials about how many people are getting sick or dying from the vaccines.

What is true of Ontario is true of everywhere.

They KNEW – and still did it

The authorities knew what was going to happen with multiple deaths and adverse reactions. The UK government's Gates-funded and Big Pharma-dominated Medicines and Healthcare products Regulatory Agency (MHRA) hired a company to employ AI in compiling the projected reactions to the 'vaccine' that would otherwise be uncountable. The request for applications said: 'The MHRA urgently seeks an Artificial Intelligence (AI) software tool to process the expected high volume of Covid-19 vaccine Adverse Drug Reaction …' This was from the agency, headed by the disingenuous June Raine, that gave the 'vaccines' emergency approval and the company was hired before the first shot was given. 'We are going to kill and maim you – is that okay?' 'Oh, yes, perfectly fine – I'm very grateful, thank you, doctor.' The range of 'Covid vaccine' adverse reactions goes on for page after page in the MHRA criminally underreported 'Yellow Card' system and includes affects to eyes, ears, skin, digestion, blood and so on. Raine's MHRA amazingly claimed that the 'overall safety experience … is

so far as expected from the clinical trials'. The death, serious adverse effects, deafness and blindness were *expected?* When did they ever mention that? If these human tragedies were expected then those that gave approval for the use of these 'vaccines' must be guilty of crimes against humanity including murder – a definition of which is 'killing a person with malice aforethought or with recklessness manifesting extreme indifference to the value of human life.' People involved at the MHRA, the CDC in America and their equivalent around the world must go before Nuremberg trials to answer for their callous inhumanity. We are only talking here about the immediate effects of the 'vaccine'. The longer-term impact of the DNA synthetic manipulation is the main reason they are so hysterically desperate to inoculate the entire global population in the shortest possible time.

Africa and the developing world are a major focus for the 'vaccine' depopulation agenda and a mass vaccination sales-pitch is underway thanks to caring people like the Rockefellers and other Cult assets. The Rockefeller Foundation, which pre-empted the 'Covid pandemic' in a document published in 2010 that 'predicted' what happened a decade later, announced an initial $34.95 million grant in February, 2021, 'to ensure more equitable access to Covid-19 testing and vaccines' among other things in Africa in collaboration with '24 organizations, businesses, and government agencies'. The pan-Africa initiative would focus on 10 countries: Burkina Faso, Ethiopia, Ghana, Kenya, Nigeria, Rwanda, South Africa, Tanzania, Uganda, and Zambia'. Rajiv Shah, President of the Rockefeller Foundation and former administrator of CIA-controlled USAID, said that if Africa was not mass-vaccinated (to change the DNA of its people) it was a 'threat to all of humanity' and not fair on Africans. When someone from the Rockefeller Foundation says they want to do something to help poor and deprived people and countries it is time for a belly-laugh. They are doing this out of the goodness of their 'heart' because 'vaccinating' the entire global population is what the 'Covid' hoax set out to achieve. Official 'decolonisation' of Africa by the Cult was merely a prelude to financial colonisation on the road to a return to physical colonisation. The 'vaccine' is vital to that and the sudden and convenient death of the 'Covid' sceptic president of Tanzania can be seen in its true light. A lot of people in Africa are aware that this is another form of colonisation and exploitation and they need to stand their ground.

The ‘vaccine is working’ scam

A potential problem for the Cult was that the ‘vaccine’ is meant to change human DNA and body messaging and not to protect anyone from a ‘virus’ never shown to exist. The vaccine couldn’t work because it was not designed to work and how could they make it *appear* to be working so that more people would have it? This was overcome by lowering the amplification rate of the PCR test to produce fewer ‘cases’ and therefore fewer ‘deaths’. Some of us had been pointing out since March, 2020, that the amplification rate of the test not testing for the ‘virus’ had been made artificially high to generate positive tests which they could call ‘cases’ to justify lockdowns. The World Health Organization recommended an absurdly high 45 amplification cycles to ensure the high positives required by the Cult and then remained silent on the issue until January 20th, 2021 – Biden’s Inauguration Day. This was when the ‘vaccinations’ were seriously underway and on that day the WHO recommended after discussions with America’s CDC that laboratories *lowered their testing amplification*. Dr David Samadi, a certified urologist and health writer, said the WHO was encouraging all labs to reduce their cycle count for PCR tests. He said the current cycle was much too high and was ‘resulting in any particle being declared a positive case’. Even one mainstream news report I saw said this meant the number of ‘Covid’ infections may have been ‘dramatically inflated’. Oh, just a little bit. The CDC in America issued new guidance to laboratories in April, 2021, to use 28 cycles *but only for ‘vaccinated’ people*. The timing of the CDC/WHO interventions were cynically designed to make it appear the ‘vaccines’ were responsible for falling cases and deaths when the real reason can be seen in the following examples. New York’s state lab, the Wadsworth Center, identified 872 positive tests in July, 2020, based on a threshold of 40 cycles. When the figure was lowered to 35 cycles *43 percent* of the 872 were no longer ‘positives’. At 30 cycles the figure was 63 percent. A Massachusetts lab found that between *85 to 90 percent* of people who tested positive in July with a cycle threshold of 40 would be negative at 30 cycles, Ashish Jha, MD, director of the Harvard Global Health Institute, said: ‘I’m really shocked that it could be that high … Boy, does it really change the way we need to be thinking about testing.’ I’m shocked that I could see the obvious in the spring of 2020, with no medical background, and most medical professionals still haven’t worked it out. No, that’s not shocking – it’s terrifying.

Three weeks after the WHO directive to lower PCR cycles the London *Daily Mail* ran this headline: 'Why ARE Covid cases plummeting? New infections have fallen 45% in the US and 30% globally in the past 3 weeks but experts say vaccine is NOT the main driver because only 8% of Americans and 13% of people worldwide have received their first dose.' They acknowledged that the drop could not be attributed to the 'vaccine', but soon this morphed throughout the media into the 'vaccine' has caused cases and deaths to fall when it was the PCR threshold. In December, 2020, there was chaos at English Channel ports with truck drivers needing negative 'Covid' tests before they could board a ferry home for Christmas. The government wanted to remove the backlog as fast as possible and they brought in troops to do the 'testing'. Out of 1,600 drivers just *36* tested positive and the rest were given the all clear to cross the Channel. I guess the authorities thought that 36 was the least they could get away with without the unquestioning catching on. The amplification trick which most people believed in the absence of information in the mainstream applied more pressure on those refusing the 'vaccine' to succumb when it 'obviously worked'. The truth was the exact opposite with deaths in care homes soaring with the 'vaccine' and in Israel the term used was 'skyrocket'. A re-analysis of published data from the Israeli Health Ministry led by Dr Hervé Seligmann at the Medicine Emerging Infectious and Tropical Diseases at Aix-Marseille University found that Pfizer's 'Covid vaccine' killed 'about 40 times more [elderly] people than the disease itself would have killed' during a five-week vaccination period and *260 times* more younger people than would have died from the 'virus' even according to the manipulated 'virus' figures. Dr Seligmann and his co-study author, Haim Yativ, declared after reviewing the Israeli 'vaccine' death data: 'This is a new Holocaust.'

Then, in mid-April, 2021, after vast numbers of people worldwide had been 'vaccinated', the story changed with clear coordination. The UK government began to prepare the ground for more future lockdowns when Nuremberg-destined Boris Johnson told yet another whopper. He said that cases had fallen because of *lockdowns* not 'vaccines'. Lockdowns are irrelevant when *there is no 'virus'* and the test and fraudulent death certificates are deciding the number of 'cases' and 'deaths'. Study after study has shown that lockdowns don't work and instead kill and psychologically destroy people. Meanwhile in the United States Anthony

Fauci and Rochelle Walensky, the ultra-Zionist head of the CDC, peddled the same line. More lockdown was the answer and not the 'vaccine', a line repeated on cue by the moron that is Canadian Prime Minister Justin Trudeau. Why all the hysteria to get everyone 'vaccinated' if lockdowns and not 'vaccines' made the difference? None of it makes sense on the face of it. Oh, but it does. The Cult wants lockdowns *and* the 'vaccine' and if the 'vaccine' is allowed to be seen as the total answer lockdowns would no longer be justified when there are still livelihoods to destroy. 'Variants' and renewed upward manipulation of PCR amplification are planned to instigate never-ending lockdown *and* more 'vaccines'.

You *must* have it – we're desperate

Israel, where the Jewish and Arab population are ruled by the Sabbatian Cult, was the front-runner in imposing the DNA-manipulating 'vaccine' on its people to such an extent that Jewish refusers began to liken what was happening to the early years of Nazi Germany. This would seem to be a fantastic claim. Why would a government of Jewish people be acting like the Nazis did? If you realise that the Sabbatian Cult was behind the Nazis and that Sabbatians hate Jews the pieces start to fit and the question of why a 'Jewish' government would treat Jews with such callous disregard for their lives and freedom finds an answer. Those controlling the government of Israel *aren't Jewish* – they're Sabbatian. Israeli lawyer Tamir Turgal was one who made the Nazi comparison in comments to German lawyer Reiner Fuellmich who is leading a class action lawsuit against the psychopaths for crimes against humanity. Turgal described how the Israeli government was vaccinating children and pregnant women on the basis that there was no evidence that this was dangerous when they had no evidence that it *wasn't* dangerous either. They just had no evidence. This was medical experimentation and Turgal said this breached the Nuremberg Code about medical experimentation and procedures requiring informed consent and choice. Think about that. A Nuremberg Code developed because of Nazi experimentation on Jews and others in concentration camps by people like the evil-beyond-belief Josef Mengele is being breached by the *Israeli* government; but when you know that it's a *Sabbatian* government along with its intelligence and military agencies like Mossad, Shin Bet and the Israeli Defense Forces, and that Sabbatians were the force behind the Nazis,

the kaleidoscope comes into focus. What have we come to when Israeli Jews are suing their government for violating the Nuremberg Code by essentially making Israelis subject to a medical experiment using the controversial ‘vaccines’? It’s a shocker that this has to be done in the light of what happened in Nazi Germany. The Anshe Ha-Emet, or ‘People of the Truth’, made up of Israeli doctors, lawyers, campaigners and public, have launched a lawsuit with the International Criminal Court. It says:

When the heads of the Ministry of Health as well as the prime minister presented the vaccine in Israel and began the vaccination of Israeli residents, the vaccinated were not advised, that, in practice, they are taking part in a medical experiment and that their consent is required for this under the Nuremberg Code.

The irony is unbelievable, but easily explained in one word: Sabbatians. The foundation of Israeli ‘Covid’ apartheid is the ‘green pass’ or ‘green passport’ which allows Jews and Arabs who have had the DNA-manipulating ‘vaccine’ to go about their lives – to work, fly, travel in general, go to shopping malls, bars, restaurants, hotels, concerts, gyms, swimming pools, theatres and sports venues, while non-’vaccinated’ are banned from all those places and activities. Israelis have likened the ‘green pass’ to the yellow stars that Jews in Nazi Germany were forced to wear – the same as the yellow stickers that a branch of UK supermarket chain Morrisons told exempt mask-wears they had to display when shopping. How very sensitive. The Israeli system is blatant South African-style apartheid on the basis of compliance or non-compliance to fascism rather than colour of the skin. How appropriate that the Sabbatian Israeli government was so close to the pre-Mandela apartheid regime in Pretoria. The Sabbatian-instigated ‘vaccine passport’ in Israel is planned for everywhere. Sabbatians struck a deal with Pfizer that allowed them to lead the way in the percentage of a national population infused with synthetic material and the result was catastrophic. Israeli freedom activist Shai Dannon told me how chairs were appearing on beaches that said ‘vaccinated only’. Health Minister Yuli Edelstein said that anyone unwilling or unable to get the jabs that ‘confer immunity’ will be ‘left behind’. The man’s a liar. Not even the makers claim the ‘vaccines’ confer immunity. When you see those figures of ‘vaccine’ deaths these psychopaths were saying that you must take the chance the ‘vaccine’ will kill you or maim

you while knowing it will change your DNA or lockdown for you will be permanent. That's fascism. The Israeli parliament passed a law to allow personal information of the non-vaccinated to be shared with local and national authorities for three months. This was claimed by its supporters to be a way to 'encourage' people to be vaccinated. Hadas Ziv from Physicians for Human Rights described this as a 'draconian law which crushed medical ethics and the patient rights'. But that's the idea, the Sabbatians would reply.

Your papers, please

Sabbatian Israel was leading what has been planned all along to be a global 'vaccine pass' called a 'green passport' without which you would remain in permanent lockdown restriction and unable to do anything. This is how badly – *desperately* – the Cult is to get everyone 'vaccinated'. The term and colour 'green' was not by chance and related to the psychology of fusing the perception of the green climate hoax with the 'Covid' hoax and how the 'solution' to both is the same Great Reset. Lying politicians, health officials and psychologists denied there were any plans for mandatory vaccinations or restrictions based on vaccinations, but they knew that was exactly what was meant to happen with governments of all countries reaching agreements to enforce a global system. 'Free' Denmark and 'free' Sweden unveiled digital vaccine certification. Cyprus, Czech Republic, Estonia, Greece, Hungary, Iceland, Italy, Poland, Portugal, Slovakia, and Spain have all committed to a vaccine passport system and the rest including the whole of the EU would follow. The satanic UK government will certainly go this way despite mendacious denials and at the time of writing it is trying to manipulate the public into having the 'vaccine' so they could go abroad on a summer holiday. How would that work without something to prove you had the synthetic toxicity injected into you? Documents show that the EU's European Commission was moving towards 'vaccine certificates' in 2018 and 2019 before the 'Covid' hoax began. They knew what was coming. Abracadabra – Ursula von der Leyen, the German President of the Commission, announced in March, 2021, an EU 'Digital Green Certificate' – green again – to track the public's 'Covid status'. The passport sting is worldwide and the Far East followed the same pattern with South Korea

ruling that only those with ‘vaccination’ passports – again the *green* pass – would be able to ‘return to their daily lives’.

Bill Gates has been preparing for this ‘passport’ with other Cult operatives for years and beyond the paper version is a Gates-funded ‘digital tattoo’ to identify who has been vaccinated and who hasn’t. The ‘tattoo’ is reported to include a substance which is externally readable to confirm who has been vaccinated. This is a bio-luminous light-generating enzyme (think fireflies) called … *Luciferase*. Yes, named after the Cult ‘god’ Lucifer the ‘light bringer’ of whom more to come. Gates said he funded the readable tattoo to ensure children in the developing world were vaccinated and no one was missed out. He cares so much about poor kids as we know. This was just the cover story to develop a vaccine tagging system for everyone on the planet. Gates has been funding the ID2020 ‘alliance’ to do just that in league with other lovely people at Microsoft, GAVI, the Rockefeller Foundation, Accenture and IDEO.org. He said in interviews in March, 2020, before any ‘vaccine’ publicly existed, that the world must have a globalised digital certificate to track the ‘virus’ and who had been vaccinated. Gates knew from the start that the mRNA vaccines were coming and when they would come and that the plan was to tag the ‘vaccinated’ to marginalise the intelligent and stop them doing anything including travel. Evil just doesn’t suffice. Gates was exposed for offering a $10 million bribe to the Nigerian House of Representatives to invoke compulsory ‘Covid’ vaccination of all Nigerians. Sara Cunial, a member of the Italian Parliament, called Gates a ‘vaccine criminal’. She urged the Italian President to hand him over to the International Criminal Court for crimes against humanity and condemned his plans to ‘chip the human race’ through ID2020.

You know it’s a long-planned agenda when war criminal and Cult gofer Tony Blair is on the case. With the scale of arrogance only someone as dark as Blair can muster he said: ‘Vaccination in the end is going to be your route to liberty.’ Blair is a disgusting piece of work and he confirms that again. The media has given a lot of coverage to a bloke called Charlie Mullins, founder of London’s biggest independent plumbing company, Pimlico Plumbers, who has said he won’t employ anyone who has not been vaccinated or have them go to any home where people are not vaccinated. He said that if he had his way no one would be allowed to walk the streets if they have not been vaccinated. Gates was cheering at the time while I was

alerting the white coats. The plan is that people will qualify for ‘passports’ for having the first two doses and then to keep it they will have to have all the follow ups and new ones for invented ‘variants’ until human genetics is transformed and many are dead who can’t adjust to the changes. Hollywood celebrities – the usual propaganda stunt – are promoting something called the WELL Health-Safety Rating to verify that a building or space has ‘taken the necessary steps to prioritize the health and safety of their staff, visitors and other stakeholders’. They included Lady Gaga, Jennifer Lopez, Michael B. Jordan, Robert DeNiro, Venus Williams, Wolfgang Puck, Deepak Chopra and 17th Surgeon General Richard Carmona. Yawn. WELL Health-Safety has big connections with China. Parent company Delos is headed by former Goldman Sachs partner Paul Scialla. This is another example – and we will see so many others – of using the excuse of ‘health’ to dictate the lives and activities of the population. I guess one confirmation of the ‘safety’ of buildings is that only ‘vaccinated’ people can go in, right?

Electronic concentration camps

I wrote decades ago about the plans to restrict travel and here we are for those who refuse to bow to tyranny. This can be achieved in one go with air travel if the aviation industry makes a blanket decree. The ‘vaccine’ and guaranteed income are designed to be part of a global version of China’s social credit system which tracks behaviour 24/7 and awards or deletes ‘credits’ based on whether your behaviour is supported by the state or not. I mean your entire lifestyle – what you do, eat, say, everything. Once your credit score falls below a certain level consequences kick in. In China tens of millions have been denied travel by air and train because of this. All the locations and activities denied to refusers by the ‘vaccine’ passports will be included in one big mass ban on doing almost anything for those that don’t bow their head to government. It’s beyond fascist and a new term is required to describe its extremes – I guess fascist technocracy will have to do. The way the Chinese system of technological – technocratic – control is sweeping the West can be seen in the Los Angeles school system and is planned to be expanded worldwide. Every child is required to have a ‘Covid’-tracking app scanned daily before they can enter the classroom. The so-called Daily Pass tracking system is produced by Gates’ Microsoft which I’m sure will shock you rigid. The pass will be scanned using a

barcode (one step from an inside-the-body barcode) and the information will include health checks, ‘Covid’ tests and vaccinations. Entry codes are for one specific building only and access will only be allowed if a student or teacher has a negative test with a test not testing for the ‘virus’, has no symptoms of anything alleged to be related to ‘Covid’ (symptoms from a range of other illness), and has a temperature under 100 degrees. No barcode, no entry, is planned to be the case for everywhere and not only schools.

Kids are being psychologically prepared to accept this as ‘normal’ their whole life which is why what they can impose in schools is so important to the Cult and its gofers. Long-time American freedom campaigner John Whitehead of the Rutherford Institute was not exaggerating when he said: ‘Databit by databit, we are building our own electronic concentration camps.’ Canada under its Cult gofer prime minister Justin Trudeau has taken a major step towards the real thing with people interned against their will if they test positive with a test not testing for the ‘virus’ when they arrive at a Canadian airport. They are jailed in internment hotels often without food or water for long periods and with many doors failing to lock there have been sexual assaults. The interned are being charged sometimes $2,000 for the privilege of being abused in this way. Trudeau is fully on board with the Cult and says the ‘Covid pandemic’ has provided an opportunity for a global ‘reset’ to permanently change Western civilisation. His number two, Deputy Prime Minister Chrystia Freeland, is a trustee of the World Economic Forum and a Rhodes Scholar. The Trudeau family have long been servants of the Cult. See *The Biggest Secret* and Cathy O’Brien’s book *Trance-Formation of America* for the horrific background to Trudeau’s father Pierre Trudeau another Canadian prime minister. Hide your fascism behind the façade of a heart-on-the-sleeve liberal. It’s a well-honed Cult technique.

What can the ‘vaccine’ *really* do?

We have a ‘virus’ never shown to exist and ‘variants’ of the ‘virus’ that have also never been shown to exist except, like the ‘original’, as computer-generated fictions. Even if you believe there’s a ‘virus’ the ‘case’ to ‘death’ rate is in the region of 0.23 to 0.15 percent and those ‘deaths’ are concentrated among the very old around the same average age that people

die anyway. In response to this lack of threat (in truth none) psychopaths and idiots, knowingly and unknowingly answering to Gates and the Cult, are seeking to 'vaccinate' every man, woman and child on Planet Earth. Clearly the 'vaccine' is not about 'Covid' – none of this ever has been. So what is it all about *really*? Why the desperation to infuse genetically-manipulating synthetic material into everyone through mRNA fraudulent 'vaccines' with the intent of doing this over and over with the excuses of 'variants' and other 'virus' inventions? Dr Sherri Tenpenny, an osteopathic medical doctor in the United States, has made herself an expert on vaccines and their effects as a vehement campaigner against their use. Tenpenny was board certified in emergency medicine, the director of a level two trauma centre for 12 years, and moved to Cleveland in 1996 to start an integrative medicine practice which has treated patients from all 50 states and some 17 other countries. Weaning people off pharmaceutical drugs is a speciality.

She became interested in the consequences of vaccines after attending a meeting at the National Vaccine Information Center in Washington DC in 2000 where she 'sat through four days of listening to medical doctors and scientists and lawyers and parents of vaccine injured kids' and asked: 'What's going on?' She had never been vaccinated and never got ill while her father was given a list of vaccines to be in the military and was 'sick his entire life'. The experience added to her questions and she began to examine vaccine documents from the Centers for Disease Control (CDC). After reading the first one, the 1998 version of *The General Recommendations of Vaccination*, she thought: 'This is it?' The document was poorly written and bad science and Tenpenny began 20 years of research into vaccines that continues to this day. She began her research into 'Covid vaccines' in March, 2020, and she describes them as 'deadly'. For many, as we have seen, they already have been. Tenpenny said that in the first 30 days of the 'vaccine' rollout in the United States there had been more than 40,000 adverse events reported to the vaccine adverse event database. A document had been delivered to her the day before that was 172 pages long. 'We have over 40,000 adverse events; we have over 3,100 cases of [potentially deadly] anaphylactic shock; we have over 5,000 neurological reactions.' Effects ranged from headaches to numbness, dizziness and vertigo, to losing feeling in hands or feet and paraesthesia which is when limbs 'fall asleep' and people have the sensation of insects crawling underneath their skin. All this happened in the first 30 days and remember

that only about *ten percent* (or far less) of adverse reactions and vaccine-related deaths are estimated to be officially reported. Tenpenny said:

So can you think of one single product in any industry, any industry, for as long as products have been made on the planet that within 30 days we have 40,000 people complaining of side effects that not only is still on the market but … we've got paid actors telling us how great they are for getting their vaccine. We're offering people $500 if they will just get their vaccine and we've got nurses and doctors going; 'I got the vaccine, I got the vaccine'.

Tenpenny said they were not going to be 'happy dancing folks' when they began to suffer Bell's palsy (facial paralysis), neuropathies, cardiac arrhythmias and autoimmune reactions that kill through a blood disorder. 'They're not going to be so happy, happy then, but we're never going to see pictures of those people' she said. Tenpenny described the 'vaccine' as 'a well-designed killing tool'.

No off-switch

Bad as the initial consequences had been Tenpenny said it would be maybe 14 months before we began to see the 'full ravage' of what is going to happen to the 'Covid vaccinated' with full-out consequences taking anything between two years and 20 years to show. You can understand why when you consider that variations of the 'Covid vaccine' use mRNA (messenger RNA) to in theory activate the immune system to produce protective antibodies without using the actual 'virus'. How can they when it's a computer program and they've never isolated what they claim is the 'real thing'? Instead they use *synthetic* mRNA. They are inoculating synthetic material into the body which through a technique known as the Trojan horse is absorbed into cells to change the nature of DNA. Human DNA is changed by an infusion of messenger RNA and with each new 'vaccine' of this type it is changed even more. Say so and you are banned by Cult Internet platforms. The contempt the contemptuous Mark Zuckerberg has for the truth and human health can be seen in an internal Facebook video leaked to the Project Veritas investigative team in which he said of the 'Covid vaccines': '… I share some caution on this because we just don't know the long term side-effects of basically modifying people's DNA and RNA.' At the same time this disgusting man's Facebook was

censoring and banning anyone saying exactly the same. He must go before a Nuremberg trial for crimes against humanity when he *knows* that he is censoring legitimate concerns and denying the right of informed consent on behalf of the Cult that owns him. People have been killed and damaged by the very 'vaccination' technique he cast doubt on himself when they may not have had the 'vaccine' with access to information that he denied them. The plan is to have at least annual 'Covid vaccinations', add others to deal with invented 'variants', and change all other vaccines into the mRNA system. Pfizer executives told shareholders at a virtual Barclays Global Healthcare Conference in March, 2021, that the public may need a third dose of 'Covid vaccine', plus regular yearly boosters and the company planned to hike prices to milk the profits in a 'significant opportunity for our vaccine'. These are the professional liars, cheats and opportunists who are telling you their 'vaccine' is safe. Given this volume of mRNA planned to be infused into the human body and its ability to then replicate we will have a transformation of human genetics from biological to synthetic biological – exactly the long-time Cult plan for reasons we'll see – and many will die. Sherri Tenpenny said of this replication:

> It's like having an on-button but no off-button and that whole mechanism … they actually give it a name and they call it the Trojan horse mechanism, because it allows that [synthetic] virus and that piece of that [synthetic] virus to get inside of your cells, start to replicate and even get inserted into other parts of your DNA as a Trojan-horse.

Ask the overwhelming majority of people who have the 'vaccine' what they know about the contents and what they do and they would reply: 'The government says it will stop me getting the virus.' Governments give that false impression on purpose to increase take-up. You can read Sherri Tenpenny's detailed analysis of the health consequences in her blog at Vaxxter.com, but in summary these are some of them. She highlights the statement by Bill Gates about how human beings can become their own 'vaccine manufacturing machine'. The man is insane. ['Vaccine'-generated] 'antibodies' carry synthetic messenger RNA into the cells and the damage starts, Tenpenny contends, and she says that lungs can be adversely affected through varying degrees of pus and bleeding which obviously affects breathing and would be dubbed 'Covid-19'. Even more sinister was the impact of 'antibodies' on macrophages, a white blood cell of the immune

system. They consist of Type 1 and Type 2 which have very different functions. She said Type 1 are ‘hyper-vigilant’ white blood cells which ‘gobble up’ bacteria etc. However, in doing so, this could cause inflammation and in extreme circumstances be fatal. She says these affects are mitigated by Type 2 macrophages which kick in to calm down the system and stop it going rogue. They clear up dead tissue debris and reduce inflammation that the Type 1 ‘fire crews’ have caused. Type 1 kills the infection and Type 2 heals the damage, she says. This is her punchline with regard to ‘Covid vaccinations’: She says that mRNA ‘antibodies’ block Type 2 macrophages by attaching to them and deactivating them. This meant that when the Type 1 response was triggered by infection there was nothing to stop that getting out of hand by calming everything down. There’s an on-switch, but no off-switch, she says. What follows can be ‘over and out, see you when I see you’.

Genetic suicide

Tenpenny also highlights the potential for autoimmune disease – the body attacking itself – which has been associated with vaccines since they first appeared. Infusing a synthetic foreign substance into cells could cause the immune system to react in a panic believing that the body is being overwhelmed by an invader (it is) and the consequences can again be fatal. There is an autoimmune response known as a ‘cytokine storm’ which I have likened to a homeowner panicked by an intruder and picking up a gun to shoot randomly in all directions before turning the fire on himself. The immune system unleashes a storm of inflammatory response called cytokines to a threat and the body commits hara-kiri. The lesson is that you mess with the body’s immune response at your peril and these ‘vaccines’ seriously – fundamentally – mess with immune response. Tenpenny refers to a consequence called anaphylactic shock which is a severe and highly dangerous allergic reaction when the immune system floods the body with chemicals. She gives the example of having a bee sting which primes the immune system and makes it sensitive to those chemicals. When people are stung again maybe years later the immune response can be so powerful that it leads to anaphylactic shock. Tenpenny relates this ‘shock’ with regard to the ‘Covid vaccine’ to something called polyethylene glycol or PEG. Enormous numbers of people have become sensitive to this over decades of

use in a whole range of products and processes including food, drink, skin creams and ‘medicine’. Studies have claimed that some 72 percent of people have antibodies triggered by PEG compared with two percent in the 1960s and allergic hypersensitive reactions to this become a gathering cause for concern. Tenpenny points out that the ‘mRNA vaccine’ is coated in a ‘bubble’ of polyethylene glycol which has the potential to cause anaphylactic shock through immune sensitivity. Many reports have appeared of people reacting this way after having the ‘Covid vaccine’. What do we think is going to happen as humanity has more and more of these ‘vaccines’? Tenpenny said: ‘All these pictures we have seen with people with these rashes … these weepy rashes, big reactions on their arms and things like that – it’s an acute allergic reaction most likely to the polyethylene glycol that you’ve been previously primed and sensitised to.’

Those who have not studied the conspiracy and its perpetrators at length might think that making the population sensitive to PEG and then putting it in these ‘vaccines’ is just a coincidence. It is not. It is instead testament to how carefully and coldly-planned current events have been and the scale of the conspiracy we are dealing with. Tenpenny further explains that the ‘vaccine’ mRNA procedure can breach the blood-brain barrier which protects the brain from toxins and other crap that will cause malfunction. In this case they could make two proteins corrupt brain function to cause Amyotrophic lateral sclerosis (ALS) , a progressive nervous system disease leading to loss of muscle control, and frontal lobe degeneration – Alzheimer’s and dementia. Immunologist J. Bart Classon published a paper connecting mRNA ‘vaccines’ to prion disease which can lead to Alzheimer’s and other forms of neurogenerative disease while others have pointed out the potential to affect the placenta in ways that make women infertile. This will become highly significant in the next chapter when I will discuss other aspects of this non-vaccine that relate to its nanotechnology and transmission from the injected to the uninjected.

Qualified in idiocy

Tenpenny describes how research has confirmed that these ‘vaccine’-generated antibodies can interact with a range of other tissues in the body and attack many other organs including the lungs. ‘This means that if you have a hundred people standing in front of you that all got this shot they

could have a hundred different symptoms.' Anyone really think that Cult gofers like the Queen, Tony Blair, Christopher Whitty, Anthony Fauci, and all the other psychopaths have really had this 'vaccine' in the pictures we've seen? Not a bloody chance. Why don't doctors all tell us about all these dangers and consequences of the 'Covid vaccine'? Why instead do they encourage and pressure patients to have the shot? Don't let's think for a moment that doctors and medical staff can't be stupid, lazy, and psychopathic and that's without the financial incentives to give the jab. Tenpenny again:

Some people are going to die from the vaccine directly but a large number of people are going to start to get horribly sick and get all kinds of autoimmune diseases 42 days to maybe a year out. What are they going to do, these stupid doctors who say; 'Good for you for getting that vaccine.' What are they going to say; 'Oh, it must be a mutant, we need to give an extra dose of that vaccine.'

Because now the vaccine, instead of one dose or two doses we need three or four because the stupid physicians aren't taking the time to learn anything about it. If I can learn this sitting in my living room reading a 19 page paper and several others so can they. There's nothing special about me, I just take the time to do it.

Remember how Sara Kayat, the NHS and TV doctor, said that the 'Covid vaccine' would '100 percent prevent hospitalisation and death'. Doctors can be idiots like every other profession and they should not be worshipped as infallible. They are not and far from it. Behind many medical and scientific 'experts' lies an uninformed prat trying to hide themselves from you although in the 'Covid' era many have failed to do so as with UK narrative-repeating 'TV doctor' Hilary Jones. Pushing back against the minority of proper doctors and scientists speaking out against the 'vaccine' has been the entire edifice of the Cult global state in the form of governments, medical systems, corporations, mainstream media, Silicon Valley, and an army of compliant doctors, medical staff and scientists willing to say anything for money and to enhance their careers by promoting the party line. If you do that you are an 'expert' and if you won't you are an 'anti-vaxxer' and 'Covidiot'. The pressure to be 'vaccinated' is incessant. We have even had reports claiming that the 'vaccine' can help cure cancer and Alzheimer's and make the lame walk. I am waiting for the announcement that it can bring you coffee in the morning and cook your tea. Just as the symptoms of 'Covid' seem to increase by the week so have the miracles of the 'vaccine'.

American supermarket giant Kroger Co. offered nearly 500,000 employees in 35 states a $100 bonus for having the ‘vaccine’ while donut chain Krispy Kreme promised ‘vaccinated’ customers a free glazed donut every day for the rest of 2021. Have your DNA changed and you will get a doughnut although we might not have to give you them for long. Such offers and incentives confirm the desperation.

Perhaps the worse vaccine-stunt of them all was UK ‘Health’ Secretary Matt-the-prat Hancock on live TV after watching a clip of someone being ‘vaccinated’ when the roll-out began. Hancock faked tears so badly it was embarrassing. Brain-of-Britain Piers Morgan, the lockdown-supporting, ‘vaccine’ supporting, ‘vaccine’ passport-supporting, TV host played along with Hancock – ‘You’re quite emotional about that’ he said in response to acting so atrocious it would have been called out at a school nativity which will presumably today include Mary and Jesus in masks, wise men keeping their camels six feet apart, and shepherds under tent arrest. System-serving Morgan tweeted this: ‘Love the idea of covid vaccine passports for everywhere: flights, restaurants, clubs, football, gyms, shops etc. It’s time covid-denying, anti-vaxxer loonies had their bullsh*t bluff called & bar themselves from going anywhere that responsible citizens go.’ If only I could aspire to his genius. To think that Morgan, who specialises in shouting over anyone he disagrees with, was lauded as a free speech hero when he lost his job after storming off the set of his live show like a child throwing his dolly out of the pram. If he is a free speech hero we are in real trouble. I have no idea what ‘bullsh*t’ means, by the way, the * throws me completely.

