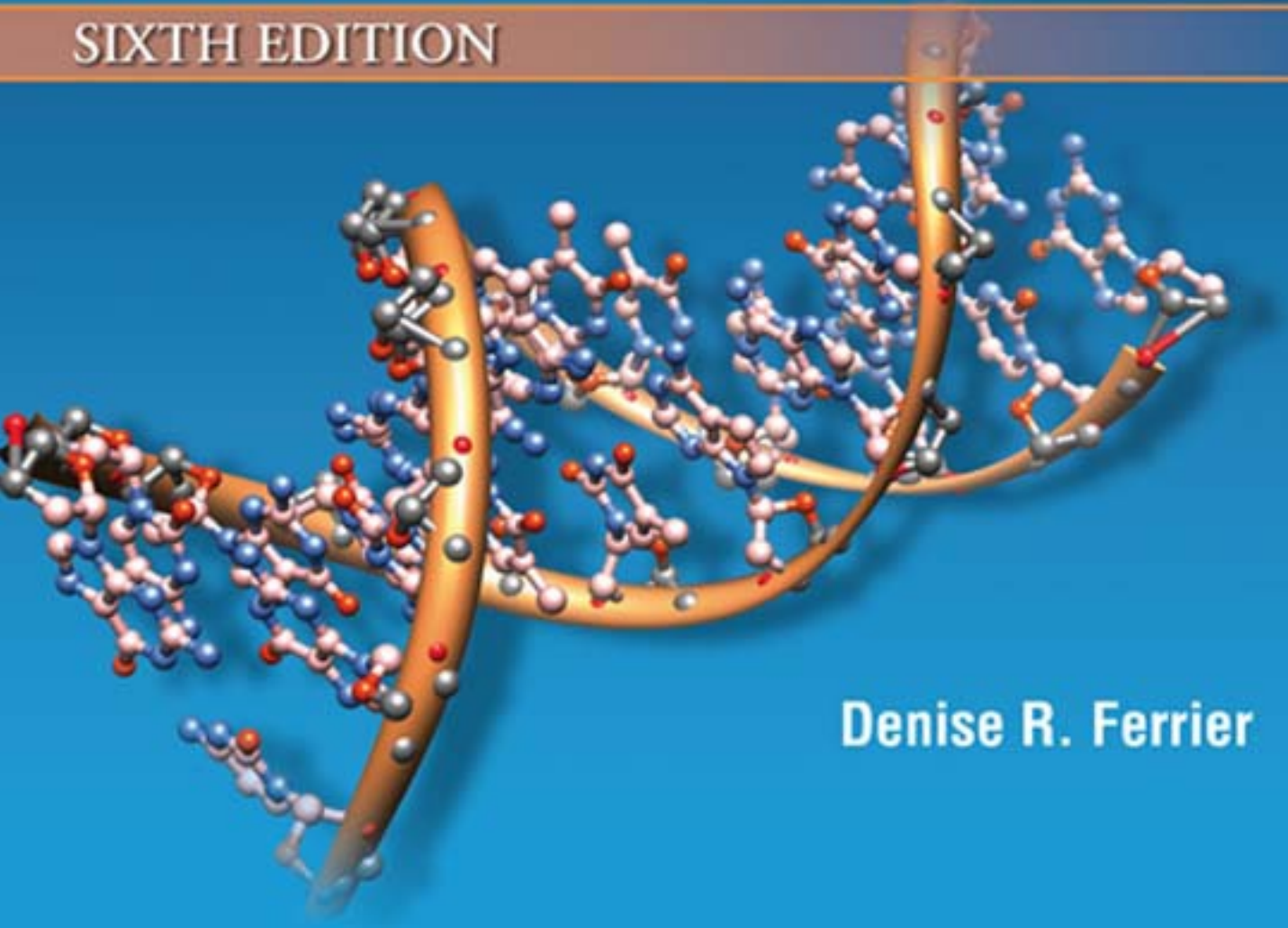
Series Editor:
Richard A. Harvey

Lippincott's Illustrated Reviews

Biochemistry

SIXTH EDITION



Denise R. Ferrier

Wolters Kluwer Health | Lippincott Williams & Wilkins

Lippincott's Illustrated Reviews: Biochemistr

Sixth Edition

Lippincott's Illustrated Reviews: Biochemistr

Sixth Edition

Denise R. Ferrier, PhD
Professor
Department of Biochemistry and Molecular Biology
Drexel University College of Medicine
Philadelphia, Pennsylvania

Health
Philadelphia • Baltimore • New York • London
Buenos Aires • Hong Kong • Sydney • Tokyo

Acquisitions Editor: Susan Rhyner
Product Manager: Angela Collins
Development Editor: Kelly Horvath
Marketing Manager: Joy Fisher-Williams
Production Manager: David Saltzberg
Art and Page Design: Debbie McQuade
Cover Design: Holly McLaughlin

Sixth Edition

351 West Camden Street
Baltimore, MD 21201

Two Commerce Square; 2001 Market Street
Philadelphia, PA 19103

Printed in China

9 8 7 6 5 4 3 2 1

Library of Congress Cataloging-in-Publication Data

Ferrier, Denise R.
Biochemistry / Denise R. Ferrier. -- 6th ed.
p. ; cm. -- (Lippincott's illustrated reviews)
Rev. ed. of: Biochemistry / Richard A. Harvey, Denise R. Ferrier. 5th ed. c2011.
Includes bibliographical references and index.
ISBN 978-1-4511-7562-2 (alk. paper)
I. Title. II. Series: Lippincott's illustrated reviews.
[DNLM: 1. Biochemistry--Examination Questions. QU 18.2]

612.3'9--dc23

2012025941

the professional responsibility of the practitioner; the clinical treatments described and recommended may not be considered absolute and universal recommendations.

The authors, editors, and publisher have exerted every effort to ensure that drug selection and dosage set forth in this text are in accordance with the current recommendations and practice at the time of publication. However, in view of ongoing research, changes in government regulations, and the constant flow of information relating to drug therapy and drug reactions, the reader is urged to check the package insert for each drug for any change in indications and dosage and for added warnings and precautions. This is particularly important when the recommended agent is a new or infrequently employed drug.

Some drugs and medical devices presented in this publication have Food and Drug Administration (FDA) clearance for limited use in restricted research settings. It is the responsibility of the health care provider to ascertain the FDA status of each drug or device planned for use in their clinical practice.

To purchase additional copies of this book, call our customer service department at **(800) 638-3030** or fax orders to **(301) 223-2320**. International customers should call **(301) 223-2300**.

Visit Lippincott Williams & Wilkins on the Internet: http://www.lww.com. Lippincott Williams & Wilkins customer service representatives are available from 8:30 am to 6:00 pm, EST.

Acknowledgments

I am grateful to my colleagues at Drexel University College of Medicine who generously shared their expertise to help make this book as accurate and as useful to medical students as possible. I am particularly appreciative of the many helpful comments of Dr. Åke Rökaeus of the Karolinska Institute as they have enhanced the accuracy of this work. In addition, the author thanks Dr. Susan K. Fried and Dr. Richard B. Horenstein for their valuable contributions to the Obesity chapter in previous editions of this text. A special thank you to Dr. Alan Katz for his helpful comments on the clinical aspects of the cases in the Appendix. Ms. Barbara Engle was an invaluable sounding board throughout the process.

The editors and production staff of Lippincott Williams & Wilkins were an important source of encouragement. I particularly want to acknowledge the contributions of Susan Ryner, the Acquisitions Editor, and Angela Collins, the Managing Editor. Many thanks are due to Kelly Horvath, Development Editor, for her assistance in the final editing of this book. I also want to thank Deborah McQuade for her work in the assembly of the 6th edition.

This book is dedicated to my husband John, whose loving support made the task possible; to my students, who have taught me so much over the last 20 years; and to Richard Harvey and the late Pamela Champe, who helped me develop as an author.

Contents

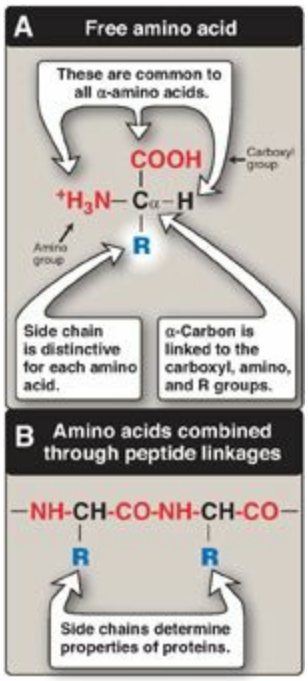
UNIT VI: Storage and Expression of Genetic Information

Amino Acids 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 plasma albumin, shuttle 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. 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.

Figure 1.1 Structural features of amino acids (shown in their fully protonated form).



II. STRUCTURE

Although more than 300 different amino acids have been described in nature, only 20 are commonly found as constituents of mammalian proteins. [Note: These are the only amino acids that are coded for by DNA, the genetic material in the cell (see p. 395).] 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 ("R group") bonded to the α-carbon atom (Figure 1.1A). At physiologic pH (approximately 7.4), the carboxyl group is dissociated, forming the negatively charged carboxylate ion ($-COO^-$), and the amino group is protonated ($-NH_3^+$). 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 formation (Figure 1.1B). Thus, it is the nature of the side chains that ultimately dictates the role an amino acid plays in a protein. It is, therefore, useful to classify the amino acids according to the properties of their side chains, that is, whether they are nonpolar (have an even distribution of electrons) or polar (have an uneven distribution of electrons, such as acids and bases) as shown in Figures 1.2 and 1.3.

A. Amino acids with nonpolar side chains

Each of these amino acids has a nonpolar side chain that does not gain or lose protons or participate in hydrogen or ionic bonds (see Figure 1.2). The side chains of these amino acids can be thought of as "oily" or lipid-like, a property that promotes hydrophobic inter-actions (see Figure 2.10, p. 19).

1. **Location of nonpolar amino acids in proteins:** In proteins found in aqueous solutions (a polar environment) the side chains of the nonpolar amino acids tend to cluster together in the interior of the protein (Figure 1.4). This phenomenon, known as the hydrophobic effect, is the result of the hydrophobicity of the nonpolar R groups, which act much like droplets of oil that coalesce in an aqueous environment. The nonpolar R groups, thus, fill up the interior of the folded protein and help give it its three-dimensional shape. However, for proteins that are located in a hydrophobic environment, such as a membrane, the nonpolar R groups are found on the outside surface of the protein, interacting with the lipid environment see Figure 1.4. The importance of these hydrophobic interactions in stabilizing protein structure is discussed on p. 19.

Figure 1.2 Classification of the 20 amino acids commonly found in proteins, 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 print. The pK values for the α-carboxyl and α-amino groups of the nonpolar amino acids are similar to those shown for glycine.

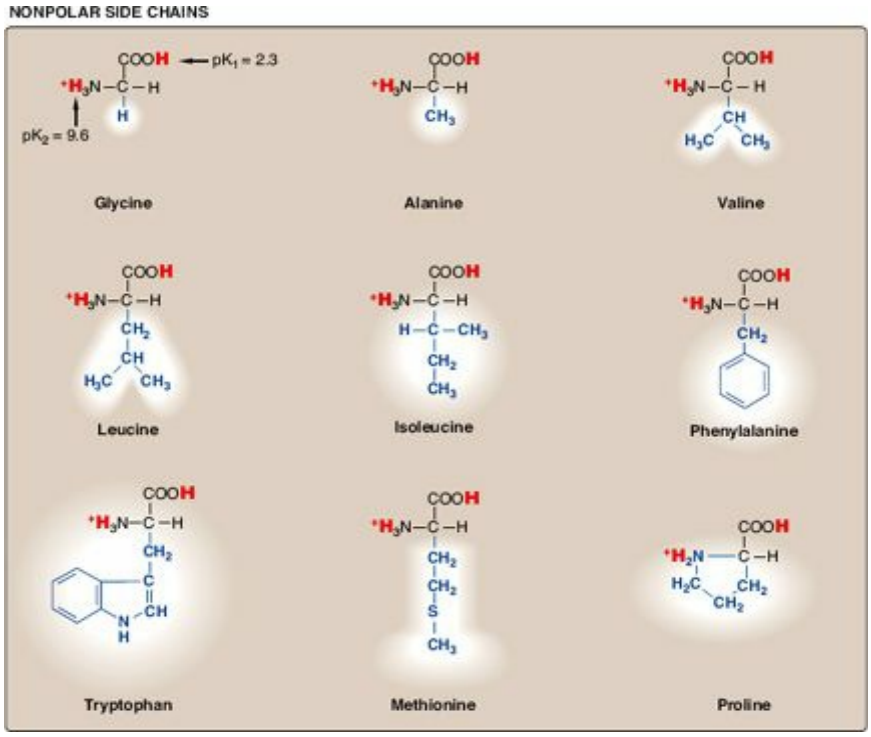


Figure 1.3 Classification of the 20 amino acids commonly found in proteins, according to the charge and polarity of their side chains at acidic pH (continued from Figure 1.2).

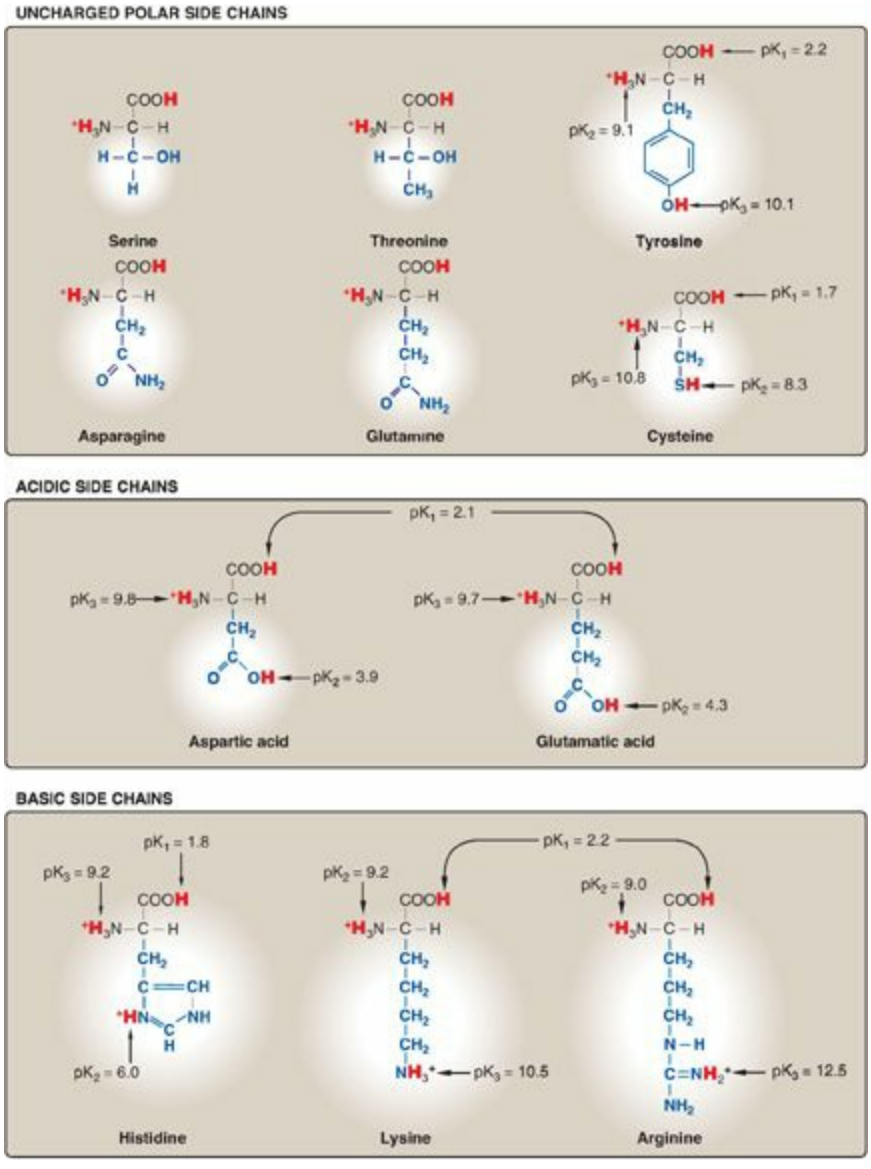
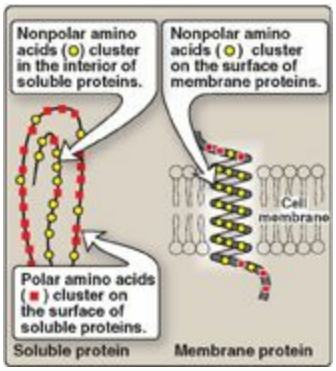
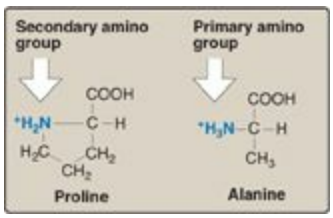


Figure 1.4 Location of nonpolar amino acids in soluble and membrane proteins.



> Sickle cell anemia, a sickling disease of red blood cells, results from the replacement of polar glutamate with nonpolar valine at the sixth position in the β subunit of hemoglobin (see p. 36).

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.



2. **Proline:** Proline differs from other amino acids in that its side chain and α-amino N form a rigid, five-membered ring structure (Figure 1.5). Proline, then, has a secondary (rather than a primary) amino group. It is frequently referred to as an "imino acid." The unique geometry of proline contributes to the formation of the fibrous structure of collagen (see p. 45) and often interrupts the α-helices found in globular proteins (see p. 26).

B. Amino acids with uncharged polar side chains

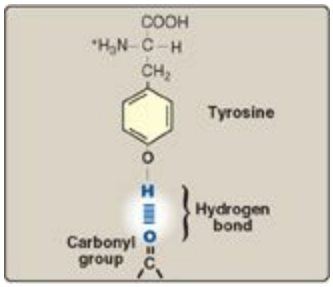
These amino acids have zero net charge at physiologic pH, although the side chains of cysteine and tyrosine can lose a proton at an alkaline pH (see Figure 1.3). Serine, threonine, and tyrosine each contain a polar hydroxyl group that can participate in hydrogen bond formation (Figure 1.6). The side 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:** The side chain of cysteine contains a sulfhydryl (thiol) group (–SH), which is an important component of 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 disulfide-linked cysteines are referred to as "cystine." (See p. 19 for a further discussion of disulfide bond formation.)

> Many extracellular proteins are stabilized by disulfide bonds. Albumin, a blood protein that functions as a transporter for a variety of molecules, is an example.

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



2. **Side chains as sites of attachment for other compounds:** The polar hydroxyl group of serine; threonine; and, rarely, tyrosine, can serve as a site of attachment for structures such as a phosphate group. 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 p. 165).

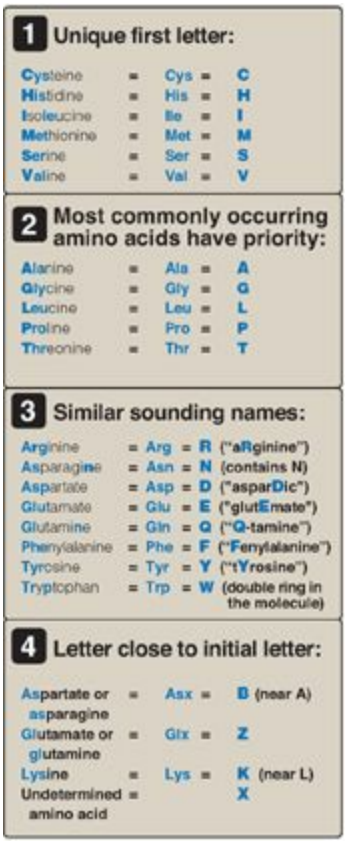
C. Amino acids with acidic side chains

The amino acids aspartic 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 ($–COO^–$). They are, therefore, called aspartate or glutamate to emphasize that these amino acids are negatively charged at physiologic pH (see Figure 1.3).

D. Amino acids with basic side chains

The side chains of the basic amino acids accept protons (see Figure 1.3). At physiologic pH, the R groups of lysine and arginine are fully ionized and positively charged. In contrast, histidine is weakly basic, and the free amino acid is 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 is an important property of histidine that contributes to the buffering role it plays in the functioning of proteins such as hemoglobin (see p. 31). [Note: Histidine is the only amino acid with a side chain that can ionize within the physiologic pH range.]