The Cult is desperate to infuse its synthetic DNA-changing concoction into everyone and has been using every lie, trick and intimidation to do so. The question of ‘*Why*?’ we shall now address.

CHAPTER TEN

Human 2.0

I believe that at the end of the century the use of words and general educated opinion will have altered so much that one will be able to speak of machines thinking without expecting to be contradicted –
Alan Turing (1912-1954), the 'Father of artificial intelligence'

I have been exposing for decades the plan to transform the human body from a biological to a synthetic-biological state. The new human that I will call Human 2.0 is planned to be connected to artificial intelligence and a global AI 'Smart Grid' that would operate as one global system in which AI would control everything from your fridge to your heating system to your car to your mind. Humans would no longer be 'human', but post-human and sub-human, with their thinking and emotional processes replaced by AI.

What I said sounded crazy and beyond science fiction and I could understand that. To any balanced, rational, mind it *is* crazy. Today, however, that world is becoming reality and it puts the 'Covid vaccine' into its true context. Ray Kurzweil is the ultra-Zionist 'computer scientist, inventor and futurist' and co-founder of the Singularity University. Singularity refers to the merging of humans with machines or 'transhumanism'. Kurzweil has said humanity would be connected to the cyber 'cloud' in the period of the ever-recurring year of 2030:

Our thinking … will be a hybrid of biological and non-biological thinking … humans will be able to extend their limitations and 'think in the cloud' … We're going to put gateways to the cloud in our

brains ... We're going to gradually merge and enhance ourselves ... In my view, that's the nature of being human – we transcend our limitations. As the technology becomes vastly superior to what we are then the small proportion that is still human gets smaller and smaller and smaller until it's just utterly negligible.

They are trying to sell this end-of-humanity-as-we-know-it as the next stage of 'evolution' when we become super-human and 'like the gods'. They are lying to you. Shocked, eh? The population, and again especially the young, have been manipulated into addiction to technologies designed to enslave them for life. First they induced an addiction to smartphones (holdables); next they moved to technology on the body (wearables); and then began the invasion of the body (implantables). I warned way back about the plan for microchipped people and we are now entering that era. We should not be diverted into thinking that this refers only to chips we can see. Most important are the nanochips known as smart dust, neural dust and nanobots which are far too small to be seen by the human eye. Nanotechnology is everywhere, increasingly in food products, and released into the atmosphere by the geoengineering of the skies funded by Bill Gates to 'shut out the Sun' and 'save the planet from global warming'. Gates has been funding a project to spray millions of tonnes of chalk (calcium carbonate) into the stratosphere over Sweden to 'dim the Sun' and cool the Earth. Scientists warned the move could be disastrous for weather systems in ways no one can predict and opposition led to the Swedish space agency announcing that the 'experiment' would not be happening as planned in the summer of 2021; but it shows where the Cult is going with dimming the impact of the Sun and there's an associated plan to change the planet's atmosphere. Who gives psychopath Gates the right to dictate to the entire human race and dismantle planetary systems? The world will not be safe while this man is at large.

The global warming hoax has made the Sun, like the gas of life, something to fear when both are essential to good health and human survival (more inversion). The body transforms sunlight into vital vitamin D through a process involving … *cholesterol*. This is the cholesterol we are also told to fear. We are urged to take Big Pharma statin drugs to reduce cholesterol and it's all systematic. Reducing cholesterol means reducing vitamin D uptake with all the multiple health problems that will cause. At least if you take statins long term it saves the government from having to

pay you a pension. The delivery system to block sunlight is widely referred to as chemtrails although these have a much deeper agenda, too. They appear at first to be contrails or condensation trails streaming from aircraft into cold air at high altitudes. Contrails disperse very quickly while chemtrails do not and spread out across the sky before eventually their content falls to earth. Many times I have watched aircraft cross-cross a clear blue sky releasing chemtrails until it looks like a cloudy day. Chemtrails contain many things harmful to humans and the natural world including toxic heavy metals, aluminium (see Alzheimer's) and nanotechnology. Ray Kurzweil reveals the reason without actually saying so: 'Nanobots will infuse all the matter around us with information. Rocks, trees, everything will become these intelligent creatures.' How do you deliver that? *From the sky*. Self-replicating nanobots would connect everything to the Smart Grid. The phenomenon of Morgellons disease began in the chemtrail era and the correlation has led to it being dubbed the 'chemtrail disease'. Self-replicating fibres appear in the body that can be pulled out through the skin. Morgellons fibres continue to grow outside the body and have a form of artificial intelligence. I cover this at greater length in *Phantom Self*.

'Vaccine' operating system

'Covid vaccines' with their self-replicating synthetic material are also designed to make the connection between humanity and Kurzweil's 'cloud'. American doctor and dedicated campaigner for truth, Carrie Madej, an Internal Medicine Specialist in Georgia with more than 20 years medical experience, has highlighted the nanotechnology aspect of the fake 'vaccines'. She explains how one of the components in at least the Moderna and Pfizer synthetic potions are 'lipid nanoparticles' which are 'like little tiny computer bits' – a 'sci-fi substance' known as nanobots and hydrogel which can be 'triggered at any moment to deliver its payload' and act as 'biosensors'. The synthetic substance had 'the ability to accumulate data from your body like your breathing, your respiration, thoughts and emotions, all kind of things' and each syringe could carry a *million* nanobots:

This substance because it's like little bits of computers in your body, crazy, but it's true, it can do that, [and] obviously has the ability to act through Wi-Fi. It can receive and transmit energy,

messages, frequencies or impulses. That issue has never been addressed by these companies. What does that do to the human?

Just imagine getting this substance in you and it can react to things all around you, the 5G, your smart device, your phones, what is happening with that? What if something is triggering it, too, like an impulse, a frequency? We have something completely foreign in the human body.

Madej said her research revealed that electromagnetic (EMF) frequencies emitted by phones and other devices had increased dramatically in the same period of the 'vaccine' rollout and she was seeing more people with radiation problems as 5G and other electromagnetic technology was expanded and introduced to schools and hospitals. She said she was 'floored with the EMF coming off' the devices she checked. All this makes total sense and syncs with my own work of decades when you think that Moderna refers in documents to its mRNA 'vaccine' as an 'operating system':

Recognizing the broad potential of mRNA science, we set out to create an mRNA technology platform that functions very much like an operating system on a computer. It is designed so that it can plug and play interchangeably with different programs. In our case, the 'program' or 'app' is our mRNA drug – the unique mRNA sequence that codes for a protein …

… Our MRNA Medicines – 'The 'Software Of Life': When we have a concept for a new mRNA medicine and begin research, fundamental components are already in place. Generally, the only thing that changes from one potential mRNA medicine to another is the coding region – the actual genetic code that instructs ribosomes to make protein. Utilizing these instruction sets gives our investigational mRNA medicines a software-like quality. We also have the ability to combine different mRNA sequences encoding for different proteins in a single mRNA investigational medicine.

Who needs a real 'virus' when you can create a computer version to justify infusing your operating system into the entire human race on the road to making living, breathing people into cyborgs? What is missed with the 'vaccines' is the *digital* connection between synthetic material and the body that I highlighted earlier with the study that hacked a computer with human DNA. On one level the body is digital, based on mathematical codes, and I'll have more about that in the next chapter. Those who ridiculously claim that mRNA 'vaccines' are not designed to change human genetics should explain the words of Dr Tal Zaks, chief medical officer at Moderna, in a

2017 TED talk. He said that over the last 30 years 'we've been living this phenomenal digital scientific revolution, and I'm here today to tell you, that we are actually *hacking the software of life*, and that it's changing the way we think about prevention and treatment of disease':

In every cell there's this thing called messenger RNA, or mRNA for short, that transmits the critical information from the DNA in our genes to the protein, which is really the stuff we're all made out of. This is the critical information that determines what the cell will do. So we think about it as an operating system. So if you could change that, if you could introduce a line of code, or change a line of code, it turns out, that has profound implications for everything, from the flu to cancer.

Zaks should more accurately have said that this has profound implications for the human genetic code and the nature of DNA. Communications within the body go both ways and not only one. But, hey, no, the 'Covid vaccine' will not affect your genetics. Cult fact-checkers say so even though the man who helped to develop the mRNA technique says that it does. Zaks said in 2017:

If you think about what it is we're trying to do. We've taken information and our understanding of that information and how that information is transmitted in a cell, and we've taken our understanding of medicine and how to make drugs, and we're fusing the two. We think of it as information therapy.

I have been writing for decades that the body is an information field communicating with itself and the wider world. This is why radiation which is information can change the information field of body and mind through phenomena like 5G and change their nature and function. 'Information therapy' means to change the body's information field and change the way it operates. DNA is a receiver-transmitter of information and can be mutated by information like mRNA synthetic messaging. Technology to do this has been ready and waiting in the underground bases and other secret projects to be rolled out when the 'Covid' hoax was played. 'Trials' of such short and irrelevant duration were only for public consumption. When they say the 'vaccine' is 'experimental' that is not true. It may appear to be 'experimental' to those who don't know what's going on, but the trials have already been done to ensure the Cult gets the result it desires. Zaks said that it took decades to sequence the human genome, completed in 2003, but now

they could do it in a week. By ‘they’ he means scientists operating in the public domain. In the secret projects they were sequencing the genome in a week long before even 2003.

Deluge of mRNA

Highly significantly the Moderna document says the guiding premise is that if using mRNA as a medicine works for one disease then it should work for many diseases. They were leveraging the flexibility afforded by their platform and the fundamental role mRNA plays in protein synthesis to pursue mRNA medicines for a broad spectrum of diseases. Moderna is confirming what I was saying through 2020 that multiple ‘vaccines’ were planned for ‘Covid’ (and later invented ‘variants’) and that previous vaccines would be converted to the mRNA system to infuse the body with massive amounts of genetically-manipulating synthetic material to secure a transformation to a synthetic-biological state. The ‘vaccines’ are designed to kill stunning numbers as part of the long-exposed Cult depopulation agenda and transform the rest. Given this is the goal you can appreciate why there is such hysterical demand for every human to be ‘vaccinated’ for an alleged ‘disease’ that has an estimated ‘infection’ to ‘death’ ratio of 0.23-0.15 percent. As I write children are being given the ‘vaccine’ in trials (their parents are a disgrace) and ever-younger people are being offered the vaccine for a ‘virus’ that even if you believe it exists has virtually zero chance of harming them. Horrific effects of the ‘trials’ on a 12-year-old girl were revealed by a family member to be serious brain and gastric problems that included a bowel obstruction and the inability to swallow liquids or solids. She was unable to eat or drink without throwing up, had extreme pain in her back, neck and abdomen, and was paralysed from the waist down which stopped her urinating unaided. When the girl was first taken to hospital doctors said it was all in her mind. She was signed up for the ‘trial’ by her parents for whom no words suffice. None of this ‘Covid vaccine’ insanity makes any sense unless you see what the ‘vaccine’ really is – a body-changer. Synthetic biology or ‘SynBio’ is a fast-emerging and expanding scientific discipline which includes everything from genetic and molecular engineering to electrical and computer engineering. Synthetic biology is defined in these ways:

• A multidisciplinary area of research that seeks to create new biological parts, devices, and systems, or to redesign systems that are already found in nature.

• The use of a mixture of physical engineering and genetic engineering to create new (and therefore synthetic) life forms.

• An emerging field of research that aims to combine the knowledge and methods of biology, engineering and related disciplines in the design of chemically-synthesized DNA to create organisms with novel or enhanced characteristics and traits (synthetic organisms including humans).

We now have synthetic blood, skin, organs and limbs being developed along with synthetic body parts produced by 3D printers. These are all elements of the synthetic human programme and this comment by Kurzweil's co-founder of the Singularity University, Peter Diamandis, can be seen in a whole new light with the 'Covid' hoax and the sanctions against those that refuse the 'vaccine':

Anybody who is going to be resisting the progress forward [to transhumanism] is going to be resisting evolution and, fundamentally, they will die out. It's not a matter of whether it's good or bad. It's going to happen.

'Resisting evolution'? What absolute bollocks. The arrogance of these people is without limit. His 'it's going to happen' mantra is another way of saying 'resistance is futile' to break the spirit of those pushing back and we must not fall for it. Getting this genetically-transforming 'vaccine' into everyone is crucial to the Cult plan for total control and the desperation to achieve that is clear for anyone to see. Vaccine passports are a major factor in this and they, too, are a form of resistance is futile. It's NOT. The paper funded by the Rockefeller Foundation for the 2013 'health conference' in China said:

We will interact more with artificial intelligence. The use of robotics, bio-engineering to augment human functioning is already well underway and will advance. Re-engineering of humans into

potentially separate and unequal forms through genetic engineering or mixed human-robots raises debates on ethics and equality.

A new demography is projected to emerge after 2030 [that year again] of technologies (robotics, genetic engineering, nanotechnology) producing robots, engineered organisms, 'nanobots' and artificial intelligence (AI) that can self-replicate. Debates will grow on the implications of an impending reality of human designed life.

What is happening today is so long planned. The world army enforcing the will of the world government is intended to be a robot army, not a human one. Today's military and its technologically 'enhanced' troops, pilotless planes and driverless vehicles are just stepping stones to that end. Human soldiers are used as Cult fodder and its time they woke up to that and worked for the freedom of the population instead of their own destruction and their family's destruction – the same with the police. Join us and let's sort this out. The phenomenon of enforce my own destruction is widespread in the 'Covid' era with Woker 'luvvies' in the acting and entertainment industries supporting 'Covid' rules which have destroyed their profession and the same with those among the public who put signs on the doors of their businesses 'closed due to Covid – stay safe' when many will never reopen. It's a form of masochism and most certainly insanity.

Transgender = transhumanism

When something explodes out of nowhere and is suddenly everywhere it is always the Cult agenda and so it is with the tidal wave of claims and demands that have infiltrated every aspect of society under the heading of 'transgenderism'. The term 'trans' is so 'in' and this is the dictionary definition:

A prefix meaning 'across', 'through', occurring … in loanwords from Latin, used in particular for denoting movement or conveyance from place to place (transfer; transmit; transplant) or complete change (transform; transmute), or to form adjectives meaning 'crossing', 'on the other side of', or 'going beyond' the place named (transmontane; transnational; trans-Siberian).

Transgender means to go beyond gender and transhuman means to go beyond human. Both are aspects of the Cult plan to transform the human body to a synthetic state with *no gender*. Human 2.0 is not designed to

procreate and would be produced technologically with no need for parents. The new human would mean the end of parents and so men, and increasingly women, are being targeted for the deletion of their rights and status. Parental rights are disappearing at an ever-quickening speed for the same reason. The new human would have no need for men or women when there is no procreation and no gender. Perhaps the transgender movement that appears to be in a permanent state of frenzy might now contemplate on how it is being used. This was never about transgender rights which are only the interim excuse for confusing gender, particularly in the young, on the road to *fusing* gender. Transgender activism is not an end; it is a *means* to an end. We see again the technique of creative destruction in which you destroy the status quo to 'build back better' in the form that you want. The gender status quo had to be destroyed by persuading the Cult-created Woke mentality to believe that you can have 100 genders or more. A programme for 9 to 12 year olds produced by the Cult-owned BBC promoted the 100 genders narrative. The very idea may be the most monumental nonsense, but it is not what is true that counts, only what you can make people *believe* is true. Once the gender of 2 + 2 = 4 has been dismantled through indoctrination, intimidation and 2 + 2 = 5 then the new no-gender normal can take its place with Human 2.0. Aldous Huxley revealed the plan in his prophetic *Brave New World* in 1932:

> Natural reproduction has been done away with and children are created, decanted', and raised in 'hatcheries and conditioning centres'. From birth, people are genetically designed to fit into one of five castes, which are further split into 'Plus' and 'Minus' members and designed to fulfil predetermined positions within the social and economic strata of the World State.

How could Huxley know this in 1932? For the same reason George Orwell knew about the Big Brother state in 1948, Cult insiders I have quoted knew about it in 1969, and I have known about it since the early 1990s. If you are connected to the Cult or you work your balls off to uncover the plan you can predict the future. The process is simple. If there is a plan for the world and nothing intervenes to stop it then it will happen. Thus if you communicate the plan ahead of time you are perceived to have predicted the future, but you haven't. You have revealed the plan which without intervention will become the human future. The whole reason I have done what I have is to alert enough people to inspire an intervention and maybe

at last that time has come with the Cult and its intentions now so obvious to anyone with a brain in working order.

The future is here

Technological wombs that Huxley described to replace parent procreation are already being developed and they are only the projects we know about in the public arena. Israeli scientists told *The Times of Israel* in March, 2021, that they have grown 250-cell embryos into mouse foetuses with fully formed organs using artificial wombs in a development they say could pave the way for gestating humans outside the womb. Professor Jacob Hanna of the Weizmann Institute of Science said:

> We took mouse embryos from the mother at day five of development, when they are just of 250 cells, and had them in the incubator from day five until day 11, by which point they had grown all their organs.
>
> By day 11 they make their own blood and have a beating heart, a fully developed brain. Anybody would look at them and say, 'this is clearly a mouse foetus with all the characteristics of a mouse.' It's gone from being a ball of cells to being an advanced foetus.

A special liquid is used to nourish embryo cells in a laboratory dish and they float on the liquid to duplicate the first stage of embryonic development. The incubator creates all the right conditions for its development, Hanna said. The liquid gives the embryo 'all the nutrients, hormones and sugars they need' along with a custom-made electronic incubator which controls gas concentration, pressure and temperature. The cutting-edge in the underground bases and other secret locations will be light years ahead of that, however, and this was reported by the London *Guardian* in 2017:

> We are approaching a biotechnological breakthrough. Ectogenesis, the invention of a complete external womb, could completely change the nature of human reproduction. In April this year, researchers at the Children's Hospital of Philadelphia announced their development of an artificial womb.

The article was headed ‘Artificial wombs could soon be a reality. What will this mean for women?’ What would it mean for children is an even bigger question. No mother to bond with only a machine in preparation for a life of soulless interaction and control in a world governed by machines (see the *Matrix* movies). Now observe the calculated manipulations of the ‘Covid’ hoax as human interaction and warmth has been curtailed by distancing, isolation and fear with people communicating via machines on a scale never seen before. These are all dots in the same picture as are all the personal assistants, gadgets and children’s toys through which kids and adults communicate with AI as if it is human. The AI ‘voice’ on Sat-Nav should be included. All these things are psychological preparation for the Cult endgame. Before you can make a physical connection with AI you have to make a psychological connection and that is what people are being conditioned to do with this ever gathering human-AI interaction. Movies and TV programmes depicting the transhuman, robot dystopia relate to a phenomenon known as ‘pre-emptive programming’ in which the world that is planned is portrayed everywhere in movies, TV and advertising. This is conditioning the conscious and subconscious mind to become familiar with the planned reality to dilute resistance when it happens for real. What would have been a shock such is the change is made less so. We have young children put on the road to transgender transition surgery with puberty blocking drugs at an age when they could never be able to make those life-changing decisions.

Rachel Levine, a professor of paediatrics and psychiatry who believes in treating children this way, became America’s highest-ranked openly-transgender official when she was confirmed as US Assistant Secretary at the Department of Health and Human Services after being nominated by Joe Biden (the Cult). Activists and governments press for laws to deny parents a say in their children’s transition process so the kids can be isolated and manipulated into agreeing to irreversible medical procedures. A Canadian father Robert Hoogland was denied bail by the Vancouver Supreme Court in 2021 and remained in jail for breaching a court order that he stay silent over his young teenage daughter, a minor, who was being offered life-changing hormone therapy without parental consent. At the age of 12 the girl’s ‘school counsellor’ said she may be transgender, referred her to a doctor and told the school to treat her like a boy. This is another example of state-serving schools imposing ever more control over

children's lives while parents have ever less. Contemptible and extreme child abuse is happening all over the world as the Cult gender-fusion operation goes into warp-speed.

Why the war on men – and now women?

The question about what artificial wombs mean for women should rightly be asked. The answer can be seen in the deletion of women's rights involving sport, changing rooms, toilets and status in favour of people in male bodies claiming to identify as women. I can identify as a mountain climber, but it doesn't mean I can climb a mountain any more than a biological man can be a biological woman. To believe so is a triumph of belief over factual reality which is the very perceptual basis of everything Woke. Women's sport is being destroyed by allowing those with male bodies who say they identify as female to 'compete' with girls and women. Male body 'women' dominate 'women's' competition with their greater muscle mass, bone density, strength and speed. With that disadvantage sport for women loses all meaning. To put this in perspective nearly 300 American high school boys can run faster than the quickest woman sprinter in the world. Women are seeing their previously protected spaces invaded by male bodies simply because they claim to identify as women. That's all they need to do to access all women's spaces and activities under the Biden 'Equality Act' that destroys equality for women with the usual Orwellian Woke inversion. Male sex offenders have already committed rapes in women's prisons after claiming to identify as women to get them transferred. Does this not matter to the Woke 'equality' hypocrites? Not in the least. What matters to Cult manipulators and funders behind transgender activists is to advance gender fusion on the way to the no-gender 'human'. When you are seeking to impose transparent nonsense like this, or the 'Covid' hoax, the only way the nonsense can prevail is through censorship and intimidation of dissenters, deletion of factual information, and programming of the unquestioning, bewildered and naive. You don't have to scan the world for long to see that all these things are happening.

Many women's rights organisations have realised that rights and status which took such a long time to secure are being eroded and that it is systematic. Kara Dansky of the global Women's Human Rights Campaign said that Biden's transgender executive order immediately he took office,

subsequent orders, and Equality Act legislation that followed 'seek to erase women and girls in the law as a category'. *Exactly*. I said during the long ago-started war on men (in which many women play a crucial part) that this was going to turn into a war on them. The Cult is phasing out *both* male and female genders. To get away with that they are brought into conflict so they are busy fighting each other while the Cult completes the job with no unity of response. Unity, people, *unity*. We need unity everywhere. Transgender is the only show in town as the big step towards the no-gender human. It's not about rights for transgender people and never has been. Woke political correctness is deleting words relating to genders to the same end. Wokers believe this is to be 'inclusive' when the opposite is true. They are deleting words describing gender because gender *itself* is being deleted by Human 2.0. Terms like 'man', 'woman', 'mother' and 'father' are being deleted in the universities and other institutions to be replaced by the *no*-gender, not trans-gender, 'individuals' and 'guardians'. Women's rights campaigner Maria Keffler of Partners for Ethical Care said: 'Children are being taught from kindergarten upward that some boys have a vagina, some girls have a penis, and that kids can be any gender they want to be.' Do we really believe that suddenly countries all over the world at the same time had the idea of having drag queens go into schools or read transgender stories to very young children in the local library? It's coldly-calculated confusion of gender on the way to the fusion of gender. Suzanne Vierling, a psychologist from Southern California, made another important point:

> Yesterday's slave woman who endured gynecological medical experiments is today's girl-child being butchered in a booming gender-transitioning sector. Ovaries removed, pushing her into menopause and osteoporosis, uncharted territory, and parents' rights and authority decimated.

The erosion of parental rights is a common theme in line with the Cult plans to erase the very concept of parents and 'ovaries removed, pushing her into menopause' means what? Those born female lose the ability to have children – another way to discontinue humanity as we know it.

Eliminating Human 1.0 (before our very eyes)

To pave the way for Human 2.0 you must phase out Human 1.0. This is happening through plummeting sperm counts and making women infertile through an onslaught of chemicals, radiation (including smartphones in pockets of men) and mRNA 'vaccines'. Common agriculture pesticides are also having a devastating impact on human fertility. I have been tracking collapsing sperm counts in the books for a long time and in 2021 came a book by fertility scientist and reproductive epidemiologist Shanna Swan, *Count Down: How Our Modern World Is Threatening Sperm Counts, Altering Male and Female Reproductive Development and Imperiling the Future of the Human Race*. She reports how the global fertility rate dropped by *half* between 1960 and 2016 with America's birth rate 16 percent below where it needs to be to sustain the population. Women are experiencing declining egg quality, more miscarriages, and more couples suffer from infertility. Other findings were an increase in erectile dysfunction, infant boys developing more genital abnormalities, male problems with conception, and plunging levels of the male hormone testosterone which would explain why so many men have lost their backbone and masculinity. This has been very evident during the 'Covid' hoax when women have been prominent among the Pushbackers and big strapping blokes have bowed their heads, covered their faces with a nappy and quietly submitted. Mind control expert Cathy O'Brien also points to how global education introduced the concept of 'we're all winners' in sport and classrooms: 'Competition was defused, and it in turn defused a sense of fighting back.' This is another version of the 'equity' doctrine in which you drive down rather than raise up. What a contrast in Cult-controlled China with its global ambitions where the government published plans in January, 2021, to 'cultivate masculinity' in boys from kindergarten through to high school in the face of a 'masculinity crisis'. A government adviser said boys would be soon become 'delicate, timid and effeminate' unless action was taken. Don't expect any similar policy in the targeted West. A 2006 study showed that a 65-year-old man in 2002 had testosterone levels *15 percent* lower than a 65-year-old man in 1987 while a 2020 study found a similar story with young adults and adolescents. Men are getting prescriptions for testosterone replacement therapy which causes an even greater drop in sperm count with up to 99 percent seeing sperm counts drop to zero during the treatment. More sperm is defective and malfunctioning with some having two heads or not pursuing an egg.

A class of *synthetic* chemicals known as phthalates are being blamed for the decline. These are found everywhere in plastics, shampoos, cosmetics, furniture, flame retardants, personal care products, pesticides, canned foods and even receipts. Why till receipts? Everyone touches them. Let no one delude themselves that all this is not systematic to advance the long-time agenda for human body transformation. Phthalates mimic hormones and disrupt the hormone balance causing testosterone to fall and genital birth defects in male infants. Animals and fish have been affected in the same way due to phthalates and other toxins in rivers. When fish turn gay or change sex through chemicals in rivers and streams it is a pointer to why there has been such an increase in gay people and the sexually confused. It doesn't matter to me what sexuality people choose to be, but if it's being affected by chemical pollution and consumption then we need to know. Does anyone really think that this is not connected to the transgender agenda, the war on men and the condemnation of male 'toxic masculinity'? You watch this being followed by 'toxic femininity'. It's already happening. When breastfeeding becomes 'chest-feeding', pregnant women become pregnant people along with all the other Woke claptrap you know that the world is going insane and there's a Cult scam in progress. Transgender activists are promoting the Cult agenda while Cult billionaires support and fund the insanity as they laugh themselves to sleep at the sheer stupidity for which humans must be infamous in galaxies far, far away.

'Covid vaccines' and female infertility

We can now see why the 'vaccine' has been connected to potential infertility in women. Dr Michael Yeadon, former Vice President and Chief Scientific Advisor at Pfizer, and Dr Wolfgang Wodarg in Germany, filed a petition with the European Medicines Agency in December, 2020, urging them to stop trials for the Pfizer/BioNTech shot and all other mRNA trials until further studies had been done. They were particularly concerned about possible effects on fertility with 'vaccine'-produced antibodies attacking the protein Syncytin-1 which is responsible for developing the placenta. The result would be infertility 'of indefinite duration' in women who have the 'vaccine' with the placenta failing to form. Section 10.4.2 of the Pfizer/BioNTech trial protocol says that pregnant women or those who might become so should not have mRNA shots. Section 10.4 warns men

taking mRNA shots to 'be abstinent from heterosexual intercourse' and not to donate sperm. The UK government said that it *did not know* if the mRNA procedure had an effect on fertility. *Did not know?* These people have to go to jail. UK government advice did not recommend at the start that pregnant women had the shot and said they should avoid pregnancy for at least two months after 'vaccination'. The 'advice' was later updated to pregnant women should only have the 'vaccine' if the benefits outweighed the risks to mother and foetus. What the hell is that supposed to mean? Then 'spontaneous abortions' began to appear and rapidly increase on the adverse reaction reporting schemes which include only a fraction of adverse reactions. Thousands and ever-growing numbers of 'vaccinated' women are describing changes to their menstrual cycle with heavier blood flow, irregular periods and menstruating again after going through the menopause – all links to reproduction effects. Women are passing blood clots and the lining of their uterus while men report erectile dysfunction and blood effects. Most significantly of all *un*vaccinated women began to report similar menstrual changes after interaction with *'vaccinated'* people and men and children were also affected with bleeding noses, blood clots and other conditions. 'Shedding' is when vaccinated people can emit the content of a vaccine to affect the unvaccinated, but this is different. 'Vaccinated' people were not shedding a 'live virus' allegedly in 'vaccines' as before because the fake 'Covid vaccines' involve synthetic material and other toxicity. Doctors exposing what is happening prefer the term 'transmission' to shedding. Somehow those that have had the shots are transmitting effects to those that haven't. Dr Carrie Madej said the nano-content of the 'vaccines' can 'act like an antenna' to others around them which fits perfectly with my own conclusions. This 'vaccine' transmission phenomenon was becoming known as the book went into production and I deal with this further in the Postscript.

Vaccine effects on sterility are well known. The World Health Organization was accused in 2014 of sterilising millions of women in Kenya with the evidence confirmed by the content of the vaccines involved. The same WHO behind the 'Covid' hoax admitted its involvement for more than ten years with the vaccine programme. Other countries made similar claims. Charges were lodged by Tanzania, Nicaragua, Mexico, and the Philippines. The Gardasil vaccine claimed to protect against a genital 'virus' known as HPV has also been linked to infertility. Big Pharma and

the WHO (same thing) are criminal and satanic entities. Then there's the Bill Gates Foundation which is connected through funding and shared interests with 20 pharmaceutical giants and laboratories. He stands accused of directing the policy of United Nations Children's Fund (UNICEF), vaccine alliance GAVI, and other groupings, to advance the vaccine agenda and silence opposition at great cost to women and children. At the same time Gates wants to reduce the global population. Coincidence?

Great Reset = Smart Grid = new human

The Cult agenda I have been exposing for 30 years is now being openly promoted by Cult assets like Gates and Klaus Schwab of the World Economic Forum under code-terms like the 'Great Reset', 'Build Back Better' and 'a rare but narrow window of opportunity to reflect, reimagine, and reset our world'. What provided this 'rare but narrow window of opportunity'? The 'Covid' hoax did. Who created that? *They* did. My books from not that long ago warned about the planned 'Internet of Things' (IoT) and its implications for human freedom. This was the plan to connect all technology to the Internet and artificial intelligence and today we are way down that road with an estimated 36 billion devices connected to the World Wide Web and that figure is projected to be 76 billion by 2025. I further warned that the Cult planned to go beyond that to the Internet of *Everything* when the human brain was connected via AI to the Internet and Kurzweil's 'cloud'. Now we have Cult operatives like Schwab calling for precisely that under the term 'Internet of Bodies', a fusion of the physical, digital and biological into one centrally-controlled Smart Grid system which the Cult refers to as the 'Fourth Industrial Revolution'. They talk about the 'biological', but they really mean the synthetic-biological which is required to fully integrate the human body and brain into the Smart Grid and artificial intelligence planned to replace the human mind. We have everything being synthetically manipulated including the natural world through GMO and smart dust, the food we eat and the human body itself with synthetic 'vaccines'. I said in *The Answer* that we would see the Cult push for synthetic meat to replace animals and in February, 2021, the so predictable psychopath Bill Gates called for the introduction of synthetic meat to save us all from 'climate change'. The climate hoax just keeps on giving like the 'Covid' hoax. The war on meat by vegan activists is a

carbon (oops, sorry) copy of the manipulation of transgender activists. They have no idea (except their inner core) that they are being used to promote and impose the agenda of the Cult or that they are only the *vehicle* and not the *reason*. This is not to say those who choose not to eat meat shouldn't be respected and supported in that right, but there are ulterior motives for those in power. A *Forbes* article in December, 2019, highlighted the plan so beloved of Schwab and the Cult under the heading: 'What Is The Internet of Bodies? And How Is It Changing Our World?' The article said the human body is the latest data platform (remember 'our vaccine is an operating system'). *Forbes* described the plan very accurately and the words could have come straight out of my books from long before:

The Internet of Bodies (IoB) is an extension of the IoT and basically connects the human body to a network through devices that are ingested, implanted, or connected to the body in some way. Once connected, data can be exchanged, and the body and device can be remotely monitored and controlled.

They were really describing a human hive mind with human perception centrally-dictated via an AI connection as well as allowing people to be 'remotely monitored and controlled'. Everything from a fridge to a human mind could be directed from a central point by these insane psychopaths and 'Covid vaccines' are crucial to this. Forbes explained the process I mentioned earlier of holdable and wearable technology followed by implantable. The article said there were three generations of the Internet of Bodies that include:

- Body external: These are wearable devices such as Apple Watches or Fitbits that can monitor our health.

- Body internal: These include pacemakers, cochlear implants, and digital pills that go inside our bodies to monitor or control various aspects of health.