Figure 1.7 Abbreviations and symbols for the commonly occurring amino acids.



E. Abbreviations and symbols for commonly occurring amino acids

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

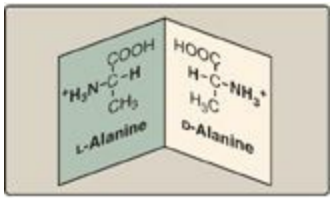
1. **Unique first letter:** If only one amino acid begins with a given letter, then that letter is used as its symbol. For example, V = valine.

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, or W = tryptophan ("twyptophan" as Elmer Fudd would say).

4. **Letter close to initial letter:** For the remaining amino acids, a one-letter symbol is assigned that is as 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, and X is assigned to an unidentified amino acid.

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



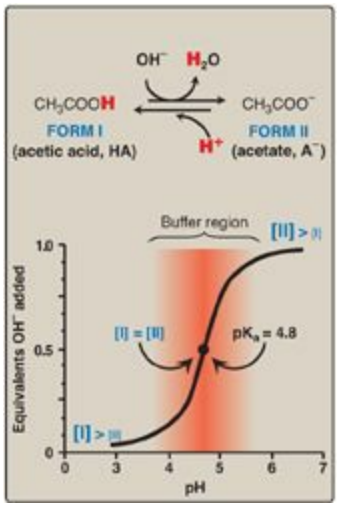
F. Optical properties of amino acids

The α-carbon of an amino acid is attached to four different chemical groups (asymmetric) and is, therefore, a chiral, or optically active carbon atom. Glycine is the exception because its α-carbon has two hydrogen substituents. Amino acids with an asymmetric center at the α-carbon can exist in two forms, designated D and L, that are mirror images of each other (Figure 1.8). The two forms in each pair are termed stereoisomers, optical isomers, or enantiomers. All amino acids found in proteins are of the L configuration. However, D-amino acids are found in some antibiotics and in bacterial cell walls. (See p. 252 for a discussion of D-amino acids.)

III. ACIDIC AND BASIC PROPERTIES OF AMINO ACIDS

Amino acids in 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. Recall that acids may be defined as proton donors and bases as proton acceptors. Acids (or bases) described as "weak" ionize to only a limited extent. The concentration of protons in aqueous solution is expressed as pH, where pH = log 1/[H^+] or –log [H^+]. The quantitative relationship between the pH of the solution and concentration of a weak acid (HA) and its conjugate base ($A^–$) is described by the Henderson-Hasselbalch equation.

Figure 1.9 Titration curve of acetic acid.



A. Derivation of the equation

Consider the release of a proton by a weak acid represented by HA:

$$\underset{\text{weak acid}}{HA} \rightleftarrows \underset{\text{proton}}{H^+} + \underset{\text{salt form or conjugate base}}{A^-}$$

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

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

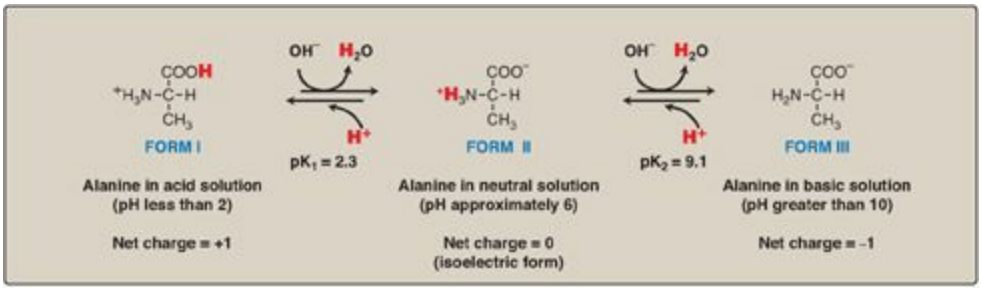
[Note: The larger the K_a, the stronger the acid, because most of the HA has dissociated into H^+ and $A^–$. Conversely, the smaller the K_a, the less acid has dissociated and, therefore, the weaker the acid.] By solving for the [H^+] in the above equation, taking the logarithm of both sides of the equation, multiplying both sides of the equation by –1, and substituting pH = –log [H^+] and pK_a = –log K_a, we obtain the Henderson-Hasselbalch equation:

$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$

B. Buffers

A buffer is a solution that resists change in pH following the addition of an acid or base. A buffer can be created by mixing a weak acid (HA) with its conjugate base (A^-). If an acid such as HCl is added to a buffer, A^- can neutralize it, being converted to HA in the process. If a base is added, HA can 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 pK_a of 4.8 resists a change in pH from pH 3.8 to 5.8, with maximum buffering at pH 4.8. At pH values less than the pK_a, the protonated acid form (CH_3–COOH) is the predominant species in solution. At pH values greater than the pK_a, the deprotonated base form (CH_3–COO^-) is the predominant species.

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



C. Titration of an amino acid

1. **Dissociation of the carboxyl group:** The titration curve of an amino acid can be analyzed in the same way as described for acetic acid. Consider alanine, for example, which contains an ionizable α-carboxyl and α-amino group. [Note: Its –CH_3 R group is nonionizable.] At a low (acidic) pH, both of these groups are protonated (shown in Figure 1.10). As the pH of the solution is raised, the –COOH group of form I can dissociate by donating a proton to the medium. The release of a proton results in the formation of the carboxylate group, –COO^-. This structure is shown as form II, which is the dipolar form of the molecule (see Figure 1.10). This form, also called a zwitterion, is the isoelectric form of alanine, that is, it has an overall (net) charge of zero.

2. **Application of the Henderson-Hasselbalch equation:** 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 in the same way as described for acetic acid:

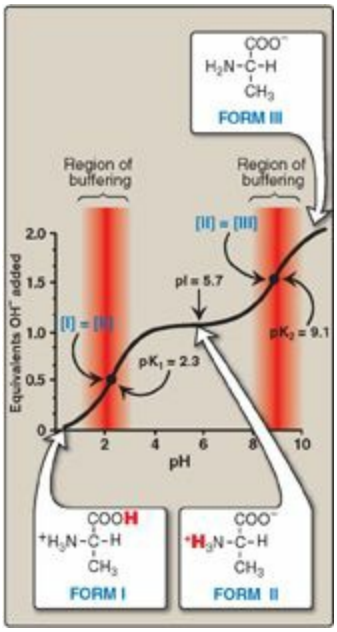
$$K_1 = \frac{[H^+][II]}{[I]}$$

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

$$pH = pK_1 + \log \frac{[II]}{[I]}$$

3. **Dissociation of the amino group:** The second titratable group of alanine is the amino (–NH_3^+) group shown in Figure 1.10. This is a much weaker acid than the –COOH group and, therefore, has a much smaller dissociation constant, K_2. [Note: Its pK_a is, therefore, larger.] Release of a proton from the protonated amino group of form II results in the fully deprotonated form of alanine, form III (see Figure 1.10).

Figure 1.11 The titration curve of alanine.



4. **pKs of alanine:** The sequential dissociation of protons from the carboxyl and amino groups of alanine is summarized in Figure 1.10. Each titratable group has a pK_a that is numerically equal to the pH at which exactly one half of the protons 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 approximately 2, whereas that of the α-amino is approximately 9.]

5. **Titration curve of alanine:** By applying the Henderson-Hasselbalch equation to each dissociable acidic 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 completely deprotonated form (III). Note the following:

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

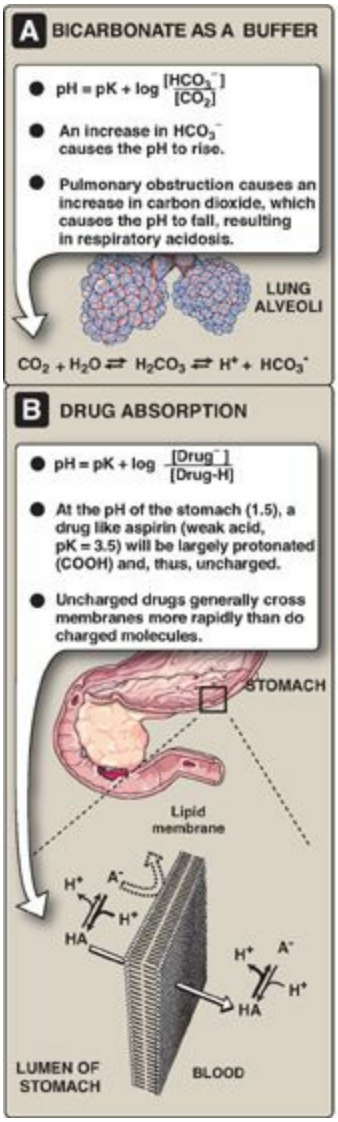
   b. **When pH = pK:** When the pH is equal to pK_1 (2.3), equal amounts of forms I and II of alanine exist in solution. When the pH is equal to pK_2 (9.1), equal amounts of forms II and III are present in solution.

   c. **Isoelectric point:** 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, in which the sum of the positive charges equals the sum of the negative charges. For an amino acid, such as alanine, that has 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).

> Separation of plasma proteins by charge typically is done at a pH above the pI of the major proteins. Thus, 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.

6. **Net charge of amino acids at neutral pH:** At physiologic pH, amino acids have a negatively charged group ($-\mathbf{I}COO^-$) and a positively charged group ($-\mathbf{I}NH_3^+$), both attached to the α-carbon. [Note: 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 defined as amphoteric and are referred to as ampholytes (amphoteric electrolytes).

Figure 1.12 The Henderson-Hasselbalch equation is used to predict: A, changes in pH as the concentrations of HCO_3^- or CO_2 are altered, or B, the ionic forms of drugs.



D. Other applications of the Henderson-Hasselbalch equation

The Henderson-Hasselbalch equation can be used to calculate how the pH of a physiologic solution responds to changes in the concentration of a weak acid and/or its corresponding "salt" form. For example, in the bicarbonate buffer system, the Henderson-Hasselbalch equation predicts how shifts in the bicarbonate ion concentration, $[HCO_3^-]$, and CO_2 influence pH (Figure 1.12A). The equation is also useful for calculating the abundance of ionic forms of acidic and basic drugs. For example, most drugs are either weak acids or weak bases (Figure 1.12B). Acidic drugs (HA) release a proton (H^+), causing a charged anion (A^-) to form.

$$HA \rightleftarrows H^+ + A^-$$

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).

$$BH^+ \rightleftarrows B + H^+$$

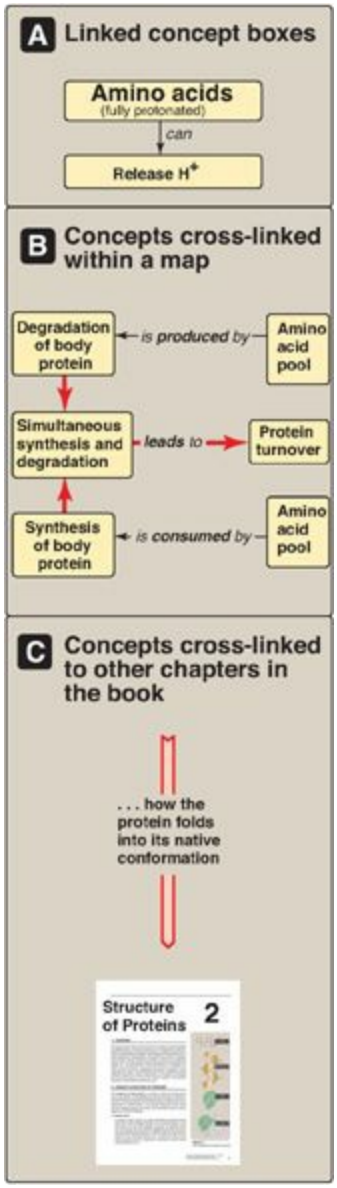
A drug passes through membranes more readily if it is uncharged. Thus, for a weak acid, such as aspirin, the uncharged HA can permeate through membranes, but A^-

cannot. For a weak base, such as morphine, the uncharged form, B, penetrates through the cell membrane, but BH^+ does not. Therefore, the effective concentration of the permeable form of each drug at its absorption site is determined by the relative concentrations of the charged (impermeant) and uncharged (permeant) forms. The ratio between the two forms is determined by the pH at the site of absorption, and by the strength of the weak acid or base, which is represented by the pK_a of the ionizable group. The Henderson-Hasselbalch equation is useful in determining how much drug is found on either side of a membrane that separates two compartments that differ in pH, for example, the stomach (pH 1.0–1.5) and blood plasma (pH 7.4).

IV. 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. Details provided to enrich understanding of these concepts inadvertently turn into distractions. What seems to be missing is a road map—a guide that provides the student with an understanding of how various topics fit together to make sense. Therefore, a series of biochemical concept maps have been created to graphically illustrate relationships between ideas presented in a chapter and 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 hierarchic 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 integrate new information into knowledge they already possess.

Figure 1.13 Symbols used in concept maps.



A. How is a concept map constructed?

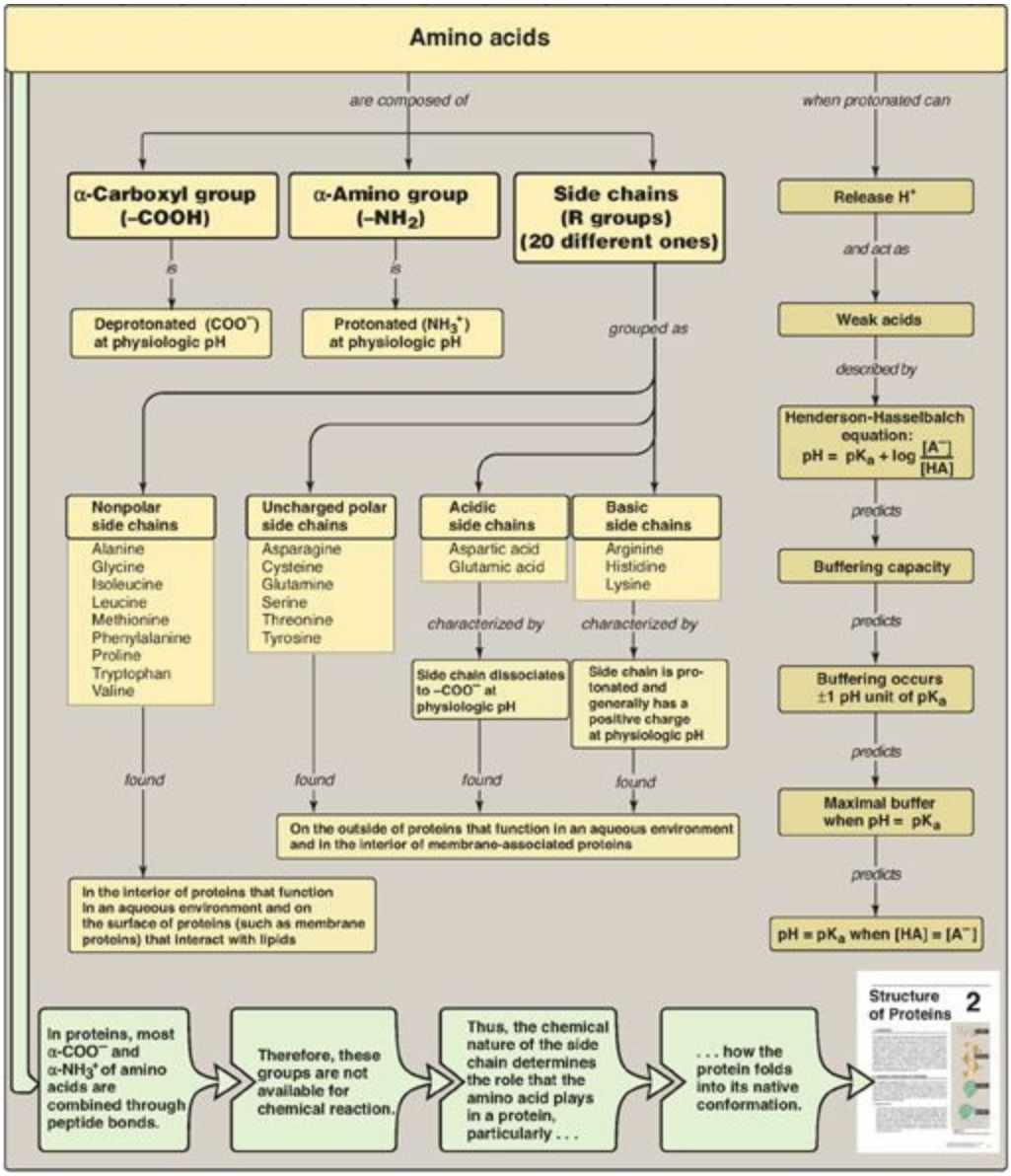
1. **Concept boxes and links:** Educators define concepts as "perceived regularities in events or objects." In the biochemical maps, concepts include abstractions (for example, free energy), processes (for example, oxidative phosphorylation), and compounds (for example, glucose 6-phosphate). These broadly 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 (Figure 1.13A). 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, that is, the connection creates meaning. The lines with arrowheads indicate in which direction the connection should be read (Figure 1.14).

2. **Cross-links:** 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 (Figure 1.13B), or between the map and other chapters in this book (Figure 1.13C). Cross-links can, thus, identify concepts that are central to more than one topic in biochemistry, empowering students to be effective in clinical situations and on the United States Medical Licensure Examination (USMLE) or other examinations that require integration of material. Students learn to visually perceive nonlinear relationships between facts, in contrast to cross-referencing within linear text.

V. 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**). At physiologic pH, the α-carboxyl group is dissociated, forming the negatively charged carboxylate ion (–**I** COO^-), and the α-amino group is protonated (–**I** 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 α-carbon of each amino acid (except glycine) is attached to four different chemical groups and is, therefore, a **chiral**, or **optically active** carbon atom. The L-form of amino acids is found in proteins synthesized by the human body.

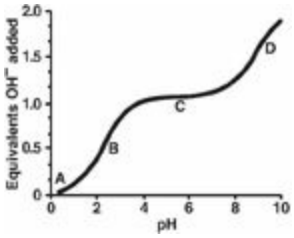
Figure 1.14 Key concept map for amino acids.


Amino acids
are composed of
when protonated can
α-Carboxyl group (–COOH)
α-Amino group (–NH2)
Side chains (R groups) (20 different ones)
Release H+
and act as
is
is
Deprotonated (COO−) at physiologic pH
Protonated (NH3+) at physiologic pH
grouped as
Weak acids
described by
Henderson-Hasselbalch equation: pH = pKa + log [A−]/[HA]
Nonpolar side chains
Alanine
Glycine
Isoleucine
Leucine
Methionine
Phenylalanine
Proline
Tryptophan
Valine
Uncharged polar side chains
Asparagine
Cysteine
Glutamine
Serine
Threonine
Tyrosine
Acidic side chains
Aspartic acid
Glutamic acid
Basic side chains
Arginine
Histidine
Lysine
predicts
Buffering capacity
characterized by
characterized by
predicts
Side chain dissociates to –COO− at physiologic pH
Side chain is protonated and generally has a positive charge at physiologic pH
Buffering occurs ±1 pH unit of pKa
found
found
found
found
predicts
On the outside of proteins that function in an aqueous environment and in the interior of membrane-associated proteins
Maximal buffer when pH = pKa
predicts
In the interior of proteins that function in an aqueous environment and on the surface of proteins (such as membrane proteins) that interact with lipids
pH = pKa when [HA] = [A−]
In proteins, most α-COO− and α-NH3+ of amino acids are combined through peptide bonds.
Therefore, these groups are not available for chemical reaction.
Thus, the chemical nature of the side chain determines the role that the amino acid plays in a protein, particularly . . .
. . . how the protein folds into its native conformation.
Structure of Proteins 2

Study Questions

Choose the ONE best answer.

1.1 Which one of the following statements concerning the titration curve for a nonpolar amino acid is correct? 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. 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 has an ionizable side chain.