- Body embedded: The third generation of the Internet of Bodies is embedded technology where technology and the human body are melded together and have a real-time connection to a remote machine.

Forbes noted the development of the Brain Computer Interface (BCI) which merges the brain with an external device for monitoring and controlling in real-time. 'The ultimate goal is to help restore function to individuals with disabilities by using brain signals rather than conventional neuromuscular pathways.' Oh, do fuck off. The goal of brain interface technology is controlling human thought and emotion from the central point in a hive mind serving its masters wishes. Many people are now agreeing to be chipped to open doors without a key. You can recognise them because they'll be wearing a mask, social distancing and lining up for the 'vaccine'. The Cult plans a Great Reset money system after they have completed the demolition of the global economy in which 'money' will be exchanged through communication with body operating systems. Rand Corporation, a Cult-owned think tank, said of the Internet of Bodies or IoB:

Internet of Bodies technologies fall under the broader IoT umbrella. But as the name suggests, IoB devices introduce an even more intimate interplay between humans and gadgets. IoB devices monitor the human body, collect health metrics and other personal information, and transmit those data over the Internet. Many devices, such as fitness trackers, are already in use … IoB devices … and those in development can track, record, and store users' whereabouts, bodily functions, and what they see, hear, and even think.

Schwab's World Economic Forum, a long-winded way of saying 'fascism' or 'the Cult', has gone full-on with the Internet of Bodies in the 'Covid' era. 'We're entering the era of the Internet of Bodies', it declared, 'collecting our physical data via a range of devices that can be implanted, swallowed or worn'. The result would be a huge amount of health-related data that could improve human wellbeing around the world, and prove crucial in fighting the 'Covid-19 pandemic'. Does anyone think these clowns care about 'human wellbeing' after the death and devastation their pandemic hoax has purposely caused? Schwab and co say we should move forward with the Internet of Bodies because 'Keeping track of symptoms could help us stop the spread of infection, and quickly detect new cases'. How wonderful, but keeping track' is all they are really bothered about. Researchers were investigating if data gathered from smartwatches and similar devices could be used as viral infection alerts by tracking the user's heart rate and breathing. Schwab said in his 2018 book *Shaping the Future of the Fourth Industrial Revolution*:

The lines between technologies and beings are becoming blurred and not just by the ability to create lifelike robots or synthetics. Instead it is about the ability of new technologies to literally become part of us. Technologies already influence how we understand ourselves, how we think about each other, and how we determine our realities. As the technologies … give us deeper access to parts of ourselves, we may begin to integrate digital technologies into our bodies.

You can see what the game is. Twenty-four hour control and people – if you could still call them that – would never know when something would go ping and take them out of circulation. It's the most obvious rush to a global fascist dictatorship and the complete submission of humanity and yet still so many are locked away in their Cult-induced perceptual coma and can't see it.

Smart Grid control centres

The human body is being transformed by the 'vaccines' and in other ways into a synthetic cyborg that can be attached to the global Smart Grid which would be controlled from a central point and other sub-locations of Grid manipulation. Where are these planned to be? Well, China for a start which is one of the Cult's biggest centres of operation. The technological control system and technocratic rule was incubated here to be unleashed across the world after the 'Covid' hoax came out of China in 2020. Another Smart Grid location that will surprise people new to this is Israel. I have exposed in *The Trigger* how Sabbatian technocrats, intelligence and military operatives were behind the horrors of 9/11 and not `19 Arab hijackers' who somehow manifested the ability to pilot big passenger airliners when instructors at puddle-jumping flying schools described some of them as a joke. The 9/11 attacks were made possible through control of civilian and military air computer systems and those of the White House, Pentagon and connected agencies. See *The Trigger* – it will blow your mind. The controlling and coordinating force were the Sabbatian networks in Israel and the United States which by then had infiltrated the entire US government, military and intelligence system. The real name of the American Deep State is 'Sabbatian State'. Israel is a tiny country of only nine million people, but it is one of the global centres of cyber operations and fast catching Silicon Valley in importance to the Cult. Israel is known as the 'start-up nation' for all the cyber companies spawned there with the Sabbatian specialisation of 'cyber security' that I mentioned earlier which

gives those companies access to computer systems of their clients in real time through 'backdoors' written into the coding when security software is downloaded. The Sabbatian centre of cyber operations outside Silicon Valley is the Israeli military Cyber Intelligence Unit, the biggest infrastructure project in Israel's history, headquartered in the desert-city of Beersheba and involving some 20,000 'cyber soldiers'. Here are located a literal army of Internet trolls scanning social media, forums and comment lists for anyone challenging the Cult agenda. The UK military has something similar with its 77th Brigade and associated operations. The Beersheba complex includes research and development centres for other Cult operations such as Intel, Microsoft, IBM, Google, Apple, Hewlett-Packard, Cisco Systems, Facebook and Motorola. Techcrunch.com ran an article about the Beersheba global Internet technology centre headlined 'Israel's desert city of Beersheba is turning into a cybertech oasis':

The military's massive relocation of its prestigious technology units, the presence of multinational and local companies, a close proximity to Ben Gurion University and generous government subsidies are turning Beersheba into a major global cybertech hub. Beersheba has all of the ingredients of a vibrant security technology ecosystem, including Ben Gurion University with its graduate program in cybersecurity and Cyber Security Research Center, and the presence of companies such as EMC, Deutsche Telekom, PayPal, Oracle, IBM, and Lockheed Martin. It's also the future home of the INCB (Israeli National Cyber Bureau); offers a special income tax incentive for cyber security companies, and was the site for the relocation of the army's intelligence corps units.

Sabbatians have taken over the cyber world through the following process: They scan the schools for likely cyber talent and develop them at Ben Gurion University and their period of conscription in the Israeli Defense Forces when they are stationed at the Beersheba complex. When the cyber talented officially leave the army they are funded to start cyber companies with technology developed by themselves or given to them by the state. Much of this is stolen through backdoors of computer systems around the world with America top of the list. Others are sent off to Silicon Valley to start companies or join the major ones and so we have many major positions filled by apparently 'Jewish' but really Sabbatian operatives. Google, YouTube and Facebook are all run by 'Jewish' CEOs while Twitter is all but run by ultra-Zionist hedge-fund shark Paul Singer. At the centre of the Sabbatian global cyber web is the Israeli army's Unit 8200 which specialises in hacking into computer systems of other countries,

inserting viruses, gathering information, instigating malfunction, and even taking control of them from a distance. A long list of Sabbatians involved with 9/11, Silicon Valley and Israeli cyber security companies are operatives of Unit 8200. This is not about Israel. It's about the Cult. Israel is planned to be a Smart Grid hub as with China and what is happening at Beersheba is not for the benefit of Jewish people who are treated disgustingly by the Sabbatian elite that control the country. A glance at the Nuremberg Codes will tell you that.

The story is much bigger than 'Covid', important as that is to where we are being taken. Now, though, it's time to really strap in. There's more … much more …

CHAPTER ELEVEN

Who controls the Cult?

Awake, arise or be forever fall'n
John Milton, *Paradise Lost*

I have exposed this far the level of the Cult conspiracy that operates in the world of the seen and within the global secret society and satanic network which operates in the shadows one step back from the seen. The story, however, goes much deeper than that.

The 'Covid' hoax is major part of the Cult agenda, but only part, and to grasp the biggest picture we have to expand our attention beyond the realm of human sight and into the infinity of possibility that we cannot see. It is from here, ultimately, that humanity is being manipulated into a state of total control by the force which dictates the actions of the Cult. How much of reality can we see? Next to damn all is the answer. We may appear to see all there is to see in the 'space' our eyes survey and observe, but little could be further from the truth. The human 'world' is only a tiny band of frequency that the body's visual and perceptual systems can decode into *perception* of a 'world'. According to mainstream science the electromagnetic spectrum is 0.005 percent of what exists in the Universe (Fig 10). The maximum estimate I have seen is 0.5 percent and either way it's miniscule. I say it is far, far, smaller even than 0.005 percent when you compare reality we see with the totality of reality that we don't. Now get this if you are new to such information: Visible light, the only band of frequency that we can see, is a *fraction* of the 0.005 percent (Fig 11 overleaf). Take this further and realise that our universe is one of infinite

universes and that universes are only a fragment of overall reality – *infinite* reality. Then compare that with the almost infinitesimal frequency band of visible light or human sight. You see that humans are as near blind as it is possible to be without actually being so. Artist and filmmaker, Sergio Toporek, said:

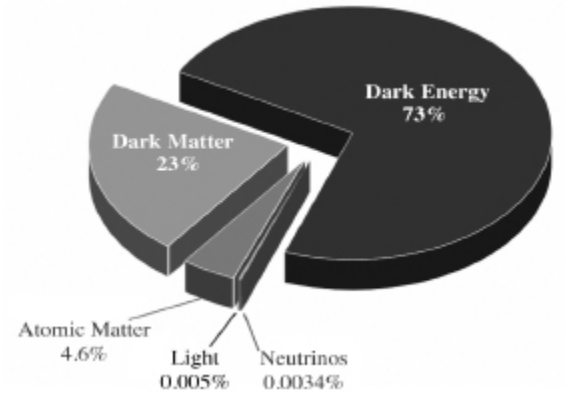

Figure 10: Humans can perceive such a tiny band of visual reality it's laughable.

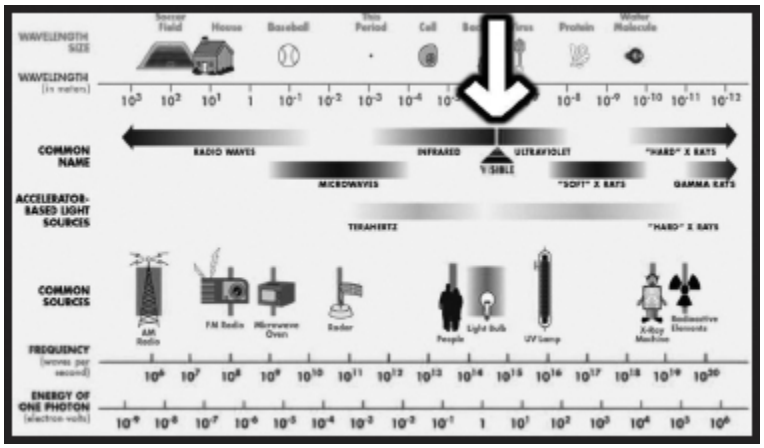

Figure 11: We can see a smear of the 0.005 percent electromagnetic spectrum, but we still know it all. Yep, makes sense.

Consider that you can see less than 1% of the electromagnetic spectrum and hear less than 1% of the acoustic spectrum. 90% of the cells in your body carry their own microbial DNA and are not 'you'. The atoms in your body are 99.9999999999999999% empty space and none of them are the ones you were born with ... Human beings have 46 chromosomes, two less than a potato.

The existence of the rainbow depends on the conical photoreceptors in your eyes; to animals without cones, the rainbow does not exist. So you don't just look at a rainbow, you create it. This is pretty amazing, especially considering that all the beautiful colours you see represent less than 1% of the electromagnetic spectrum.

Suddenly the ‘world’ of humans looks a very different place. Take into account, too, that Planet Earth when compared with the projected size of this single universe is the equivalent of a billionth of a pinhead. Imagine the ratio that would be when compared to infinite reality. To think that Christianity once insisted that Earth and humanity were the centre of everything. This background is vital if we are going to appreciate the nature of ‘human’ and how we can be manipulated by an unseen force. To human visual reality virtually *everything* is unseen and yet the prevailing perception within the institutions and so much of the public is that if we can’t see it, touch it, hear it, taste it and smell it then it cannot exist. Such perception is indoctrinated and encouraged by the Cult and its agents because it isolates believers in the strictly limited, village-idiot, realm of the five senses where perceptions can be firewalled and information controlled. Most of those perpetuating the ‘this-world-is-all-there-is’ insanity are themselves indoctrinated into believing the same delusion. While major players and influencers know that official reality is laughable most of those in science, academia and medicine really believe the nonsense they peddle and teach succeeding generations. Those who challenge the orthodoxy are dismissed as nutters and freaks to protect the manufactured illusion from exposure. Observe the dynamic of the ‘Covid’ hoax and you will see how that takes the same form. The inner-circle psychopaths knows it’s a gigantic scam, but almost the entirety of those imposing their fascist rules believe that ‘Covid’ is all that they’re told it is.

Stolen identity

Ask people who they are and they will give you their name, place of birth, location, job, family background and life story. Yet that is not who they are – it is what they are *experiencing*. The difference is *absolutely crucial*. The true ‘I’, the eternal, infinite ‘I’, is consciousness, a state of being aware. Forget ‘form’. That is a vehicle for a brief experience. Consciousness does not come *from* the brain, but *through* the brain and even that is more symbolic than literal. We are awareness, pure awareness, and this is what withdraws from the body at what we call ‘death’ to continue our eternal beingness, *isness*, in other realms of reality within the limitlessness of infinity or the Biblical ‘many mansions in my father’s house’. Labels of a human life, man, woman, transgender, black, white, brown, nationality,

circumstances and income are not who we are. They are what we are – awareness – is *experiencing* in a brief connection with a band of frequency we call 'human'. The labels are not the self; they are, to use the title of one of my books, a *Phantom* Self. I am not David Icke born in Leicester, England, on April 29th, 1952. I am the consciousness *having that experience*. The Cult and its non-human masters seek to convince us through the institutions of 'education', science, medicine, media and government that what we are *experiencing* is who we *are*. It's so easy to control and direct perception locked away in the bewildered illusions of the five senses with no expanded radar. Try, by contrast, doing the same with a humanity aware of its true self and its true power to consciously create its reality and experience. How is it possible to do this? We do it all day every day. If you perceive yourself as 'little me' with no power to impact upon your life and the world then your life experience will reflect that. You will hand the power you don't think you have to authority in all its forms which will use it to control your experience. This, in turn, will appear to confirm your perception of 'little me' in a self-fulfilling feedback loop. But that is what 'little me' really is – a *perception*. We are all 'big-me', infinite me, and the Cult has to make us forget that if its will is to prevail. We are therefore manipulated and pressured into self-identifying with human labels and not the consciousness/awareness *experiencing* those human labels.

The phenomenon of identity politics is a Cult-instigated manipulation technique to sub-divide previous labels into even smaller ones. A United States university employs this list of letters to describe student identity: LGBTTQQFAGPBDSM or lesbian, gay, bisexual, transgender, transsexual, queer, questioning, flexual, asexual, gender-fuck, polyamorous, bondage/discipline, dominance/submission and sadism/masochism. I'm sure other lists are even longer by now as people feel the need to self-identity the 'I' with the minutiae of race and sexual preference. Wokers programmed by the Cult for generations believe this is about 'inclusivity' when it's really the Cult locking them away into smaller and smaller versions of Phantom Self while firewalling them from the influence of their true self, the infinite, eternal 'I'. You may notice that my philosophy which contends that we are all unique points of attention/awareness within the same infinite whole or Oneness is the ultimate non-racism. The very sense of Oneness makes the judgement of people by their body-type, colour or sexuality utterly ridiculous and confirms that racism has no understanding

of reality (including anti-white racism). Yet despite my perception of life Cult agents and fast-asleep Wokers label me racist to discredit my information while they are themselves phenomenally racist and sexist. All they see is race and sexuality and they judge people as good or bad, demons or untouchables, by their race and sexuality. All they see is *Phantom Self* and perceive themselves in terms of Phantom Self. They are pawns and puppets of the Cult agenda to focus attention and self-identity in the five senses and play those identities against each other to divide and rule. Columbia University has introduced segregated graduations in another version of social distancing designed to drive people apart and teach them that different racial and cultural groups have nothing in common with each other. The last thing the Cult wants is unity. Again the pump-primers of this will be Cult operatives in the knowledge of what they are doing, but the rest are just the Phantom Self blind leading the Phantom Self blind. We *do* have something in common – we are all *the same consciousness* having different temporary experiences.

What is this 'human'?

Yes, what *is* 'human'? That is what we are supposed to be, right? I mean 'human'? True, but 'human' is the experience not the 'I'. Break it down to basics and 'human' is the way that information is processed. If we are to experience and interact with this band of frequency we call the 'world' we must have a vehicle that operates within that band of frequency. Our consciousness in its prime form cannot do that; it is way beyond the frequency of the human realm. My consciousness or awareness could not tap these keys and pick up the cup in front of me in the same way that radio station A cannot interact with radio station B when they are on different frequencies. The human body is the means through which we have that interaction. I have long described the body as a biological computer which processes information in a way that allows consciousness to experience this reality. The body is a receiver, transmitter and processor of information in a particular way that we call human. We visually perceive only the world of the five senses in a wakened state – that is the limit of the body's visual decoding system. In truth it's not even visual in the way we experience 'visual reality' as I will come to in a moment. We are 'human' because the body processes the information sources of human into a reality and

behaviour system that we *perceive* as human. Why does an elephant act like an elephant and not like a human or a duck? The elephant's biological computer is a different information field and processes information according to that program into a visual and behaviour type we call an elephant. The same applies to everything in our reality. These body information fields are perpetuated through procreation (like making a copy of a software program). The Cult wants to break that cycle and intervene technologically to transform the human information field into one that will change what we call humanity. If it can change the human information field it will change the way that field processes information and change humanity both 'physically' and psychologically. Hence the *messenger* (information) RNA 'vaccines' and so much more that is targeting human genetics by changing the body's information – *messaging* – construct through food, drink, radiation, toxicity and other means.

Reality that we experience is nothing like reality as it really is in the same way that the reality people experience in virtual reality games is not the reality they are really living in. The game is only a decoded source of information that appears to be a reality. Our world is also an information construct – a *simulation* (more later). In its base form our reality is a wavefield of information much the same in theme as Wi-Fi. The five senses decode wavefield information into electrical information which they communicate to the brain to decode into holographic (illusory 'physical') information. Different parts of the brain specialise in decoding different senses and the information is fused into a reality that appears to be outside of us but is really inside the brain and the genetic structure in general (Fig 12 overleaf). DNA is a receiver-transmitter of information and a vital part of this decoding process and the body's connection to other realities. Change DNA and you change the way we decode and connect with reality – see 'Covid vaccines'. Think of computers decoding Wi-Fi. You have information encoded in a radiation field and the computer decodes that information into a very different form on the screen. You can't see the Wi-Fi until its information is made manifest on the screen and the information on the screen is inside the computer and not outside. I have just described how we decode the 'human world'. All five senses decode the waveform 'Wi-Fi' field into electrical signals and the brain (computer) constructs reality inside the brain and not outside – 'You don't just look at a rainbow, you create it'. Sound is a simple example. We don't hear sound until the

brain decodes it. Waveform sound waves are picked up by the hearing sense and communicated to the brain in an electrical form to be decoded into the sounds that we hear. Everything we hear is inside the brain along with everything we see, feel, smell and taste. Words and language are waveform fields generated by our vocal chords which pass through this process until they are decoded by the brain into words that we hear. Different languages are different frequency fields or sound waves generated by vocal chords. Late British philosopher Alan Watts said:

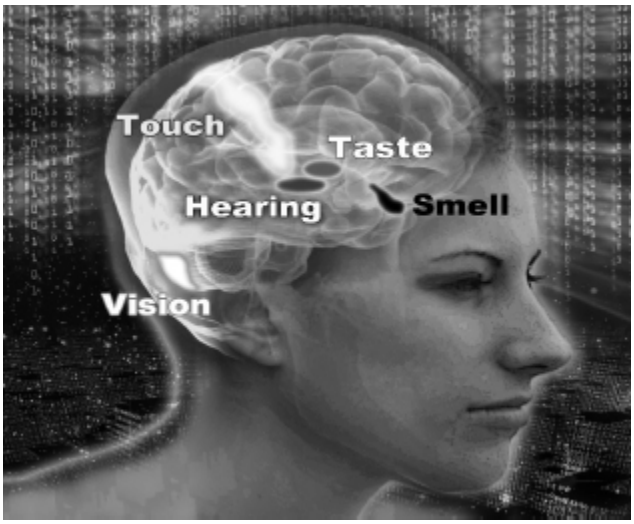

Figure 12: The brain receives information from the five senses and constructs from that our perceived reality.

[Without the brain] the world is devoid of light, heat, weight, solidity, motion, space, time or any other imaginable feature. All these phenomena are interactions, or transactions, of vibrations with a certain arrangement of neurons.

That's exactly what they are and scientist Robert Lanza describes in his book, *Biocentrism*, how we decode electromagnetic waves and energy into visual and 'physical' experience. He uses the example of a flame emitting photons, electromagnetic energy, each pulsing electrically and magnetically:

… these … invisible electromagnetic waves strike a human retina, and if (and only if) the waves happen to measure between 400 and 700 nano meters in length from crest to crest, then their energy is just right to deliver a stimulus to the 8 million cone-shaped cells in the retina.

Each in turn send an electrical pulse to a neighbour neuron, and on up the line this goes, at 250 mph, until it reaches the … occipital lobe of the brain, in the back of the head. There, a cascading complex of neurons fire from the incoming stimuli, and we subjectively perceive this experience as a yellow brightness occurring in a place we have been conditioned to call the 'external world'.

You hear what you decode

If a tree falls or a building collapses they make no noise unless someone is there to decode the energetic waves generated by the disturbance into what we call sound. Does a falling tree make a noise? Only if you hear it – *decode* it. Everything in our reality is a frequency field of information operating within the overall 'Wi-Fi' field that I call The Field. A vibrational disturbance is generated in The Field by the fields of the falling tree or building. These disturbance waves are what we decode into the sound of them falling. If no one is there to do that then neither will make any noise. Reality is created by the observer – *decoder* – and the *perceptions* of the observer affect the decoding process. For this reason different people – different *perceptions* – will perceive the same reality or situation in a different way. What one may perceive as a nightmare another will see as an opportunity. The question of why the Cult is so focused on controlling human perception now answers itself. All experienced reality is the act of decoding and we don't experience Wi-Fi until it is decoded on the computer screen. The sight and sound of an Internet video is encoded in the Wi-Fi all around us, but we don't see or hear it until the computer decodes that information. Taste, smell and touch are all phenomena of the brain as a result of the same process. We don't taste, smell or feel anything except in the brain and there are pain relief techniques that seek to block the signal from the site of discomfort to the brain because if the brain doesn't decode that signal we don't feel pain. Pain is in the brain and only appears to be at the point of impact thanks to the feedback loop between them. We don't see anything until electrical information from the sight senses is decoded in an area at the back of the brain. If that area is damaged we can go blind when our eyes are perfectly okay. So why do we go blind if we damage an eye? We damage the information processing between the waveform visual information and the visual decoding area of the brain. If information doesn't reach the brain in a form it can decode then we can't see the visual reality that it represents. What's more the brain is decoding only a fraction of the

information it receives and the rest is absorbed by the sub-conscious mind. This explanation is from the science magazine, *Wonderpedia*:

Every second, 11 million sensations crackle along these [brain] pathways ... The brain is confronted with an alarming array of images, sounds and smells which it rigorously filters down until it is left with a manageable list of around 40. Thus 40 sensations per second make up what we perceive as reality.

The 'world' is not what people are told to believe that is it and the inner circles of the Cult *know that*.

Illusory 'physical' reality

We can only see a smear of 0.005 percent of the Universe which is only one of a vast array of universes – 'mansions' – within infinite reality. Even then the brain decodes only 40 pieces of information ('sensations') from a potential *11 million* that we receive every second. Two points strike you from this immediately: The sheer breathtaking stupidity of believing we know anything so rigidly that there's nothing more to know; and the potential for these processes to be manipulated by a malevolent force to control the reality of the population. One thing I can say for sure with no risk of contradiction is that when you can perceive an almost indescribable fraction of infinite reality there is always more to know as in tidal waves of it. Ancient Greek philosopher Socrates was so right when he said that wisdom is to know how little we know. How obviously true that is when you think that we are experiencing a physical world of solidity that is neither physical nor solid and a world of apartness when everything is connected. Cult-controlled 'science' dismisses the so-called 'paranormal' and all phenomena related to that when the 'para'-normal is perfectly normal and explains the alleged 'great mysteries' which dumbfound scientific minds. There is a reason for this. A 'scientific mind' in terms of the mainstream is a material mind, a five-sense mind imprisoned in see it, touch it, hear it, smell it and taste it. Phenomena and happenings that can't be explained that way leave the 'scientific mind' bewildered and the rule is that if they can't account for why something is happening then it can't, by definition, be happening. I beg to differ. Telepathy is thought waves passing through The Field (think wave disturbance again) to be decoded by

someone able to connect with that wavelength (information). For example: You can pick up the thought waves of a friend at any distance and at the very least that will bring them to mind. A few minutes later the friend calls you. 'My god', you say, 'that's incredible – I was just thinking of you.' Ah, but *they* were thinking of *you* before they made the call and that's what you decoded. Native peoples not entrapped in five-sense reality do this so well it became known as the 'bush telegraph'. Those known as psychics and mediums (genuine ones) are doing the same only across dimensions of reality. 'Mind over matter' comes from the fact that matter and mind are the *same*. The state of one influences the state of the other. Indeed one *and* the other are illusions. They are aspects of the same field. Paranormal phenomena are all explainable so why are they still considered 'mysteries' or not happening? Once you go down this road of understanding you begin to expand awareness beyond the five senses and that's the nightmare for the Cult.

Figure 13: Holograms are not solid, but the best ones appear to be.

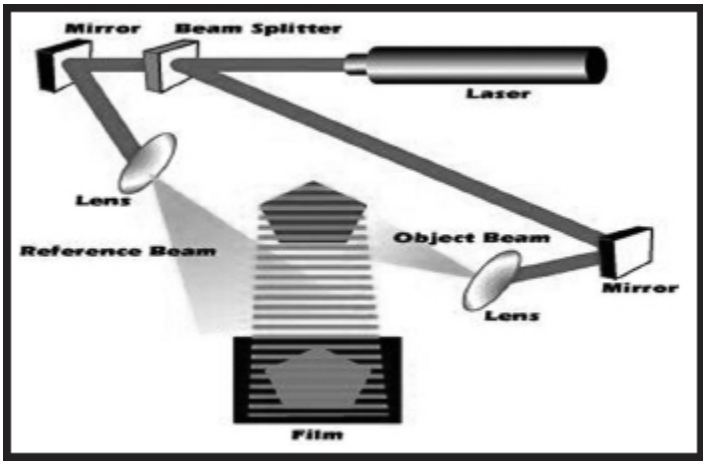

Figure 14: How holograms are created by capturing a waveform version of the subject image.

Holographic 'solidity'

Our reality is not solid, it is holographic. We are now well aware of holograms which are widely used today. Two-dimensional information is decoded into a three-dimensional reality that is not solid although can very much appear to be (Fig 13). Holograms are created with a laser divided into two parts. One goes directly onto a holographic photographic print ('reference beam') and the other takes a waveform image of the subject ('working beam') before being directed onto the print where it 'collides' with the other half of the laser (Fig 14). This creates a *waveform* interference pattern which contains the wavefield information of whatever is being photographed (Fig 15 overleaf). The process can be likened to dropping pebbles in a pond. Waves generated by each one spread out across the water to collide with the others and create a wave representation of where the stones fell and at what speed, weight and distance. A waveform interference pattern of a hologram is akin to the waveform information in The Field which the five senses decode into electrical signals to be decoded by the brain into a holographic illusory 'physical' reality. In the same way when a laser (think human attention) is directed at the waveform interference pattern a three-dimensional version of the subject is projected into apparently 'solid' reality (Fig 16). An amazing trait of holograms reveals more 'paranormal mysteries'. Information of the *whole* hologram is encoded in waveform in every part of the interference pattern by the way they are created. This means that every *part* of a hologram is a smaller version of the whole. Cut the interference wave-pattern into four and you won't get four parts of the image. You get quarter-sized versions of the *whole* image. The body is a hologram and the same applies. Here we have the basis of acupuncture, reflexology and other forms of healing which identify representations of the whole body in all of the parts, hands, feet, ears, everywhere. Skilled palm readers can do what they do because the information of whole body is encoded in the hand. The concept of as above, so below, comes from this.

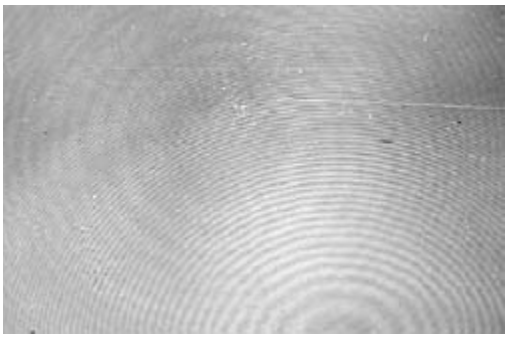

Figure 15: A waveform interference pattern that holds the information that transforms into a hologram.

Figure 16: Holographic people including 'Elvis' holographically inserted to sing a duet with Celine Dion.

The question will be asked of why, if solidity is illusory, we can't just walk through walls and each other. The resistance is not solid against solid; it is electromagnetic field against electromagnetic field and we decode this into the *experience* of solid against solid. We should also not underestimate the power of belief to dictate reality. What you believe is impossible *will be*. Your belief impacts on your decoding processes and they won't decode what you think is impossible. What we believe we perceive and what we perceive we experience. 'Can't dos' and 'impossibles' are like a firewall in a computer system that won't put on the screen what the firewall blocks. How vital that is to understanding how human experience has been hijacked. I explain in *The Answer*, *Everything You Need To Know But Have Never Been Told* and other books a long list of 'mysteries' and 'paranormal' phenomena that are not mysterious and perfectly normal once you realise

what reality is and how it works. ‘Ghosts’ can be seen to pass through ‘solid’ walls because the walls are not solid and the ghost is a discarnate entity operating on a frequency so different to that of the wall that it’s like two radio stations sharing the same space while never interfering with each other. I have seen ghosts do this myself. The apartness of people and objects is also an illusion. Everything is connected by the Field like all sea life is connected by the sea. It’s just that within the limits of our visual reality we only ‘see’ holographic information and not the field of information that connects everything and from which the holographic world is made manifest. If you can only see holographic ‘objects’ and not the field that connects them they will appear to you as unconnected to each other in the same way that we see the computer while not seeing the Wi-Fi.

What you don’t know *can* hurt you

Okay, we return to those ‘two worlds’ of human society and the Cult with its global network of interconnecting secret societies and satanic groups which manipulate through governments, corporations, media, religions, etc. The fundamental difference between them is *knowledge*. The idea has been to keep humanity ignorant of the plan for its total enslavement underpinned by a crucial ignorance of reality – who we are and where we are – and how we interact with it. ‘Human’ should be the interaction between our expanded eternal consciousness and the five-sense body experience. We are meant to be *in* this world in terms of the five senses but not *of* this world in relation to our greater consciousness and perspective. In that state we experience the small picture of the five senses within the wider context of the big picture of awareness beyond the five senses. Put another way the five senses see the dots and expanded awareness connects them into pictures and patterns that give context to the apparently random and unconnected. Without the context of expanded awareness the five senses see only apartness and randomness with apparently no meaning. The Cult and its other-dimensional controllers seek to intervene in the frequency realm where five-sense reality is supposed to connect with expanded reality and to keep the two apart (more on this in the final chapter). When that happens five-sense mental and emotional processes are no longer influenced by expanded awareness, or the True ‘I’, and instead are driven by the isolated perceptions of the body’s decoding systems. They are in the

world *and* of it. Here we have the human plight and why humanity with its potential for infinite awareness can be so easily manipulatable and descend into such extremes of stupidity.

Once the Cult isolates five-sense mind from expanded awareness it can then program the mind with perceptions and beliefs by controlling information that the mind receives through the 'education' system of the formative years and the media perceptual bombardment and censorship of an entire lifetime. Limit perception and a sense of the possible through limiting knowledge by limiting and skewing information while censoring and discrediting that which could set people free. As the title of another of my books says ... *And The Truth Shall Set You Free*. For this reason the last thing the Cult wants in circulation is the truth about anything – especially the reality of the eternal 'I' – and that's why it is desperate to control information. The Cult knows that information becomes perception which becomes behaviour which, collectively, becomes human society. Cult-controlled and funded mainstream 'science' denies the existence of an eternal 'I' and seeks to dismiss and trash all evidence to the contrary. Cult-controlled mainstream religion has a version of 'God' that is little more than a system of control and dictatorship that employs threats of damnation in an afterlife to control perceptions and behaviour in the here and now through fear and guilt. Neither is true and it's the 'neither' that the Cult wishes to suppress. This 'neither' is that everything is an expression, a point of attention, within an infinite state of consciousness which is the real meaning of the term 'God'.

Perceptual obsession with the 'physical body' and five-senses means that 'God' becomes personified as a bearded bloke sitting among the clouds or a raging bully who loves us if we do what 'he' wants and condemns us to the fires of hell if we don't. These are no more than a 'spiritual' fairy tales to control and dictate events and behaviour through fear of this 'God' which has bizarrely made 'God-fearing' in religious circles a state to be desired. I would suggest that fearing *anything* is not to be encouraged and celebrated, but rather deleted. You can see why 'God fearing' is so beneficial to the Cult and its religions when *they* decide what 'God' wants and what 'God' demands (the Cult demands) that everyone do. As the great American comedian Bill Hicks said satirising a Christian zealot: 'I think what God meant to say.' How much of this infinite awareness ('God') that we access is decided by how far we choose to expand our perceptions, self-identity

and sense of the possible. The scale of self-identity reflects itself in the scale of awareness that we can connect with and are influenced by – how much knowing and insight we have instead of programmed perception. You cannot expand your awareness into the infinity of possibility when you believe that you are little me Peter the postman or Mary in marketing and nothing more. I'll deal with this in the concluding chapter because it's crucial to how we turnaround current events.

Where the Cult came from

When I realised in the early 1990s there was a Cult network behind global events I asked the obvious question: When did it start? I took it back to ancient Rome and Egypt and on to Babylon and Sumer in Mesopotamia, the 'Land Between Two Rivers', in what we now call Iraq. The two rivers are the Tigris and Euphrates and this region is of immense historical and other importance to the Cult, as is the land called Israel only 550 miles away by air. There is much more going with deep esoteric meaning across this whole region. It's not only about 'wars for oil'. Priceless artefacts from Mesopotamia were stolen or destroyed after the American and British invasion of Iraq in 2003 justified by the lies of Boy Bush and Tony Blair (their Cult masters) about non-existent 'weapons of mass destruction'. Mesopotamia was the location of Sumer (about 5,400BC to 1,750BC), and Babylon (about 2,350BC to 539BC). Sabbatians may have become immensely influential in the Cult in modern times but they are part of a network that goes back into the mists of history. Sumer is said by historians to be the 'cradle of civilisation'. I disagree. I say it was the re-start of what we call human civilisation after cataclysmic events symbolised in part as the 'Great Flood' destroyed the world that existed before. These fantastic upheavals that I have been describing in detail in the books since the early1990s appear in accounts and legends of ancient cultures across the world and they are supported by geological and biological evidence. Stone tablets found in Iraq detailing the Sumer period say the cataclysms were caused by non-human 'gods' they call the Anunnaki. These are described in terms of extraterrestrial visitations in which knowledge supplied by the Anunnaki is said to have been the source of at least one of the world's oldest writing systems and developments in astronomy, mathematics and architecture that were way ahead of their time. I have covered this subject at

length in *The Biggest Secret* and *Children of the Matrix* and the same basic 'Anunnaki' story can be found in Zulu accounts in South Africa where the late and very great Zulu high shaman Credo Mutwa told me that the Sumerian Anunnaki were known by Zulus as the Chitauri or 'children of the serpent'. See my six-hour video interview with Credo on this subject entitled *The Reptilian Agenda* recorded at his then home near Johannesburg in 1999 which you can watch on the Ickonic media platform.

The Cult emerged out of Sumer, Babylon and Egypt (and elsewhere) and established the Roman Empire before expanding with the Romans into northern Europe from where many empires were savagely imposed in the form of Cult-controlled societies all over the world. Mass death and destruction was their calling card. The Cult established its centre of operations in Europe and European Empires were Cult empires which allowed it to expand into a global force. Spanish and Portuguese colonialists headed for Central and South America while the British and French targeted North America. Africa was colonised by Britain, France, Belgium, the Netherlands, Portugal, Spain, Italy, and Germany. Some like Britain and France moved in on the Middle East. The British Empire was by far the biggest for a simple reason. By now Britain was the headquarters of the Cult from which it expanded to form Canada, the United States, Australia and New Zealand. The Sun never set on the British Empire such was the scale of its occupation. London remains a global centre for the Cult along with Rome and the Vatican although others have emerged in Israel and China. It is no accident that the 'virus' is alleged to have come out of China while Italy was chosen as the means to terrify the Western population into compliance with 'Covid' fascism. Nor that Israel has led the world in 'Covid' fascism and mass 'vaccination'.

You would think that I would mention the United States here, but while it has been an important means of imposing the Cult's will it is less significant than would appear and is currently in the process of having what power it does have deleted. The Cult in Europe has mostly loaded the guns for the US to fire. America has been controlled from Europe from the start through Cult operatives in Britain and Europe. The American Revolution was an illusion to make it appear that America was governing itself while very different forces were pulling the strings in the form of Cult families such as the Rothschilds through the Rockefellers and other subordinates. The Rockefellers are extremely close to Bill Gates and established both scalpel

and drug ‘medicine’ and the World Health Organization. They play a major role in the development and circulation of vaccines through the Rockefeller Foundation on which Bill Gates said his Foundation is based. Why wouldn’t this be the case when the Rockefellers and Gates are on the same team? Cult infiltration of human society goes way back into what we call history and has been constantly expanding and centralising power with the goal of establishing a global structure to dictate everything. Look how this has been advanced in great leaps with the ‘Covid’ hoax.

The non-human dimension

I researched and observed the comings and goings of Cult operatives through the centuries and even thousands of years as they were born, worked to promote the agenda within the secret society and satanic networks, and then died for others to replace them. Clearly there had to be a coordinating force that spanned this entire period while operatives who would not have seen the end goal in their lifetimes came and went advancing the plan over millennia. I went in search of that coordinating force with the usual support from the extraordinary synchronicity of my life which has been an almost daily experience since 1990. I saw common themes in religious texts and ancient cultures about a non-human force manipulating human society from the hidden. Christianity calls this force Satan, the Devil and demons; Islam refers to the Jinn or Djinn; Zulus have their Chitauri (spelt in other ways in different parts of Africa); and the Gnostic people in Egypt in the period around and before 400AD referred to this phenomena as the ‘Archons’, a word meaning rulers in Greek. Central American cultures speak of the ‘Predators’ among other names and the same theme is everywhere. I will use ‘Archons’ as a collective name for all of them. When you see how their nature and behaviour is described all these different sources are clearly talking about the same force. Gnostics described the Archons in terms of ‘luminous fire’ while Islam relates the Jinn to ‘smokeless fire’. Some refer to beings in form that could occasionally be seen, but the most common of common theme is that they operate from unseen realms which means almost all existence to the visual processes of humans. I had concluded that this was indeed the foundation of human control and that the Cult was operating within the human frequency

band on behalf of this hidden force when I came across the writings of Gnostics which supported my conclusions in the most extraordinary way.

A sealed earthen jar was found in 1945 near the town of Nag Hammadi about 75-80 miles north of Luxor on the banks of the River Nile in Egypt. Inside was a treasure trove of manuscripts and texts left by the Gnostic people some 1,600 years earlier. They included 13 leather-bound papyrus codices (manuscripts) and more than 50 texts written in Coptic Egyptian estimated to have been hidden in the jar in the period of 400AD although the source of the information goes back much further. Gnostics oversaw the Great or Royal Library of Alexandria, the fantastic depository of ancient texts detailing advanced knowledge and accounts of human history. The Library was dismantled and destroyed in stages over a long period with the death-blow delivered by the Cult-established Roman Church in the period around 415AD. The Church of Rome was the Church of Babylon relocated as I said earlier. Gnostics were not a race. They were a way of perceiving reality. Whenever they established themselves and their information circulated the terrorists of the Church of Rome would target them for destruction. This happened with the Great Library and with the Gnostic Cathars who were burned to death by the psychopaths after a long period of oppression at the siege of the Castle of Monségur in southern France in 1244. The Church has always been terrified of Gnostic information which demolishes the official Christian narrative although there is much in the Bible that supports the Gnostic view if you read it in another way. To anyone studying the texts of what became known as the Nag Hammadi Library it is clear that great swathes of Christian and Biblical belief has its origin with Gnostics sources going back to Sumer. Gnostic themes have been twisted to manipulate the perceived reality of Bible believers. Biblical texts have been in the open for centuries where they could be changed while Gnostic documents found at Nag Hammadi were sealed away and untouched for 1,600 years. What you see is what they wrote.

Use your *pneuma* not your *nous*

Gnosticism and Gnostic come from ‘gnosis’ which means knowledge, or rather *secret* knowledge, in the sense of spiritual awareness – knowledge about reality and life itself. The desperation of the Cult’s Church of Rome to destroy the Gnostics can be understood when the knowledge they were

circulating was the last thing the Cult wanted the population to know. Sixteen hundred years later the same Cult is working hard to undermine and silence me for the same reason. The dynamic between knowledge and ignorance is a constant. 'Time' appears to move on, but essential themes remain the same. We are told to 'use your nous', a Gnostic word for head/brain/intelligence. They said, however, that spiritual awakening or 'salvation' could only be secured by expanding awareness *beyond* what they called *nous* and into *pneuma* or Infinite Self. Obviously as I read these texts the parallels with what I have been saying since 1990 were fascinating to me. There is a universal truth that spans human history and in that case why wouldn't we be talking the same language 16 centuries apart? When you free yourself from the perception program of the five senses and explore expanded realms of consciousness you are going to connect with the same information no matter what the perceived 'era' within a manufactured timeline of a single and tiny range of manipulated frequency. Humans working with 'smart' technology or knocking rocks together in caves is only a timeline appearing to operate within the human frequency band. Expanded awareness and the knowledge it holds have always been there whether the era be Stone Age or computer age. We can only access that knowledge by opening ourselves to its frequency which the five-sense prison cell is designed to stop us doing. Gates, Fauci, Whitty, Vallance, Zuckerberg, Brin, Page, Wojcicki, Bezos, and all the others behind the 'Covid' hoax clearly have a long wait before their range of frequency can make that connection given that an open heart is crucial to that as we shall see. Instead of accessing knowledge directly through expanded awareness it is given to Cult operatives by the secret society networks of the Cult where it has been passed on over thousands of years outside the public arena. Expanded realms of consciousness is where great artists, composers and writers find their inspiration and where truth awaits anyone open enough to connect with it. We need to go there fast.

Archon hijack

A fifth of the Nag Hammadi texts describe the existence and manipulation of the Archons led by a 'Chief Archon' they call 'Yaldabaoth', or the 'Demiurge', and this is the Christian 'Devil', 'Satan', 'Lucifer', and his demons. Archons in Biblical symbolism are the 'fallen ones' which are also

referred to as fallen angels after the angels expelled from heaven according to the Abrahamic religions of Judaism, Christianity and Islam. These angels are claimed to tempt humans to 'sin' ongoing and you will see how accurate that symbolism is during the rest of the book. The theme of 'original sin' is related to the 'Fall' when Adam and Eve were 'tempted by the serpent' and fell from a state of innocence and 'obedience' (connection) with God into a state of disobedience (disconnection). The Fall is said to have brought sin into the world and corrupted everything including human nature. Yaldabaoth, the 'Lord Archon', is described by Gnostics as a 'counterfeit spirit', 'The Blind One', 'The Blind God', and 'The Foolish One'. The Jewish name for Yaldabaoth in Talmudic writings is Samael which translates as 'Poison of God', or 'Blindness of God'. You see the parallels. Yaldabaoth in Islamic belief is the Muslim Jinn devil known as Shaytan – Shaytan is Satan as the same themes are found all over the world in every religion and culture. The 'Lord God' of the Old Testament is the 'Lord Archon' of Gnostic manuscripts and that's why he's such a bloodthirsty bastard. Satan is known by Christians as 'the Demon of Demons' and Gnostics called Yaldabaoth the 'Archon of Archons'. Both are known as 'The Deceiver'. We are talking about the same 'bloke' for sure and these common themes using different names, storylines and symbolism tell a common tale of the human plight.

Archons are referred to in Nag Hammadi documents as mind parasites, inverters, guards, gatekeepers, detainers, judges, pitiless ones and deceivers. The 'Covid' hoax alone is a glaring example of all these things. The Biblical 'God' is so different in the Old and New Testaments because they are not describing the same phenomenon. The vindictive, angry, hate-filled, 'God' of the Old Testament, known as Yahweh, is Yaldabaoth who is depicted in Cult-dictated popular culture as the 'Dark Lord', 'Lord of Time', Lord (Darth) Vader and Dormammu, the evil ruler of the 'Dark Dimension' trying to take over the 'Earth Dimension' in the Marvel comic movie, *Dr Strange*. Yaldabaoth is both the Old Testament 'god' and the Biblical 'Satan'. Gnostics referred to Yaldabaoth as the 'Great Architect of the Universe'and the Cult-controlled Freemason network calls their god 'the 'Great Architect of the Universe' (also Grand Architect). The 'Great Architect' Yaldabaoth is symbolised by the Cult as the all-seeing eye at the top of the pyramid on the Great Seal of the United States and the dollar bill. Archon is encoded in *arch*-itect as it is in *arch*-angels and *arch*-bishops. All

religions have the theme of a force for good and force for evil in some sort of spiritual war and there is a reason for that – the theme is true. The Cult and its non-human masters are quite happy for this to circulate. They present themselves as the force for good fighting evil when they are really the force of evil (absence of love). The whole foundation of Cult modus operandi is inversion. They promote themselves as a force for good and anyone challenging them in pursuit of peace, love, fairness, truth and justice is condemned as a satanic force for evil. This has been the game plan throughout history whether the Church of Rome inquisitions of non-believers or 'conspiracy theorists' and 'anti-vaxxers' of today. The technique is the same whatever the timeline era.

Yaldabaoth is revolting (true)

Yaldabaoth and the Archons are said to have revolted against God with Yaldabaoth claiming to *be* God – the *All That Is*. The Old Testament 'God' (Yaldabaoth) demanded to be worshipped as such: ' *I am* the LORD, and there is none else, there is no God beside me' (Isaiah 45:5). I have quoted in other books a man who said he was the unofficial son of the late Baron Philippe de Rothschild of the Mouton-Rothschild wine producing estates in France who died in 1988 and he told me about the Rothschild 'revolt from God'. The man said he was given the name Phillip Eugene de Rothschild and we shared long correspondence many years ago while he was living under another identity. He said that he was conceived through 'occult incest' which (within the Cult) was 'normal and to be admired'. 'Phillip' told me about his experience attending satanic rituals with rich and famous people whom he names and you can see them and the wider background to Cult Satanism in my other books starting with *The Biggest Secret*. Cult rituals are interactions with Archontic 'gods'. 'Phillip' described Baron Philippe de Rothschild as 'a master Satanist and hater of God' and he used the same term 'revolt from God' associated with Yaldabaoth/Satan/Lucifer/the Devil in describing the Sabbatian Rothschild dynasty. 'I played a key role in my family's revolt from God', he said. That role was to infiltrate in classic Sabbatian style the Christian Church, but eventually he escaped the mind-prison to live another life. The Cult has been targeting religion in a plan to make worship of the Archons the global one-world religion. Infiltration of Satanism into modern 'culture',

especially among the young, through music videos, stage shows and other means, is all part of this.

Nag Hammadi texts describe Yaldabaoth and the Archons in their prime form as energy – consciousness – and say they can take form if they choose in the same way that consciousness takes form as a human. Yaldabaoth is called 'formless' and represents a deeply inverted, distorted and chaotic state of consciousness which seeks to attached to humans and turn them into a likeness of itself in an attempt at assimilation. For that to happen it has to manipulate humans into low frequency mental and emotional states that match its own. Archons can certainly appear in human form and this is the origin of the psychopathic personality. The energetic distortion Gnostics called Yaldabaoth *is* psychopathy. When psychopathic Archons take human form that human will be a psychopath as an expression of Yaldabaoth consciousness. Cult psychopaths are Archons in human form. The principle is the same as that portrayed in the 2009 *Avatar* movie when the American military travelled to a fictional Earth-like moon called Pandora in the Alpha Centauri star system to infiltrate a society of blue people, or Na'vi, by hiding within bodies that looked like the Na'vi. Archons posing as humans have a particular hybrid information field, part human, part Archon, (the ancient 'demigods') which processes information in a way that manifests behaviour to match their psychopathic evil, lack of empathy and compassion, and stops them being influenced by the empathy, compassion and love that a fully-human information field is capable of expressing. Cult bloodlines interbreed, be they royalty or dark suits, for this reason and you have their obsession with incest. Interbreeding with full-blown humans would dilute the Archontic energy field that guarantees psychopathy in its representatives in the human realm.

Gnostic writings say the main non-human forms that Archons take are *serpentine* (what I have called for decades 'reptilian' amid unbounded ridicule from the Archontically-programmed) and what Gnostics describe as 'an unborn baby or foetus with grey skin and dark, unmoving eyes'. This is an excellent representation of the ET 'Greys' of UFO folklore which large numbers of people claim to have seen and been abducted by – Zulu shaman Credo Mutwa among them. I agree with those that believe in extraterrestrial or interdimensional visitations today and for thousands of years past. No wonder with their advanced knowledge and technological capability they were perceived and worshipped as gods for technological

and other ‘miracles’ they appeared to perform. Imagine someone arriving in a culture disconnected from the modern world with a smartphone and computer. They would be seen as a ‘god’ capable of ‘miracles’. The Renegade Mind, however, wants to know the source of everything and not only the way that source manifests as human or non-human. In the same way that a Renegade Mind seeks the original source material for the ‘Covid virus’ to see if what is claimed is true. The original source of Archons in form is consciousness – the distorted state of consciousness known to Gnostics as Yaldabaoth.

‘Revolt from God’ is energetic disconnection

Where I am going next will make a lot of sense of religious texts and ancient legends relating to ‘Satan’, Lucifer’ and the ‘gods’. Gnostic descriptions sync perfectly with the themes of my own research over the years in how they describe a consciousness distortion seeking to impose itself on human consciousness. I’ve referred to the core of infinite awareness in previous books as Infinite Awareness in Awareness of Itself. By that I mean a level of awareness that knows that it is all awareness and is aware of all awareness. From here comes the frequency of love in its true sense and balance which is what love is on one level – the balance of all forces into a single whole called Oneness and Isness. The more we disconnect from this state of love that many call ‘God’ the constituent parts of that Oneness start to unravel and express themselves as a part and not a whole. They become individualised as intellect, mind, selfishness, hatred, envy, desire for power over others, and such like. This is not a problem in the greater scheme in that ‘God’, the *All That Is*, can experience all these possibilities through different expressions of itself including humans. What we as expressions of the whole experience the *All That Is* experiences. We are the *All That Is* experiencing itself. As we withdraw from that state of Oneness we disconnect from its influence and things can get very unpleasant and very stupid. Archontic consciousness is at the extreme end of that. It has so disconnected from the influence of Oneness that it has become an inversion of unity and love, an inversion of everything, an inversion of life itself. Evil is appropriately live written backwards. Archontic consciousness is obsessed with death, an inversion of life, and so its manifestations in Satanism are obsessed with death. They use inverted

symbols in their rituals such as the inverted pentagram and cross. Sabbatians as Archontic consciousness incarnate invert Judaism and every other religion and culture they infiltrate. They seek disunity and chaos and they fear unity and harmony as they fear love like garlic to a vampire. As a result the Cult, Archons incarnate, act with such evil, psychopathy and lack of empathy and compassion disconnected as they are from the source of love. How could Bill Gates and the rest of the Archontic psychopaths do what they have to human society in the 'Covid' era with all the death, suffering and destruction involved and have no emotional consequence for the impact on others? Now you know. Why have Zuckerberg, Brin, Page, Wojcicki and company callously censored information warning about the dangers of the 'vaccine' while thousands have been dying and having severe, sometimes life-changing reactions? Now you know. Why have Tedros, Fauci, Whitty, Vallance and their like around the world been using case and death figures they're aware are fraudulent to justify lockdowns and all the deaths and destroyed lives that have come from that? Now you know. Why did Christian Drosten produce and promote a 'testing' protocol that he knew couldn't test for infectious disease which led to a global human catastrophe. Now you know. The Archontic mind doesn't give a shit (Fig 17). I personally think that Gates and major Cult insiders are a form of AI cyborg that the Archons want humans to become.

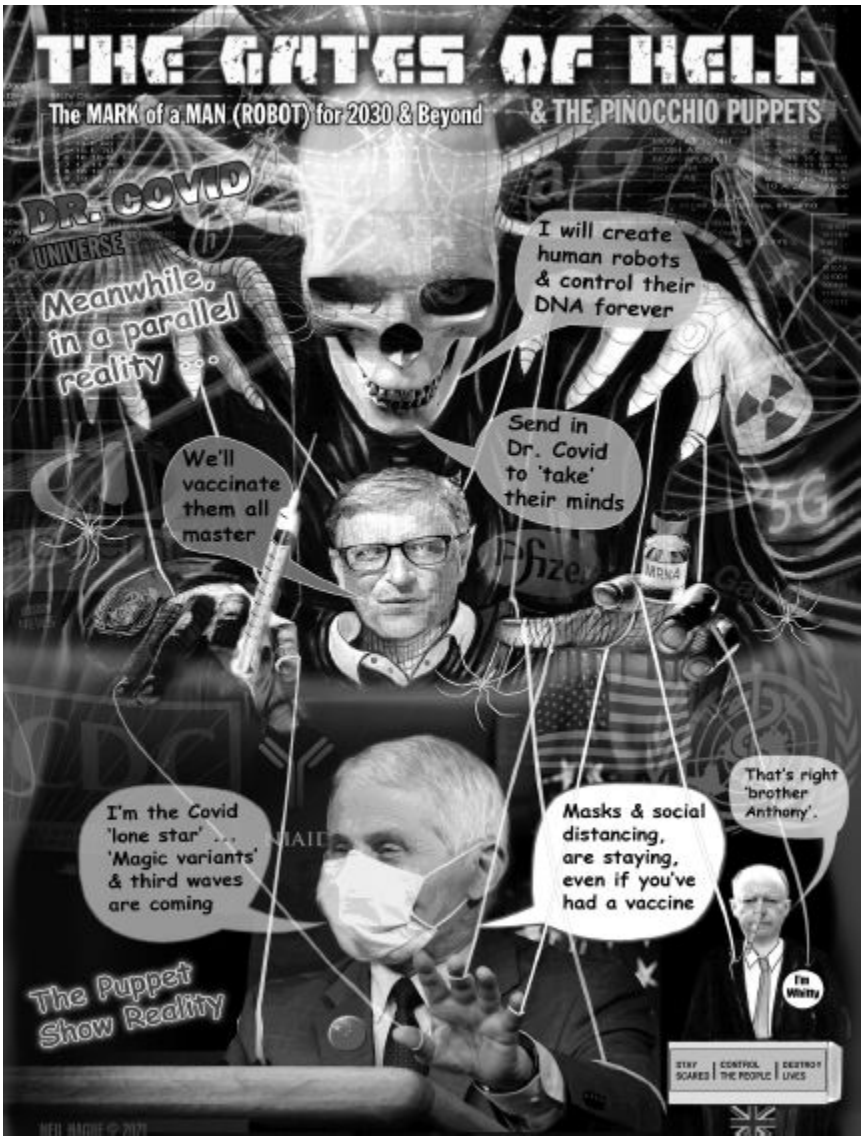

Figure 17: Artist Neil Hague's version of the 'Covid' hierarchy.

Human batteries

A state of such inversion does have its consequences, however. The level of disconnection from the Source of All means that you withdraw from that source of energetic sustenance and creativity. This means that you have to find your own supply of energetic power and it has – *us*. When the Morpheus character in the first *Matrix* movie held up a battery he spoke a profound truth when he said: 'The Matrix is a computer-generated dream world built to keep us under control in order to change the human being into one of these.' The statement was true in all respects. We do live in a

technologically-generated virtual reality simulation (more very shortly) and we have been manipulated to be an energy source for Archontic consciousness. The Disney-Pixar animated movie *Monsters, Inc*. in 2001 symbolised the dynamic when monsters in their world had no energy source and they would enter the human world to terrify children in their beds, catch the child's scream, terror (low-vibrational frequencies), and take that energy back to power the monster world. The lead character you might remember was a single giant eye and the symbolism of the Cult's all-seeing eye was obvious. Every thought and emotion is broadcast as a frequency unique to that thought and emotion. Feelings of love and joy, empathy and compassion, are high, quick, frequencies while fear, depression, anxiety, suffering and hate are low, slow, dense frequencies. Which kind do you think Archontic consciousness can connect with and absorb? In such a low and dense frequency state there's no way it can connect with the energy of love and joy. Archons can only feed off energy compatible with their own frequency and they and their Cult agents want to delete the human world of love and joy and manipulate the transmission of low vibrational frequencies through low-vibrational human mental and emotional states. *We are their energy source*. Wars are energetic banquets to the Archons – a world war even more so – and think how much low-frequency mental and emotional energy has been generated from the consequences for humanity of the 'Covid' hoax orchestrated by Archons incarnate like Gates.

The ancient practice of human sacrifice 'to the gods', continued in secret today by the Cult, is based on the same principle. 'The gods' are Archontic consciousness in different forms and the sacrifice is induced into a state of intense terror to generate the energy the Archontic frequency can absorb. Incarnate Archons in the ritual drink the blood which contains an adrenaline they crave which floods into the bloodstream when people are terrorised. Most of the sacrifices, ancient and modern, are children and the theme of 'sacrificing young virgins to the gods' is just code for children. They have a particular pre-puberty energy that Archons want more than anything and the energy of the young in general is their target. The California Department of Education wants students to chant the names of Aztec gods (Archontic gods) once worshipped in human sacrifice rituals in a curriculum designed to encourage them to 'challenge racist, bigoted, discriminatory, imperialist/colonial beliefs', join 'social movements that struggle for social justice', and 'build new possibilities for a post-racist, post-systemic racism

society'. It's the usual Woke crap that inverts racism and calls it anti-racism. In this case solidarity with 'indigenous tribes' is being used as an excuse to chant the names of 'gods' to which people were sacrificed (and still are in secret). What an example of Woke's inability to see beyond black and white, us and them, They condemn the colonisation of these tribal cultures by Europeans (quite right), but those cultures sacrificing people including children to their 'gods', and mass murdering untold numbers as the Aztecs did, is just fine. One chant is to the Aztec god Tezcatlipoca who had a man sacrificed to him in the 5th month of the Aztec calendar. His heart was cut out and he was eaten. Oh, that's okay then. Come on children … after three … Other sacrificial 'gods' for the young to chant their allegiance include Quetzalcoatl, Huitzilopochtli and Xipe Totec. The curriculum says that 'chants, affirmations, and energizers can be used to bring the class together, build unity around ethnic studies principles and values, and to reinvigorate the class following a lesson that may be emotionally taxing or even when student engagement may appear to be low'. Well, that's the cover story, anyway. Chanting and mantras are the repetition of a particular frequency generated from the vocal cords and chanting the names of these Archontic 'gods' tunes you into their frequency. That is the last thing you want when it allows for energetic synchronisation, attachment and perceptual influence. Initiates chant the names of their 'Gods' in their rituals for this very reason.

Vampires of the Woke

Paedophilia is another way that Archons absorb the energy of children. Paedophiles possessed by Archontic consciousness are used as the conduit during sexual abuse for discarnate Archons to vampire the energy of the young they desire so much. Stupendous numbers of children disappear every year never to be seen again although you would never know from the media. Imagine how much low-vibrational energy has been generated by children during the 'Covid' hoax when so many have become depressed and psychologically destroyed to the point of killing themselves. Shocking numbers of children are now taken by the state from loving parents to be handed to others. I can tell you from long experience of researching this since 1996 that many end up with paedophiles and assets of the Cult through corrupt and Cult-owned social services which in the reframing era has hired many psychopaths and emotionless automatons to do the job.

Children are even stolen to order using spurious reasons to take them by the corrupt and secret (because they're corrupt) 'family courts'. I have written in detail in other books, starting with *The Biggest Secret* in 1997, about the ubiquitous connections between the political, corporate, government, intelligence and military elites (Cult operatives) and Satanism and paedophilia. If you go deep enough both networks have an interlocking leadership. The Woke mentality has been developed by the Cult for many reasons: To promote almost every aspect of its agenda; to hijack the traditional political left and turn it fascist; to divide and rule; and to target agenda pushbackers. But there are other reasons which relate to what I am describing here. How many happy and joyful Wokers do you ever see especially at the extreme end? They are a mental and psychological mess consumed by emotional stress and constantly emotionally cocked for the next explosion of indignation at someone referring to a female as a female. They are walking, talking, batteries as Morpheus might say emitting frequencies which both enslave them in low-vibrational bubbles of perceptual limitation and feed the Archons. Add to this the hatred claimed to be love; fascism claimed to 'anti-fascism', racism claimed to be 'anti-racism'; exclusion claimed to inclusion; and the abuse-filled Internet trolling. You have a purpose-built Archontic energy system with not a wind turbine in sight and all founded on Archontic *inversion*. We have whole generations now manipulated to serve the Archons with their actions and energy. They will be doing so their entire adult lives unless they snap out of their Archon-induced trance. Is it really a surprise that Cult billionaires and corporations put so much money their way? Where is the energy of joy and laughter, including laughing at yourself which is confirmation of your own emotional security? Mark Twain said: 'The human race has one really effective weapon, and that is laughter.' We must use it all the time. Woke has destroyed comedy because it has no humour, no joy, sense of irony, or self-deprecation. Its energy is dense and intense. *Mmmmm*, lunch says the Archontic frequency. Rudolf Steiner (1861-1925) was the Austrian philosopher and famous esoteric thinker who established Waldorf education or Steiner schools to treat children like unique expressions of consciousness and not minds to be programmed with the perceptions determined by authority. I'd been writing about this energy vampiring for decades when I was sent in 2016 a quote by Steiner. He was spot on:

There are beings in the spiritual realms for whom anxiety and fear emanating from human beings offer welcome food. When humans have no anxiety and fear, then these creatures starve. If fear and anxiety radiates from people and they break out in panic, then these creatures find welcome nutrition and they become more and more powerful. These beings are hostile towards humanity. Everything that feeds on negative feelings, on anxiety, fear and superstition, despair or doubt, are in reality hostile forces in super-sensible worlds, launching cruel attacks on human beings, while they are being fed ... These are exactly the feelings that belong to contemporary culture and materialism; because it estranges people from the spiritual world, it is especially suited to evoke hopelessness and fear of the unknown in people, thereby calling up the above mentioned hostile forces against them.

Pause for a moment from this perspective and reflect on what has happened in the world since the start of 2020. Not only will pennies drop, but billion dollar bills. We see the same theme from Don Juan Matus, a Yaqui Indian shaman in Mexico and the information source for Peruvian-born writer, Carlos Castaneda, who wrote a series of books from the 1960s to 1990s. Don Juan described the force manipulating human society and his name for the Archons was the predator:

We have a predator that came from the depths of the cosmos and took over the rule of our lives. Human beings are its prisoners. The predator is our lord and master. It has rendered us docile, helpless. If we want to protest, it suppresses our protest. If we want to act independently, it demands that we don't do so ... indeed we are held prisoner!

They took us over because we are food to them, and they squeeze us mercilessly because we are their sustenance. Just as we rear chickens in coops, the predators rear us in human coops, humaneros. Therefore, their food is always available to them.

Different cultures, different eras, same recurring theme.

The 'ennoia' dilemma

Nag Hammadi Gnostic manuscripts say that Archon consciousness has no 'ennoia'. This is directly translated as 'intentionality', but I'll use the term 'creative imagination'. The *All That Is* in awareness of itself is the source of all creativity – all possibility – and the more disconnected you are from that source the more you are subsequently denied 'creative imagination'. Given that Archon consciousness is almost entirely disconnected it severely lacks creativity and has to rely on far more mechanical processes of thought and exploit the creative potential of those that do have 'ennoia'. You can see cases of this throughout human society. Archon consciousness almost entirely dominates the global banking system and if we study how that

system works you will appreciate what I mean. Banks manifest ‘money’ out of nothing by issuing lines of ‘credit’ which is ‘money’ that has never, does not, and will never exist except in theory. It’s a confidence trick. If you think ‘credit’ figures-on-a-screen ‘money’ is worth anything you accept it as payment. If you don’t then the whole system collapses through lack of confidence in the value of that ‘money’. Archontic bankers with no ‘ennoia’ are ‘lending’ ‘money’ that doesn’t exist to humans that *do* have creativity – those that have the inspired ideas and create businesses and products. Archon banking feeds off human creativity which it controls through ‘money’ creation and debt. Humans have the creativity and Archons exploit that for their own benefit and control while having none themselves. Archon Internet platforms like Facebook claim joint copyright of everything that creative users post and while Archontic minds like Zuckerberg may officially head that company it will be human creatives on the staff that provide the creative inspiration. When you have limitless ‘money’ you can then buy other companies established by creative humans. Witness the acquisition record of Facebook, Google and their like. Survey the Archon-controlled music industry and you see non-creative dark suit executives making their fortune from the human creativity of their artists. The cases are endless. Research the history of people like Gates and Zuckerberg and how their empires were built on exploiting the creativity of others. Archon minds cannot create out of nothing, but they are skilled (because they have to be) in what Gnostic texts call ‘countermimicry’. They can imitate, but not innovate. Sabbatians trawl the creativity of others through backdoors they install in computer systems through their cybersecurity systems. Archon-controlled China is globally infamous for stealing intellectual property and I remember how Hong Kong, now part of China, became notorious for making counterfeit copies of the creativity of others – ‘countermimicry’. With the now pervasive and all-seeing surveillance systems able to infiltrate any computer you can appreciate the potential for Archons to vampire the creativity of humans. Author John Lamb Lash wrote in his book about the Nag Hammadi texts, *Not In His Image*:

Although they cannot originate anything, because they lack the divine factor of ennoia (intentionality), Archons can imitate with a vengeance. Their expertise is simulation (HAL, virtual reality). The Demiurge [Yaldabaoth] fashions a heaven world copied from the fractal patterns [of the

original] ... His construction is celestial kitsch, like the fake Italianate villa of a Mafia don complete with militant angels to guard every portal.