1.2 Which one of the following statements concerning the peptide shown below is correct?

Val-Cys-Glu-Ser-Asp-Arg-Cys

A. The peptide contains asparagine.

B. The peptide contains a side chain with a secondary amino group.

C. The peptide contains a side chain that can be phosphorylated.

D. The peptide cannot form an internal disulfide bond.

E. The peptide would move to the cathode (negative electrode) during electrophoresis at pH 5.

> Correct answer = C. The hydroxyl group of serine can accept a phosphate group. Asp is aspartate. Proline contains a secondary amino group. The two cysteine residues can, under oxidizing conditions, form

a disulfide (covalent) bond. The net charge on the peptide at pH 5 is negative, and it would move to the anode.

1.3 A 2-year-old child presents with metabolic acidosis after ingesting an unknown number of flavored aspirin tablets. At presentation, her blood pH was 7.0. Given that the pK_a of aspirin (salicylic acid) is 3, calculate the ratio of its ionized to un-ionized forms at pH 7.0.

Correct answer = 10,000 to 1.

$pH = pK_a + \log [A–]/[HA]$. Therefore, $7 = 3 + \times$ and $\times = 4$. The ratio of A– (ionized) to HA (un-ionized), then, is 10,000 to 1 because the log of 10,000 is 4.

Structure of Proteins 2

I. OVERVIEW

The 20 amino acids commonly found in proteins are joined together by peptide bonds. The linear sequence of the linked amino acids contains the information necessary to generate a protein molecule with a unique three-dimensional shape. The complexity of protein structure is best analyzed by considering the molecule in terms of four organizational levels: primary, secondary, tertiary, and quaternary (Figure 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 p. 19).

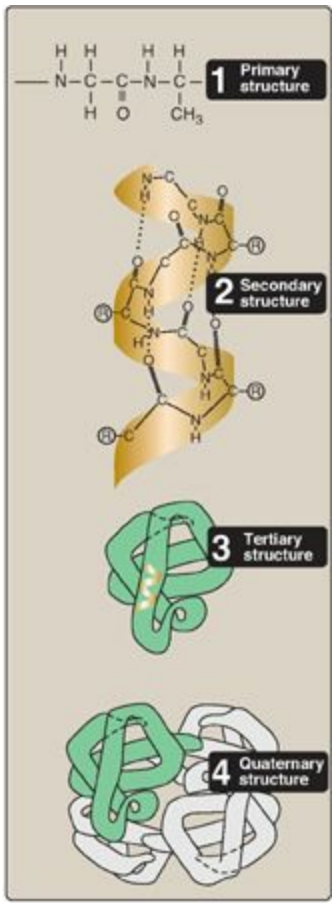
II. PRIMARY STRUCTURE OF PROTEINS

The sequence of amino acids in a protein is called the primary structure of the protein. Understanding the primary structure of proteins is important because many genetic diseases result in proteins with abnormal amino acid sequences, which cause 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, 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 another. For example, valine and alanine can form the dipeptide valylalanine through the formation of a peptide bond (Figure 2.2). Peptide bonds are resistant to conditions that denature proteins, such as heating and high concentrations of urea (see p. 20). Prolonged exposure to a strong acid or base at elevated temperatures is required to break these bonds nonenzymically.

Figure 2.1 Four hierarchies of protein structure.



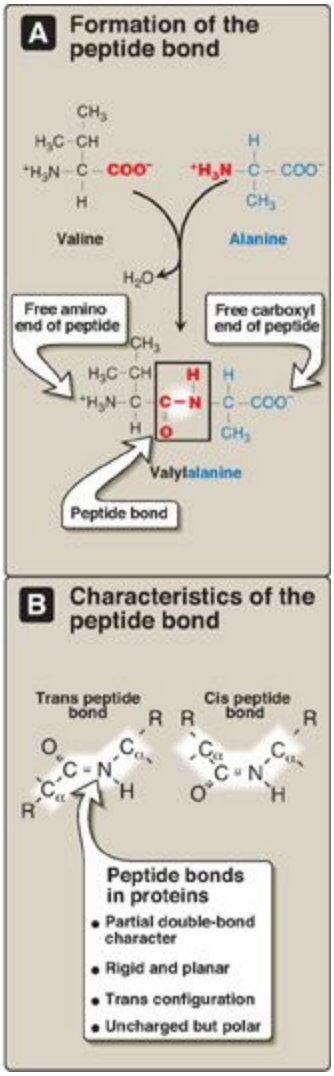
1. **Naming the peptide:** By convention, the free amino end (N-terminal) of the peptide chain is written to the left and the free carboxyl end (C-terminal) to the right. Therefore, all amino acid sequences are read from the N- to the C-terminal

end of the peptide. For example, in Figure 2.2A, the order of the amino acids is "valine, alanine." Linkage of many amino acids through peptide bonds results in an unbranched chain called a polypeptide. Each component amino acid in a polypeptide is called a "residue" because it is the portion of the amino acid remaining after the atoms of water are lost in the formation of the peptide bond. When a polypeptide 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.

2. **Characteristics of the peptide bond:** The peptide bond has a partial double-bond character, that is, it is shorter than a single bond and is rigid and planar (Figure 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 be freely rotated (although they are limited by the size and character of the R groups). This allows the polypeptide chain to assume a variety of possible configurations. The peptide bond is almost always a trans bond (instead of cis, see Figure 2.2B), in large part because of steric interference of the R groups when in the cis position.

3. **Polarity of the peptide bond:** 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 2–12. Thus, 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. The –C=O and –NH groups of the peptide bond are polar, however, and are involved in hydrogen bonds (for example, in α-helices and β-sheets), as described on pp. 16–17.

Figure 2.2 A. Formation of a peptide bond, showing the structure of the dipeptide valylalanine.
B. Characteristics of the peptide bond.



B. Determination of the amino acid composition of a polypeptide

The first step in determining the primary structure of a polypeptide is to identify and quantitate its constituent amino acids. A purified sample of the polypeptide to be analyzed is first hydrolyzed by strong acid at 110°C for 24 hours. This treatment cleaves the peptide bonds and releases the individual amino acids, which can be separated by cation-exchange chromatography. In this technique, a mixture of amino acids is applied to a column that contains a resin to which a negatively charged group is tightly attached. [Note: If the attached group is positively charged, the column becomes an anion-exchange column.] The amino acids bind to the column with different affinities, depending on their charges, hydrophobicity, and other characteristics. Each amino acid is sequentially released from the chromatography column by eluting with solutions of increasing ionic strength and pH (Figure 2.3). The separated amino acids contained in the eluate from the column are quantitated by heating them with ninhydrin (a reagent that forms a purple compound with most amino acids, ammonia, and amines). The amount of each amino acid is determined spectrophotometrically by measuring the amount of light absorbed by the ninhydrin derivative. The analysis described above is performed using an amino acid analyzer, an automated machine whose components are depicted in Figure 2.3.

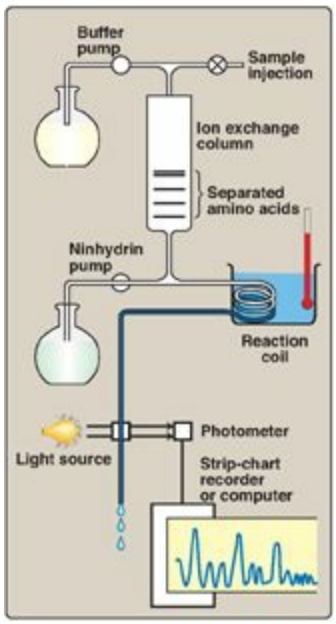
C. Sequencing of the peptide from its N-terminal end

Sequencing is a stepwise process of identifying the specific amino acid at each position in the peptide chain, beginning at the N-terminal end. Phenylisothiocyanate, known as Edman reagent, is used to label the amino-terminal residue under mildly alkaline conditions (Figure 2.4). The resulting phenylthiohydantoin (PTH) derivative introduces an instability in the N-terminal peptide bond such that it can be hydrolyzed without cleaving the other peptide bonds. The identity of the amino acid derivative can then be determined. Edman reagent can be applied repeatedly to the shortened peptide obtained in each previous cycle. The process is now automated.

D. Cleavage of 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. By using more than one cleaving agent (enzymes and/or chemicals) on separate samples of the purified polypeptide, overlapping fragments can be generated that permit the proper ordering of the sequenced fragments, thereby providing a complete amino acid sequence of the large polypeptide (Figure 2.5). Enzymes that hydrolyze peptide bonds are termed peptidases (proteases). [Note: Exopeptidases cut at the ends of proteins and are divided into aminopeptidases and carboxypeptidases. Carboxypeptidases are used in determining the C-terminal amino acid. Endopeptidases cleave within a protein.]

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

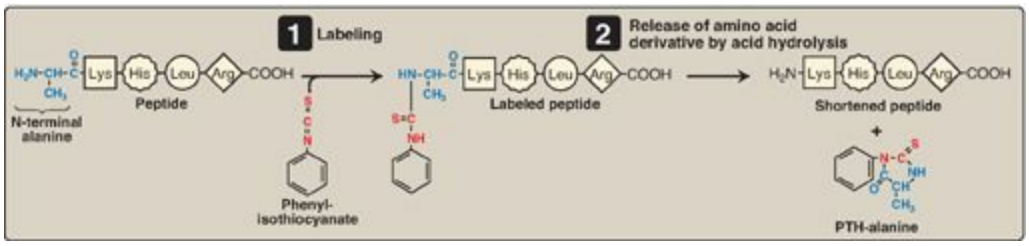


E. Determination of a protein's primary structure by DNA sequencing

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, it is possible, from knowledge of the genetic code (see p. 432), to translate the sequence of nucleotides 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, see p. 443). Therefore, direct protein sequencing is an extremely important tool for determining the true character of the primary sequence of many polypeptides.

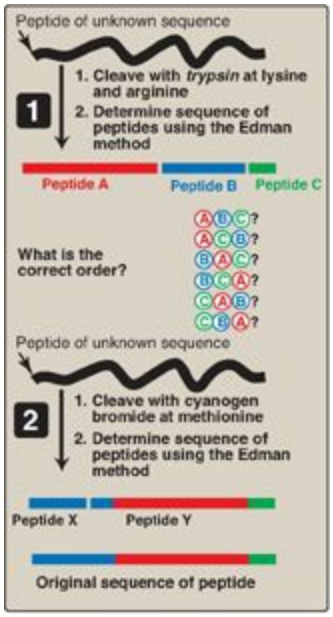
Figure 2.4 Determination of the amino (N)-terminal residue of a polypeptide by Edman degradation. PTH = phenylthiohydantoin.



III. SECONDARY STRUCTURE OF PROTEINS

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 (β-turn) are examples of secondary structures commonly encountered in proteins. [Note: The collagen α-chain helix, another example of secondary structure, is discussed on p. 45.]

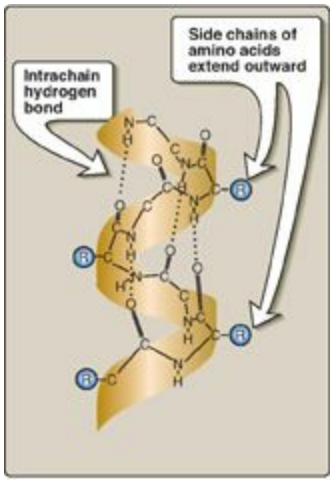
Figure 2.5 Overlapping of peptides produced by the action of trypsin and cyanogen bromide.



A. α-Helix

Several different polypeptide helices are found in nature, but the α-helix is the most common. It is a spiral structure, consisting of a tightly packed, coiled polypeptide backbone core, with the side chains of the component amino acids extending outward from the central axis to avoid interfering sterically with each other (Figure 2.6). A very diverse group of proteins contains α-helices. For example, the keratins are a family of closely related, fibrous proteins whose structure is nearly entirely α-helical. They are a major component of tissues such as hair and skin, and their rigidity is determined by the number of disulfide bonds between the constituent polypeptide chains. In contrast to keratin, myoglobin, whose structure is also highly α-helical, is a globular, flexible molecule (see p. 26).

Figure 2.6 α-Helix showing peptide backbone.

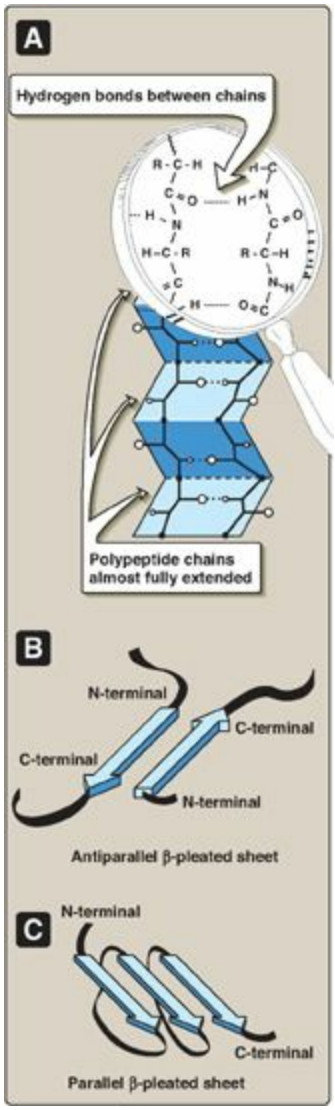


1. **Hydrogen bonds:** An α-helix is stabilized by extensive hydrogen bonding between the peptide-bond carbonyl oxygens and amide hydrogens that are part of the polypeptide backbone (see Figure 2.6). The hydrogen bonds extend up and are parallel to the spiral from the carbonyl oxygen of one peptide bond to the –NH– group of a peptide linkage four residues ahead in the polypeptide. This insures 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 acid residues spaced three or four residues apart in the primary sequence are spatially close together when folded in the α-helix.

3. **Amino acids that disrupt an α-helix:** Proline disrupts an α-helix because its 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. Large numbers of charged amino acids (for example, glutamate, aspartate, histidine, lysine, and arginine) also disrupt the helix by forming ionic bonds or by electrostatically repelling each other. Finally, amino acids with bulky side chains, such as tryptophan, or amino acids, such as valine or isoleucine, that branch at the β-carbon (the first carbon in the R group, next to the α-carbon) can interfere with formation of the α-helix if they are present in large numbers.

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.



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 (Figure 2.7A). The surfaces of β-sheets appear "pleated," and these structures are, therefore, often called β-pleated sheets. When illustrations are made of protein structure, β-strands are often visualized as broad arrows (Figure 2.7B).

1. **Comparison of a β-sheet and an α-helix:** Unlike the α-helix, β-sheets are composed of two or more peptide chains (β-strands), or segments of polypeptide chains, which are almost fully extended. Note also that the hydrogen bonds are perpendicular to the polypeptide backbone in β-sheets (see Figure 2.7A).

2. **Parallel and antiparallel sheets:** A β-sheet can be formed from two or more separate polypeptide chains or segments of polypeptide chains that are arranged either antiparallel to each other (with the N-terminal and C-terminal ends of the β-strands alternating as shown in Figure 2.7B) or parallel to each other (with all the N-termini of the β-strands together as shown in Figure 2.7C). When the hydrogen bonds are formed between the polypeptide backbones of separate polypeptide chains, they are termed interchain bonds. A β-sheet can also be formed by a single

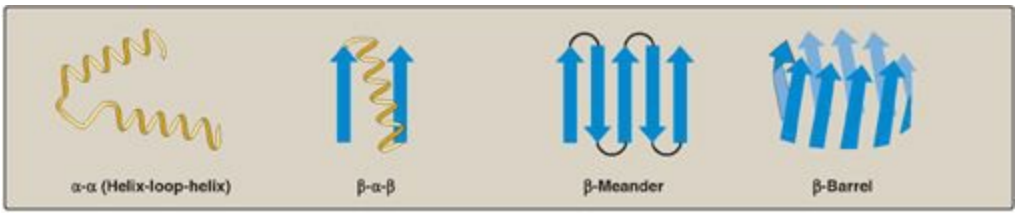
polypeptide chain folding back on itself (see Figure 2.7C). In this case, the hydrogen bonds are intrachain bonds. In globular proteins, β-sheets always have a right-handed curl, or twist, when viewed along the polypeptide backbone. [Note: Twisted β-sheets often form the core of globular proteins.]

> The α-helix and β-sheet structures provide maximal hydrogen bonding for peptide bond components within the interior of polypeptides.

C. β-Bends (reverse turns, β-turns)

β-Bends 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. [Note: β-Bends were given this name because they often connect successive strands of antiparallel β-sheets.] β-Bends 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 and ionic bonds.

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



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. [Note: The term "random coil" refers to the disordered structure obtained when proteins are denatured (see p. 20).]

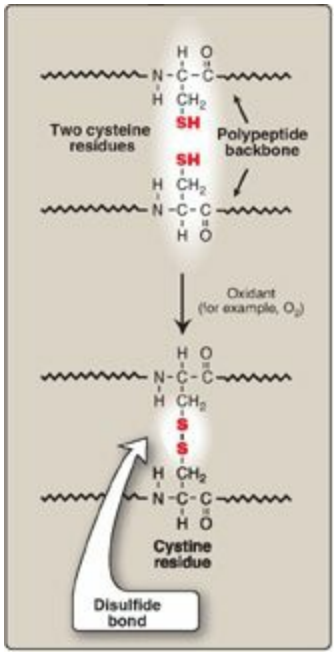
E. Supersecondary structures (motifs)

Globular proteins are constructed by combining secondary structural elements (that is, 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 (for example, 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. Thus, 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.

> 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 p. 450).

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



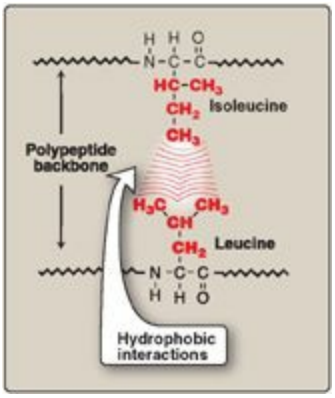
IV. TERTIARY STRUCTURE OF GLOBULAR PROTEINS

The primary structure of a polypeptide chain determines its tertiary structure. "Tertiary" refers both to the folding of domains (the basic units of structure and function, see discussion below), and to the final arrangement of domains in the polypeptide. The 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 greater 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 2.10 Hydrophobic interactions between amino acids with nonpolar side chains.



B. Interactions stabilizing tertiary structure

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 is a covalent linkage formed from the sulfhydryl group (–SH) of each of two cysteine residues to produce a cystine residue (Figure 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 polypeptide chains. The folding of the polypeptide chain(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

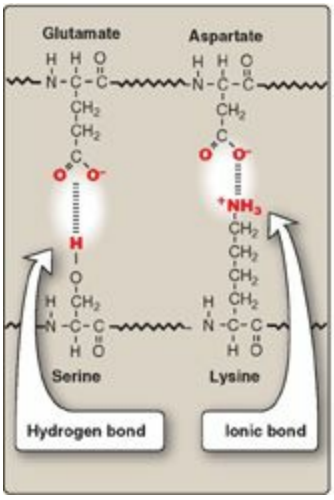
molecule and prevents it from becoming denatured in the extracellular environment. For example, many disulfide bonds are found in proteins such as immunoglobulins that are secreted by cells.

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 (Figure 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. [Note: Recall that proteins located in nonpolar (lipid) environments, such as a membrane, exhibit the reverse arrangement (see Figure 1.4, p. 4).] 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 (Figure 2.11; see also Figure 1.6, p. 4). 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 Figure 2.11).

Figure 2.11 Interactions of side chains of amino acids through hydrogen bonds and ionic bonds (salt bridges).



C. Protein folding

Interactions between the side chains of amino acids determine how a long 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 (that is, 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 (Figure 2.12). [Note: Some biologically active proteins or segments thereof lack a stable tertiary structure. They are referred to as "intrinsically disordered" proteins.]