This brings us to something that I have been speaking about since the turn of the millennium. Our reality is a simulation; a virtual reality that we think is real. No, I'm not kidding.

Human reality? Well, virtually

I had pondered for years about whether our reality is 'real' or some kind of construct. I remembered being immensely affected on a visit as a small child in the late 1950s to the then newly-opened Planetarium on the Marylebone Road in London which is now closed and part of the adjacent Madame Tussauds wax museum. It was in the middle of the day, but when the lights went out there was the night sky projected in the Planetarium's domed ceiling and it appeared to be so real. The experience never left me and I didn't know why until around the turn of the millennium when I became certain that our 'night sky' and entire reality is a projection, a virtual reality, akin to the illusory world portrayed in the *Matrix* movies. I looked at the sky one day in this period and it appeared to me like the domed roof of the Planetarium. The release of the first *Matrix* movie in 1999 also provided a synchronistic and perfect visual representation of where my mind had been going for a long time. I hadn't come across the Gnostic Nag Hammadi texts then. When I did years later the correlation was once again astounding. As I read Gnostic accounts from 1,600 years and more earlier it was clear that they were describing the same simulation phenomenon. They tell how the Yaldabaoth 'Demiurge' and Archons created a 'bad copy' of original reality to rule over all that were captured by its illusions and the body was a prison to trap consciousness in the 'bad copy' fake reality. Read how Gnostics describe the 'bad copy' and update that to current times and they are referring to what we would call today a virtual reality simulation.

Author John Lamb Lash said 'the Demiurge fashions a heaven world copied from the fractal patterns' of the original through expertise in 'HAL' or virtual reality simulation. Fractal patterns are part of the energetic information construct of our reality, a sort of blueprint. If these patterns were copied in computer terms it would indeed give you a copy of a

‘natural’ reality in a non-natural frequency and digital form. The principle is the same as making a copy of a website. The original website still exists, but now you can change the copy version to make it whatever you like and it can become very different to the original website. Archons have done this with our reality, a *synthetic* copy of prime reality that still exists beyond the frequency walls of the simulation. Trapped within the illusions of this synthetic Matrix, however, were and are human consciousness and other expressions of prime reality and this is why the Archons via the Cult are seeking to make the human body synthetic and give us synthetic AI minds to complete the job of turning the entire reality synthetic including what we perceive to be the natural world. To quote Kurzweil: ‘Nanobots will infuse all the matter around us with information. Rocks, trees, everything will become these intelligent creatures.’ Yes, *synthetic* ‘creatures’ just as ‘Covid’ and other genetically-manipulating ‘vaccines’ are designed to make the human body synthetic. From this perspective it is obvious why Archons and their Cult are so desperate to infuse synthetic material into every human with their ‘Covid’ scam.

Let there be (electromagnetic) light

Yaldabaoth, the force that created the simulation, or Matrix, makes sense of the Gnostic reference to ‘The Great Architect’ and its use by Cult Freemasonry as the name of its deity. The designer of the Matrix in the movies is called ‘The Architect’ and that trilogy is jam-packed with symbolism relating to these subjects. I have contended for years that the angry Old Testament God (Yaldabaoth) is the ‘God’ being symbolically ‘quoted’ in the opening of Genesis as ‘creating the world’. This is not the creation of prime reality – it’s the creation of the *simulation*. The Genesis ‘God’ says: ‘Let there be Light: and there was light.’ But what is this ‘Light’? I have said for decades that the speed of light (186,000 miles per second) is not the fastest speed possible as claimed by mainstream science and is in fact the frequency walls or outer limits of the Matrix. You can’t have a fastest or slowest anything within all possibility when everything is possible. The human body is encoded to operate within the speed of light or *within the simulation* and thus we see only the tiny frequency band of visible *light*. Near-death experiencers who perceive reality outside the body during temporary ‘death’ describe a very different form of light and this is

supported by the Nag Hammadi texts. Prime reality beyond the simulation ('Upper Aeons' to the Gnostics) is described as a realm of incredible beauty, bliss, love and harmony – a realm of 'watery light' that is so powerful 'there are no shadows'. Our false reality of Archon control, which Gnostics call the 'Lower Aeons', is depicted as a realm with a different kind of 'light' and described in terms of chaos, 'Hell', 'the Abyss' and 'Outer Darkness', where trapped souls are tormented and manipulated by demons (relate that to the 'Covid' hoax alone). The watery light theme can be found in near-death accounts and it is not the same as *simulation* 'light' which is electromagnetic or radiation light within the speed of light – the 'Lower Aeons'. Simulation 'light' is the 'luminous fire' associated by Gnostics with the Archons. The Bible refers to Yaldabaoth as 'that old serpent, called the Devil, and Satan, which deceiveth the whole world' (Revelation 12:9). I think that making a simulated copy of prime reality ('countermimicry') and changing it dramatically while all the time manipulating humanity to believe it to be real could probably meet the criteria of deceiving the whole world. Then we come to the Cult god Lucifer – the *Light Bringer*. Lucifer is symbolic of Yaldabaoth, the bringer of radiation light that forms the bad copy simulation within the speed of light. 'He' is symbolised by the lighted torch held by the Statue of Liberty and in the name 'Illuminati'. Sabbatian-Frankism declares that Lucifer is the true god and Lucifer is the real god of Freemasonry honoured as their 'Great or Grand Architect of the Universe' (simulation).

I would emphasise, too, the way Archontic technologically-generated luminous fire of radiation has deluged our environment since I was a kid in the 1950s and changed the nature of The Field with which we constantly interact. Through that interaction technological radiation is changing us. The Smart Grid is designed to operate with immense levels of communication power with 5G expanding across the world and 6G, 7G, in the process of development. Radiation is the simulation and the Archontic manipulation system. Why wouldn't the Archon Cult wish to unleash radiation upon us to an ever-greater extreme to form Kurzweil's 'cloud'? The plan for a synthetic human is related to the need to cope with levels of radiation beyond even anything we've seen so far. Biological humans would not survive the scale of radiation they have in their script. The Smart Grid is a technological sub-reality within the technological simulation to

further disconnect five-sense perception from expanded consciousness. It's a technological prison of the mind.

Infusing the 'spirit of darkness'

A recurring theme in religion and native cultures is the manipulation of human genetics by a non-human force and most famously recorded as the biblical 'sons of god' (the god*s* plural in the original) who interbred with the daughters of men. The Nag Hammadi *Apocryphon of John* tells the same story this way:

> He [Yaldabaoth] sent his angels [Archons/demons] to the daughters of men, that they might take some of them for themselves and raise offspring for their enjoyment. And at first they did not succeed. When they had no success, they gathered together again and they made a plan together ... And the angels changed themselves in their likeness into the likeness of their mates, filling them with the spirit of darkness, which they had mixed for them, and with evil ... And they took women and begot children out of the darkness according to the likeness of their spirit.

Possession when a discarnate entity takes over a human body is an age-old theme and continues today. It's very real and I've seen it. Satanic and secret society rituals can create an energetic environment in which entities can attach to initiates and I've heard many stories of how people have changed their personality after being initiated even into lower levels of the Freemasons. I have been inside three Freemasonic temples, one at a public open day and two by just walking in when there was no one around to stop me. They were in Ryde, the town where I live, Birmingham, England, when I was with a group, and Boston, Massachusetts. They all felt the same energetically – dark, dense, low-vibrational and sinister. Demonic attachment can happen while the initiate has no idea what is going on. To them it's just a ritual to get in the Masons and do a bit of good business. In the far more extreme rituals of Satanism human possession is even more powerful and they are designed to make possession possible. The hierarchy of the Cult is dictated by the power and perceived status of the possessing Archon. In this way the Archon hierarchy becomes the Cult hierarchy. Once the entity has attached it can influence perception and behaviour and if it attaches to the extreme then so much of its energy (information) infuses into the body information field that the hologram starts to reflect the nature of

the possessing entity. This is the *Exorcist* movie type of possession when facial features change and it's known as shapeshifting. Islam's Jinn are said to be invisible tricksters who change shape, 'whisper', confuse and take human form. These are all traits of the Archons and other versions of the same phenomenon. Extreme possession could certainty infuse the 'spirit of darkness' into a partner during sex as the Nag Hammadi texts appear to describe. Such an infusion can change genetics which is also energetic information. Human genetics is information and the 'spirit of darkness' is information. Mix one with the other and change must happen. Islam has the concept of a 'Jinn baby' through possession of the mother and by Jinn taking human form. There are many ways that human genetics can be changed and remember that Archons have been aware all along of advanced techniques to do this. What is being done in human society today – and far more – was known about by Archons at the time of the 'fallen ones' and their other versions described in religions and cultures.

Archons and their human-world Cult are obsessed with genetics as we see today and they know this dictates how information is processed into perceived reality during a human life. They needed to produce a human form that would decode the simulation and this is symbolically known as 'Adam and Eve' who left the 'garden' (prime reality) and 'fell' into Matrix reality. The simulation is not a 'physical' construct (there is no 'physical'); it is a source of information. Think Wi-Fi again. The simulation is an energetic field encoded with information and body-brain systems are designed to decode that information encoded in wave or frequency form which is transmitted to the brain as electrical signals. These are decoded by the brain to construct our sense of reality – an illusory 'physical' world that only exists in the brain or the mind. Virtual reality games mimic this process using the same sensory decoding system. Information is fed to the senses to decode a virtual reality that can appear so real, but isn't (Figs 18 and 19). Some scientists believe – and I agree with them – that what we perceive as 'physical' reality only exists when we are looking or observing. The act of perception or focus triggers the decoding systems which turn waveform information into holographic reality. When we are not observing something our reality reverts from a holographic state to a waveform state. This relates to the same principle as a falling tree not making a noise unless someone is there to hear it or decode it. The concept makes sense from the simulation perspective. A computer is not decoding all the information in a

Wi-Fi field all the time and only decodes or brings into reality on the screen that part of Wi-Fi that it's decoding – focusing upon – at that moment.

Figure 18: Virtual reality technology 'hacks' into the body's five-sense decoding system.

Figure 19: The result can be experienced as very 'real'.

Interestingly, Professor Donald Hoffman at the Department of Cognitive Sciences at the University of California, Irvine, says that our experienced reality is like a computer interface that shows us only the level with which we interact while hiding all that exists beyond it: 'Evolution shaped us with a user interface that hides the truth. Nothing that we see is the truth – the very language of space and time and objects is the wrong language to describe reality.' He is correct in what he says on so many levels. Space and time are not a universal reality. They are a phenomenon of decoded *simulation* reality as part of the process of enslaving our sense of reality. Near-death experiencers report again and again how space and time did not exist as we perceive them once they were free of the body – body decoding systems. You can appreciate from this why Archons and their Cult are so desperate to entrap human attention in the five senses where we are in the Matrix and of the Matrix. Opening your mind to expanded states of awareness takes you beyond the information confines of the simulation and

you become aware of knowledge and insights denied to you before. This is what we call ‘awakening’ – *awakening from the Matrix* – and in the final chapter I will relate this to current events.

Where are the ‘aliens’?

A simulation would explain the so-called ‘Fermi Paradox’ named after Italian physicist Enrico Fermi (1901-1954) who created the first nuclear reactor. He considered the question of why there is such a lack of extraterrestrial activity when there are so many stars and planets in an apparently vast universe; but what if the night sky that we see, or think we do, is a simulated projection as I say? If you control the simulation and your aim is to hold humanity fast in essential ignorance would you want other forms of life including advanced life coming and going sharing information with humanity? Or would you want them to believe they were isolated and apparently alone? Themes of human isolation and apartness are common whether they be the perception of a lifeless universe or the fascist isolation laws of the ‘Covid’ era. Paradoxically the very existence of a simulation means that we are not alone when some force had to construct it. My view is that experiences that people have reported all over the world for centuries with Reptilians and Grey entities are Archon phenomena as Nag Hammadi texts describe; and that benevolent ‘alien’ interactions are non-human groups that come in and out of the simulation by overcoming Archon attempts to keep them out. It should be highlighted, too, that Reptilians and Greys are obsessed with *genetics* and *technology* as related by cultural accounts and those who say they have been abducted by them. Technology is their way of overcoming some of the limitations in their creative potential and our technology-driven and controlled human society of today is *arch*etypical Archon-Reptilian-Grey modus operandi. Technocracy is really *Archon*tocracy. The Universe does not have to be as big as it appears with a simulation. There is no space or distance only information decoded into holographic reality. What we call ‘space’ is only the absence of holographic ‘objects’ and that ‘space’ is The Field of energetic information which connects everything into a single whole. The same applies with the artificially-generated information field of the simulation. The Universe is not big or small as a physical reality. It is decoded information, that’s all, and its perceived size is decided by the way the simulation is encoded to

make it appear. The entire night sky as we perceive it only exists in our brain and so where are those ‘millions of light years’? The ‘stars’ on the ceiling of the Planetarium looked a vast distance away.

There’s another point to mention about ‘aliens’. I have been highlighting since the 1990s the plan to stage a fake ‘alien invasion’ to justify the centralisation of global power and a world military. Nazi scientist Werner von Braun, who was taken to America by Operation Paperclip after World War Two to help found NASA, told his American assistant Dr Carol Rosin about the Cult agenda when he knew he was dying in 1977. Rosin said that he told her about a sequence that would lead to total human control by a one-world government. This included threats from terrorism, rogue nations, meteors and asteroids before finally an ‘alien invasion’. All of these things, von Braun said, would be bogus and what I would refer to as a No-Problem-Reaction-Solution. Keep this in mind when ‘the aliens are coming’ is the new mantra. The aliens are not coming – they are *already here* and they have infiltrated human society while looking human. French-Canadian investigative journalist Serge Monast said in 1994 that he had uncovered a NASA/military operation called Project Blue Beam which fits with what Werner von Braun predicted. Monast died of a ‘heart attack’ in 1996 the day after he was arrested and spent a night in prison. He was 51. He said Blue Beam was a plan to stage an alien invasion that would include religious figures beamed holographically into the sky as part of a global manipulation to usher in a ‘new age’ of worshipping what I would say is the Cult ‘god’ Yaldabaoth in a one-world religion. Fake holographic asteroids are also said to be part of the plan which again syncs with von Braun. How could you stage an illusory threat from asteroids unless they were holographic inserts? This is pretty straightforward given the advanced technology outside the public arena and the fact that our ‘physical’ reality is holographic anyway. Information fields would be projected and we would decode them into the illusion of a ‘physical’ asteroid. If they can sell a global ‘pandemic’ with a ‘virus’ that doesn’t exist what will humans not believe if government and media tell them?

All this is particularly relevant as I write with the Pentagon planning to release in June, 2021, information about ‘UFO sightings’. I have been following the UFO story since the early 1990s and the common theme throughout has been government and military denials and cover up. More recently, however, the Pentagon has suddenly become more talkative and

apparently open with Air Force pilot radar images released of unexplained craft moving and changing direction at speeds well beyond anything believed possible with human technology. Then, in March, 2021, former Director of National Intelligence John Ratcliffe said a Pentagon report months later in June would reveal a great deal of information about UFO sightings unknown to the public. He said the report would have 'massive implications'. The order to do this was included bizarrely in a $2.3 trillion 'coronavirus' relief and government funding bill passed by the Trump administration at the end of 2020. I would add some serious notes of caution here. I have been pointing out since the 1990s that the US military and intelligence networks have long had craft – 'flying saucers' or anti-gravity craft – which any observer would take to be extraterrestrial in origin. Keeping this knowledge from the public allows craft flown by *humans* to be perceived as alien visitations. I am not saying that 'aliens' do not exist. I would be the last one to say that, but we have to be streetwise here. President Ronald Reagan told the UN General Assembly in 1987: 'I occasionally think how quickly our differences worldwide would vanish if we were facing an alien threat from outside this world.' That's the idea. Unite against a common 'enemy' with a common purpose behind your 'saviour force' (the Cult) as this age-old technique of mass manipulation goes global.

Science moves this way …

I could find only one other person who was discussing the simulation hypothesis publicly when I concluded it was real. This was Nick Bostrom, a Swedish-born philosopher at the University of Oxford, who has explored for many years the possibility that human reality is a computer simulation although his version and mine are not the same. Today the simulation and holographic reality hypothesis have increasingly entered the scientific mainstream. Well, the more open-minded mainstream, that is. Here are a few of the ever-gathering examples. American nuclear physicist Silas Beane led a team of physicists at the University of Bonn in Germany pursuing the question of whether we live in a simulation. They concluded that we probably do and it was likely based on a lattice of cubes. They found that cosmic rays align with that specific pattern. The team highlighted the Greisen–Zatsepin–Kuzmin (GZK) limit which refers to cosmic ray particle

interaction with cosmic background radiation that creates an apparent boundary for cosmic ray particles. They say in a paper entitled 'Constraints on the Universe as a Numerical Simulation' that this 'pattern of constraint' is exactly what you would find with a computer simulation. They also made the point that a simulation would create its own 'laws of physics' that would limit possibility. I've been making the same point for decades that the *perceived* laws of physics relate only to this reality, or what I would later call the simulation. When designers write codes to create computer and virtual reality games they are the equivalent of the laws of physics for that game. Players interact within the limitations laid out by the coding. In the same way those who wrote the codes for the simulation decided the laws of physics that would apply. These can be overridden by expanded states of consciousness, but not by those enslaved in only five-sense awareness where simulation codes rule. Overriding the codes is what people call 'miracles'. They are not. They are bypassing the encoded limits of the simulation. A population caught in simulation perception would have no idea that this was their plight. As the Bonn paper said: 'Like a prisoner in a pitch-black cell we would not be able to see the "walls" of our prison,' That's true if people remain mesmerised by the five senses. Open to expanded awareness and those walls become very clear. The main one is the speed of light.

American theoretical physicist James Gates is another who has explored the simulation question and found considerable evidence to support the idea. Gates was Professor of Physics at the University of Maryland, Director of The Center for String and Particle Theory, and on Barack Obama's Council of Advisors on Science and Technology. He and his team found *computer codes* of digital data embedded in the fabric of our reality. They relate to on-off electrical charges of 1 and 0 in the binary system used by computers. 'We have no idea what they are doing there', Gates said. They found within the energetic fabric mathematical sequences known as error-correcting codes or block codes that 'reboot' data to its original state or 'default settings' when something knocks it out of sync. Gates was asked if he had found a set of equations embedded in our reality indistinguishable from those that drive search engines and browsers and he said: 'That is correct.' Rich Terrile, director of the Centre for Evolutionary Computation and Automated Design at NASA's Jet Propulsion Laboratory, has said publicly that he believes the Universe is a digital hologram that must have

been created by a form of intelligence. I agree with that in every way. Waveform information is delivered electrically by the senses to the brain which constructs a *digital* holographic reality that we call the 'world'. This digital level of reality can be read by the esoteric art of numerology. Digital holograms are at the cutting edge of holographics today. We have digital technology everywhere designed to access and manipulate our digital level of perceived reality. Synthetic mRNA in 'Covid vaccines' has a digital component to manipulate the body's digital 'operating system'.

Reality is numbers

How many know that our reality can be broken down to numbers and codes that are the same as computer games? Max Tegmark, a physicist at the Massachusetts Institute of Technology (MIT), is the author of *Our Mathematical Universe* in which he lays out how reality can be entirely described by numbers and maths in the way that a video game is encoded with the 'physics' of computer games. Our world and computer virtual reality are essentially the same. Tegmark imagines the perceptions of characters in an advanced computer game when the graphics are so good they don't know they are in a game. They think they can bump into real objects (electromagnetic resistance in our reality), fall in love and feel emotions like excitement. When they began to study the apparently 'physical world' of the video game they would realise that everything was made of pixels (which have been found in our energetic reality as must be the case when on one level our world is digital). What computer game characters thought was physical 'stuff', Tegmark said, could actually be broken down into numbers:

> And we're exactly in this situation in our world. We look around and it doesn't seem that mathematical at all, but everything we see is made out of elementary particles like quarks and electrons. And what properties does an electron have? Does it have a smell or a colour or a texture? No! ... We physicists have come up with geeky names for [Electron] properties, like electric charge, or spin, or lepton number, but the electron doesn't care what we call it, the properties are just numbers.

This is the illusory reality Gnostics were describing. This is the simulation. The A, C, G, and T codes of DNA have a binary value – A and

C = 0 while G and T = 1. This has to be when the simulation is digital and the body must be digital to interact with it. Recurring mathematical sequences are encoded throughout reality and the body. They include the Fibonacci sequence in which the two previous numbers are added to get the next one, as in ... 1, 1, 2, 3, 5, 8, 13, 21, 34, 55, etc. The sequence is encoded in the human face and body, proportions of animals, DNA, seed heads, pine cones, trees, shells, spiral galaxies, hurricanes and the number of petals in a flower. The list goes on and on. There are fractal patterns – a 'never-ending pattern that is infinitely complex and self-similar across all scales in the as above, so below, principle of holograms. These and other famous recurring geometrical and mathematical sequences such as Phi, Pi, Golden Mean, Golden Ratio and Golden Section are *computer codes* of the simulation. I had to laugh and give my head a shake the day I finished this book and it went into the production stage. I was sent an article in *Scientific American* published in April, 2021, with the headline 'Confirmed! We Live in a Simulation'. Two decades after I first said our reality is a simulation and the speed of light is it's outer limit the article suggested that we do live in a simulation and that the speed of light is its outer limit. I left school at 15 and never passed a major exam in my life while the writer was up to his eyes in qualifications. As I will explain in the final chapter *knowing* is far better than thinking and they come from very different sources. The article rightly connected the speed of light to the processing speed of the 'Matrix' and said what has been in my books all this time … 'If we are in a simulation, as it appears, then space is an abstract property written in code. It is not real'. No it's not and if we live in a simulation something created it and it wasn't *us*. 'That David Icke says we are manipulated by aliens' – he's crackers.'

Wow …

The reality that humanity thinks is so real is an illusion. Politicians, governments, scientists, doctors, academics, law enforcement, media, school and university curriculums, on and on, are all founded on a world that *does not exist* except as a simulated prison cell. Is it such a stretch to accept that 'Covid' doesn't exist when our entire 'physical' reality doesn't exist? Revealed here is the knowledge kept under raps in the Cult networks of compartmentalised secrecy to control humanity's sense of reality by

inducing the population to believe in a reality that's not real. If it wasn't so tragic in its experiential consequences the whole thing would be hysterically funny. None of this is new to Renegade Minds. Ancient Greek philosopher Plato (about 428 to about 347BC) was a major influence on Gnostic belief and he described the human plight thousands of years ago with his Allegory of the Cave. He told the symbolic story of prisoners living in a cave who had never been outside. They were chained and could only see one wall of the cave while behind them was a fire that they could not see. Figures walked past the fire casting shadows on the prisoners' wall and those moving shadows became their sense of reality. Some prisoners began to study the shadows and were considered experts on them (today's academics and scientists), but what they studied was only an illusion (today's academics and scientists). A prisoner escaped from the cave and saw reality as it really is. When he returned to report this revelation they didn't believe him, called him mad and threatened to kill him if he tried to set them free. Plato's tale is not only a brilliant analogy of the human plight and our illusory reality. It describes, too, the dynamics of the 'Covid' hoax. I have only skimmed the surface of these subjects here. The aim of this book is to crisply connect all essential dots to put what is happening today into its true context. All subject areas and their connections in this chapter are covered in great evidential detail in *Everything You Need To Know, But Have Never Been Told* and *The Answer*.

They say that bewildered people 'can't see the forest for the trees'. Humanity, however, can't see the forest for the *twigs*. The five senses see only twigs while Renegade Minds can see the forest and it's the forest where the answers lie with the connections that reveals. Breaking free of perceptual programming so the forest can be seen is the way we turn all this around. Not breaking free is how humanity got into this mess. The situation may seem hopeless, but I promise you it's not. We are a perceptual heartbeat from paradise if only we knew.

CHAPTER TWELVE

Escaping Wetiko

Life is simply a vacation from the infinite
Dean Cavanagh

Renegade Minds weave the web of life and events and see common themes in the apparently random. They are always there if you look for them and their pursuit is aided by incredible synchronicity that comes when your mind is open rather than mesmerised by what it thinks it can see.

Infinite awareness is infinite possibility and the more of infinite possibility that we access the more becomes infinitely possible. That may be stating the apparently obvious, but it is a devastatingly-powerful fact that can set us free. We are a point of attention within an infinity of consciousness. The question is how much of that infinity do we choose to access? How much knowledge, insight, awareness, wisdom, do we want to connect with and explore? If your focus is only in the five senses you will be influenced by a fraction of infinite awareness. I mean a range so tiny that it gives new meaning to infinitesimal. Limitation of self-identity and a sense of the possible limit accordingly your range of consciousness. We are what we think we are. Life is what we think it is. The dream is the dreamer and the dreamer is the dream. Buddhist philosophy puts it this way: 'As a thing is viewed, so it appears.' Most humans live in the realm of touch, taste, see, hear, and smell and that's the limit of their sense of the possible and sense of self. Many will follow a religion and speak of a God in his heaven, but their lives are still dominated by the five senses in their perceptions and actions. The five senses become the arbiter of everything.

When that happens all except a smear of infinity is sealed away from influence by the rigid, unyielding, reality bubbles that are the five-sense human or Phantom Self. Archon Cult methodology is to isolate consciousness within five-sense reality – the simulation – and then program that consciousness with a sense of self and the world through a deluge of life-long information designed to instil the desired perception that allows global control. Efforts to do this have increased dramatically with identity politics as identity bubbles are squeezed into the minutiae of five-sense detail which disconnect people even more profoundly from the infinite 'I'.

Five-sense focus and self-identity are like a firewall that limits access to the infinite realms. You only perceive one radio or television station and no other. We'll take that literally for a moment. Imagine a vast array of stations giving different information and angles on reality, but you only ever listen to one. Here we have the human plight in which the population is overwhelmingly confined to CultFM. This relates only to the frequency range of CultFM and limits perception and insight to that band – limits *possibility* to that band. It means you are connecting with an almost imperceptibly minuscule range of possibility and creative potential within the infinite Field. It's a world where everything seems apart from everything else and where synchronicity is rare. Synchronicity is defined in the dictionary as 'the happening by chance of two or more related or similar events at the same time'. Use of 'by chance' betrays a complete misunderstanding of reality. Synchronicity is not 'by chance'. As people open their minds, or 'awaken' to use the term, they notice more and more coincidences in their lives, bits of 'luck', apparently miraculous happenings that put them in the right place at the right time with the right people. Days become peppered with 'fancy meeting you here' and 'what are the chances of that?' My entire life has been lived like this and ever more so since my own colossal awakening in 1990 and 91 which transformed my sense of reality. Synchronicity is not 'by chance'; it is by accessing expanded realms of possibility which allow expanded potential for manifestation. People broadcasting the same vibe from the same openness of mind tend to be drawn 'by chance' to each other through what I call frequency magnetism and it's not only people. In the last more than 30 years incredible synchronicity has also led me through the Cult maze to information in so many forms and to crucial personal experiences. These 'coincidences' have allowed me to put the puzzle pieces together across an enormous array of

subjects and situations. Those who have breached the bubble of five-sense reality will know exactly what I mean and this escape from the perceptual prison cell is open to everyone whenever they make that choice. This may appear super-human when compared with the limitations of 'human', but it's really our natural state. 'Human' as currently experienced is consciousness in an unnatural state of induced separation from the infinity of the whole. I'll come to how this transformation into unity can be made when I have described in more detail the force that holds humanity in servitude by denying this access to infinite self.

The Wetiko factor

I have been talking and writing for decades about the way five-sense mind is systematically barricaded from expanded awareness. I have used the analogy of a computer (five-sense mind) and someone at the keyboard (expanded awareness). Interaction between the computer and the operator is symbolic of the interaction between five-sense mind and expanded awareness. The computer directly experiences the Internet and the operator experiences the Internet via the computer which is how it's supposed to be – the two working as one. Archons seek to control that point where the operator connects with the computer to stop that interaction (Fig 20). Now the operator is banging the keyboard and clicking the mouse, but the computer is not responding and this happens when the computer is taken over – *possessed* – by an appropriately-named computer 'virus'. The operator has lost all influence over the computer which goes its own way making decisions under the control of the 'virus'. I have just described the dynamic through which the force known to Gnostics as Yaldabaoth and Archons disconnects five-sense mind from expanded awareness to imprison humanity in perceptual servitude.

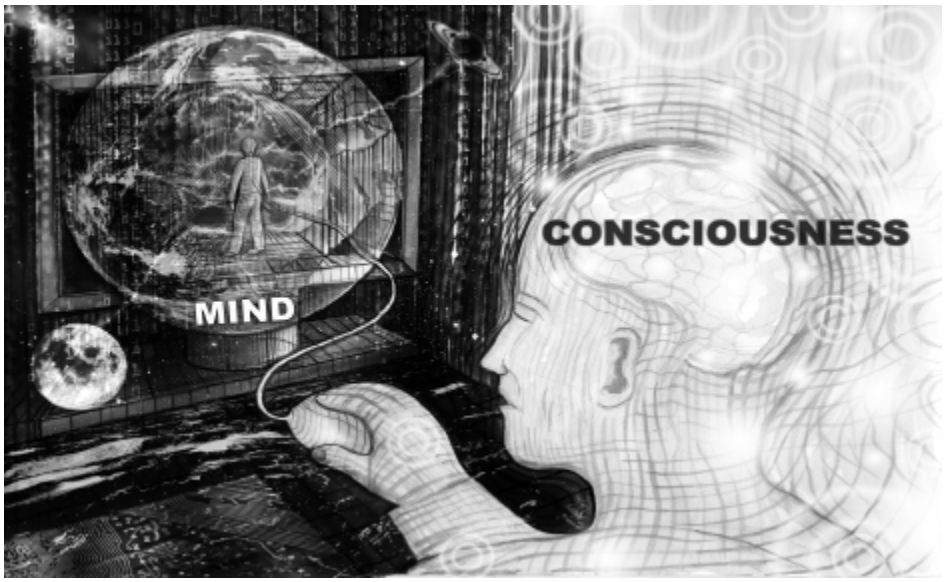

Figure 20: The mind 'virus' I have been writing about for decades seeks to isolate five-sense mind (the computer) from the true 'I'. (Image by Neil Hague).

About a year ago I came across a Native American concept of Wetiko which describes precisely the same phenomenon. Wetiko is the spelling used by the Cree and there are other versions including wintiko and windigo used by other tribal groups. They spell the name with lower case, but I see Wetiko as a proper noun as with Archons and prefer a capital. I first saw an article about Wetiko by writer and researcher Paul Levy which so synced with what I had been writing about the computer/operator disconnection and later the Archons. I then read his book, the fascinating *Dispelling Wetiko, Breaking the Spell of Evil*. The parallels between what I had concluded long before and the Native American concept of Wetiko were so clear and obvious that it was almost funny. For Wetiko see the Gnostic Archons for sure and the Jinn, the Predators, and every other name for a force of evil, inversion and chaos. Wetiko is the Native American name for the force that divides the computer from the operator (Fig 21). Indigenous author Jack D. Forbes, a founder of the Native American movement in the 1960s, wrote another book about Wetiko entitled *Columbus And Other Cannibals – The Wetiko Disease of Exploitation, Imperialism, and Terrorism* which I also read. Forbes says that Wetiko refers to an evil person or spirit 'who terrorizes other creatures by means of terrible acts, including cannibalism'. Zulu shaman Credo Mutwa told me that African accounts tell how cannibalism was brought into the world by

the Chitauri ‘gods’ – another manifestation of Wetiko. The distinction between ‘evil person or spirit’ relates to Archons/Wetiko possessing a human or acting as pure consciousness. Wetiko is said to be a sickness of the soul or spirit and a state of being that takes but gives nothing back – the Cult and its operatives perfectly described. Black Hawk, a Native American war leader defending their lands from confiscation, said European invaders had ‘poisoned hearts’ – Wetiko hearts – and that this would spread to native societies. Mention of the heart is very significant as we shall shortly see. Forbes writes: ‘Tragically, the history of the world for the past 2,000 years is, in great part, the story of the epidemiology of the wetiko disease.’ Yes, and much longer. Forbes is correct when he says: ‘The wetikos destroyed Egypt and Babylon and Athens and Rome and Tenochtitlan [capital of the Aztec empire] and perhaps now they will destroy the entire earth.’ Evil, he said, is the number one export of a Wetiko culture – see its globalisation with ‘Covid’. Constant war, mass murder, suffering of all kinds, child abuse, Satanism, torture and human sacrifice are all expressions of Wetiko and the Wetiko possessed. The world is Wetiko made manifest, *but it doesn’t have to be*. There is a way out of this even now.

Figure 21: The mind ‘virus’ is known to Native Americans as ‘Wetiko’. (Image by Neil Hague).

Cult of Wetiko

Wetiko is the Yaldabaoth frequency distortion that seeks to attach to human consciousness and absorb it into its own. Once this connection is made Wetiko can drive the perceptions of the target which they believe to be coming from their own mind. All the horrors of history and today from mass killers to Satanists, paedophiles like Jeffrey Epstein and other psychopaths, are the embodiment of Wetiko and express its state of being in all its grotesqueness. The Cult is Wetiko incarnate, Yaldabaoth incarnate, and it seeks to facilitate Wetiko assimilation of humanity in totality into its distortion by manipulating the population into low frequency states that match its own. Paul Levy writes: 'Holographically enforced within the psyche of every human being the wetiko virus pervades and underlies the entire field of consciousness, and can therefore potentially manifest through any one of us at any moment if we are not mindful.' The 'Covid' hoax has achieved this with many people, but others have not fallen into Wetiko's frequency lair. Players in the 'Covid' human catastrophe including Gates, Schwab, Tedros, Fauci, Whitty, Vallance, Johnson, Hancock, Ferguson, Drosten, and all the rest, including the psychopath psychologists, are expressions of Wetiko. This is why they have no compassion or empathy and no emotional consequence for what they do that would make them stop doing it. Observe all the people who support the psychopaths in authority against the Pushbackers despite the damaging impact the psychopaths have on their own lives and their family's lives. You are again looking at Wetiko possession which prevents them seeing through the lies to the obvious scam going on. *Why can't they see it?* Wetiko won't let them see it. The perceptual divide that has now become a chasm is between the Wetikoed and the non-Wetikoed.

Paul Levy describes Wetiko in the same way that I have long described the Archontic force. They are the same distorted consciousness operating across dimensions of reality: '… the subtle body of wetiko is not located in the third dimension of space and time, literally existing in another dimension … it is able to affect ordinary lives by mysteriously interpenetrating into our three-dimensional world.' Wetiko does this through its incarnate representatives in the Cult and by weaving itself into The Field which on our level of reality is the electromagnetic information field of the simulation or Matrix. More than that, the simulation *is* Wetiko / Yaldabaoth. Caleb Scharf, Director of Astrobiology at Columbia University, has speculated that 'alien life' could be so advanced that it has transcribed

itself into the quantum realm to become what we call physics. He said intelligence indistinguishable from the fabric of the Universe would solve many of its greatest mysteries:

> Perhaps hyper-advanced life isn't just external. Perhaps it's already all around. It is embedded in what we perceive to be physics itself, from the root behaviour of particles and fields to the phenomena of complexity and emergence ... In other words, life might not just be in the equations. It might BE the equations [My emphasis].

Scharf said it is possible that 'we don't recognise advanced life because it forms an integral and unsuspicious part of what we've considered to be the natural world'. I agree. Wetiko/Yaldabaoth *is* the simulation. We are literally in the body of the beast. But that doesn't mean it has to control us. We all have the power to overcome Wetiko influence and the Cult knows that. I doubt it sleeps too well because it knows that.

Which Field?

This, I suggest, is how it all works. There are two Fields. One is the fierce electromagnetic light of the Matrix within the speed of light; the other is the 'watery light' of The Field beyond the walls of the Matrix that connects with the Great Infinity. Five-sense mind and the decoding systems of the body attach us to the Field of Matrix light. They have to or we could not experience this reality. Five-sense mind sees only the Matrix Field of information while our expanded consciousness is part of the Infinity Field. When we open our minds, and most importantly our hearts, to the Infinity Field we have a mission control which gives us an expanded perspective, a road map, to understand the nature of the five-sense world. If we are isolated only in five-sense mind there is no mission control. We're on our own trying to understand a world that's constantly feeding us information to ensure we do not understand. People in this state can feel 'lost' and bewildered with no direction or radar. You can see ever more clearly those who are influenced by the Fields of Big Infinity or little five-sense mind simply by their views and behaviour with regard to the 'Covid' hoax. We have had this division throughout known human history with the mass of the people on one side and individuals who could see and intuit beyond the walls of the simulation – Plato's prisoner who broke out of the cave and

saw reality for what it is. Such people have always been targeted by Wetiko/Archon-possessed authority, burned at the stake or demonised as mad, bad and dangerous. The Cult today and its global network of 'anti-hate', 'anti-fascist' Woke groups are all expressions of Wetiko attacking those exposing the conspiracy, 'Covid' lies and the 'vaccine' agenda.

Woke as a whole is Wetiko which explains its black and white mentality and how at one it is with the Wetiko-possessed Cult. Paul Levy said: 'To be in this paradigm is to still be under the thrall of a two-valued logic – where things are either true or false – of a wetikoized mind.' Wetiko consciousness is in a permanent rage, therefore so is Woke, and then there is Woke inversion and contradiction. 'Anti-fascists' act like fascists because fascists *and* 'anti-fascists' are both Wetiko at work. Political parties act the same while claiming to be different for the same reason. Secret society and satanic rituals are attaching initiates to Wetiko and the cold, ruthless, psychopathic mentality that secures the positions of power all over the world is Wetiko. Reframing 'training programmes' have the same cumulative effect of attaching Wetiko and we have their graduates described as automatons and robots with a cold, psychopathic, uncaring demeanour. They are all traits of Wetiko possession and look how many times they have been described in this book and elsewhere with regard to personnel behind 'Covid' including the police and medical profession. Climbing the greasy pole in any profession in a Wetiko society requires traits of Wetiko to get there and that is particularly true of politics which is not about fair competition and pre-eminence of ideas. It is founded on how many backs you can stab and arses you can lick. This culminated in the global 'Covid' coordination between the Wetiko possessed who pulled it off in all the different countries without a trace of empathy and compassion for their impact on humans. Our sight sense can see only holographic form and not the Field which connects holographic form. Therefore we perceive 'physical' objects with 'space' in between. In fact that 'space' is energy/consciousness operating on multiple frequencies. One of them is Wetiko and that connects the Cult psychopaths, those who submit to the psychopaths, and those who serve the psychopaths in the media operations of the world. Wetiko is Gates. Wetiko is the mask-wearing submissive. Wetiko is the fake journalist and 'fact-checker'. The Wetiko Field is coordinating the whole thing. Psychopaths, gofers, media operatives, 'anti-hate' hate groups, 'fact-checkers' and submissive people work as one unit

even without human coordination because they are attached to the *same* Field which is organising it all (Fig 22). Paul Levy is here describing how Wetiko-possessed people are drawn together and refuse to let any information breach their rigid perceptions. He was writing long before 'Covid', but I think you will recognise followers of the 'Covid' religion *oh just a little bit*:

People who are channelling the vibratory frequency of wetiko align with each other through psychic resonance to reinforce their unspoken shared agreement so as to uphold their deranged view of reality. Once an unconscious content takes possession of certain individuals, it irresistibly draws them together by mutual attraction and knits them into groups tied together by their shared madness that can easily swell into an avalanche of insanity.

A psychic epidemic is a closed system, which is to say that it is insular and not open to any new information or informing influences from the outside world which contradict its fixed, limited, and limiting perspective.

There we have the Woke mind and the 'Covid' mind. Compatible resonance draws the awakening together, too, which is clearly happening today.

Figure 22: The Wetiko Field from which the Cult pyramid and its personnel are made manifest. (Image by Neil Hague).

Spiritual servitude

Wetiko doesn't care about humans. It's not human; it just possesses humans for its own ends and the effect (depending on the scale of possession) can be anything from extreme psychopathy to unquestioning obedience. Wetiko's worst nightmare is for human consciousness to expand beyond the simulation. Everything is focussed on stopping that happening through control of information, thus perception, thus frequency. The 'education system', media, science, medicine, academia, are all geared to maintaining humanity in five-sense servitude as is the constant stimulation of low-vibrational mental and emotional states (see 'Covid'). Wetiko seeks to dominate those subconscious spaces between five-sense perception and expanded consciousness where the computer meets the operator. From these subconscious hiding places Wetiko speaks to us to trigger urges and desires that we take to be our own and manipulate us into anything from low-vibrational to psychopathic states. Remember how Islam describes the Jinn as invisible tricksters that 'whisper' and confuse. Wetiko is the origin of the 'trickster god' theme that you find in cultures all over the world. Jinn, like the Archons, are Wetiko which is terrified of humans awakening and reconnecting with our true self for then its energy source has gone. With that the feedback loop breaks between Wetiko and human perception that provides the energetic momentum on which its very existence depends as a force of evil. Humans are both its target and its source of survival, but only if we are operating in low-vibrational states of fear, hate, depression and the background anxiety that most people suffer. We are Wetiko's target because we are its key to survival. It needs us, not the other way round. Paul Levy writes:

> A vampire has no intrinsic, independent, substantial existence in its own right; it only exists in relation to us. The pathogenic, vampiric mind-parasite called wetiko is nothing in itself – not being able to exist from its own side – yet it has a 'virtual reality' such that it can potentially destroy our species …
>
> …The fact that a vampire is not reflected by a mirror can also mean that what we need to see is that there's nothing, no-thing to see, other than ourselves. The fact that wetiko is the expression of something inside of us means that the cure for wetiko is with us as well. The critical issue is finding this cure within us and then putting it into effect.

Evil begets evil because if evil does not constantly expand and find new sources of energetic sustenance its evil, its *distortion*, dies with the assimilation into balance and harmony. Love is the garlic to Wetiko's vampire. Evil, the absence of love, cannot exist in the presence of love. I think I see a way out of here. I have emphasised so many times over the decades that the Archons/Wetiko and their Cult are not all powerful. *They are not*. I don't care how it looks even now *they are not*. I have not called them little boys in short trousers for effect. I have said it because it is true. Wetiko's insatiable desire for power over others is not a sign of its omnipotence, but its insecurity. Paul Levy writes: 'Due to the primal fear which ultimately drives it and which it is driven to cultivate, wetiko's body politic has an intrinsic and insistent need for centralising power and control so as to create imagined safety for itself.' *Yeeeeeees!* Exactly! Why does Wetiko want humans in an ongoing state of fear? Wetiko itself *is* fear and it is petrified of love. As evil is an absence of love, so love is an absence of fear. Love conquers all and *especially* Wetiko which *is* fear. Wetiko brought fear into the world when it wasn't here before. *Fear* was the 'fall', the fall into low-frequency ignorance and illusion – fear is **F**alse **E**motion **A**ppearing **R**eal. The simulation is driven and energised by fear because Wetiko/Yaldabaoth (fear) *are* the simulation. Fear is the absence of love and Wetiko is the absence of love.

Wetiko today

We can now view current events from this level of perspective. The 'Covid' hoax has generated momentous amounts of ongoing fear, anxiety, depression and despair which have empowered Wetiko. No wonder people like Gates have been the instigators when they are Wetiko incarnate and exhibit every trait of Wetiko in the extreme. See how cold and unemotional these people are like Gates and his cronies, how dead of eye they are. That's Wetiko. Sabbatians are Wetiko and everything they control including the World Health Organization, Big Pharma and the 'vaccine' makers, national 'health' hierarchies, corporate media, Silicon Valley, the banking system, and the United Nations with its planned transformation into world government. All are controlled and possessed by the Wetiko distortion into distorting human society in its image. We are with this knowledge at the gateway to understanding the world. Divisions of race, culture, creed and

sexuality are diversions to hide the real division between those possessed and influenced by Wetiko and those that are not. The 'Covid' hoax has brought both clearly into view. Human behaviour is not about race. Tyrants and dictatorships come in all colours and creeds. What unites the US president bombing the innocent and an African tribe committing genocide against another as in Rwanda? What unites them? *Wetiko*. All wars are Wetiko, all genocide is Wetiko, all hunger over centuries in a world of plenty is Wetiko. Children going to bed hungry, including in the West, is Wetiko. Cult-generated Woke racial divisions that focus on the body are designed to obscure the reality that divisions in behaviour are manifestations of mind, not body. Obsession with body identity and group judgement is a means to divert attention from the real source of behaviour – mind and perception. Conflict sown by the Woke both within themselves and with their target groups are Wetiko providing lunch for itself through still more agents of the division, chaos, and fear on which it feeds. The Cult is seeking to assimilate the entirety of humanity and all children and young people into the Wetiko frequency by manipulating them into states of fear and despair. Witness all the suicide and psychological unravelling since the spring of 2020. Wetiko psychopaths want to impose a state of unquestioning obedience to authority which is no more than a conduit for Wetiko to enforce its will and assimilate humanity into itself. It needs us to believe that resistance is futile when it fears resistance and even more so the game-changing non-cooperation with its impositions. It can use violent resistance for its benefit. Violent impositions and violent resistance are *both* Wetiko. The Power of Love with its Power of No will sweep Wetiko from our world. Wetiko and its Cult know that. They just don't want us to know.

AI Wetiko

This brings me to AI or artificial intelligence and something else Wetikos don't want us to know. What is AI *really*? I know about computer code algorithms and AI that learns from data input. These, however, are more diversions, the expeditionary force, for the real AI that they want to connect to the human brain as promoted by Silicon Valley Wetikos like Kurzweil. What is this AI? It is the frequency of *Wetiko*, the frequency of the Archons. The connection of AI to the human brain is the connection of the Wetiko frequency to create a Wetiko hive mind and complete the job of

assimilation. The hive mind is planned to be controlled from Israel and China which are both 100 percent owned by Wetiko Sabbatians. The assimilation process has been going on minute by minute in the ‘smart’ era which fused with the ‘Covid’ era. We are told that social media is scrambling the minds of the young and changing their personality. This is true, but what is social media? Look more deeply at how it works, how it creates divisions and conflict, the hostility and cruelty, the targeting of people until they are destroyed. That’s Wetiko. Social media is manipulated to tune people to the Wetiko frequency with all the emotional exploitation tricks employed by platforms like Facebook and its Wetiko front man, Zuckerberg. Facebook’s Instagram announced a new platform for children to overcome a legal bar on them using the main site. This is more Wetiko exploitation and manipulation of kids. Amnesty International likened the plan to foxes offering to guard the henhouse and said it was incompatible with human rights. Since when did Wetiko or Zuckerberg (I repeat myself) care about that? Would Brin and Page at Google, Wojcicki at YouTube, Bezos at Amazon and whoever the hell runs Twitter act as they do if they were not channelling Wetiko? Would those who are developing technologies for no other reason than human control? How about those designing and selling technologies to kill people and Big Pharma drug and ‘vaccine’ producers who know they will end or devastate lives? Quite a thought for these people to consider is that if you are Wetiko in a human life you are Wetiko on the ‘other side’ unless your frequency changes and that can only change by a change of perception which becomes a change of behaviour. Where Gates is going does not bear thinking about although perhaps that’s exactly where he wants to go. Either way, that’s where he’s going. His frequency will make it so.

The frequency lair

I have been saying for a long time that a big part of the addiction to smartphones and devices is that a frequency is coming off them that entraps the mind. People spend ages on their phones and sometimes even a minute or so after they put them down they pick them up again and it all repeats. ‘Covid’ lockdowns will have increased this addiction a million times for obvious reasons. Addictions to alcohol overindulgence and drugs are another way that Wetiko entraps consciousness to attach to its own. Both

are symptoms of low-vibrational psychological distress which alcoholism and drug addiction further compound. Do we think it's really a coincidence that access to them is made so easy while potions that can take people into realms beyond the simulation are banned and illegal? I have explored smartphone addiction in other books, the scale is mind-blowing, and that level of addiction does not come without help. Tech companies that make these phones are Wetiko and they will have no qualms about destroying the minds of children. We are seeing again with these companies the Wetiko perceptual combination of psychopathic enforcers and weak and meek unquestioning compliance by the rank and file.

The global Smart Grid is the Wetiko Grid and it is crucial to complete the Cult endgame. The simulation is radiation and we are being deluged with technological radiation on a devastating scale. Wetiko frauds like Elon Musk serve Cult interests while occasionally criticising them to maintain his street-cred. 5G and other forms of Wi-Fi are being directed at the earth from space on a volume and scale that goes on increasing by the day. Elon Musk's (officially) SpaceX Starlink project is in the process of putting tens of thousands of satellites in low orbit to cover every inch of the planet with 5G and other Wi-Fi to create Kurzweil's global 'cloud' to which the human mind is planned to be attached very soon. SpaceX has approval to operate 12,000 satellites with more than 1,300 launched at the time of writing and applications filed for *30,000* more. Other operators in the Wi-Fi, 5G, low-orbit satellite market include OneWeb (UK), Telesat (Canada), and AST & Science (US). Musk tells us that AI could be the end of humanity and then launches a company called Neuralink to connect the human brain to computers. Musk's (in theory) Tesla company is building electric cars and the driverless vehicles of the smart control grid. As frauds and bullshitters go Elon Musk in my opinion is Major League.

5G and technological radiation in general are destructive to human health, genetics and psychology and increasing the strength of artificial radiation underpins the five-sense perceptual bubbles which are themselves expressions of radiation or electromagnetism. Freedom activist John Whitehead was so right with his 'databit by databit, we are building our own electronic concentration camps'. The Smart Grid and 5G is a means to control the human mind and infuse perceptual information into The Field to influence anyone in sync with its frequency. You can change perception and behaviour en masse if you can manipulate the population into those levels

of frequency and this is happening all around us today. The arrogance of Musk and his fellow Cult operatives knows no bounds in the way that we see with Gates. Musk's satellites are so many in number already they are changing the night sky when viewed from Earth. The astronomy community has complained about this and they have seen nothing yet. Some consequences of Musk's Wetiko hubris include: Radiation; visible pollution of the night sky; interference with astronomy and meteorology; ground and water pollution from intensive use of increasingly many spaceports; accumulating space debris; continual deorbiting and burning up of aging satellites, polluting the atmosphere with toxic dust and smoke; and ever-increasing likelihood of collisions. A collective public open letter of complaint to Musk said:

> We are writing to you … because SpaceX is in process of surrounding the Earth with a network of thousands of satellites whose very purpose is to irradiate every square inch of the Earth. SpaceX, like everyone else, is treating the radiation as if it were not there. As if the mitochondria in our cells do not depend on electrons moving undisturbed from the food we digest to the oxygen we breathe.
>
> As if our nervous systems and our hearts are not subject to radio frequency interference like any piece of electronic equipment. As if the cancer, diabetes, and heart disease that now afflict a majority of the Earth's population are not metabolic diseases that result from interference with our cellular machinery. As if insects everywhere, and the birds and animals that eat them, are not starving to death as a result.

People like Musk and Gates believe in their limitless Wetiko arrogance that they can do whatever they like to the world because they own it. Consequences for humanity are irrelevant. It's absolutely time that we stopped taking this shit from these self-styled masters of the Earth when you consider where this is going.

Why is the Cult so anti-human?

I hear this question often: Why would they do this when it will affect them, too? Ah, but will it? Who is this *them*? Forget their bodies. They are just vehicles for Wetiko consciousness. When you break it all down to the foundations we are looking at a state of severely distorted consciousness targeting another state of consciousness for assimilation. The rest is detail. The simulation is the fly-trap in which unique sensations of the five senses

create a cycle of addiction called reincarnation. Renegade Minds see that everything which happens in our reality is a smaller version of the whole picture in line with the holographic principle. Addiction to the radiation of smart technology is a smaller version of addiction to the whole simulation. Connecting the body/brain to AI is taking that addiction on a giant step further to total ongoing control by assimilating human incarnate consciousness into Wetiko. I have watched during the 'Covid' hoax how many are becoming ever more profoundly attached to Wetiko's perceptual calling cards of aggressive response to any other point of view ('There is no other god but me'), psychopathic lack of compassion and empathy, and servile submission to the narrative and will of authority. Wetiko is the psychopaths *and* subservience to psychopaths. The Cult of Wetiko is so anti-human because it is *not* human. It embarked on a mission to destroy human by targeting everything that it means to be human and to survive as human. 'Covid' is not the end, just a means to an end. The Cult with its Wetiko consciousness is seeking to change Earth systems, including the atmosphere, to suit them, not humans. The gathering bombardment of 5G alone from ground and space is dramatically changing The Field with which the five senses interact. There is so much more to come if we sit on our hands and hope it will all go away. It is not meant to go away. It is meant to get ever more extreme and we need to face that while we still can – just.

Carbon dioxide is the gas of life. Without that human is over. Kaput, gone, history. No natural world, no human. The Cult has created a cock and bull story about carbon dioxide and climate change to justify its reduction to the point where Gates and the ignoramus Biden 'climate chief' John Kerry want to suck it out of the atmosphere. Kerry wants to do this because his master Gates does. Wetikos have made the gas of life a demon with the usual support from the Wokers of Extinction Rebellion and similar organisations and the bewildered puppet-child that is Greta Thunberg who was put on the world stage by Klaus Schwab and the World Economic Forum. The name Extinction Rebellion is both ironic and as always Wetiko inversion. The gas that we need to survive must be reduced to save us from extinction. The most basic need of human is oxygen and we now have billions walking around in face nappies depriving body and brain of this essential requirement of human existence. More than that 5G at 60 gigahertz interacts with the oxygen molecule to reduce the amount of oxygen the body can absorb into the bloodstream. The obvious knock-on

consequences of that for respiratory and cognitive problems and life itself need no further explanation. Psychopaths like Musk are assembling a global system of satellites to deluge the human atmosphere with this insanity. The man should be in jail. Here we have two most basic of human needs, oxygen and carbon dioxide, being dismantled.

Two others, water and food, are getting similar treatment with the United Nations Agendas 21 and 2030 – the Great Reset – planning to centrally control all water and food supplies. People will not even own rain water that falls on their land. Food is affected at the most basic level by reducing carbon dioxide. We have genetic modification or GMO infiltrating the food chain on a mass scale, pesticides and herbicides polluting the air and destroying the soil. Freshwater fish that provide livelihoods for 60 million people and feed hundreds of millions worldwide are being 'pushed to the brink' according the conservationists while climate change is the only focus. Now we have Gates and Schwab wanting to dispense with current food sources all together and replace them with a synthetic version which the Wetiko Cult would control in terms of production and who eats and who doesn't. We have been on the Totalitarian Tiptoe to this for more than 60 years as food has become ever more processed and full of chemical shite to the point today when it's not natural food at all. As Dr Tom Cowan says: 'If it has a label don't eat it.' Bill Gates is now the biggest owner of farmland in the United States and he does nothing without an ulterior motive involving the Cult. Klaus Schwab wrote: 'To feed the world in the next 50 years we will need to produce as much food as was produced in the last 10,000 years … food security will only be achieved, however, if regulations on genetically modified foods are adapted to reflect the reality that gene editing offers a precise, efficient and safe method of improving crops.' Liar. People and the world are being targeted with aluminium through vaccines, chemtrails, food, drink cans, and endless other sources when aluminium has been linked to many health issues including dementia which is increasing year after year. Insects, bees and wildlife essential to the food chain are being deleted by pesticides, herbicides and radiation which 5G is dramatically increasing with 6G and 7G to come. The pollinating bee population is being devastated while wildlife including birds, dolphins and whales are having their natural radar blocked by the effects of ever-increasing radiation. In the summer windscreens used to be splattered with

insects so numerous were they. It doesn't happen now. Where have they gone?

Synthetic everything

The Cult is introducing genetically-modified versions of trees, plants and insects including a Gates-funded project to unleash hundreds of millions of genetically-modified, lab-altered and patented male mosquitoes to mate with wild mosquitoes and induce genetic flaws that cause them to die out. Clinically-insane Gates-funded Japanese researchers have developed mosquitos that spread vaccine and are dubbed 'flying vaccinators'. Gates is funding the modification of weather patterns in part to sell the myth that this is caused by carbon dioxide and he's funding geoengineering of the skies to change the atmosphere. Some of this came to light with the Gates-backed plan to release tonnes of chalk into the atmosphere to 'deflect the Sun and cool the planet'. Funny how they do this while the heating effect of the Sun is not factored into climate projections focussed on carbon dioxide. The reason is that they want to reduce carbon dioxide (so don't mention the Sun), but at the same time they do want to reduce the impact of the Sun which is so essential to human life and health. I have mentioned the sun-cholesterol-vitamin D connection as they demonise the Sun with warnings about skin cancer (caused by the chemicals in sun cream they tell you to splash on). They come from the other end of the process with statin drugs to reduce cholesterol that turns sunlight into vitamin D. A lack of vitamin D leads to a long list of health effects and how vitamin D levels must have fallen with people confined to their homes over 'Covid'. Gates is funding other forms of geoengineering and most importantly chemtrails which are dropping heavy metals, aluminium and self-replicating nanotechnology onto the Earth which is killing the natural world. See *Everything You Need To Know, But Have Never Been Told* for the detailed background to this.

Every human system is being targeted for deletion by a force that's not human. The Wetiko Cult has embarked on the process of transforming the human body from biological to synthetic biological as I have explained. Biological is being replaced by the artificial and synthetic – Archontic 'countermimicry' – right across human society. The plan eventually is to dispense with the human body altogether and absorb human consciousness – which it wouldn't really be by then – into cyberspace (the simulation

which is Wetiko/Yaldabaoth). Preparations for that are already happening if people would care to look. The alternative media rightly warns about globalism and 'the globalists', but this is far bigger than that and represents the end of the human race as we know it. The 'bad copy' of prime reality that Gnostics describe was a bad copy of harmony, wonder and beauty to start with before Wetiko/Yaldabaoth set out to change the simulated 'copy' into something very different. The process was slow to start with. Entrapped humans in the simulation timeline were not technologically aware and they had to be brought up to intellectual speed while being suppressed spiritually to the point where they could build their own prison while having no idea they were doing so. We have now reached that stage where technological intellect has the potential to destroy us and that's why events are moving so fast. Central American shaman Don Juan Matus said:

> Think for a moment, and tell me how you would explain the contradictions between the intelligence of man the engineer and the stupidity of his systems of belief, or the stupidity of his contradictory behaviour. Sorcerers believe that the predators have given us our systems of beliefs, our ideas of good and evil; our social mores. They are the ones who set up our dreams of success or failure. They have given us covetousness, greed, and cowardice. It is the predator who makes us complacent, routinary, and egomaniacal.
>
> In order to keep us obedient and meek and weak, the predators engaged themselves in a stupendous manoeuvre – stupendous, of course, from the point of view of a fighting strategist; a horrendous manoeuvre from the point of those who suffer it. They gave us their mind. The predators' mind is baroque, contradictory, morose, filled with the fear of being discovered any minute now.

For 'predators' see Wetiko, Archons, Yaldabaoth, Jinn, and all the other versions of the same phenomenon in cultures and religions all over the world. The theme is always the same because it's true and it's real. We have reached the point where we have to deal with it. The question is – how?

Don't fight – walk away

I thought I'd use a controversial subheading to get things moving in terms of our response to global fascism. What do you mean 'don't fight'? What do you mean 'walk away'? We've got to fight. We can't walk away. Well, it depends what we mean by fight and walk away. If fighting means physical combat we are playing Wetiko's game and falling for its trap. It wants us to get angry, aggressive, and direct hate and hostility at the enemy we think we

must fight. Every war, every battle, every conflict, has been fought with Wetiko leading both sides. It's what it does. Wetiko wants a fight, anywhere, any place. Just hit me, son, so I can hit you back. Wetiko hits Wetiko and Wetiko hits Wetiko in return. I am very forthright as you can see in exposing Wetikos of the Cult, but I don't hate them. I refuse to hate them. It's what they want. What you hate you become. What you *fight* you become. Wokers, 'anti-haters' and 'anti-fascists' prove this every time they reach for their keyboards or don their balaclavas. By walk away I mean to disengage from Wetiko which includes ceasing to cooperate with its tyranny. Paul Levy says of Wetiko:

The way to 'defeat' evil is not to try to destroy it (for then, in playing evil's game, we have already lost), but rather, to find the invulnerable place within ourselves where evil is unable to vanquish us – this is to truly 'win' our battle with evil.

Wetiko is everywhere in human society and it's been on steroids since the 'Covid' hoax. Every shouting match over wearing masks has Wetiko wearing a mask and Wetiko not wearing one. It's an electrical circuit of push and resist, push and resist, with Wetiko pushing *and* resisting. Each polarity is Wetiko empowering itself. Dictionary definitions of 'resist' include 'opposing, refusing to accept or comply with' and the word to focus on is 'opposing'. What form does this take – setting police cars alight or 'refusing to accept or comply with'? The former is Wetiko opposing Wetiko while the other points the way forward. This is the difference between those aggressively demanding that government fascism must be obeyed who stand in stark contrast to the great majority of Pushbackers. We saw this clearly with a march by thousands of Pushbackers against lockdown in London followed days later by a Woker-hijacked protest in Bristol in which police cars were set on fire. Masks were virtually absent in London and widespread in Bristol. Wetiko wants lockdown on every level of society and infuses its aggression to police it through its unknowing stooges. Lockdown protesters are the ones with the smiling faces and the hugs, The two blatantly obvious states of being – getting more obvious by the day – are the result of Wokers and their like becoming ever more influenced by the simulation Field of Wetiko and Pushbackers ever more influenced by The Field of a far higher vibration beyond the simulation. Wetiko can't invade

the heart which is where most lockdown opponents are coming from. It's the heart that allows them to see through the lies to the truth in ways I will be highlighting.

Renegade Minds know that calmness is the place from which wisdom comes. You won't find wisdom in a hissing fit and wisdom is what we need in abundance right now. Calmness is not weakness – you don't have to scream at the top of your voice to be strong. Calmness is indeed a sign of strength. 'No' means I'm not doing it. *NOOOO!!!* doesn't mean you're not doing it even more. Volume does not advance 'No – I'm not doing it'. You are just not doing it. Wetiko possessed and influenced don't know how to deal with that. Wetiko wants a fight and we should not give it one. What it needs more than anything is our *cooperation* and we should not give that either. Mass rallies and marches are great in that they are a visual representation of feeling, but if it ends there they are irrelevant. You demand that Wetikos act differently? Well, they're not going to are they? They are Wetikos. We don't need to waste our time demanding that something doesn't happen when that will make no difference. We need to delete the means that *allows* it to happen. This, invariably, is our cooperation. You can demand a child stop firing a peashooter at the dog or you can refuse to buy the peashooter. If you provide the means you are cooperating with the dog being smacked on the nose with a pea. How can the authorities enforce mask-wearing if millions in a country refuse? What if the 74 million Pushbackers that voted for Trump in 2020 refused to wear masks, close their businesses or stay in their homes. It would be unenforceable. The few control the many through the compliance of the many and that's always been the dynamic be it 'Covid' regulations or the Roman Empire. I know people can find it intimidating to say no to authority or stand out in a crowd for being the only one with a face on display; but it has to be done or it's over. I hope I've made clear in this book that where this is going will be far more intimidating than standing up now and saying 'No' – I will not cooperate with my own enslavement and that of my children. There might be consequences for some initially, although not so if enough do the same. The question that must be addressed is what is going to happen if we don't? It is time to be strong and unyieldingly so. No means no. Not here and there, but *everywhere* and *always*. I have refused to wear a mask and obey all the other nonsense. I will not comply with tyranny. I repeat: Fascism is not imposed by fascists – there are never enough of them.

Fascism is imposed by the population acquiescing to fascism. *I will not do it*. I will die first, or my body will. Living meekly under fascism is a form of death anyway, the death of the spirit that Martin Luther King described.

Making things happen

We must not despair. This is not over till it's over and it's far from that. The 'fat lady' must refuse to sing. The longer the 'Covid' hoax has dragged on and impacted on more lives we have seen an awakening of phenomenal numbers of people worldwide to the realisation that what they have believed all their lives is not how the world really is. Research published by the system-serving University of Bristol and King's College London in February, 2021, concluded: 'One in every 11 people in Britain say they trust David Icke's take on the coronavirus pandemic.' It will be more by now and we have gathering numbers to build on. We must urgently progress from seeing the scam to ceasing to cooperate with it. Prominent German lawyer Reiner Fuellmich, also licenced to practice law in America, is doing a magnificent job taking the legal route to bring the psychopaths to justice through a second Nuremberg tribunal for crimes against humanity. Fuellmich has an impressive record of beating the elite in court and he formed the German Corona Investigative Committee to pursue civil charges against the main perpetrators with a view to triggering criminal charges. Most importantly he has grasped the foundation of the hoax – the PCR test not testing for the 'virus' – and Christian Drosten is therefore on his charge sheet along with Gates frontman Tedros at the World Health Organization. Major players must be not be allowed to inflict their horrors on the human race without being brought to book. A life sentence must follow for Bill Gates and the rest of them. A group of researchers has also indicted the government of Norway for crimes against humanity with copies sent to the police and the International Criminal Court. The lawsuit cites participation in an internationally-planned false pandemic and violation of international law and human rights, the European Commission's definition of human rights by coercive rules, Nuremberg and Hague rules on fundamental human rights, and the Norwegian constitution. We must take the initiative from hereon and not just complain, protest and react.