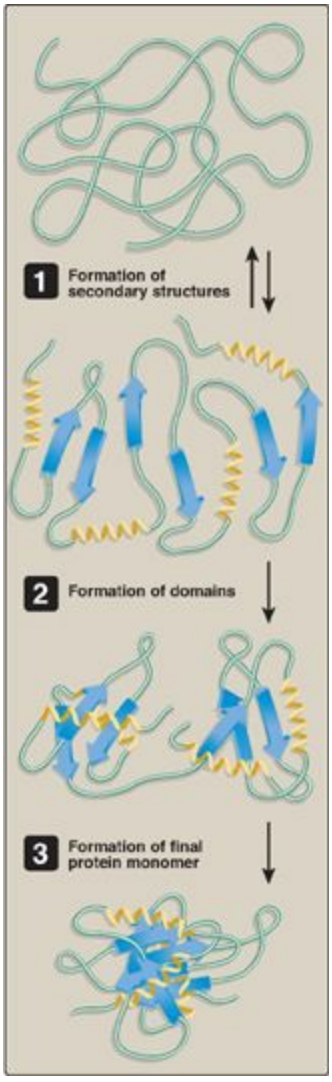
D. Denaturation of proteins

Protein 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, 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, once denatured, remain permanently disordered. Denatured proteins are often insoluble and precipitate from solution.

E. Role of chaperones in protein folding

The information needed for correct protein folding is contained in the primary structure of the polypeptide. However, most proteins when denatured do not resume their native conformations even under favorable environmental conditions. This is because, for many proteins, folding is a facilitated process that requires a specialized group of proteins, referred to as "molecular chaperones," and adenosine triphosphate hydrolysis. The chaperones, also known as "heat shock proteins" (Hsp), 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 (for example, 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 (for example, mitochondrial Hsp60). [Note: Cage-like chaperones are sometimes referred to as "chaperonins."] Chaperones, then, facilitate correct protein folding by binding to and stabilizing exposed, aggregation-prone hydrophobic regions in nascent (and denatured) polypeptides, preventing premature folding.

Figure 2.12 Steps in protein folding (simplified).


1 Formation of secondary structures
2 Formation of domains
3 Formation of final protein monomer

V. QUATERNARY STRUCTURE OF PROTEINS

Many proteins consist of a single polypeptide chain and are defined as monomeric proteins. However, others may consist of two or more polypeptide chains that may be structurally identical or totally unrelated. The arrangement of these polypeptide subunits is called the quaternary structure of the protein. Subunits are held together primarily by noncovalent interactions (for example, hydrogen bonds, ionic bonds, and hydrophobic interactions). Subunits may either 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 p. 29).

Isoforms are proteins that 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 isozymes (see p. 65).

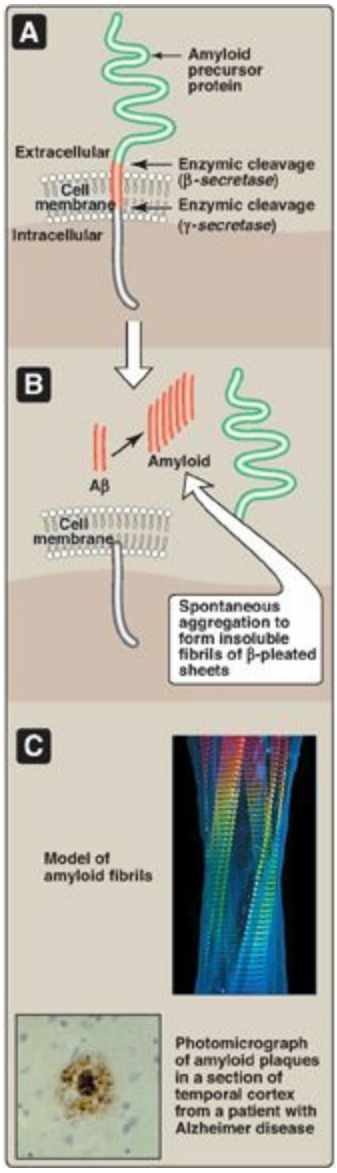
VI. PROTEIN MISFOLDING

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 p. 444). 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 conformational state that leads to the formation of long, fibrillar protein assemblies consisting of β-pleated sheets. Accumulation of these insoluble, spontaneously aggregating proteins, called amyloids, has been implicated in degenerative diseases such as Parkinson and Huntington and particularly in the age-related neurodegenerative disorder, Alzheimer disease. The dominant component of the amyloid plaque that accumulates in Alzheimer disease is amyloid β (Aβ), an extracellular peptide containing 40–42 amino acid residues. X-ray crystallography and infrared spectroscopy demonstrate a characteristic β-pleated sheet conformation in nonbranching fibrils. This peptide, when aggregated in a β-pleated sheet configuration, 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 Alzheimer disease is derived by enzymic cleavages (by secretases) from the larger amyloid precursor protein, a single transmembrane protein expressed on the cell surface in the brain and other tissues (Figure 2.13). The Aβ peptides aggregate, generating the amyloid that is found in the brain parenchyma and around blood vessels. Most cases of Alzheimer disease are not genetically based, although at least 5% of cases are familial. A second biologic factor involved in the development of Alzheimer disease is the accumulation of neurofibrillary tangles inside neurons. A key component of these tangled fibers is an abnormal form (hyperphosphorylated and insoluble) of the tau (τ) protein, which, in its healthy version, helps in the assembly of the microtubular structure. The defective τ appears to block the actions of its normal counterpart.

Figure 2.13 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.]

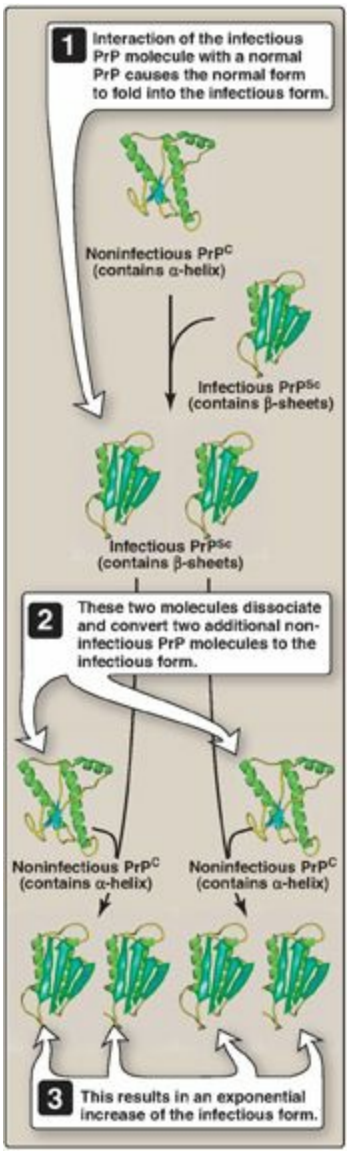


B. Prion diseases

The prion protein (PrP) has been strongly implicated as the causative agent of transmissible spongiform encephalopathies (TSEs), including Creutzfeldt-Jakob disease in humans, scrapie in sheep, and bovine spongiform encephalopathy in cattle (popularly called "mad cow" disease). After an extensive series of purification procedures, scientists were surprised to find that the infectivity of the agent causing scrapie in sheep was 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}. It has been observed that a number of α-helices present in noninfectious PrP^{C} are replaced by β-sheets in the

infectious form (Figure 2.14). It is presumably this conformational difference that 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.

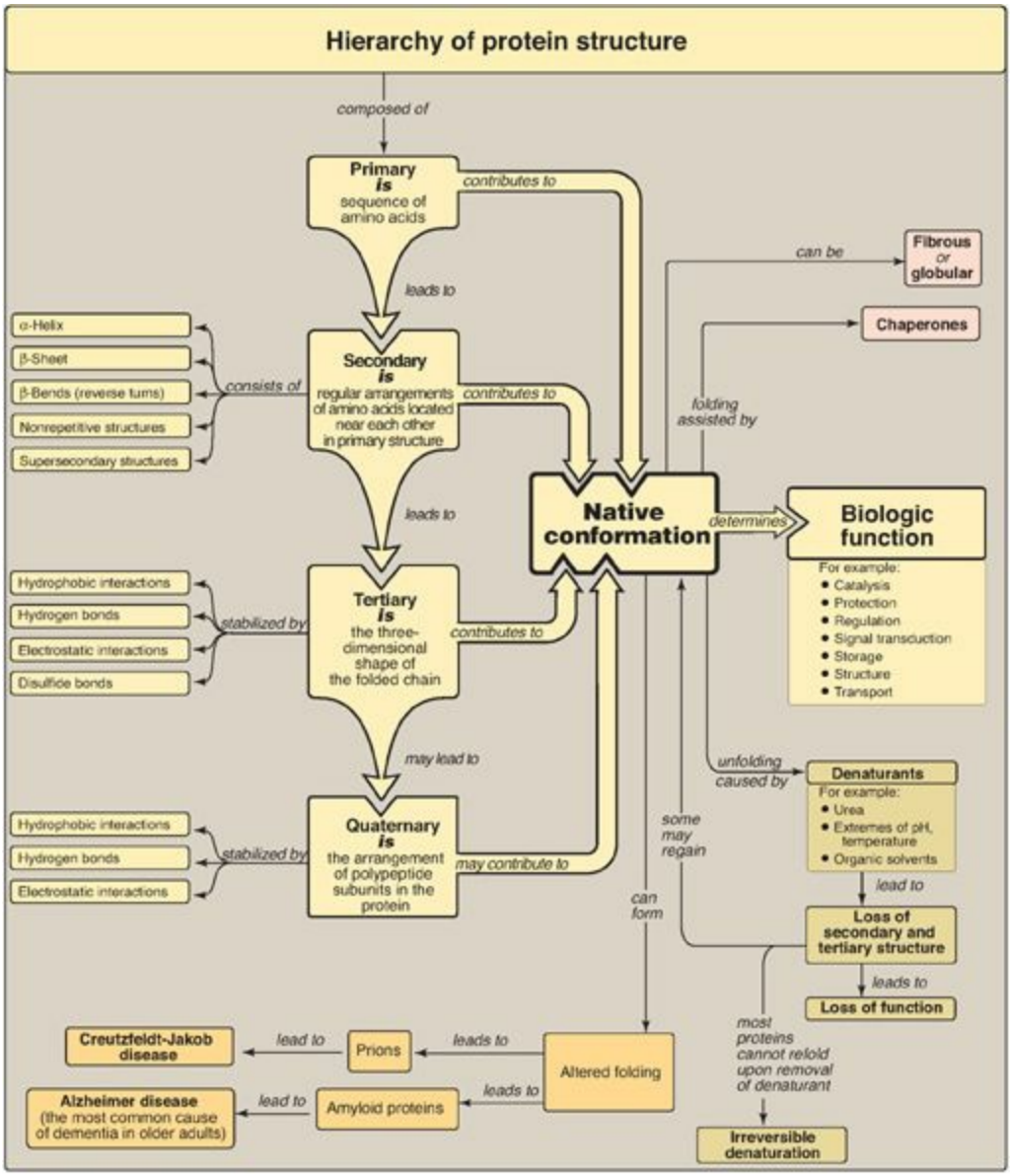
Figure 2.14 One proposed mechanism for multiplication of infectious prion agents. PrP = prion protein; PrP^{c} = prion protein cellular; PrP^{Sc} = prion protein scrapie.



VII. CHAPTER SUMMARY

Central to understanding protein structure is the concept of the **native conformation** (Figure 2.15), which is the functional, fully folded protein structure (for example, an active enzyme or structural protein). The unique three-dimensional structure of the native conformation is determined by its **primary structure**, that is, its 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. In addition, 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 are not accompanied by hydrolysis of peptide bonds. Denaturation may be reversible or, more commonly, irreversible. Disease can occur when an apparently normal protein assumes a conformation that is cytotoxic, as in the case of Alzheimer disease and the **transmissible spongiform encephalopathies** (**TSEs**), including **Creutzfeldt-Jakob disease**. In **Alzheimer disease,** 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 TSEs, 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.


Hierarchy of protein structure
composed of
Primary
is
sequence of amino acids
contributes to
leads to
can be
Fibrous or globular
Chaperones
folding assisted by
α-Helix
β-Sheet
β-Bends (reverse turns)
Nonrepetitive structures
Supersecondary structures
consists of
Secondary
is
regular arrangements of amino acids located near each other in primary structure
contributes to
leads to
Native conformation
determines
Biologic function
For example:
Catalysis
Protection
Regulation
Signal transduction
Storage
Structure
Transport
Hydrophobic interactions
Hydrogen bonds
Electrostatic interactions
Disulfide bonds
stabilized by
Tertiary
is
the three-dimensional shape of the folded chain
contributes to
may lead to
unfolding caused by
Denaturants
For example:
Urea
Extremes of pH, temperature
Organic solvents
Hydrophobic interactions
Hydrogen bonds
Electrostatic interactions
stabilized by
Quaternary
is
the arrangement of polypeptide subunits in the protein
may contribute to
some may regain
can form
lead to
Loss of secondary and tertiary structure
leads to
Loss of function
most proteins cannot refold upon removal of denaturant
Irreversible denaturation
Creutzfeldt-Jakob disease
lead to
Prions
leads to
Altered folding
Alzheimer disease
(the most common cause of dementia in older adults)
lead to
Amyloid proteins
leads to

Study Questions

Choose the ONE best answer.

2.1 Which one of the following statements concerning protein structure is correct?

A. Proteins consisting of one polypeptide have quaternary structure that is stabilized by covalent bonds.
B. The peptide bonds that link amino acids in a protein most commonly occur in the cis configuration.
C. The formation of a disulfide bond in a protein requires the participating cysteine residues to be adjacent in the primary structure.
D. The denaturation of proteins leads to irreversible loss of secondary structural elements such as the α-helix.
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 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.

2.3 In comparing the α-helix to the β-sheet, which statement is correct only for the β-sheet?

A. Extensive hydrogen bonds between the carbonyl oxygen (C=O) and the amide hydrogen (N-H) of the peptide bond are formed.
B. It may be found in typical globular proteins.
C. It is stabilized by interchain hydrogen bonds.
D. it is an example of secondary structure.
E. It may be found in supersecondary structures.

Correct answer = C. 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, B, D, and E are true for both of these secondary structural elements.

2.4 An 80-year-old man presented with impairment of higher intellectual function and alterations in mood and 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. Which one of the following best describes Alzheimer disease?

A. It is associated with β-amyloid, an abnormal protein with an altered amino acid sequence.
B. It results from accumulation of denatured proteins that have random conformations.
C. It is associated with the accumulation of amyloid precursor protein.
D. It is associated with the deposition of neurotoxic amyloid β peptide aggregates.
E. It is an environmentally produced disease not influenced by the genetics of the individual.
F. It is caused by the infectious β-sheet form of a host-cell protein.

Correct answer = D. Alzheimer disease 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 configuration that is neurotoxic. The amyloid β that is deposited in the brain in Alzheimer disease 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 Alzheimer disease are sporadic, although at least 5% of cases are familial. Prion diseases, such as Creutzfeldt-Jakob, are caused by the infectious β-sheet form (PrP^{Sc}) of a host-cell protein (PrP^{c}).

Globular Proteins 3

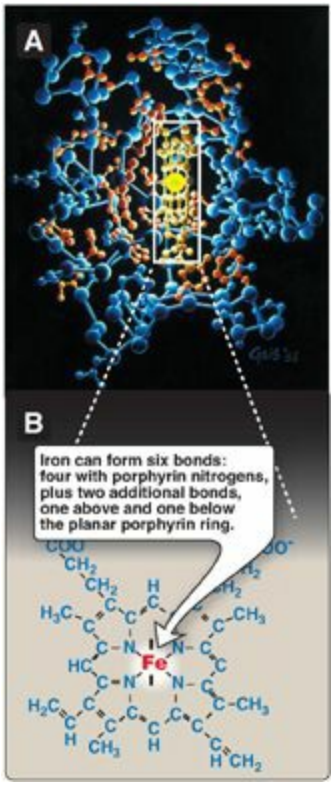
I. OVERVIEW

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 can be constructed that are capable of various specialized functions. This chapter examines the relationship between structure and function for the clinically important globular hemeproteins. Fibrous structural proteins are discussed in Chapter 4.

II. GLOBULAR HEMEPROTEINS

Hemeproteins are a group of specialized proteins that contain heme as a tightly bound prosthetic group. (See p. 54 for a discussion of prosthetic groups.) The role of the heme group is dictated by the environment created by the three-dimensional structure of the protein. For example, the heme group of a cytochrome functions as an electron carrier that is alternately oxidized and reduced (see p. 76). In contrast, the heme group of the enzyme catalase is part of the active site of the enzyme that catalyzes the breakdown of hydrogen peroxide (see p. 148). In hemoglobin and myoglobin, the two most abundant hemeproteins in humans, the heme group serves to reversibly bind oxygen.

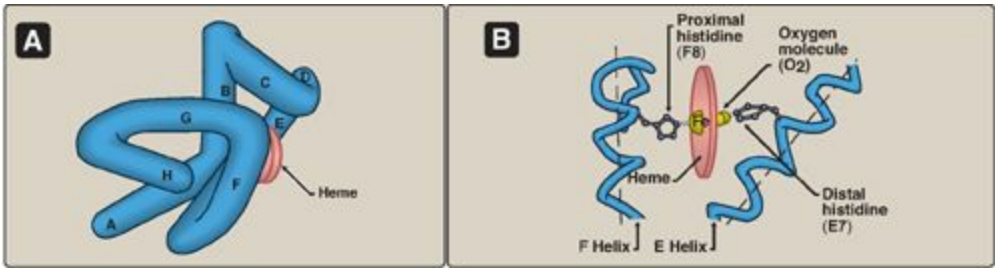
Figure 3.1 A. Hemeprotein (cytochrome c). B. Structure of heme.



A. Structure of heme

Heme is a complex of protoporphyrin IX and ferrous iron (Fe^{2+}) (Figure 3.1). The iron is held in the center of the heme molecule by bonds to the 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 myoglobin and 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 oxygen (Figure 3.2). (See pp. 278 and 282 for a discussion of the synthesis and degradation of heme.)

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



B. Structure and function of myoglobin

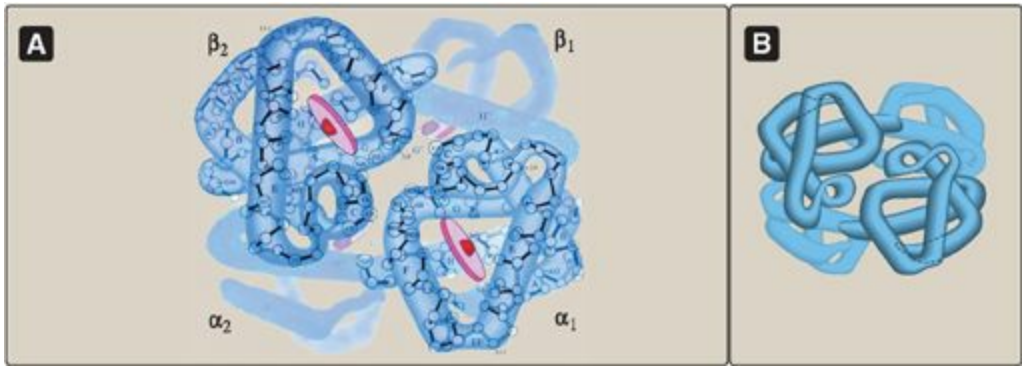
Myoglobin, a hemeprotein present in heart and skeletal muscle, functions both as a reservoir for oxygen and as an oxygen carrier that increases the rate of transport of oxygen within the muscle cell. [Note: Mouse myoglobin double knockouts (see p. 486) have, surprisingly, an apparently normal phenotype.] 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 approximately 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. 17). [Note: Ionic bonds are also termed electrostatic interactions or salt bridges.]

2. **Location of polar and nonpolar amino acid residues:** The interior of the myoglobin molecule is composed almost entirely of nonpolar amino acids. They 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.

3. **Binding of the heme group:** The heme group of the myoglobin molecule sits in a crevice, which is lined with nonpolar amino acids. Notable exceptions are two histidine residues (Figure 3.2B). One, the proximal histidine (F8), binds directly to the iron of heme. The second, or distal histidine (E7), does not directly interact with the heme group but helps stabilize the binding of oxygen to the ferrous iron. The protein, or globin, portion of myoglobin thus creates a special microenvironment for the heme that permits the reversible binding of one oxygen molecule (oxygenation). The simultaneous loss of electrons by the ferrous iron (oxidation to the ferric form) occurs only rarely.

Figure 3.3 A. Structure of hemoglobin showing the polypeptide backbone. B. Simplified drawing showing the helices.



C. Structure and function of hemoglobin

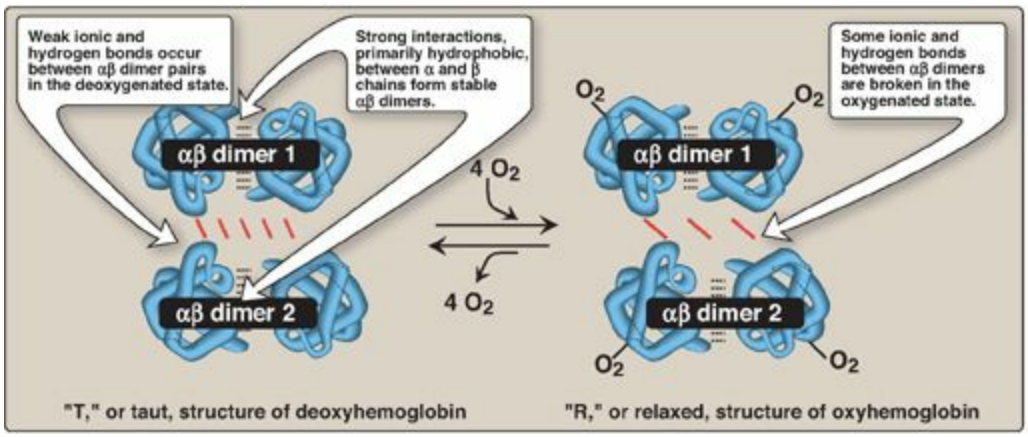
Hemoglobin is found exclusively in red blood cells (RBC), where its main function is to transport oxygen (O_2) from the lungs to the capillaries of the tissues. Hemoglobin A, the major hemoglobin in adults, is composed of four polypeptide chains (two α chains and two β chains) held together by noncovalent interactions (Figure 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 H^+ and 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. 29).

> 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 of hemoglobin:** The hemoglobin tetramer can be envisioned as being 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 (Figure 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 α-subunits and β-subunits in the dimers.] In contrast, the two dimers are held together primarily by polar bonds. The weaker interactions between the dimers allows 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 Figure 3.4). [Note: The binding of O_2 to the heme iron pulls the iron into the plane of the heme. Because the iron is also linked to the proximal histidine (F8), there is movement of the globin

chains that alters the interface between the αβ dimers.]

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 T conformation is the low-oxygen-affinity form of hemoglobin.

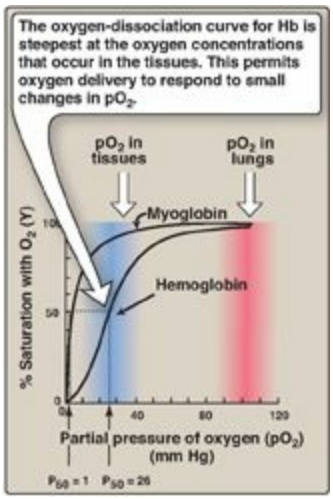
b. R form: The binding of O_2 to hemoglobin causes the rupture of some of the polar bonds between the αβ dimers, allowing movement. This leads to a structure called the "R," or relaxed form (see Figure 3.4). The R conformation is the high-oxygen-affinity form of hemoglobin.

D. Binding of oxygen 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 O_2 molecules, 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 3.5. [Note: Pulse oximetry is a noninvasive, indirect method of measuring the O_2 saturation of arterial blood based on differences in light absorption by oxyhemoglobin and deoxyhemoglobin.]

1. **Oxygen-dissociation curve:** A plot of 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 Figure 3.5). 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 approximately 1 mm Hg for myoglobin and 26 mm Hg for hemoglobin. The higher the oxygen affinity (that is, the more tightly oxygen binds), the lower the P_{50}.

Figure 3.5 Oxygen-dissociation curves for myoglobin and hemoglobin (Hb).



a. Myoglobin: The oxygen-dissociation curve for myoglobin has a hyperbolic shape (see Figure 3.5). This reflects the fact that myoglobin reversibly binds a single molecule of oxygen. Thus, oxygenated (MbO_2) and deoxygenated (Mb) myoglobin exist in a simple equilibrium:

$$Mb + O_2 \rightleftarrows MbO_2$$

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

b. Hemoglobin: The oxygen-dissociation curve for hemoglobin is sigmoidal in shape (see Figure 3.5), indicating that the subunits cooperate in binding oxygen. Cooperative binding of oxygen by the four subunits of hemoglobin means that the binding of an oxygen molecule at one heme group increases the oxygen affinity of the remaining heme groups in the same hemoglobin tetramer (Figure 3.6). This effect is referred to as heme–heme interaction (see below). Although it is more difficult for the first oxygen molecule to bind to hemoglobin, the subsequent binding of oxygen occurs with high affinity, as shown by the steep upward curve in the region near 20–30 mm Hg (see Figure 3.5).

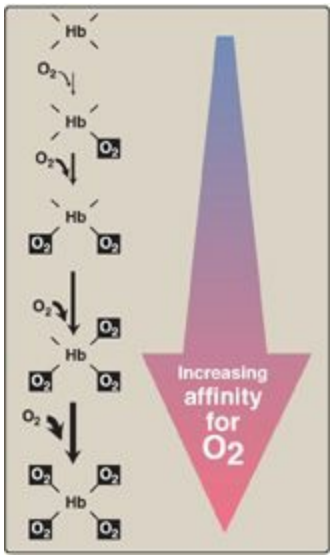
E. Allosteric effects

The ability of hemoglobin to reversibly bind oxygen is affected by the pO_2 (through heme–heme interactions as described above), the pH of the environment, the partial pressure of carbon dioxide (pCO_2) and the availability of 2,3-bisphosphoglycerate. These are collectively called allosteric ("other site") effectors, because their interaction at one site on the hemoglobin molecule affects the binding of oxygen to heme groups at other sites on the molecule. [Note: The binding of oxygen to monomeric myoglobin

is not influenced by allosteric effectors.]

1. **Heme–heme interactions:** The sigmoidal oxygen-dissociation curve reflects specific structural changes that are initiated at one heme group and transmitted to other heme groups in the hemoglobin tetramer. The net effect is that the affinity of hemoglobin for the last oxygen bound is approximately 300 times greater than its affinity for the first oxygen bound.

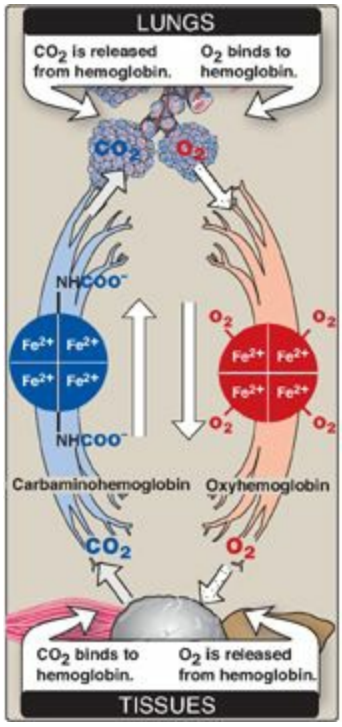
Figure 3.6 Hemoglobin (Hb) binds successive molecules of oxygen with increasing affinity.



a. **Loading and unloading oxygen:** The cooperative binding of oxygen allows hemoglobin to deliver more oxygen to the tissues in response to relatively small changes in the partial pressure of oxygen. This can be seen in Figure 3.5, which indicates pO_2 in the alveoli of the lung and the capillaries of the tissues. For example, in the lung, the concentration of oxygen is high, and hemoglobin becomes virtually saturated (or "loaded") with oxygen. In contrast, in the peripheral tissues, oxyhemoglobin releases (or "unloads") much of its oxygen for use in the oxidative metabolism of the tissues (Figure 3.7).

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 oxygen 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 oxygen release within this range of partial pressures of oxygen. Instead, it would have maximum affinity for oxygen throughout this oxygen pressure range and, therefore, would deliver no oxygen to the tissues.

Figure 3.7 Transport of oxygen and carbon dioxide by hemoglobin. Fe = iron.



2. **Bohr effect:** The release of oxygen from hemoglobin is enhanced when the pH is lowered or when the hemoglobin is in the presence of an increased pCO_2. Both result in a decreased oxygen affinity of hemoglobin and, therefore, a shift to the right in the oxygen-dissociation curve (Figure 3.8), and 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 affinity for oxygen, a shift to the left in the oxygen-dissociation curve, and stabilization of the R (oxy) form.

   a. **Source of the protons that lower the 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, CO_2 is converted by carbonic anhydrase to carbonic acid:

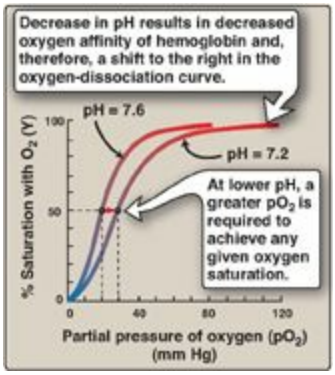
$$CO_2 + H_2O \rightleftarrows H_2CO_3$$

   which spontaneously loses a proton, becoming bicarbonate (the major blood buffer):

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

   The H^+ produced by this pair of reactions contributes to the lowering of pH. This differential pH gradient (that is, lungs having a higher pH and tissues a lower pH) favors the unloading of oxygen in the peripheral tissues and the loading of oxygen 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 oxygen.

Figure 3.8 Effect of pH on the oxygen affinity of hemoglobin. Protons are allosteric effectors of hemoglobin.



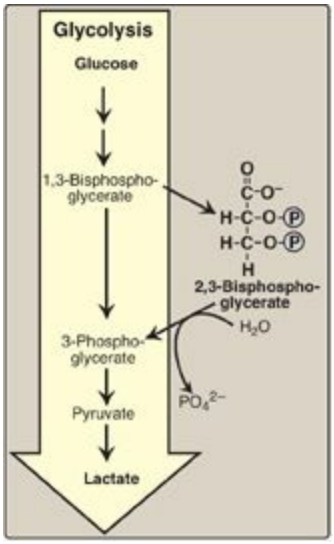
b. Mechanism of the Bohr effect: The Bohr effect reflects the fact that the deoxy form of hemoglobin has a greater affinity for protons than does oxyhemoglobin. This effect is caused by ionizable groups such as specific histidine side chains that have a higher pK_a in deoxyhemoglobin than in oxyhemoglobin. Therefore, an increase in the concentration of protons (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 the deoxy form of hemoglobin, producing a decrease in oxygen affinity. [Note: Hemoglobin, then, is an important blood buffer.]

The Bohr effect can be represented schematically as:

$$\underset{\text{oxyhemoglobin}}{HbO_2} + H^+ \rightleftarrows \underset{\text{deoxyhemoglobin}}{HbH} + O_2$$

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

Figure 3.9 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. **Effect of 2,3-bisphosphoglycerate on oxygen affinity:** 2,3-Bisphosphoglycerate (2,3-BPG) is an important regulator of the binding of oxygen to hemoglobin. It is the most abundant organic phosphate in the RBC, where its concentration is approximately that of hemoglobin. 2,3-BPG is synthesized from an intermediate of the glycolytic pathway (Figure 3.9; see p. 101 for a discussion of 2,3-BPG synthesis in glycolysis).

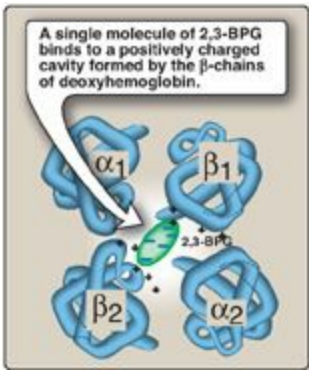
   a. **Binding of 2,3-BPG to deoxyhemoglobin:** 2,3-BPG decreases the O_2 affinity of hemoglobin by binding to deoxyhemoglobin but not to oxyhemoglobin. This preferential binding stabilizes the T conformation of deoxyhemoglobin. The effect of binding 2,3-BPG can be represented schematically as:

$$\underset{\text{oxyhemoglobin}}{HbO_2} + \text{2,3-BPG} \rightleftarrows \underset{\text{deoxyhemoglobin}}{\text{Hb–2,3-BPG}} + O_2$$

   b. **Binding site of 2,3-BPG:** One molecule of 2,3-BPG binds to a pocket, formed by the two β-globin chains, in the center of the deoxyhemoglobin tetramer (Figure 3.10). 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).] 2,3-BPG is expelled with oxygenation of the hemoglobin.

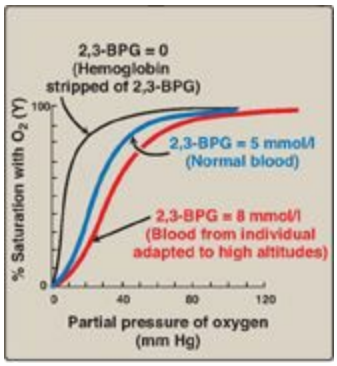
   c. **Shift of the oxygen-dissociation curve:** Hemoglobin from which 2,3-BPG has been removed has a high affinity for oxygen. However, as seen in the RBC, the presence of 2,3-BPG significantly reduces the affinity of hemoglobin for oxygen, shifting the oxygen-dissociation curve to the right (Figure 3.11). This reduced affinity enables hemoglobin to release oxygen efficiently at the partial pressures found in the tissues.

Figure 3.10 Binding of 2,3-bisphosphoglycerate (2,3-BPG) by deoxyhemoglobin.



d. Response of 2,3-BPG levels to chronic hypoxia or anemia: The concentration of 2,3-BPG in the RBC increases in response to chronic hypoxia, such as that observed in chronic obstructive pulmonary disease (COPD) like emphysema, or at high altitudes, where circulating hemoglobin may have difficulty receiving sufficient oxygen. 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 oxygen in the capillaries of the tissues (see Figure 3.11).

Figure 3.11 Allosteric effect of 2,3-bisphosphoglycerate (2,3-BPG) on the oxygen affinity of hemoglobin.



e. Role of 2,3-BPG in transfused blood: 2,3-BPG is essential for the normal oxygen transport function of hemoglobin. However, storing blood in the currently available media results in a decrease in 2,3-BPG. Stored blood displays an abnormally high oxygen affinity and fails to unload its bound oxygen properly in the tissues. Hemoglobin deficient in 2,3-BPG thus acts as an oxygen "trap" rather than as an oxygen transport system. Transfused RBC are able to restore their depleted supplies of 2,3-BPG in 6–24 hours. However, severely ill patients may be compromised if transfused with large quantities of such 2,3-BPG–"stripped" blood. [Note: The maximum storage time for RBC has been doubled (21 to 42 days, with median time of 15 days) by changes in H^+, phosphate, and hexose sugar concentration and by the addition of adenine (see p. 291). Although the content of 2,3-BPG was not greatly improved in the long-term by these changes, adenosine triphosphate production was increased and improved RBC survival.]

4. **Binding of CO_2:** Most of the CO_2 produced in metabolism is hydrated and transported as bicarbonate ion (see p. 9). However, some CO_2 is carried as carbamate bound to the N-terminal amino groups of hemoglobin (forming carbaminohemoglobin as shown in Figure 3.7), 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 affinity for oxygen (see p. 28) 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. **Binding of CO:** 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 oxygen 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 oxygen to the tissues (Figure 3.12). [Note: The affinity of hemoglobin for CO is 220 times greater than for oxygen. 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% oxygen at high pressure (hyperbaric oxygen therapy), which facilitates the dissociation of CO from the hemoglobin. [Note: CO inhibits Complex IV of the electron transport chain (see p. 76).] In addition to O_2, CO_2, and CO, nitric oxide gas (NO) also is carried by hemoglobin. NO is a potent vasodilator (see p. 151). It can be taken up (salvaged) or released from RBC, thus modulating NO availability and influencing vessel diameter.

Figure 3.12 Effect of carbon monoxide (CO) on the oxygen affinity of hemoglobin. CO-Hb = carboxyhemoglobin (carbon monoxyhemoglobin).

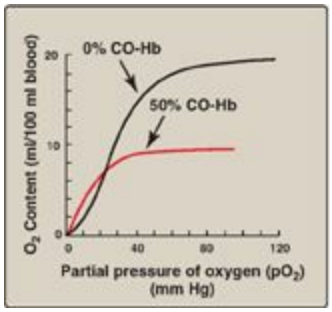


Figure 3.13 Normal adult human hemoglobins. [Note: The α-chains in these hemoglobins are identical.] Hb = hemoglobin.

Form	Chain composition	Fraction of total hemoglobin
HbA	$\alpha_2\beta_2$	90%
HbF	$\alpha_2\gamma_2$	<2%
HbA_2	$\alpha_2\delta_2$	2%–5%
HbA_{1c}	$\alpha_2\beta_2$-glucose	3%–9%

F. Minor hemoglobins

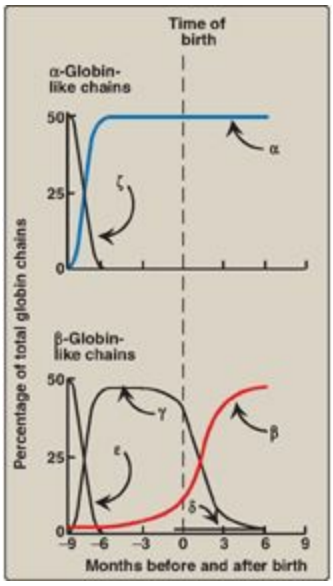
It is important to remember that human hemoglobin A (HbA) is just one member of a functionally and structurally related family of proteins, the hemoglobins (Figure 3.13). Each of these oxygen-carrying proteins is a tetramer, composed of two α-globin (or α-like) polypeptides and two β-globin (or β-like) polypeptides. Certain hemoglobins, such as HbF, are normally synthesized only during fetal development, whereas others, such as HbA_2, are synthesized in the adult, although at low levels compared with HbA. HbA can also become modified by the covalent addition of a hexose (see p. 34).

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 Figure 3.13). The γ chains are members of the β-globin gene family (see p. 35).

   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 about 60% of the total hemoglobin in the RBC during the last months of fetal life (Figure 3.14). HbA synthesis starts in the bone marrow at about the eighth month of pregnancy and gradually replaces HbF. (Figure 3.14 shows the relative production of each type of hemoglobin chain during fetal and postnatal life.) [Note: HbF represents less than 1% of the hemoglobin in most adults and is concentrated in RBC known as F cells.]

   b. **Binding of 2,3-BPG to HbF:** Under physiologic conditions, HbF has a higher affinity for oxygen than does HbA as a result of HbF only 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 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. In contrast, if both HbA and HbF are stripped of their 2,3-BPG, they then have a similar affinity for oxygen. The higher oxygen affinity of HbF facilitates the transfer of oxygen from the maternal circulation across the placenta to the RBC of the fetus.

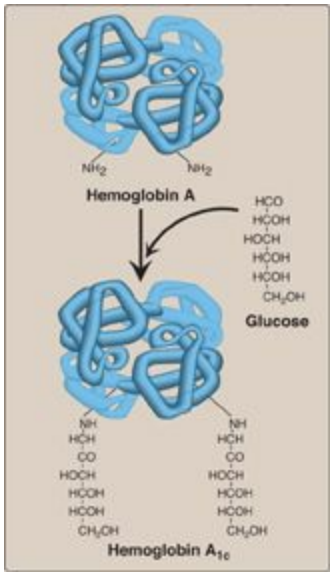
Figure 3.14 Developmental changes in hemoglobin.



2. **Hemoglobin A_2:** HbA_2 is a minor component of normal adult hemoglobin, first appearing shortly before birth and, ultimately, constituting about 2% of the total hemoglobin. It is composed of two α-globin chains and two δ-globin chains ($\alpha_2\delta_2$; see Figure 3.13).

3. **Hemoglobin A_{1c}:** Under physiologic conditions, HbA is slowly and nonenzymically glycosylated (glycated), the extent of glycosylation being dependent on the plasma concentration of a particular hexose. The most abundant form of glycosylated hemoglobin is HbA_{1c}. It has glucose residues attached predominantly to the NH_2 groups of the N-terminal valines of the β-globin chains (Figure 3.15). Increased amounts of HbA_{1c} are found in RBC 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 3.15 Nonenzymic addition of glucose to hemoglobin. The nonenzymic addition of a sugar to a protein is referred to as glycation.