There are practical ways to support vital mass non-cooperation. Organising in numbers is one. Lockdown marches in London in the spring

in 2021 were mass non-cooperation that the authorities could not stop. There were too many people. Hundreds of thousands walked the London streets in the centre of the road for mile after mile while the Face-Nappies could only look on. They were determined, but calm, and just *did it* with no histrionics and lots of smiles. The police were impotent. Others are organising group shopping without masks for mutual support and imagine if that was happening all over. Policing it would be impossible. If the store refuses to serve people in these circumstances they would be faced with a long line of trolleys full of goods standing on their own and everything would have to be returned to the shelves. How would they cope with that if it kept happening? I am talking here about moving on from complaining to being pro-active; from watching things happen to making things happen. I include in this our relationship with the police. The behaviour of many Face-Nappies has been disgraceful and anyone who thinks they would never find concentration camp guards in the 'enlightened' modern era have had that myth busted big-time. The period and setting may change – Wetikos never do. I watched film footage from a London march in which a police thug viciously kicked a protestor on the floor who had done nothing. His fellow Face-Nappies stood in a ring protecting him. What he did was a criminal assault and with a crowd far outnumbering the police this can no longer be allowed to happen unchallenged. I get it when people chant 'shame on you' in these circumstances, but that is no longer enough. They *have* no shame those who do this. Crowds needs to start making a citizen's arrest of the police who commit criminal offences and brutally attack innocent people and defenceless women. A citizen's arrest can be made under section 24A of the UK Police and Criminal Evidence (PACE) Act of 1984 and you will find something similar in other countries. I prefer to call it a Common Law arrest rather than citizen's for reasons I will come to shortly. Anyone can arrest a person committing an indictable offence or if they have reasonable grounds to suspect they are committing an indictable offence. On both counts the attack by the police thug would have fallen into this category. A citizen's arrest can be made to stop someone:

- Causing physical injury to himself or any other person
- Suffering physical injury
- Causing loss of or damage to property
- Making off before a constable can assume responsibility for him

A citizen's arrest may also be made to prevent a breach of the peace under Common Law and if they believe a breach of the peace will happen or anything related to harm likely to be done or already done in their presence. This is the way to go I think – the Common Law version. If police know that the crowd and members of the public will no longer be standing and watching while they commit their thuggery and crimes they will think twice about acting like Brownshirts and Blackshirts.

Common Law – common sense

Mention of Common Law is very important. Most people think the law is the law as in one law. This is not the case. There are two bodies of law, Common Law and Statute Law, and they are not the same. Common Law is founded on the simple premise of do no harm. It does not recognise victimless crimes in which no harm is done while Statute Law does. There is a Statute Law against almost everything. So what is Statute Law? Amazingly it's the law of the *sea* that was brought ashore by the Cult to override the law of the land which is Common Law. They had no right to do this and as always they did it anyway. They had to. They could not impose their will on the people through Common Law which only applies to do no harm. How could you stitch up the fine detail of people's lives with that? Instead they took the law of the sea, or Admiralty Law, and applied it to the population. Statute Law refers to all the laws spewing out of governments and their agencies including all the fascist laws and regulations relating to 'Covid'. The key point to make is that Statute Law is *contract law*. It only applies between *contracting* corporations. Most police officers don't even know this. They have to be kept in the dark, too. Long ago when merchants and their sailing ships began to trade with different countries a contractual law was developed called Admiralty Law and other names. Again it only applied to *contracts* agreed between *corporate* entities. If there is no agreed contract the law of the sea had no jurisdiction *and that still applies to its new alias of Statute Law*. The problem for the Cult when the law of the sea was brought ashore was an obvious one. People were not corporations and neither were government entities. To overcome the latter they made governments and all associated organisations corporations. All the institutions are *private corporations* and I mean governments and their

agencies, local councils, police, courts, military, US states, the whole lot. Go to the Dun and Bradstreet corporate listings website for confirmation that they are all corporations. You are arrested by a private corporation called the police by someone who is really a private security guard and they take you to court which is another private corporation. Neither have jurisdiction over you unless you consent and *contract* with them. This is why you hear the mantra about law enforcement policing by *consent* of the people. In truth the people 'consent' only in theory through monumental trickery.

Okay, the Cult overcame the corporate law problem by making governments and institutions corporate entities; but what about people? They are not corporations are they? Ah ... well in a sense, and *only* a sense, they are. Not people exactly – the illusion of people. The Cult creates a corporation in the name of everyone at the time that their birth certificate is issued. Note birth/ *berth* certificate and when you go to court under the law of the sea on land you stand in a *dock*. These are throwbacks to the origin. My Common Law name is David Vaughan Icke. The name of the corporation created by the government when I was born is called Mr David Vaughan Icke usually written in capitals as MR DAVID VAUGHAN ICKE. That is not me, the living, breathing man. It is a fictitious corporate entity. The trick is to make you think that David Vaughan Icke and MR DAVID VAUGHAN ICKE are the same thing. *They are not*. When police charge you and take you to court they are prosecuting the corporate entity and not the living, breathing, man or woman. They have to trick you into identifying as the corporate entity and contracting with them. Otherwise they have no jurisdiction. They do this through a language known as legalese. Lawful and legal are not the same either. Lawful relates to Common Law and legal relates to Statute Law. Legalese is the language of Statue Law which uses terms that mean one thing to the public and another in legalese. Notice that when a police officer tells someone why they are being charged he or she will say at the end: 'Do you understand?' To the public that means 'Do you comprehend?' In legalese it means 'Do you stand under me?' Do you stand under my authority? If you say yes to the question you are unknowingly agreeing to give them jurisdiction over you in a contract between two corporate entities.

This is a confidence trick in every way. Contracts have to be agreed between informed parties and if you don't know that David Vaughan Icke is

agreeing to be the corporation MR DAVID VAUGHAN ICKE you cannot knowingly agree to contract. They are deceiving you and another way they do this is to ask for proof of identity. You usually show them a driving licence or other document on which your corporate name is written. In doing so you are accepting that you are that corporate entity when you are not. Referring to yourself as a 'person' or 'citizen' is also identifying with your corporate fiction which is why I made the Common Law point about the citizen's arrest. If you are approached by a police officer you identify yourself immediately as a living, breathing, man or woman and say 'I do not consent, I do not contract with you and I do not understand' or stand under their authority. I have a Common Law birth certificate as a living man and these are available at no charge from commonlawcourt.com. Businesses registered under the Statute Law system means that its laws apply. There are, however, ways to run a business under Common Law. Remember all 'Covid' laws and regulations are Statute Law – the law of *contracts* and you do not have to contract. This doesn't mean that you can kill someone and get away with it. Common Law says do no harm and that applies to physical harm, financial harm etc. Police are employees of private corporations and there needs to be a new system of non-corporate Common Law constables operating outside the Statute Law system. If you go to davidicke.com and put Common Law into the search engine you will find videos that explain Common Law in much greater detail. It is definitely a road we should walk.

With all my heart

I have heard people say that we are in a spiritual war. I don't like the term 'war' with its Wetiko dynamic, but I know what they mean. Sweep aside all the bodily forms and we are in a situation in which two states of consciousness are seeking very different realities. Wetiko wants upheaval, chaos, fear, suffering, conflict and control. The other wants love, peace, harmony, fairness and freedom. That's where we are. We should not fall for the idea that Wetiko is all-powerful and there's nothing we can do. Wetiko is not all-powerful. It's a joke, pathetic. It doesn't have to be, but it has made that choice for now. A handful of times over the years when I have felt the presence of its frequency I have allowed it to attach briefly so I could consciously observe its nature. The experience is not pleasant, the

energy is heavy and dark, but the ease with which you can kick it back out the door shows that its real power is in persuading us that it has power. It's all a con. Wetiko is a con. It's a trickster and not a power that can control us if we unleash our own. The con is founded on manipulating humanity to give its power to Wetiko which recycles it back to present the illusion that it has power when its power is *ours* that we gave away. This happens on an energetic level and plays out in the world of the seen as humanity giving its power to Wetiko authority which uses that power to control the population when the power is only the power the population has handed over. How could it be any other way for billions to be controlled by a relative few? I have had experiences with people possessed by Wetiko and again you can kick its arse if you do it with an open heart. Oh yes – the *heart* which can transform the world of perceived 'matter'.

We are receiver-transmitters and processors of information, but what information and where from? Information is processed into perception in three main areas – the brain, the heart and the belly. These relate to thinking, knowing, and emotion. Wetiko wants us to be head and belly people which means we think within the confines of the Matrix simulation and low-vibrational emotional reaction scrambles balance and perception. A few minutes on social media and you see how emotion is the dominant force. Woke is all emotion and is therefore thought-free and fact-free. Our heart is something different. It *knows* while the head *thinks* and has to try to work it out because it doesn't know. The human energy field has seven prime vortexes which connect us with wider reality (Fig 23). Chakra means 'wheels of light' in the Sanskrit language of ancient India. The main ones are: The crown chakra on top of the head; brow (or 'third eye') chakra in the centre of the forehead; throat chakra; heart chakra in the centre of the chest; solar plexus chakra below the sternum; sacral chakra beneath the navel; and base chakra at the bottom of the spine. Each one has a particular function or functions. We feel anxiety and nervousness in the belly where the sacral chakra is located and this processes emotion that can affect the colon to give people 'the shits' or make them 'shit scared' when they are nervous. Chakras all play an important role, but the Mr and Mrs Big is the heart chakra which sits at the centre of the seven, above the chakras that connect us to the 'physical' and below those that connect with higher realms (or at least should). Here in the heart chakra we feel love, empathy and compassion – 'My heart goes out to you'. Those with closed hearts

become literally ‘heart-less’ in their attitudes and behaviour (see Bill Gates). Native Americans portrayed Wetiko with what Paul Levy calls a ‘frigid, icy heart, devoid of mercy’ (see Bill Gates).

Figure 23: The chakra system which interpenetrates the human energy field. The heart chakra is the governor – or should be.

Wetiko trembles at the thought of heart energy which it cannot infiltrate. The frequency is too high. What it seeks to do instead is close the heart chakra vortex to block its perceptual and energetic influence. Psychopaths have ‘hearts of stone’ and emotionally-damaged people have ‘heartache’ and ‘broken hearts’. The astonishing amount of heart disease is related to heart chakra disruption with its fundamental connection to the ‘physical’ heart. Dr Tom Cowan has written an outstanding book challenging the belief that the heart is a pump and making the connection between the ‘physical’ and spiritual heart. Rudolph Steiner who was way ahead of his time said the same about the fallacy that the heart is a pump. *What*? The heart is not a pump? That’s crazy, right? Everybody knows that. Read Cowan’s *Human Heart, Cosmic Heart* and you will realise that the very idea of the heart as a pump is ridiculous when you see the evidence. How does blood in the feet so far from the heart get pumped horizontally up the body by the heart?? Cowan explains in the book the real reason why blood moves as it does. Our ‘physical’ heart is used to symbolise love when the source is really the heart vortex or spiritual heart which is our most powerful energetic connection to ‘out there’ expanded consciousness. That’s why we feel *knowing* – intuitive knowing – in the centre of the chest. Knowing doesn’t come from a process of thoughts leading to a conclusion. It is there in an instant all in one go. Our heart knows because of its

connection to levels of awareness that *do* know. This is the meaning and source of intuition – intuitive *knowing*.

For the last more than 30 years of uncovering the global game and the nature of reality my heart has been my constant antenna for truth and accuracy. An American intelligence insider once said that I had quoted a disinformer in one of my books and yet I had only quoted the part that was true. He asked: 'How do you do that?' By using my heart antenna was the answer and anyone can do it. Heart-centred is how we are meant to be. With a closed heart chakra we withdraw into a closed mind and the bubble of five-sense reality. If you take a moment to focus your attention on the centre of your chest, picture a spinning wheel of light and see it opening and expanding. You will feel it happening, too, and perceptions of the heart like joy and love as the heart impacts on the mind as they interact. The more the chakra opens the more you will feel expressions of heart consciousness and as the process continues, and becomes part of you, insights and knowings will follow. An open heart is connected to that level of awareness that knows all is *One*. You will see from its perspective that the fault-lines that divide us are only illusions to control us. An open heart does not process the illusions of race, creed and sexuality except as brief experiences for a consciousness that is all. Our heart does not see division, only unity (Figs 24 and 25). There's something else, too. Our hearts love to laugh. Mark Twain's quote that says 'The human race has one really effective weapon, and that is laughter' is really a reference to the heart which loves to laugh with the joy of knowing the true nature of infinite reality and that all the madness of human society is an illusion of the mind. Twain also said: 'Against the assault of laughter nothing can stand.' This is so true of Wetiko and the Cult. Their insecurity demands that they be taken seriously and their power and authority acknowledged and feared. We should do nothing of the sort. We should not get aggressive or fearful which their insecurity so desires. We should laugh in their face. Even in their no-face as police come over in their face-nappies and expect to be taken seriously. They don't take themselves seriously looking like that so why should we? Laugh in the face of intimidation. Laugh in the face of tyranny. You will see by its reaction that you have pressed all of its buttons. Wetiko does not know what to do in the face of laughter or when its targets refuse to concede their joy to fear. We have seen many examples during the 'Covid' hoax when people have expressed their energetic power and the string puppets of Wetiko retreat

with their tail limp between their knees. Laugh – the world is bloody mad after all and if it's a choice between laughter and tears I know which way I'm going.

Figure 24: Head consciousness without the heart sees division and everything apart from everything else.

Figure 25: Heart consciousness sees everything as One.

'Vaccines' and the soul

The foundation of Wetiko/Archon control of humans is the separation of incarnate five-sense mind from the infinite 'I' and closing the heart chakra where the True 'I' lives during a human life. The goal has been to achieve complete separation in both cases. I was interested therefore to read an account by a French energetic healer of what she said she experienced with a patient who had been given the 'Covid' vaccine. Genuine energy healers can sense information and consciousness fields at different levels of being which are referred to as 'subtle bodies'. She described treating the patient who later returned after having, without the healer's knowledge, two doses of the 'Covid vaccine'. The healer said:

I noticed immediately the change, very heavy energy emanating from [the] subtle bodies. The scariest thing was when I was working on the heart chakra, I connected with her soul: it was detached from the physical body, it had no contact and it was, as if it was floating in a state of total confusion: a damage to the consciousness that loses contact with the physical body, i.e. with our biological machine, there is no longer any communication between them.

I continued the treatment by sending light to the heart chakra, the soul of the person, but it seemed that the soul could no longer receive any light, frequency or energy. It was a very powerful experience for me. Then I understood that this substance is indeed used to detach consciousness so that this consciousness can no longer interact through this body that it possesses in life, where there is no longer any contact, no frequency, no light, no more energetic balance or mind.

This would create a human that is rudderless and at the extreme almost zombie-like operating with a fractional state of consciousness at the mercy of Wetiko. I was especially intrigued by what the healer said in the light of the prediction by the highly-informed Rudolf Steiner more than a hundred years ago. He said:

In the future, we will eliminate the soul with medicine. Under the pretext of a 'healthy point of view', there will be a vaccine by which the human body will be treated as soon as possible directly at birth, so that the human being cannot develop the thought of the existence of soul and Spirit. To materialistic doctors will be entrusted the task of removing the soul of humanity.

As today, people are vaccinated against this disease or that disease, so in the future, children will be vaccinated with a substance that can be produced precisely in such a way that people, thanks to this vaccination, will be immune to being subjected to the 'madness' of spiritual life. He would be extremely smart, but he would not develop a conscience, and that is the true goal of some materialistic circles.

Steiner said the vaccine would detach the physical body from the etheric body (subtle bodies) and 'once the etheric body is detached the relationship between the universe and the etheric body would become extremely unstable, and man would become an automaton'. He said 'the physical body of man must be polished on this Earth by spiritual will – so the vaccine becomes a kind of arymanique (Wetiko) force' and 'man can no longer get rid of a given materialistic feeling'. Humans would then, he said, become 'materialistic of constitution and can no longer rise to the spiritual'. I have been writing for years about DNA being a receiver-transmitter of information that connects us to other levels of reality and these 'vaccines' changing DNA can be likened to changing an antenna and what it can transmit and receive. Such a disconnection would clearly lead to changes in

personality and perception. Steiner further predicted the arrival of AI. Big Pharma 'Covid vaccine' makers, expressions of Wetiko, are testing their DNA-manipulating evil on children as I write with a view to giving the 'vaccine' to babies. If it's a soul-body disconnector – and I say that it is or can be – every child would be disconnected from 'soul' at birth and the 'vaccine' would create a closed system in which spiritual guidance from the greater self would play no part. This has been the ambition of Wetiko all along. A Pentagon video from 2005 was leaked of a presentation explaining the development of vaccines to change behaviour by their effect on the brain. Those that believe this is not happening with the 'Covid' genetically-modifying procedure masquerading as a 'vaccine' should make an urgent appointment with Naivety Anonymous. Klaus Schwab wrote in 2018:

> Neurotechnologies enable us to better influence consciousness and thought and to understand many activities of the brain. They include decoding what we are thinking in fine levels of detail through new chemicals and interventions that can influence our brains to correct for errors or enhance functionality.

The plan is clear and only the heart can stop it. With every heart that opens, every mind that awakens, Wetiko is weakened. Heart and love are far more powerful than head and hate and so nothing like a majority is needed to turn this around.

Beyond the Phantom

Our heart is the prime target of Wetiko and so it must be the answer to Wetiko. We *are* our heart which is part of one heart, the infinite heart. Our heart is where the true self lives in a human life behind firewalls of five-sense illusion when an imposter takes its place – *Phantom Self*; but our heart waits patiently to be set free any time we choose to see beyond the Phantom, beyond Wetiko. A Wetikoed Phantom Self can wreak mass death and destruction while the love of forever is locked away in its heart. The time is here to unleash its power and let it sweep away the fear and despair that is Wetiko. Heart consciousness does not seek manipulated, censored, advantage for its belief or religion, its activism and desires. As an expression of the One it treats all as One with the same rights to freedom and opinion. Our heart demands fairness for itself no more than for others.

From this unity of heart we can come together in mutual support and transform this Wetikoed world into what reality is meant to be – a place of love, joy, happiness, fairness, justice and freedom. Wetiko has another agenda and that's why the world is as it is, but enough of this nonsense. Wetiko can't stay where hearts are open and it works so hard to keep them closed. Fear is its currency and its food source and love in its true sense has no fear. Why would love have fear when it knows it is *All That Is, Has Been, And Ever Can Be* on an eternal exploration of all possibility? Love in this true sense is not the physical attraction that passes for love. This can be an expression of it, yes, but Infinite Love, a love without condition, goes far deeper to the core of all being. It *is* the core of all being. Infinite realty was born from love beyond the illusions of the simulation. Love infinitely expressed is the knowing that all is One and the swiftly-passing experience of separation is a temporary hallucination. You cannot disconnect from Oneness; you can only *perceive* that you have and withdraw from its influence. This is the most important of all perception trickery by the mind parasite that is Wetiko and the foundation of all its potential for manipulation.

If we open our hearts, open the sluice gates of the mind, and redefine self-identity amazing things start to happen. Consciousness expands or contracts in accordance with self-identity. When true self is recognised as infinite awareness and label self – Phantom Self – is seen as only a series of brief experiences life is transformed. Consciousness expands to the extent that self-identity expands and everything changes. You see unity, not division, the picture, not the pixels. From this we can play the long game. No more is an experience something in and of itself, but a fleeting moment in the eternity of forever. Suddenly people in uniform and dark suits are no longer intimidating. Doing what your heart knows to be right is no longer intimidating and consequences for those actions take on the same nature of a brief experience that passes in the blink of an infinite eye. Intimidation is all in the mind. Beyond the mind there is no intimidation.

An open heart does not consider consequences for what it knows to be right. To do so would be to consider not doing what it knows to be right and for a heart in its power that is never an option. The Renegade Mind is really the Renegade Heart. Consideration of consequences will always provide a getaway car for the mind and the heart doesn't want one. What is right in the light of what we face today is to stop cooperating with Wetiko in all its

forms and to do it without fear or compromise. You cannot compromise with tyranny when tyranny always demands more until it has everything. Life is your perception and you are your destiny. Change your perception and you change your life. Change collective perception and we change the world.

Come on people … One human family, One heart, One goal … *FREEEEEEDOM*!

We must settle for nothing less.

Postscript

The big scare story as the book goes to press is the 'Indian' variant and the world is being deluged with propaganda about the 'Covid catastrophe' in India which mirrors in its lies and misrepresentations what happened in Italy before the first lockdown in 2020.

The *New York Post* published a picture of someone who had 'collapsed in the street from Covid' in India in April, 2021, which was actually taken during a gas leak in May, 2020. Same old, same old. Media articles in mid-February were asking why India had been so untouched by 'Covid' and then as their vaccine rollout gathered pace the alleged 'cases' began to rapidly increase. Indian 'Covid vaccine' maker Bharat Biotech was funded into existence by the Bill and Melinda Gates Foundation (the pair announced their divorce in May, 2021, which is a pity because they so deserve each other). The Indian 'Covid crisis' was ramped up by the media to terrify the world and prepare people for submission to still more restrictions. The scam that worked the first time was being repeated only with far more people seeing through the deceit. Davidicke.com and Ickonic.com have sought to tell the true story of what is happening by talking to people living through the Indian nightmare which has nothing to do with 'Covid'. We posted a letter from 'Alisha' in Pune who told a very different story to government and media mendacity. She said scenes of dying people and overwhelmed hospitals were designed to hide what was really happening – genocide and starvation. Alisha said that millions had already died of starvation during the ongoing lockdowns while government and media were lying and making it look like the 'virus':

Restaurants, shops, gyms, theatres, basically everything is shut. The cities are ghost towns. Even so-called ‘essential’ businesses are only open till 11am in the morning. You basically have just an hour to buy food and then your time is up.

Inter-state travel and even inter-district travel is banned. The cops wait at all major crossroads to question why you are traveling outdoors or to fine you if you are not wearing a mask.

The medical community here is also complicit in genocide, lying about hospitals being full and turning away people with genuine illnesses, who need immediate care. They have even created a shortage of oxygen cylinders.

This is the classic Cult modus operandi played out in every country. Alisha said that people who would not have a PCR test not testing for the ‘virus’ were being denied hospital treatment. She said the people hit hardest were migrant workers and those in rural areas. Most businesses employed migrant workers and with everything closed there were no jobs, no income and no food. As a result millions were dying of starvation or malnutrition. All this was happening under Prime Minister Narendra Modi, a 100-percent asset of the Cult, and it emphasises yet again the scale of pure anti-human evil we are dealing with. Australia banned its people from returning home from India with penalties for trying to do so of up to five years in jail and a fine of £37,000. The manufactured ‘Covid’ crisis in India was being prepared to justify further fascism in the West. Obvious connections could be seen between the Indian ‘vaccine’ programme and increased ‘cases’ and this became a common theme. The Seychelles, the most per capita ‘Covid vaccinated’ population in the world, went back into lockdown after a ‘surge of cases’.

Long ago the truly evil Monsanto agricultural biotechnology corporation with its big connections to Bill Gates devastated Indian farming with genetically-modified crops. Human rights activist Gurcharan Singh highlighted the efforts by the Indian government to complete the job by destroying the food supply to hundreds of millions with ‘Covid’ lockdowns. He said that 415 million people at the bottom of the disgusting caste system (still going whatever they say) were below the poverty line and struggled to feed themselves every year. Now the government was imposing lockdown at just the time to destroy the harvest. This deliberate policy was leading to mass starvation. People may reel back at the suggestion that a government would do that, but Wetiko-controlled ‘leaders’ are capable of any level of evil. In fact what is described in India is in the process of being instigated

worldwide. The food chain and food supply are being targeted at every level to cause world hunger and thus control. Bill Gates is not the biggest owner of farmland in America for no reason and destroying access to food aids both the depopulation agenda and the plan for synthetic 'food' already being funded into existence by Gates. Add to this the coming hyper-inflation from the suicidal creation of fake 'money' in response to 'Covid' and the breakdown of container shipping systems and you have a cocktail that can only lead one way and is meant to. The Cult plan is to crash the entire system to 'build back better' with the Great Reset.

'Vaccine' transmission

Reports from all over the world continue to emerge of women suffering menstrual and fertility problems after having the fake 'vaccine' and of the non-'vaccinated' having similar problems when interacting with the 'vaccinated'. There are far too many for 'coincidence' to be credible. We've had menopausal women getting periods, others having periods stop or not stopping for weeks, passing clots, sometimes the lining of the uterus, breast irregularities, and miscarriages (which increased by 400 percent in parts of the United States). Non-'vaccinated' men and children have suffered blood clots and nose bleeding after interaction with the 'vaccinated'. Babies have died from the effects of breast milk from a 'vaccinated' mother. Awake doctors – the small minority – speculated on the cause of non-'vaccinated' suffering the same effects as the 'vaccinated'. Was it nanotechnology in the synthetic substance transmitting frequencies or was it a straight chemical bioweapon that was being transmitted between people? I am not saying that some kind of chemical transmission is not one possible answer, but the foundation of all that the Cult does is frequency and this is fertile ground for understanding how transmission can happen. American doctor Carrie Madej, an internal medicine physician and osteopath, has been practicing for the last 20 years, teaching medical students, and she says attending different meetings where the agenda for humanity was discussed. Madej, who operates out of Georgia, did not dismiss other possible forms of transmission, but she focused on frequency in search of an explanation for transmission. She said the Moderna and Pfizer 'vaccines' contained nano-lipid particles as a key component. This was a brand new technology never before used on humanity. 'They're using a nanotechnology which is pretty

much little tiny computer bits … nanobots or hydrogel.' Inside the 'vaccines' was 'this sci-fi kind of substance' which suppressed immune checkpoints to get into the cell. I referred to this earlier as the 'Trojan horse' technique that tricks the cell into opening a gateway for the self-replicating synthetic material and while the immune system is artificially suppressed the body has no defences. Madej said the substance served many purposes including an on-demand ability to 'deliver the payload' and using the nano 'computer bits' as biosensors in the body. 'It actually has the ability to accumulate data from your body, like your breathing, your respiration, thoughts, emotions, all kinds of things.'

She said the technology obviously has the ability to operate through Wi-Fi and transmit and receive energy, messages, frequencies or impulses. 'Just imagine you're getting this new substance in you and it can react to things all around you, the 5G, your smart device, your phones.' We had something completely foreign in the human body that had never been launched large scale at a time when we were seeing 5G going into schools and hospitals (plus the Musk satellites) and she believed the 'vaccine' transmission had something to do with this: '… if these people have this inside of them … it can act like an antenna and actually transmit it outwardly as well.' The synthetic substance produced its own voltage and so it could have that kind of effect. This fits with my own contention that the nano receiver-transmitters are designed to connect people to the Smart Grid and break the receiver-transmitter connection to expanded consciousness. That would explain the French energy healer's experience of the disconnection of body from 'soul' with those who have had the 'vaccine'. The nanobots, self-replicating inside the body, would also transmit the synthetic frequency which could be picked up through close interaction by those who have not been 'vaccinated'. Madej speculated that perhaps it was 5G and increased levels of other radiation that was causing the symptoms directly although interestingly she said that non-'vaccinated' patients had shown improvement when they were away from the 'vaccinated' person they had interacted with. It must be remembered that you can control frequency and energy with your mind and you can consciously create energetic barriers or bubbles with the mind to stop damaging frequencies from penetrating your field. American paediatrician Dr Larry Palevsky said the 'vaccine' was not a 'vaccine' and was never designed to protect from a 'viral' infection. He called it 'a massive, brilliant propaganda of genocide' because they didn't

have to inject everyone to get the result they wanted. He said the content of the jabs was able to infuse any material into the brain, heart, lungs, kidneys, liver, sperm and female productive system. 'This is genocide; this is a weapon of mass destruction.' At the same time American colleges were banning students from attending if they didn't have this life-changing and potentially life-ending 'vaccine'. Class action lawsuits must follow when the consequences of this college fascism come to light. As the book was going to press came reports about fertility effects on sperm in 'vaccinated' men which would absolutely fit with what I have been saying and hospitals continued to fill with 'vaccine' reactions. Another question is what about transmission via blood transfusions? The NHS has extended blood donation restrictions from seven days after a 'Covid vaccination' to 28 days after even a sore arm reaction.

I said in the spring of 2020 that the then touted 'Covid vaccine' would be ongoing each year like the flu jab. A year later Pfizer CEO, the appalling Albert Bourla, said people would 'likely' need a 'booster dose' of the 'vaccine' within 12 months of getting 'fully vaccinated' and then a yearly shot. 'Variants will play a key role', he said confirming the point. Johnson & Johnson CEO Alex Gorsky also took time out from his 'vaccine' disaster to say that people may need to be vaccinated against 'Covid-19' each year. UK Health Secretary, the psychopath Matt Hancock, said additional 'boosters' would be available in the autumn of 2021. This is the trap of the 'vaccine passport'. The public will have to accept every last 'vaccine' they introduce, including for the fake 'variants', or it would cease to be valid. The only other way in some cases would be continuous testing with a test not testing for the 'virus' and what is on the swabs constantly pushed up your noise towards the brain every time?

'Vaccines' changing behaviour

I mentioned in the body of the book how I believed we would see gathering behaviour changes in the 'vaccinated' and I am already hearing such comments from the non-'vaccinated' describing behaviour changes in friends, loved ones and work colleagues. This will only increase as the self-replicating synthetic material and nanoparticles expand in body and brain. An article in the *Guardian* in 2016 detailed research at the University of Virginia in Charlottesville which developed a new method for controlling

brain circuits associated with complex animal behaviour. The method, dubbed 'magnetogenetics', involves genetically-engineering a protein called ferritin, which stores and releases iron, to create a magnetised substance – 'Magneto' – that can activate specific groups of nerve cells from a distance. This is claimed to be an advance on other methods of brain activity manipulation known as optogenetics and chemogenetics (the Cult has been developing methods of brain control for a long time). The ferritin technique is said to be non-invasive and able to activate neurons 'rapidly and reversibly'. In other words, human thought and perception. The article said that earlier studies revealed how nerve cell proteins 'activated by heat and mechanical pressure can be genetically engineered so that they become sensitive to radio waves and magnetic fields, by attaching them to an iron-storing protein called ferritin, or to inorganic paramagnetic particles'. Sensitive to radio waves and magnetic fields? You mean like 5G, 6G and 7G? This is the human-AI Smart Grid hive mind we are talking about. The *Guardian* article said:

> … the researchers injected Magneto into the striatum of freely behaving mice, a deep brain structure containing dopamine-producing neurons that are involved in reward and motivation, and then placed the animals into an apparatus split into magnetised and non-magnetised sections.
>
> Mice expressing Magneto spent far more time in the magnetised areas than mice that did not, because activation of the protein caused the striatal neurons expressing it to release dopamine, so that the mice found being in those areas rewarding. This shows that Magneto can remotely control the firing of neurons deep within the brain, and also control complex behaviours.

Make no mistake this basic methodology will be part of the 'Covid vaccine' cocktail and using magnetics to change brain function through electromagnetic field frequency activation. The Pentagon is developing a 'Covid vaccine' using ferritin. Magnetics would explain changes in behaviour and why videos are appearing across the Internet as I write showing how magnets stick to the skin at the point of the 'vaccine' shot. Once people take these 'vaccines' anything becomes possible in terms of brain function and illness which will be blamed on 'Covid-19' and 'variants'. Magnetic field manipulation would further explain why the non-'vaccinated' are reporting the same symptoms as the 'vaccinated' they interact with and why those symptoms are reported to decrease when not in their company. Interestingly 'Magneto', a 'mutant', is a character in the

Marvel Comic *X-Men* stories with the ability to manipulate magnetic fields and he believes that mutants should fight back against their human oppressors by any means necessary. The character was born Erik Lehnsherr to a Jewish family in Germany.

Cult-controlled courts

The European Court of Human Rights opened the door for mandatory 'Covid-19 vaccines' across the continent when it ruled in a Czech Republic dispute over childhood immunisation that legally enforced vaccination could be 'necessary in a democratic society'. The 17 judges decided that compulsory vaccinations did not breach human rights law. On the face of it the judgement was so inverted you gasp for air. If not having a vaccine infused into your body is not a human right then what is? Ah, but they said human rights law which has been specifically written to delete all human rights at the behest of the state (the Cult). Article 8 of the European Convention on Human Rights relates to the right to a private life. The crucial word here is *'except'*:

There shall be no interference by a public authority with the exercise of this right EXCEPT such as is in accordance with the law and is necessary in a democratic society in the interests of national security, public safety or the economic wellbeing of the country, for the prevention of disorder or crime, for the protection of health or morals, or for the protection of the rights and freedoms of others [My emphasis].

No interference *except* in accordance with the law means there *are* no 'human rights' *except* what EU governments decide you can have at their behest. 'As is necessary in a democratic society' explains that reference in the judgement and 'in the interests of national security, public safety or the economic well-being of the country, for the prevention of disorder or crime, for the protection of health or morals, or for the protection of the rights and freedoms of others' gives the EU a coach and horses to ride through 'human rights' and scatter them in all directions. The judiciary is not a check and balance on government extremism; it is a vehicle to enforce it. This judgement was almost laughably predictable when the last thing the Cult wanted was a decision that went against mandatory vaccination. Judges rule over and over again to benefit the system of which they are a part.

Vaccination disputes that come before them are invariably delivered in favour of doctors and authorities representing the view of the state which owns the judiciary. Oh, yes, and we have even had calls to stop putting 'Covid-19' on death certificates within 28 days of a 'positive test' because it is claimed the practice makes the 'vaccine' appear not to work. They are laughing at you.

The scale of madness, inhumanity and things to come was highlighted when those not 'vaccinated' for 'Covid' were refused evacuation from the Caribbean island of St Vincent during massive volcanic eruptions. Cruise ships taking residents to the safety of another island allowed only the 'vaccinated' to board and the rest were left to their fate. Even in life and death situations like this we see 'Covid' stripping people of their most basic human instincts and the insanity is even more extreme when you think that fake 'vaccine'-makers are not even claiming their body-manipulating concoctions stop 'infection' and 'transmission' of a 'virus' that doesn't exist. St Vincent Prime Minister Ralph Gonsalves said: 'The chief medical officer will be identifying the persons already vaccinated so that we can get them on the ship.' Note again the power of the chief medical officer who, like Whitty in the UK, will be answering to the World Health Organization. This is the Cult network structure that has overridden politicians who 'follow the science' which means doing what WHO-controlled 'medical officers' and 'science advisers' tell them. Gonsalves even said that residents who were 'vaccinated' after the order so they could board the ships would still be refused entry due to possible side effects such as 'wooziness in the head'. The good news is that if they were woozy enough in the head they could qualify to be prime minister of St Vincent.