NH2
NH2
Hemoglobin A
HCO
HCOH
HOCH
HCOH
HCOH
CH2OH
Glucose
NH
HCH
CO
HOCH
HCOH
HCOH
CH2OH
NH
HCH
CO
HOCH
HCOH
HCOH
CH2OH
Hemoglobin A1c

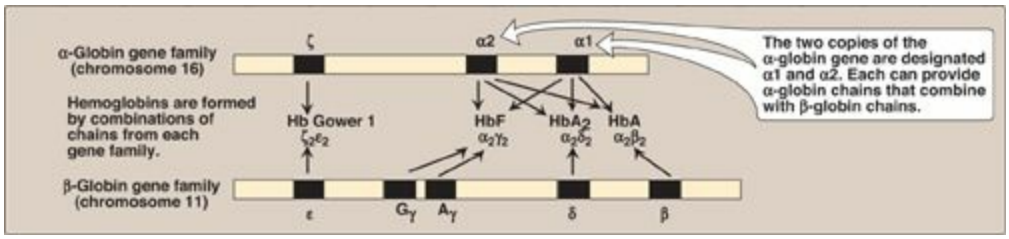
III. ORGANIZATION OF THE GLOBIN GENES

To understand diseases resulting from genetic alterations in the structure or synthesis of hemoglobins, 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.

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 (Figure 3.16). 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 hemoblobin. [Note: Globin gene famillies also contain globin-like genes that are not expressed, that is, their genetic information is not used to produce globin chains. These are called pseudogenes.]

Figure 3.16 Organization of the globin gene families. Hb = hemoglobin.



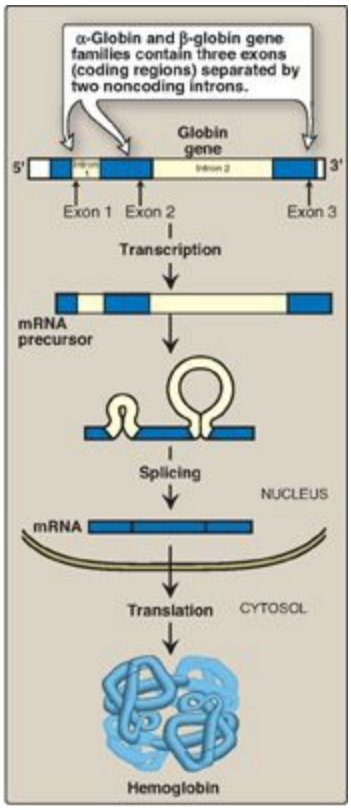
B. β-Gene family

A single gene for the β-globin chain is located on chromosome 11 (see Figure 3.16). There are an additional four β-globin-like genes: 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.

C. Steps in globin chain synthesis

Expression of a globin gene begins in the nucleus of RBC precursors, where the DNA sequence encoding the gene is transcribed. The RNA produced by transcription is actually a precursor of the messenger RNA (mRNA) that is used as a template for the synthesis of a globin chain. Before it can serve this function, two noncoding stretches of RNA (introns) must be removed from the mRNA precursor sequence and the remaining three fragments (exons) joined in a linear manner. The resulting mature mRNA enters the cytosol, where its genetic information is translated, producing a globin chain. (A summary of this process is shown in Figure 3.17. A more detailed description of gene expresion is presented in Unit VI, p. 395.)

Figure 3.17 Synthesis of globin chains. mRNA = messenger RNA.



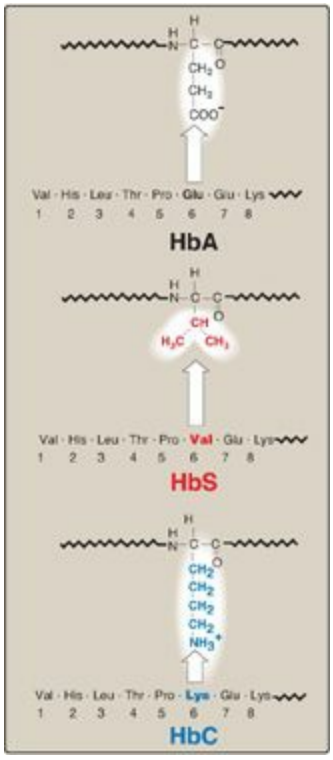
IV. HEMOGLOBINOPATHIES

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 (qualitative hemoglobinopathy), whereas the thalassemias are caused by decreased production of normal hemoglobin (quantitative hemoglobinopathy).

A. Sickle cell anemia (hemoglobin S disease)

Sickle cell anemia, the most common of the RBC sickling diseases, is a genetic disorder of the blood caused by a single nucleotide substitution (a point mutation, see p. 433) in the gene for β-globin. It is the most common inherited blood disorder in the United States, affecting 50,000 Americans. It occurs primarily in the African American population, affecting one of 500 newborn African American infants in the United States. Sickle cell anemia is an autosomal recessive disorder. It occurs in individuals who have inherited two mutant genes (one from each parent) that code for synthesis of the β chains of the globin molecules. [Note: The mutant β-globin chain is designated βS, 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 below). Sickle cell anemia is characterized by lifelong episodes of pain ("crises"); chronic hemolytic anemia with associated hyperbilirubinemia (see p. 284); and increased susceptibility to infections, usually beginning in infancy. [Note: The lifetime of a RBC in sickle cell anemia is less than 20 days, compared with 120 days for normal RBC, hence, the anemia.] Other symptoms include acute chest syndrome, stroke, splenic and renal dysfunction, and bone changes due to marrow hyperplasia. Heterozygotes, representing 1 in 12 African Americans, have one normal and one sickle cell gene. The blood cells of such heterozygotes contain both HbS and HbA. These individuals have sickle cell trait. They usually do not show clinical 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:** 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 (Figure 3.18). Therefore, during electrophoresis at alkaline pH, HbS migrates more slowly toward the anode (positive electrode) than does HbA (Figure 3.19). This altered mobility of HbS is a result of the absence of the negatively charged glutamate residues in the two β chains, thereby rendering HbS less negative than HbA. [Note: Electrophoresis of hemoglobin obtained from lysed RBC is routinely used in the diagnosis of sickle cell trait and sickle cell disease. DNA analysis also is used (see p. 472).]

2. **Sickling and tissue anoxia:** The replacement of the charged glutamate with the nonpolar valine forms a protrusion on the β chain that fits into a complementary site on the β chain of another hemoglobin molecule in the cell (Figure 3.20). At low oxygen tension, deoxyhemoglobin S polymerizes inside the RBC, forming a network of insoluble fibrous polymers that stiffen and distort the cell, producing rigid, misshapen RBC. Such sickled cells frequently block the flow of blood in the narrow capillaries. This interruption in the supply of oxygen leads to localized anoxia (oxygen deprivation) in the tissue, causing pain and eventually 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 3–4 μm. Compared to normal RBC, sickled cells have a decreased ability to deform and an increased tendency to adhere to vessel walls and so have difficulty moving through small vessels, thereby causing microvascular occlusion.]

3. **Variables that increase sickling:** The extent of sickling and, therefore, the severity of disease is enhanced by any variable that increases the proportion of HbS

in the deoxy state (that is, reduces the affinity of HbS for O_2). These variables include decreased pO_2, increased pCO_2, decreased pH, dehydration, and an increased concentration of 2,3-BPG in RBC.

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 RBC reduce the risk of stroke, but the benefits must be weighed against the complications of transfusion, which include iron overload (hemosiderosis), bloodborne 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. [Note: The morbidity and mortality associated with sickle cell anemia has led to its inclusion in newborn screening panels to allow prophylactic antibiotic therapy to begin soon after the birth of an affected child.]

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

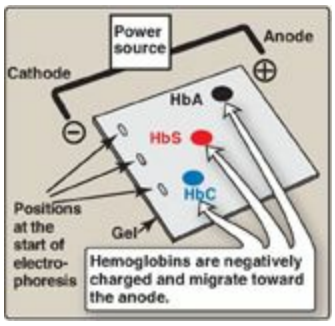
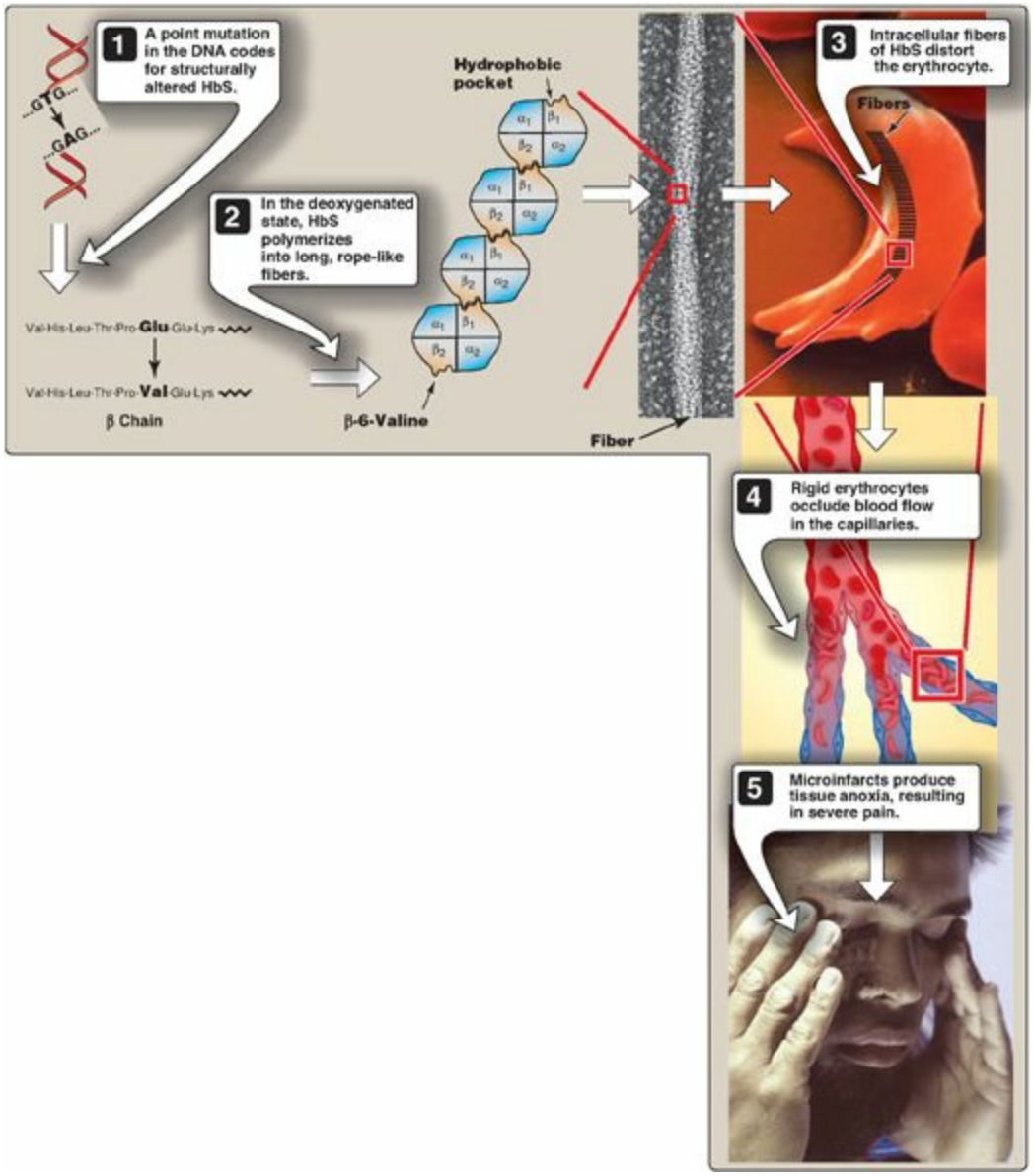


Figure 3.20 Molecular and cellular events leading to sickle cell crisis. HbS = hemoglobin S.



5. Possible selective advantage of the heterozygous state: The high frequency of the b^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 RBC. 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 fact may provide a selective advantage to heterozygotes living in regions where malaria is a major cause of death. Figure 3.21 illustrates that 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 Figure 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 Figure 3.19).] Rare patients homozygous for HbC generally have a relatively mild, chronic hemolytic anemia. These patients do not suffer from infarctive crises, and no specific therapy is required.

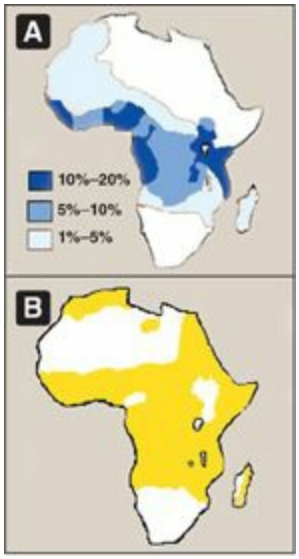
C. Hemoglobin SC disease

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 to the ferric (Fe^{3+}) state forms methemoglobin, which cannot bind O_2. This oxidation may be caused by the action of certain drugs, such as nitrates, or endogenous products such as reactive oxygen species (see p. 148). The oxidation may also result from inherited defects, for example, certain mutations in the α- or β-globin chain promote the formation of methemoglobin (HbM). Additionally, 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 HbM. [Note: The RBC of newborns have approximately half the capacity of those of adults to reduce HbM. They are, therefore, particularly susceptible to the effects of HbM-producing compounds.] The methemoglobinemias are characterized by "chocolate cyanosis" (a brownish blue coloration of the skin and mucous membranes and brown-colored blood) as a result of the dark-colored HbM. 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} is reduced.

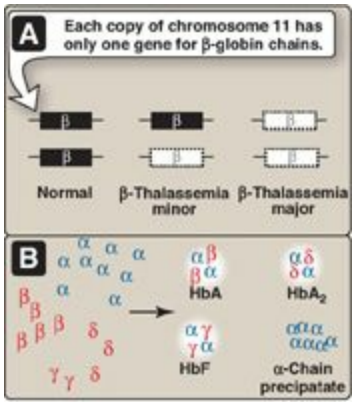
Figure 3.21 A. Distribution of sickle cell in Africa expressed as a percentage of the population with disease. B. Distribution of malaria in Africa.



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. A thalassemia can be caused by a variety of mutations, including entire gene deletions, or substitutions or deletions of one to many nucleotides in the DNA. [Note: Each thalassemia can be classified as either a disorder in which no globin chains are produced (α^o- or β^o-thalassemia), or one in which some chains are synthesized but at a reduced level (α^+- or β^+-thalassemia).]

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

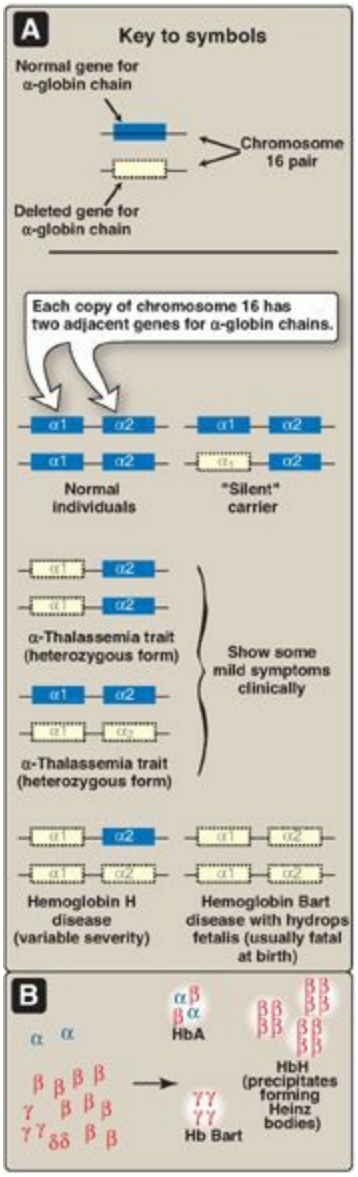


1. **β-Thalassemias:** In these disorders, synthesis of β-globin chains is decreased or absent, typically as a result of point mutations that affect the production of functional mRNA. However, α-globin chain synthesis is normal. Excess α-globin chains cannot form stable tetramers and so precipitate, causing the premature death of cells initially destined to become mature RBC. 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 (Figure 3.22). Because the β-globin gene is not expressed until late in fetal gestation, 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, those infants born with β-thalassemia major are seemingly healthy at birth but become severely anemic, usually during the first or second year of life due to ineffective erythropoiesis. 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 (a syndrome known as hemosiderosis). Use of iron chelation therapy has improved morbidity and mortality.] The only curative option available is hematopoietic stem cell transplantation.

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 (Figure 3.23). If one of the four genes is defective, the individual is termed a silent carrier of α-thalassemia, because no physical manifestations of the disease occur. If two α-globin genes are defective, the individual is designated as having α-thalassemia trait. If three α-globin genes are defective, the individual has hemoglobin H (β_4) disease, a hemolytic anemia of variable severity. If all four α-globin genes are defective, hemoglobin Bart (γ_4) disease with hydrops fetalis and fetal death results, because α-globin chains are required for the synthesis of HbF.

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


A
Key to symbols
Normal gene for α-globin chain
Chromosome 16 pair
Deleted gene for α-globin chain
Each copy of chromosome 16 has two adjacent genes for α-globin chains.
α1 α2
α1 α2
Normal individuals
α1 α2
α2
"Silent" carrier
α1 α2
α1 α2
α-Thalassemia trait (heterozygous form)
Show some mild symptoms clinically
α1 α2
α1
α-Thalassemia trait (heterozygous form)
α1 α2
α1 α2
Hemoglobin H disease (variable severity)
α1 α2
α1 α2
Hemoglobin Bart disease with hydrops fetalis (usually fatal at birth)
B
α α
αβ βα
HbA
β β β β
γ β β β
γγ δδ β β
γγ γγ
Hb Bart
ββ ββ ββ ββ
HbH (precipitates forming Heinz bodies)

V. CHAPTER SUMMARY

Hemoglobin A (HbA), the major hemoglobin (Hb) in adults, is composed of four polypeptide chains (two α chains and two β chains, $\alpha_2\beta_2$) held together by noncovalent interactions (Figure 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 O_2 to Hb 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. **Cooperative binding** of O_2 by the four subunits of Hb means that the binding of an O_2 molecule at one heme group increases the oxygen affinity of the remaining heme groups in the same Hb molecule. Hb's **ability to bind O_2 reversibly** is affected by the **partial pressure of O_2** (**pO_2**) (through heme-heme interactions), the **pH** of the environment, the partial pressure of CO_2 (**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. **Carbon monoxide** (**CO**) binds tightly (but reversibly) to the Hb iron, forming **carboxyhemoglobin**. **Hemoglobinopathies** are disorders caused either by production of a **structurally abnormal Hb** molecule; synthesis of **insufficient quantities** of normal Hb subunits, or, rarely, both (Figure 3.25). The sickling diseases **sickle cell anemia** (hemoglobin S disease) and **hemoglobin SC disease** as well as **hemoglobin C disease** and the **thalassemias** are representative hemoglobinopathies that can have severe clinical consequences.

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 approximately 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 glycosylated form of HbA, formed nonenzymically in red cells. HbA_2 is a minor component of normal adult hemoglobin, first appearing shortly before birth and rising to adult levels (about 2% of the total hemoglobin) by age 6 months.

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 affinity of hemoglobin for O_2.
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. A decrease in pH (acidosis) causes the oxygen-dissociation curve to shift to the right, indicating a decreased affinity for oxygen. 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 hemoglobins.

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 affinity for oxygen at higher pH values.

B. Carbon dioxide increases the oxygen affinity of hemoglobin by binding to the C-terminal groups of the polypeptide chains.

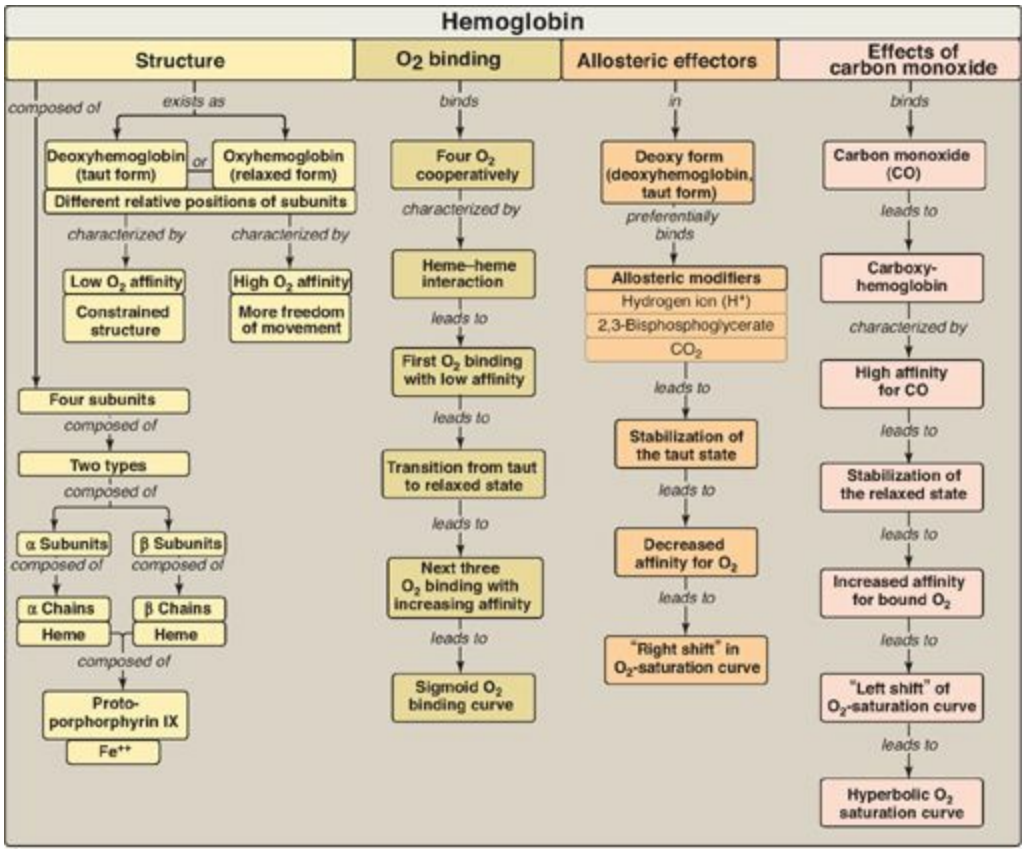
C. The oxygen affinity of hemoglobin increases as the percentage saturation increases.

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 affinity for oxygen. 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 and, therefore, is a weaker acid.

Figure 3.24 Key concept map for hemoglobin structure and function.



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

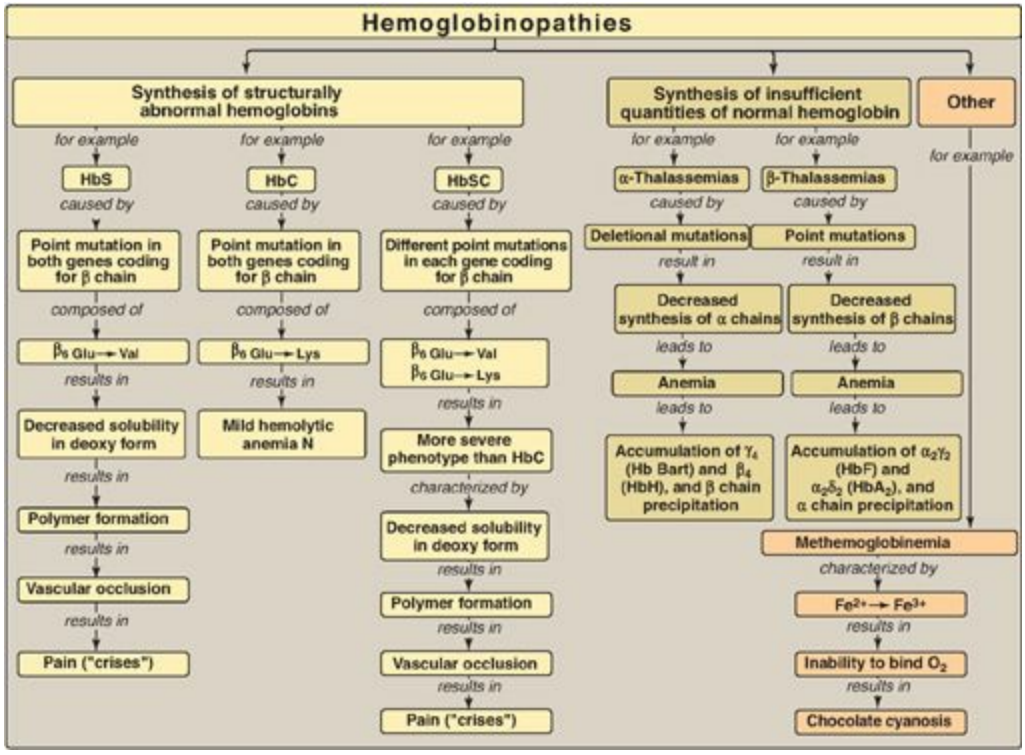
A. It should be stabilized in the taut, rather than the relaxed, form.
B. It should have increased O_2 affinity and, consequently, decreased delivery of O_2 to tissues.
C. Its O_2-dissociation curve should be shifted to the right relative to HbA.
D. It results in anemia.

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

3.5 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.

Figure 3.25 Key concept map for hemoglobinopathies. Hb = hemoglobin.



3.6 A 67-year-old man presented to the emergency department with a 1-week history of angina and shortness of breath. He complained that his face and extremities had a "blue color." His medical history included chronic stable angina treated with isosorbide dinitrate and nitroglycerin. Blood obtained for analysis was brown colored. 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 heme component of hemoglobin to the ferric (Fe^{3+}) state forms methemoglobin. This may be caused by the action of certain drugs such as nitrates. The methemoglobinemias are characterized by chocolate cyanosis (a brownish 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.]

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

Decreased. HbF reduces HbS concentration. It also inhibits polymerization of deoxy HbS.

Fibrous Proteins 4

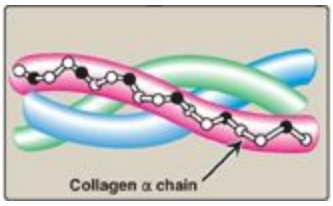
I. OVERVIEW

Collagen and elastin are examples of common, well-characterized fibrous proteins of the extracellular matrix that serve structural functions in the body. For example, collagen and elastin are found as components of 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 are obtained by combining specific amino acids into regular, secondary structural elements. This is in contrast to globular proteins, whose shapes are the result of complex interactions between secondary, tertiary, and, sometimes, quaternary structural elements.

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 (Figure 4.1). Although these molecules are found throughout the body, their types and organization are 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, as in the extracellular matrix 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.

Figure 4.1 Triple-stranded helix of collagen.



A. Types

The collagen superfamily of proteins includes more than 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 (approximately 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 ($\alpha1_2\alpha2$), whereas type II collagen contains three α1 chains ($\alpha1_3$). The collagens can be organized into three groups, based on their location and functions in the body (Figure 4.2).

Figure 4.2 The most abundant types of collagen. *Known as FACITs: fibril-associated collagens with interrupted triple helices.

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

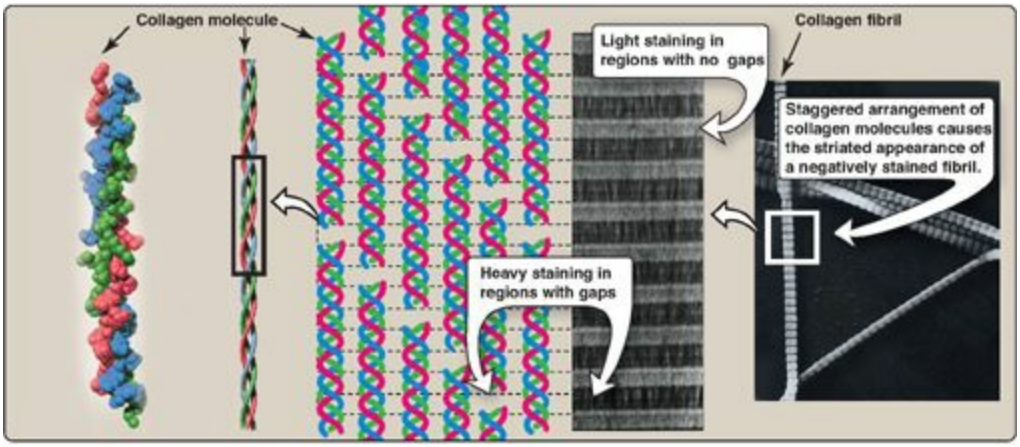
1. **Fibril-forming collagens:** Types I, II, and III are the fibrillar collagens and have the rope-like structure described above for a typical collagen molecule. In the electron microscope, these linear polymers of fibrils have characteristic banding patterns, reflecting the regular staggered packing of the individual collagen molecules in the fibril (Figure 4.3). Type I collagen fibers (composed of collagen fibrils) are found in supporting elements of high tensile strength (for example, tendon and cornea), 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 (Figure 4.4). For example, type IV molecules assemble into a sheet or meshwork that constitutes a major part of basement membranes.

> 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 extracellular matrix (see Figure 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.

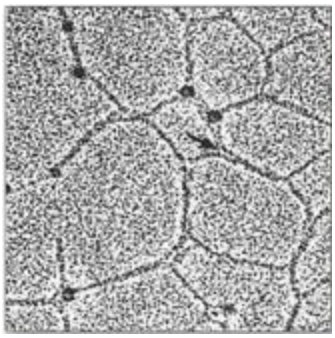


B. Structure

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 the polypeptide chain. It 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, Figure 4.5). Thus, most of the α chain can be regarded as a polytripeptide whose sequence can be represented as $(–Gly–Pro–Hyp–)_{333}$.

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

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



3. **Hydroxyproline and hydroxylysine:** Collagen contains hydroxyproline and hydroxylysine, which are not present in most other proteins. These residues result from the hydroxylation of some of the proline and lysine residues after their incorporation into polypeptide chains (Figure 4.6). The hydroxylation is, thus, an example of posttranslational modification (see p. 443). [Note: Generation of

hydroxyproline maximizes formation of interchain hydrogen bonds that stabilize the triple-helical structure.]

4. **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 (Figure 4.7).

Figure 4.5 Amino acid sequence of a portion of the α1 chain of collagen. [Note: Hyp is hydroxyproline, and Hyl is hydroxylysine.]

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

1. **Formation of pro-α chains:** 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 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 RER to yield a precursor of collagen called a pro-α chain (see Figure 4.7).

Figure 4.6 Hydroxylation of proline residues of pro-α chains of collagen by prolyl hydroxylase.

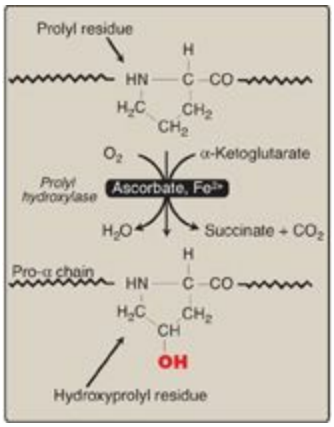
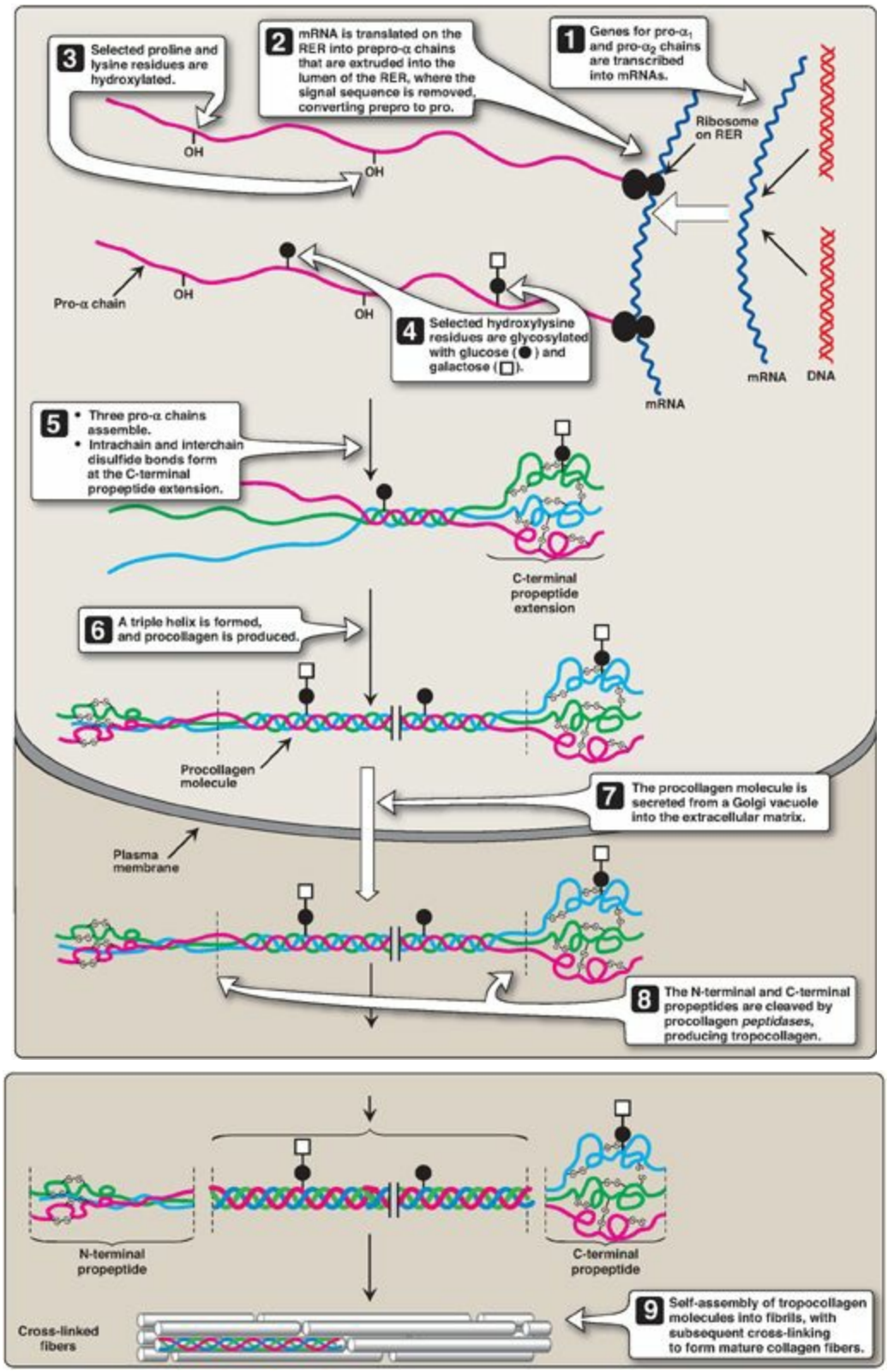


Figure 4.7 Synthesis of collagen. RER = rough endoplasmic reticulum; mRNA = messenger RNA. Synthesis of collagen.



2. **Hydroxylation:** The pro-α chains are processed by a number of enzymic steps within the lumen of the RER while the polypeptides are still being synthesized (see Figure 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, Fe^{2+}, and the reducing agent vitamin C (ascorbic acid, see p. 377), without which the hydroxylating

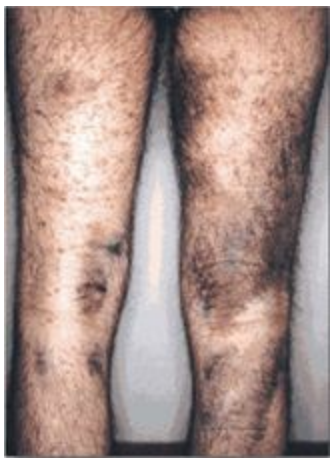
enzymes, prolyl hydroxylase and lysyl hydroxylase, are unable to function (see Figure 4.6). In the case of ascorbic acid deficiency (and, therefore, a lack of proline and lysine hydroxylation), interchain H-bond formation is impaired, as is formation of a stable triple helix. Additionally, collagen fibrils cannot be cross-linked (see below), greatly decreasing the tensile strength of the assembled fiber. The resulting deficiency disease is known as scurvy. Patients with ascorbic acid deficiency also often show bruises on the limbs as a result of subcutaneous extravasation (leakage) of blood due to capillary fragility (Figure 4.8).

3. **Glycosylation:** Some hydroxylysine residues are modified by glycosylation with glucose or glucosyl-galactose (see Figure 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 amino- and carboxyl-terminal extensions called propeptides (see Figure 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 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.

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

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

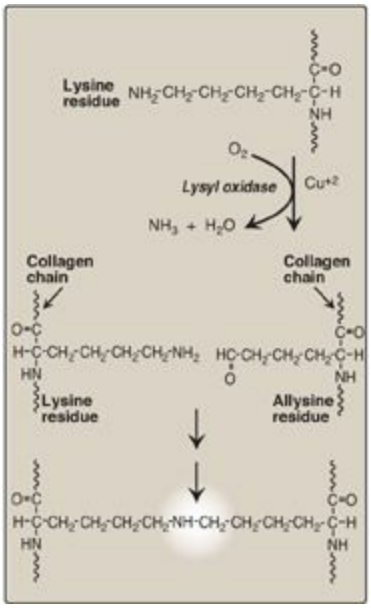


6. **Formation of collagen fibrils:** Tropocollagen molecules spontaneously associate to form collagen fibrils. They form an ordered, overlapping, parallel array, with adjacent collagen molecules arranged in a staggered pattern, each overlapping its neighbor by a length approximately three-quarters of a molecule (see Figure 4.7).

7. **Cross-link formation:** The fibrillar array of collagen molecules serves as a substrate for lysyl oxidase. This Cu^{2+}-containing extracellular enzyme oxidatively

deaminates some of the lysine and hydroxylysine residues in collagen. The reactive aldehydes that result (allysine and hydroxyallysine) can condense with lysine or hydroxylysine residues in neighboring collagen molecules to form covalent cross-links and, thus, mature collagen fibers (Figure 4.9).

Figure 4.9 Formation of cross-links in collagen. [Note: Lysyl oxidase is irreversibly inhibited by a toxin from plants in the genus Lathyrus, leading to a condition known as lathyrism.]



> Lysyl oxidase is one of several copper-containing enzymes. Others include cytochrome oxidase (see p. 76), dopamine hydroxylase (see p. 286), superoxide dismutase (see p.148), and tyrosinase (see p. 273). Disruption in copper homeostasis causes copper deficiency (X-linked Menkes disease) or overload (Wilson disease).

D. Degradation

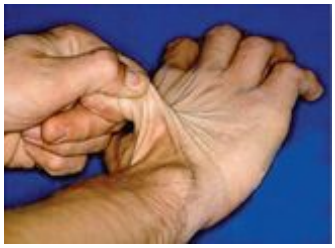
Normal collagens are highly stable molecules, having half-lives as long as several years. However, connective tissue is dynamic and is constantly being remodeled, often in response to growth or injury of the tissue. Breakdown of collagen fibers is dependent on the proteolytic action of collagenases, 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. Collagen diseases: 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

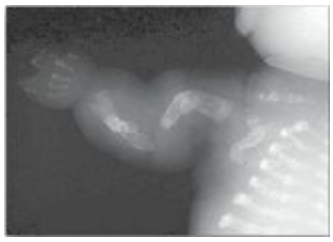
inability to provide tissues with the needed tensile strength normally provided by collagen. More than 1,000 mutations have been identified in 23 genes coding for 13 of the collagen types. The following are examples of diseases that are the result of defective collagen synthesis.

Figure 4.10 Stretchy skin of classic Ehlers-Danlos syndrome.



1. **Ehlers-Danlos syndrome:** Ehlers-Danlos syndrome (EDS) is a heterogeneous group of connective tissue disorders that result from inheritable defects in the metabolism of fibrillar collagen molecules. EDS can be caused by a deficiency of collagen-processing enzymes (for example, lysyl hydroxylase or N-procollagen peptidase) or from mutations in the amino acid sequences of collagen types I, III, or V. The classic form of EDS, caused by defects in type V collagen, is characterized by skin extensibility and fragility and joint hypermobility (Figure 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 containing mutant chains may have altered structure, secretion, or distribution. 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.].