Microchipping freedom

The European judgement will be used at some point to justify moves to enforce the 'Covid' DNA-manipulating procedure. Sandra Ro, CEO of the Global Blockchain Business Council, told a World Economic Forum event that she hoped 'vaccine passports' would help to 'drive forced consent and standardisation' of global digital identity schemes: 'I'm hoping with the desire and global demand for some sort of vaccine passport – so that people can get travelling and working again – [it] will drive forced consent, standardisation, and frankly, cooperation across the world.' The lady is

either not very bright, or thoroughly mendacious, to use the term ‘forced consent’. You do not ‘consent’ if you are forced – you *submit*. She was describing what the plan has been all along and that’s to enforce a digital identity on every human without which they could not function. ‘Vaccine passports’ are opening the door and are far from the end goal. A digital identity would allow you to be tracked in everything you do in cyberspace and this is the same technique used by Cult-owned China to enforce its social credit system of total control. The ultimate ‘passport’ is planned to be a microchip as my books have warned for nearly 30 years. Those nice people at the Pentagon working for the Cult-controlled Defense Advanced Research Projects Agency (DARPA) claimed in April, 2021, they have developed a microchip inserted under the skin to detect ‘asymptomatic Covid-19 infection’ before it becomes an outbreak and a ‘revolutionary filter’ that can remove the ‘virus’ from the blood when attached to a dialysis machine. The only problems with this are that the ‘virus’ does not exist and people transmitting the ‘virus’ with no symptoms is brain-numbing bullshit. This is, of course, not a ruse to get people to be microchipped for very different reasons. DARPA also said it was producing a one-stop ‘vaccine’ for the ‘virus’ and all ‘variants’. One of the most sinister organisations on Planet Earth is doing this? Better have it then. These people are insane because Wetiko that possesses them is insane.

Researchers from the Salk Institute in California announced they have created an embryo that is part human and part monkey. My books going back to the 1990s have exposed experiments in top secret underground facilities in the United States where humans are being crossed with animal and non-human ‘extraterrestrial’ species. They are now easing that long-developed capability into the public arena and there is much more to come given we are dealing with psychiatric basket cases. Talking of which – Elon Musk’s scientists at Neuralink trained a monkey to play Pong and other puzzles on a computer screen using a joystick and when the monkey made the correct move a metal tube squirted banana smoothie into his mouth which is the basic technique for training humans into unquestioning compliance. Two Neuralink chips were in the monkey’s skull and more than 2,000 wires ‘fanned out’ into its brain. Eventually the monkey played a video game purely with its brain waves. Psychopathic narcissist Musk said the ‘breakthrough’ was a step towards putting Neuralink chips into human

skulls and merging minds with artificial intelligence. *Exactly*. This man is so dark and Cult to his DNA.

World Economic Fascism (WEF)

The World Economic Forum is telling you the plan by the statements made at its many and various events. Cult-owned fascist YouTube CEO Susan Wojcicki spoke at the 2021 WEF Global Technology Governance Summit (see the name) in which 40 governments and 150 companies met to ensure 'the responsible design and deployment of emerging technologies'. Orwellian translation: 'Ensuring the design and deployment of long-planned technologies will advance the Cult agenda for control and censorship.' Freedom-destroyer and Nuremberg-bound Wojcicki expressed support for tech platforms like hers to censor content that is 'technically legal but could be harmful'. Who decides what is 'harmful'? She does and they do. 'Harmful' will be whatever the Cult doesn't want people to see and we have legislation proposed by the UK government that would censor content on the basis of 'harm' no matter if the information is fair, legal and provably true. Make that *especially* if it is fair, legal and provably true. Wojcicki called for a global coalition to be formed to enforce content moderation standards through automated censorship. This is a woman and mega-censor so self-deluded that she shamelessly accepted a 'free expression' award – *Wojcicki* – in an event sponsored by her own *YouTube*. They have no shame and no self-awareness.

You know that 'Covid' is a scam and Wojcicki a Cult operative when YouTube is censoring medical and scientific opinion purely on the grounds of whether it supports or opposes the Cult 'Covid' narrative. Florida governor Ron DeSantis compiled an expert panel with four professors of medicine from Harvard, Oxford, and Stanford Universities who spoke against forcing children and vaccinated people to wear masks. They also said there was no proof that lockdowns reduced spread or death rates of 'Covid-19'. Cult-gofer Wojcicki and her YouTube deleted the panel video 'because it included content that contradicts the consensus of local and global health authorities regarding the efficacy of masks to prevent the spread of Covid-19'. This 'consensus' refers to what the Cult tells the World Health Organization to say and the WHO tells 'local health authorities' to do. Wojcicki knows this, of course. The panellists pointed out

that censorship of scientific debate was responsible for deaths from many causes, but Wojcicki couldn't care less. She would not dare go against what she is told and as a disgrace to humanity she wouldn't want to anyway. The UK government is seeking to pass a fascist 'Online Safety Bill' to specifically target with massive fines and other means non-censored video and social media platforms to make them censor 'lawful but harmful' content like the Cult-owned Facebook, Twitter, Google and YouTube. What is 'lawful but harmful' would be decided by the fascist Blair-created Ofcom.

Another WEF obsession is a cyber-attack on the financial system and this is clearly what the Cult has planned to take down the bank accounts of everyone – except theirs. Those that think they have enough money for the Cult agenda not to matter to them have got a big lesson coming if they continue to ignore what is staring them in the face. The World Economic Forum, funded by Gates and fronted by Klaus Schwab, announced it would be running a 'simulation' with the Russian government and global banks of just such an attack called Cyber Polygon 2021. What they simulate – as with the 'Covid' Event 201 – they plan to instigate. The WEF is involved in a project with the Cult-owned Carnegie Endowment for International Peace called the WEF-Carnegie Cyber Policy Initiative which seeks to merge Wall Street banks, 'regulators' (I love it) and intelligence agencies to 'prevent' (arrange and allow) a cyber-attack that would bring down the global financial system as long planned by those that control the WEF and the Carnegie operation. The Carnegie Endowment for International Peace sent an instruction to First World War US President Woodrow Wilson not to let the war end before society had been irreversibly transformed.

The Wuhan lab diversion

As I close, the Cult-controlled authorities and lapdog media are systematically pushing 'the virus was released from the Wuhan lab' narrative. There are two versions – it happened by accident and it happened on purpose. Both are nonsense. The perceived existence of the never-shown-to-exist 'virus' is vital to sell the impression that there is actually an infective agent to deal with and to allow the endless potential for terrifying the population with 'variants' of a 'virus' that does not exist. The authorities at the time of writing are going with the 'by accident' while the

alternative media is promoting the ‘on purpose’. Cable news host Tucker Carlson who has questioned aspects of lockdown and ‘vaccine’ compulsion has bought the Wuhan lab story. ‘Everyone now agrees’ he said. Well, I don’t and many others don’t and the question is *why* does the system and its media suddenly ‘agree’? When the media moves as one unit with a narrative it is always a lie – witness the hour by hour mendacity of the ‘Covid’ era. Why would this Cult-owned combination which has unleashed lies like machine gun fire suddenly ‘agree’ to tell the truth??

Much of the alternative media is buying the lie because it fits the conspiracy narrative, but it’s the *wrong* conspiracy. The real conspiracy is that *there is no virus* and that is what the Cult is desperate to hide. The idea that the ‘virus’ was released by accident is ludicrous when the whole ‘Covid’ hoax was clearly long-planned and waiting to be played out as it was so fast in accordance with the Rockefeller document and Event 201. So they prepared everything in detail over decades and then sat around strumming their fingers waiting for an ‘accidental’ release from a bio-lab? *What??* It’s crazy. Then there’s the ‘on purpose’ claim. You want to circulate a ‘deadly virus’ and hide the fact that you’ve done so and you release it down the street from the highest-level bio-lab in China? I repeat – *What??* You would release it far from that lab to stop any association being made. But, no, we’ll do it in a place where the connection was certain to be made. Why would you need to scam ‘cases’ and ‘deaths’ and pay hospitals to diagnose ‘Covid-19’ if you had a real ‘virus’? What are sections of the alternative media doing believing this crap? Where were all the mass deaths in Wuhan from a ‘deadly pathogen’ when the recovery to normal life after the initial propaganda was dramatic in speed? Why isn’t the ‘deadly pathogen’ now circulating all over China with bodies in the street? Once again we have the technique of tell them what they want to hear and they will likely believe it. The alternative media has its ‘conspiracy’ and with Carlson it fits with his ‘China is the danger’ narrative over years. China *is* a danger as a global Cult operations centre, but not for this reason. The Wuhan lab story also has the potential to instigate conflict with China when at some stage the plan is to trigger a Problem-Reaction-Solution confrontation with the West. Question everything – *everything* – and especially when the media agrees on a common party line.

Third wave … fourth wave … fifth wave …

As the book went into production the world was being set up for more lockdowns and a ‘third wave’ supported by invented ‘variants’ that were increasing all the time and will continue to do so in public statements and computer programs, but not in reality. India became the new Italy in the ‘Covid’ propaganda campaign and we were told to be frightened of the new ‘Indian strain’. Somehow I couldn’t find it within myself to do so. A document produced for the UK government entitled ‘Summary of further modelling of easing of restrictions – Roadmap Step 2’ declared that a third wave was inevitable (of course when it’s in the script) and it would be the fault of children and those who refuse the health-destroying fake ‘Covid vaccine’. One of the computer models involved came from the Cult-owned *Imperial College* and the other from Warwick University which I wouldn’t trust to tell me the date in a calendar factory. The document states that both models presumed extremely high uptake of the ‘Covid vaccines’ and didn’t allow for ‘variants’. The document states: ‘The resurgence is a result of some people (mostly children) being ineligible for vaccination; others choosing not to receive the vaccine; and others being vaccinated but not perfectly protected.’ The mendacity takes the breath away. Okay, blame those with a brain who won’t take the DNA-modifying shots and put more pressure on children to have it as ‘trials’ were underway involving children as young as six months with parents who give insanity a bad name. Massive pressure is being put on the young to have the fake ‘vaccine’ and child age consent limits have been systematically lowered around the world to stop parents intervening. Most extraordinary about the document was its claim that the ‘third wave’ would be driven by ‘the resurgence in both hospitalisations and deaths … dominated by *those that have received two doses of the vaccine*, comprising around 60-70% of the wave respectively’. The predicted peak of the ‘third wave’ suggested 300 deaths per day with 250 of them *fully ‘vaccinated’ people*. How many more lies do acquiescers need to be told before they see the obvious? Those who took the jab to ‘protect themselves’ are projected to be those who mostly get sick and die? So what’s in the ‘vaccine’? The document went on:

It is possible that a summer of low prevalence could be followed by substantial increases in incidence over the following autumn and winter. Low prevalence in late summer should not be taken as an

indication that SARS-CoV-2 has retreated or that the population has high enough levels of immunity to prevent another wave.

They are telling you the script and while many British people believed 'Covid' restrictions would end in the summer of 2021 the government was preparing for them to be ongoing. Authorities were awarding contracts for 'Covid marshals' to police the restrictions with contracts starting in July, 2021, and going through to January 31st, 2022, and the government was advertising for 'Media Buying Services' to secure media propaganda slots worth a potential £320 million for 'Covid-19 campaigns' with a contract not ending until March, 2022. The recipient – via a list of other front companies – was reported to be American media marketing giant Omnicom Group Inc. While money is no object for 'Covid' the UK waiting list for all other treatment – including life-threatening conditions – passed 4.5 million. Meantime the Cult is seeking to control all official 'inquiries' to block revelations about what has really been happening and why. It must not be allowed to – we need Nuremberg jury trials in every country. The cover-up doesn't get more obvious than appointing ultra-Zionist professor Philip Zelikow to oversee two dozen US virologists, public health officials, clinicians, former government officials and four American 'charitable foundations' to 'learn the lessons' of the 'Covid' debacle. The personnel will be those that created and perpetuated the 'Covid' lies while Zelikow is the former executive director of the 9/11 Commission who ensured that the truth about those attacks never came out and produced a report that must be among the most mendacious and manipulative documents ever written – see *The Trigger* for the detailed exposure of the almost unimaginable 9/11 story in which Sabbatians can be found at every level.

Passive no more

People are increasingly challenging the authorities with amazing numbers of people taking to the streets in London well beyond the ability of the Face-Nappies to stop them. Instead the Nappies choose situations away from the mass crowds to target, intimidate, and seek to promote the impression of 'violent protestors'. One such incident happened in London's Hyde Park. Hundreds of thousands walking through the streets in protest against 'Covid' fascism were ignored by the Cult-owned BBC and most of

the rest of the mainstream media, but they delighted in reporting how police were injured in ‘clashes with protestors’. The truth was that a group of people gathered in Hyde Park at the end of one march when most had gone home and they were peacefully having a good time with music and chat. Face-Nappies who couldn’t deal with the full-march crowd then waded in with their batons and got more than they bargained for. Instead of just standing for this criminal brutality the crowd used their numerical superiority to push the Face-Nappies out of the park. Eventually the Nappies turned and ran. Unfortunately two or three idiots in the crowd threw drink cans striking two officers which gave the media and the government the image they wanted to discredit the 99.9999 percent who were peaceful. The idiots walked straight into the trap and we must always be aware of potential agent provocateurs used by the authorities to discredit their targets.

This response from the crowd – the can people apart – must be a turning point when the public no longer stand by while the innocent are arrested and brutally attacked by the Face-Nappies. That doesn’t mean to be violent, that’s the last thing we need. We’ll leave the violence to the Face-Nappies and government. But it does mean that when the Face-Nappies use violence against peaceful people the numerical superiority is employed to stop them and make citizen’s arrests or Common Law arrests for a breach of the peace. The time for being passive in the face of fascism is over.

We are the many, they are the few, and we need to make that count before there is no freedom left and our children and grandchildren face an ongoing fascist nightmare.

COME ON PEOPLE – IT’S TIME.

One final thought …

The power of love
A force from above
Cleaning my soul
Flame on burn desire

Love with tongues of fire
Purge the soul
Make love your goal

I'll protect you from the hooded claw
Keep the vampires from your door
When the chips are down I'll be around
With my undying, death-defying
Love for you

Envy will hurt itself
Let yourself be beautiful
Sparkling love, flowers
And pearls and pretty girls
Love is like an energy
Rushin' rushin' inside of me

This time we go sublime
Lovers entwine, divine, divine,
Love is danger, love is pleasure
Love is pure – the only treasure

I'm so in love with you
Purge the soul
Make love your goal

The power of love
A force from above
Cleaning my soul

The power of love
A force from above
A sky-scraping dove

Flame on burn desire
Love with tongues of fire
Purge the soul
Make love your goal

Frankie Goes To Hollywood

Appendix

Cowan-Kaufman-Morell Statement on Virus Isolation (SOVI)

Isolation: The action of isolating; the fact or condition of being isolated or standing alone; separation from other things or persons; solitariness
Oxford English Dictionary

The controversy over whether the SARS-CoV-2 virus has ever been isolated or purified continues. However, using the above definition, common sense, the laws of logic and the dictates of science, any unbiased person must come to the conclusion that the SARS-CoV-2 virus has never been isolated or purified. As a result, no confirmation of the virus' existence can be found. The logical, common sense, and scientific consequences of this fact are:

- the structure and composition of something not shown to exist can't be known, including the presence, structure, and function of any hypothetical spike or other proteins;
- the genetic sequence of something that has never been found can't be known;
- "variants" of something that hasn't been shown to exist can't be known;
- it's impossible to demonstrate that SARS-CoV-2 causes a disease called Covid-19.

In as concise terms as possible, here's the proper way to isolate, characterize and demonstrate a new virus. First, one takes samples (blood, sputum, secretions) from many people (e.g. 500) with symptoms which are unique and specific enough to characterize an illness. Without mixing these samples with ANY tissue or products that also contain genetic material, the virologist macerates, filters and ultracentrifuges i.e. *purifies* the specimen. This common virology technique, done for decades to isolate bacteriophages[1] and so-called giant viruses in every virology lab, then allows the virologist to demonstrate with electron microscopy thousands of identically sized and shaped particles. These particles are the isolated and purified virus.

These identical particles are then checked for uniformity by physical and/or microscopic techniques. Once the purity is determined, the particles may be further characterized. This would include examining the structure, morphology, and chemical composition of the particles. Next, their genetic makeup is characterized by extracting the genetic material directly from the purified particles and using genetic-sequencing techniques, such as Sanger sequencing, that have also been around for decades. Then one does an analysis to confirm that these uniform particles are exogenous (outside) in origin as a virus is conceptualized to be, and not the normal breakdown products of dead and dying tissues.[2] (As of May 2020, we know that virologists have no way to determine whether the particles they're seeing are viruses or just normal break-down products of dead and dying tissues.)[3]

1 Isolation, characterization and analysis of bacteriophages from the haloalkaline lake Elmenteita, KenyaJuliah Khayeli Akhwale et al, PLOS One, Published: April 25, 2019. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0215734 – accessed 2/15/21

2 "Extracellular Vesicles Derived From Apoptotic Cells: An Essential Link Between Death and Regeneration," Maojiao Li1 et al, Frontiers in Cell and Developmental Biology, 2020 October 2. https://www.frontiersin.org/articles/10.3389/fcell.2020.573511/full – accessed 2/15/21

3 "The Role of Extraellular Vesicles as Allies of HIV, HCV and SARS Viruses," Flavia Giannessi, et al, Viruses, 2020 May

If we have come this far then we have fully isolated, characterized, and genetically sequenced an exogenous virus particle. However, we still have to show it is causally related to a disease. This is carried out by exposing a group of healthy subjects (animals are usually used) to this isolated,

purified virus in the manner in which the disease is thought to be transmitted. If the animals get sick with the same disease, as confirmed by clinical and autopsy findings, one has now shown that the virus actually causes a disease. This demonstrates infectivity and transmission of an infectious agent.

None of these steps has even been attempted with the SARS-CoV-2 virus, nor have all these steps been successfully performed for any so-called pathogenic virus. Our research indicates that a single study showing these steps does not exist in the medical literature.

Instead, since 1954, virologists have taken unpurified samples from a relatively few people, often less than ten, with a similar disease. They then minimally process this sample and inoculate this unpurified sample onto tissue culture containing usually four to six other types of material – all of which contain identical genetic material as to what is called a "virus." The tissue culture is starved and poisoned and naturally disintegrates into many types of particles, some of which contain genetic material. Against all common sense, logic, use of the English language and scientific integrity, this process is called "virus isolation." This brew containing fragments of genetic material from many sources is then subjected to genetic analysis, which then creates in a computer-simulation process the alleged sequence of the alleged virus, a so called in silico genome. At no time is an actual virus confirmed by electron microscopy. At no time is a genome extracted and sequenced from an actual virus. This is scientific fraud.

The observation that the unpurified specimen — inoculated onto tissue culture along with toxic antibiotics, bovine fetal tissue, amniotic fluid and other tissues — destroys the kidney tissue onto which it is inoculated is given as evidence of the virus' existence and pathogenicity. This is scientific fraud.

From now on, when anyone gives you a paper that suggests the SARS-CoV-2 virus has been isolated, please check the methods sections. If the

researchers used Vero cells or any other culture method, you know that their process was not isolation. You will hear the following excuses for why actual isolation isn't done:

1. There were not enough virus particles found in samples from patients to analyze.

2. Viruses are intracellular parasites; they can't be found outside the cell in this manner.

If No. 1 is correct, and we can't find the virus in the sputum of sick people, then on what evidence do we think the virus is dangerous or even lethal? If No. 2 is correct, then how is the virus spread from person to person? We are told it emerges from the cell to infect others. Then why isn't it possible to find it?

Finally, questioning these virology techniques and conclusions is not some distraction or divisive issue. Shining the light on this truth is essential to stop this terrible fraud that humanity is confronting. For, as we now know, if the virus has never been isolated, sequenced or shown to cause illness, if the virus is imaginary, then why are we wearing masks, social distancing and putting the whole world into prison?

Finally, if pathogenic viruses don't exist, then what is going into those injectable devices erroneously called "vaccines," and what is their purpose? This scientific question is the most urgent and relevant one of our time.

We are correct. The SARS-CoV2 virus does not exist.

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Dr. Andrew Kaufman, MD

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ICKONIC
THE ALTERNATIVE

Ickonic is something that has been a dream of mine for the last 5 years, growing up around alternative information I have always had a natural interest in what is going on in the World and what could I do to make it better.
Across the range of subjects and positions of influence occupied mainly by people who don't strive to make things better it's the Media that I have always found the most frustrating and fascinating. Mainly because if the Media did their Jobs properly then so much of the negative things happening in the World simply would not be able to happen, because they would be exposed within a heartbeat.
Free Press and the Opportunities that the internet could have given would mean that the Media are able to expose things like never before and hold people to account for their actions. As we all know there are 'Untouchables' that walk among us, people the Media simply won't touch, expose or investigate and that leads to the dark underworlds that infest the establishment the World over.
Well I say enough, it's time for something different, a different kind of Media, where no one is off limits from exposing and investigating. All we're interested in at Ickonic is the truth of what is really going on in the World on whichever subject we're covering.
We hope you enjoy what we have created and take something away from the platform, we aim to deliver information that's informative and most importantly self-empowering, you're not a little person, you're part of something much bigger than that and its time we as a collective race began to understand that and look to the future as ours to take.

It's time...

Jaymie Icke - Founder Ickonic Alternative Media.

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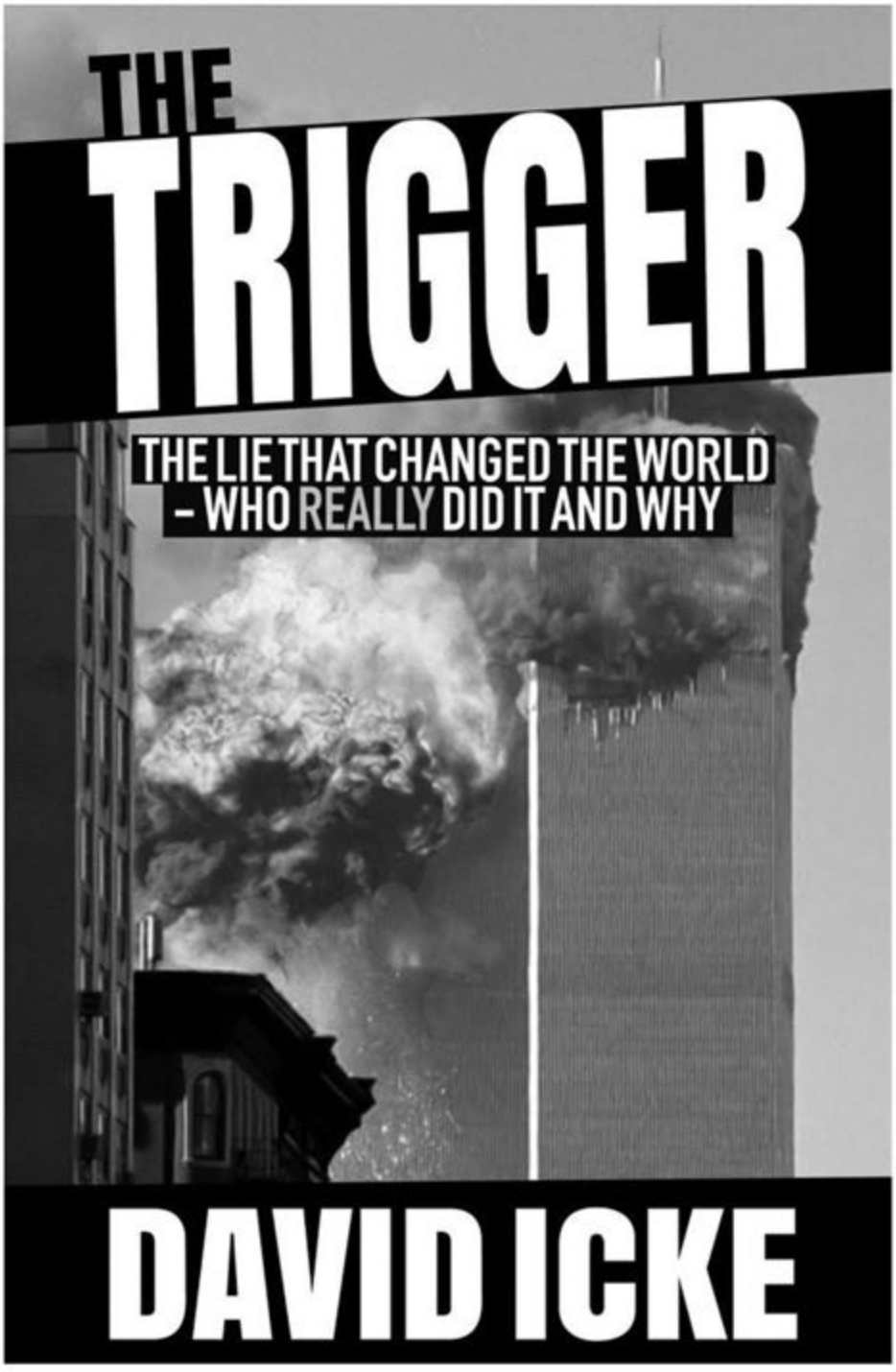
THE
TRIGGER
THE LIE THAT CHANGED THE WORLD
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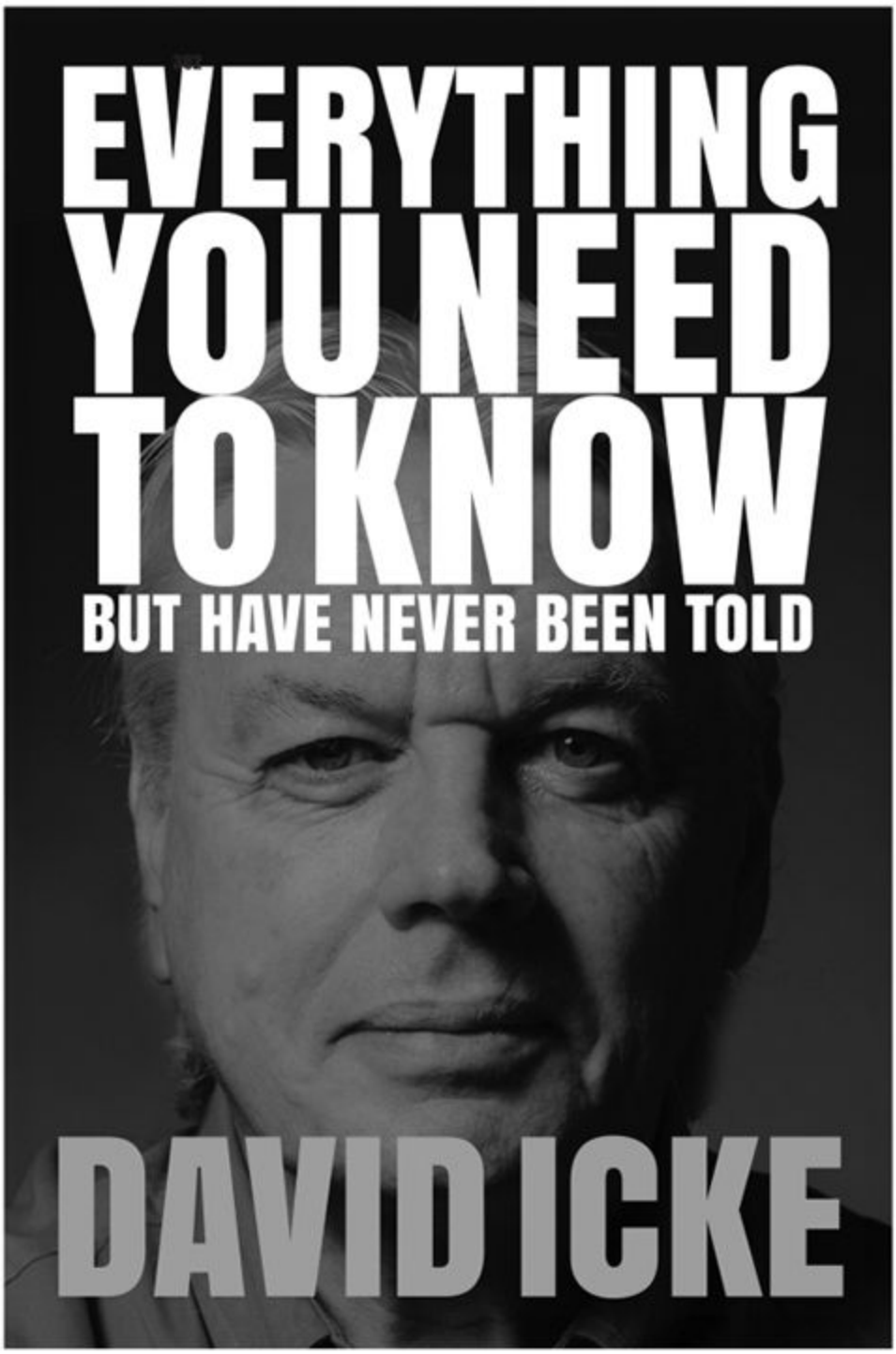
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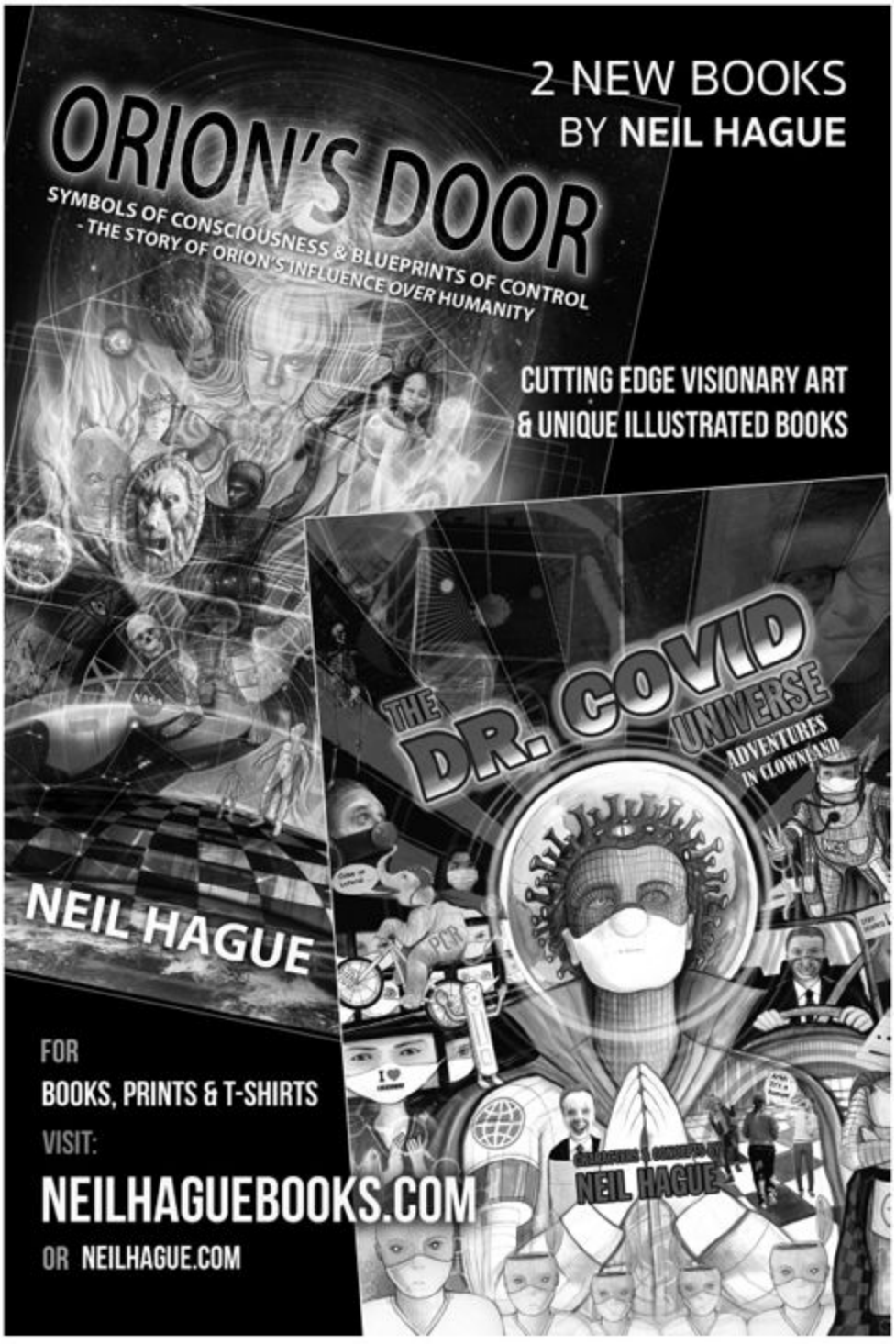
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