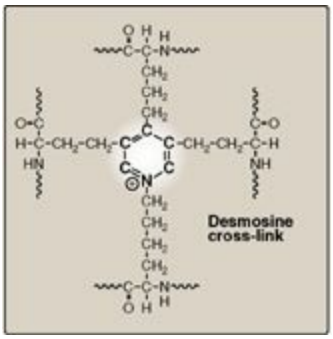
Figure 4.11 Lethal form (type II) of osteogenesis imperfecta in which the fractures appear in utero, as revealed by this radiograph of a stillborn fetus.



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 (Figure 4.11). Over 80% of cases of osteogenesis imperfecta (OI) are caused by dominant mutations to the genes that code for 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 (see Figure 4.11). Type III is also a severe form. It 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.

Figure 4.12 Desmosine cross-link in elastin.



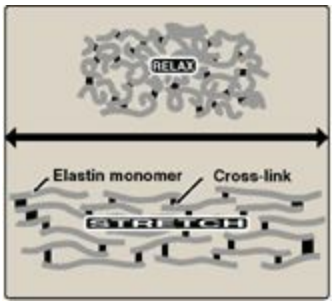
III. ELASTIN

In contrast to collagen, which forms fibers that are tough and have high tensile strength, elastin is a connective tissue protein with rubber-like properties. 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.

A. Structure

Elastin is an insoluble protein polymer synthesized from a precursor, tropoelastin, which is a linear polypeptide composed of about 700 amino acids that are primarily small and nonpolar (for example, glycine, alanine, and valine). Elastin is also rich in proline and lysine but contains scant hydroxyproline and hydroxylysine. Tropoelastin is secreted by the cell into the extracellular space. 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 (Figure 4.12). This produces elastin, an extensively interconnected, rubbery network that can stretch and bend in any direction when stressed, giving connective tissue elasticity (Figure 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. [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. Role of α_1-antitrypsin in elastin degradation

1. **α_1-Antitrypsin:** Blood and other body fluids contain a protein, α_1-antitrypsin (AAT or A1AT), which inhibits a number of proteolytic enzymes (called proteases or proteinases) that hydrolyze and destroy proteins. [Note: The inhibitor was originally named α1-antitrypsin because it inhibits the activity of trypsin, a proteolytic enzyme

synthesized as trypsinogen by the pancreas (see p. 248).] 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 (Figure 4.14). Most of the AAT found in plasma is synthesized and secreted by the liver. AAT comprises more than 90% of the α_1-globulin fraction of normal plasma. Extrahepatic synthesis occurs in monocytes and alveolar macrophages, and may be important in the prevention of local tissue injury by elastase.

2. **Role of α_1-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 (see Figure 4.14). Because lung tissue cannot regenerate, the destruction of the connective tissue of alveolar walls results in emphysema.

3. **Emphysema resulting from α_1-antitrypsin deficiency:** In the United States, approximately 2%–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. The mutation causes the normally monomeric AAT to polymerize within the endoplasmic reticulum of hepatocytes, resulting in decreased secretion of AAT by the liver. Consequently, blood levels of AAT are reduced, decreasing the amount that gets to the alveoli. The polymer that accumulates in the liver may result in cirrhosis (scarring of the liver). 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 gene, 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 airborne pathogens.

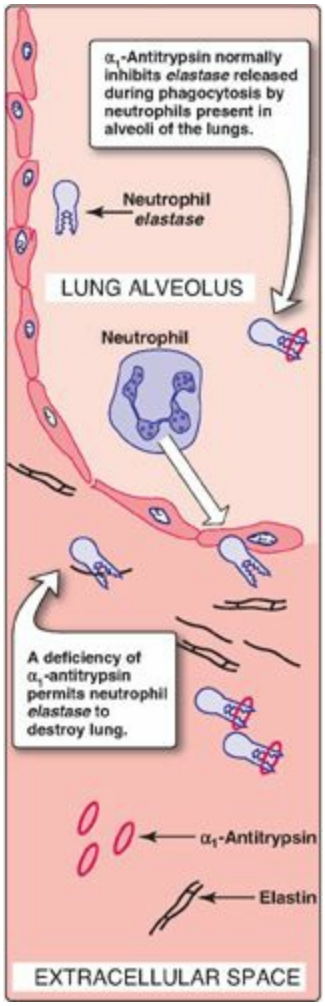
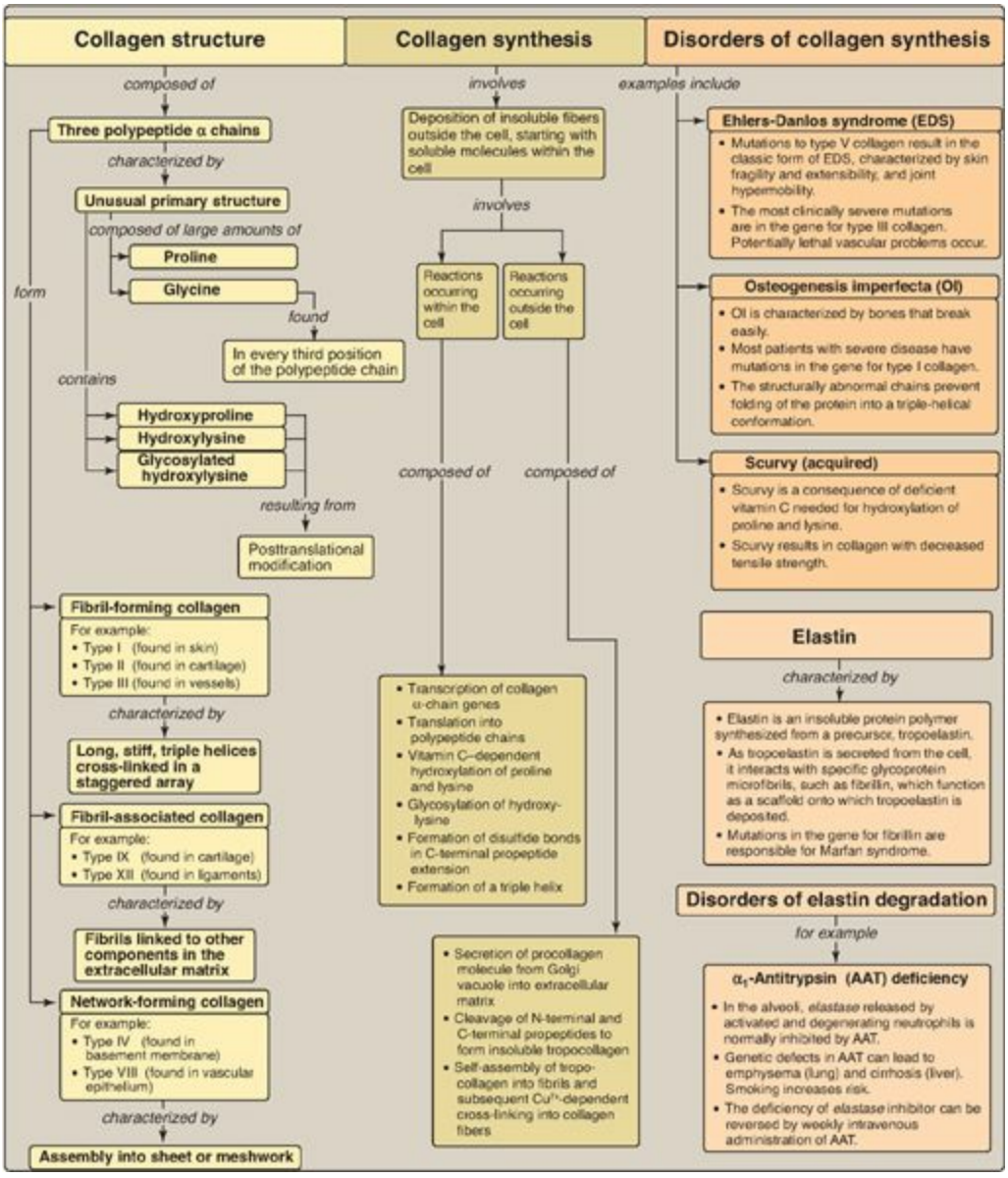


Figure 4.15 Key concept map for the fibrous proteins collagen and elastin.


Collagen structure
Collagen synthesis
Disorders of collagen synthesis
composed of
Three polypeptide α chains
characterized by
Unusual primary structure
composed of large amounts of
Proline
Glycine
found
In every third position of the polypeptide chain
form
contains
Hydroxyproline
Hydroxylysine
Glycosylated hydroxylysine
resulting from
Posttranslational modification
Fibril-forming collagen
For example:
• Type I (found in skin)
• Type II (found in cartilage)
• Type III (found in vessels)
characterized by
Long, stiff, triple helices cross-linked in a staggered array
Fibril-associated collagen
For example:
• Type IX (found in cartilage)
• Type XII (found in ligaments)
characterized by
Fibrils linked to other components in the extracellular matrix
Network-forming collagen
For example:
• Type IV (found in basement membrane)
• Type VIII (found in vascular epithelium)
characterized by
Assembly into sheet or meshwork
involves
Deposition of insoluble fibers outside the cell, starting with soluble molecules within the cell
involves
Reactions occurring within the cell
Reactions occurring outside the cell
composed of
• Transcription of collagen α-chain genes
• Translation into polypeptide chains
• Vitamin C–dependent hydroxylation of proline and lysine
• Glycosylation of hydroxylysine
• Formation of disulfide bonds in C-terminal propeptide extension
• Formation of a triple helix
composed of
• Secretion of procollagen molecule from Golgi vacuole into extracellular matrix
• Cleavage of N-terminal and C-terminal propeptides to form insoluble tropocollagen
• Self-assembly of tropocollagen into fibrils and subsequent Cu^{2+}-dependent cross-linking into collagen fibers
examples include
Ehlers-Danlos syndrome (EDS)
• Mutations to type V collagen result in the classic form of EDS, characterized by skin fragility and extensibility, and joint hypermobility.
• The most clinically severe mutations are in the gene for type III collagen. Potentially lethal vascular problems occur.
Osteogenesis imperfecta (OI)
• OI is characterized by bones that break easily.
• Most patients with severe disease have mutations in the gene for type I collagen.
• The structurally abnormal chains prevent folding of the protein into a triple-helical conformation.
Scurvy (acquired)
• Scurvy is a consequence of deficient vitamin C needed for hydroxylation of proline and lysine.
• Scurvy results in collagen with decreased tensile strength.
Elastin
characterized by
• Elastin is an insoluble protein polymer synthesized from a precursor, tropoelastin.
• As tropoelastin is secreted from the cell, it interacts with specific glycoprotein microfibrils, such as fibrillin, which function as a scaffold onto which tropoelastin is deposited.
• Mutations in the gene for fibrillin are responsible for Marfan syndrome.
Disorders of elastin degradation
for example
α_1-Antitrypsin (AAT) deficiency
• In the alveoli, elastase released by activated and degenerating neutrophils is normally inhibited by AAT.
• Genetic defects in AAT can lead to emphysema (lung) and cirrhosis (liver). Smoking increases risk.
• The deficiency of elastase inhibitor can be reversed by weekly intravenous administration of AAT.

IV. CHAPTER SUMMARY

Collagen and elastin are fibrous proteins (Figure 4.15). Collagen molecules contain an abundance of **proline**, **lysine**, and **glycine**, the latter occurring at every third position in the primary structure. Collagen also contains **hydroxyproline**, **hydroxylysine**, and **glycosylated hydroxylysine**, each formed by posttranslational modification. Collagen molecules typically form **fibrils** containing a long, stiff, triple-stranded helical structure, in which three collagen polypeptide chains are wound around one another in a rope-like superhelix (**triple helix**). Other types of collagen form mesh-like networks. **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 but also by monocytes and alveolar macrophages, prevents elastase-catalyzed degradation of elastin in the alveolar walls. A deficiency of AAT can cause **emphysema** and, in some cases, cirrhosis of the liver.

Study Questions

Choose the ONE best answer.

4.1 A 30-year-old woman 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?

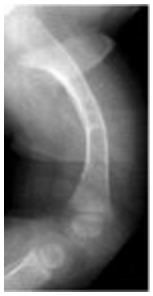
A. Deficiency in dietary vitamin C
B. Deficiency of α_1-antitrypsin
C. Deficiency of prolyl hydroxylase
D Decreased elastase activity
E. Increased collagenase activity

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 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 protease inhibitor) in the lung such that elastase (a protease) is unopposed.

4.3 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 (OI)
D. Type V collagen and Ehlers-Danlos syndrome (EDS)
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 EDS characterized by skin extensibility and fragility and joint hypermobility. Vitamin C deficiency is characterized by capillary fragility.

4.4 How and why is proline hydroxylated in collagen?

Proline is hydroxlyated by prolyl hydroxylase, an enzyme of the rough endoplasmic reticulum that requires O_2, Fe^{2+}, and vitamin C. Hydroxylation increases interchain hydrogen bond formation, strengthening the triple helix of collagen. Vitamin C deficiency impairs hydroxylation.

Enzymes 5

I. OVERVIEW

Virtually all reactions in the body are mediated by enzymes, which are protein catalysts 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 (called substrates) into useful pathways. Enzymes thus direct all metabolic events. This chapter examines the nature of these catalytic molecules and their mechanism 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.

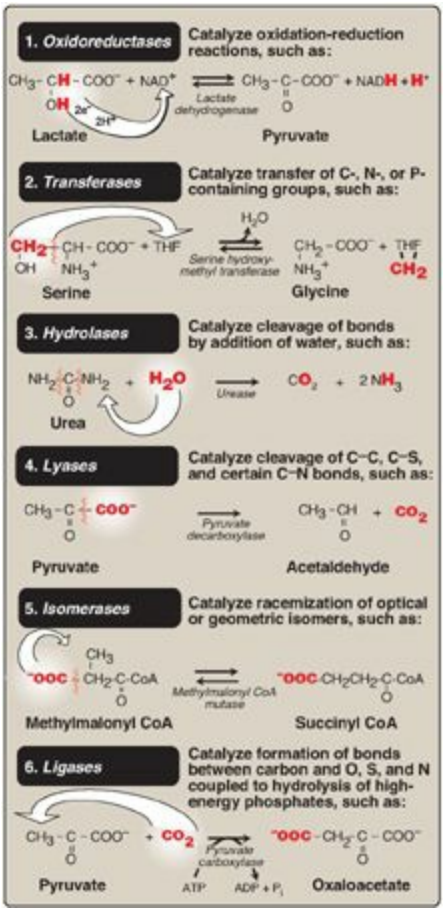
A. Recommended name

Most commonly used enzyme names have the suffix "-ase" attached to the substrate of the reaction (for example, glucosidase and urease) or to a description of the action performed (for example, lactate dehydrogenase and adenylyl cyclase). [Note: Some enzymes retain their original trivial names, which give no hint of the associated enzymic reaction, for example, trypsin and pepsin.]

B. Systematic name

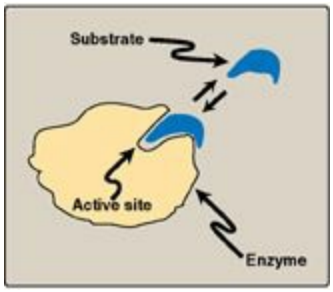
In the systematic naming system, enzymes are divided into six major classes (Figure 5.1), each with numerous subgroups. For a given enzyme, the suffix -ase is attached to a fairly complete description of the chemical reaction catalyzed, including the names of all the substrates, for example, lactate:NAD^+ oxidoreductase. [Note: Each enzyme is also assigned a classification number. Lactate:NAD^+ oxidoreductase, for example, 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.



Potentially confusing enzyme nomenclature: synthetase (requires ATP), synthase (no ATP required); phosphatase (uses water to remove phosphoryl group), phosphorylase (uses P_i to break a bond and generate a phosphorylated product); dehydrogenase (NAD^+/FAD is an electron acceptor in a redox reaction), oxidase (O_2 is the acceptor, and oxygen atoms are not incorporated into substrate), oxygenase (one or both oxygen atoms are incorporated).

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



III. PROPERTIES

Enzymes are protein catalysts that increase the velocity of a chemical reaction and are not consumed during the reaction. [Note: Some RNAs can act like enzymes, usually catalyzing the cleavage and synthesis of phosphodiester bonds. RNAs with catalytic activity are called ribozymes (see p. 439) and are much less commonly encountered than protein catalysts.]

A. Active sites

Enzyme molecules contain a special pocket or cleft called the active site. The active site, formed by folding of the protein, contains amino acid side chains that participate in substrate binding and catalysis (Figure 5.2). The substrate 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 catalysis. ES is converted to an enzyme–product (EP) complex that subsequently dissociates to enzyme and product.

B. Catalytic efficiency

Enzyme-catalyzed reactions are highly efficient, proceeding from 10^3–10^8 times faster than uncatalyzed reactions. The number of molecules of substrate converted to product per enzyme molecule per second is called the turnover number, or k_{cat}, and typically is 10^2–$10^4 s^{-1}$.

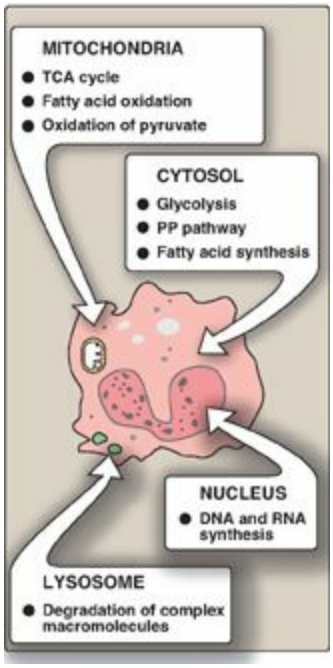
C. Specificity

Enzymes are highly specific, interacting with one or a few substrates and catalyzing only one type of chemical reaction. The set of enzymes made in a cell determines which reactions occur in that cell.

D. Holoenzymes, apoenzymes, cofactors, and coenzymes

Some enzymes require molecules other than proteins for enzymic activity. The term holoenzyme refers to the active enzyme with its nonprotein component, whereas the enzyme without its nonprotein moiety is termed an apoenzyme and is inactive. If the nonprotein moiety is a metal ion, such as Zn^{2+} or Fe^{2+}, it is called a cofactor. If it is a small organic molecule, it is termed a coenzyme. Coenzymes that only transiently associate with the enzyme are called cosubstrates. Cosubstrates dissociate from the enzyme in an altered state (NAD^+ is an example, see p. 101). If the coenzyme is permanently associated with the enzyme and returned to its original form, it is called a prosthetic group (FAD is an example, see p. 110). Coenzymes commonly are derived from vitamins. For example, NAD^+ contains niacin, and FAD contains riboflavin (see Chapter 28).

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



E. Regulation

Enzyme activity can be regulated, that is, increased or decreased, so that the rate of product formation responds to cellular need.

F. Location within the cell

Many enzymes are localized in specific organelles within the cell (Figure 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. HOW ENZYMES WORK

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 free energy of activation, is the energy difference between that of the reactants and a high-energy intermediate that occurs during the formation of product. For example, 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 (high-energy intermediate), T*:

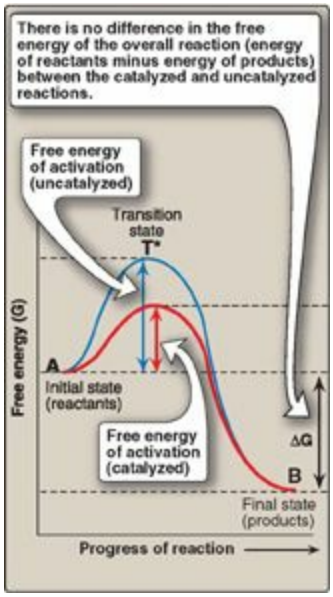
$$A \rightleftarrows T^* \rightleftarrows B$$

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

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 free energy of activation, 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 free energy of activation (see Figure 5.4). The enzyme does not change the free energies of the reactants or products and, therefore, does not change the equilibrium of the reaction (see p. 70). It does, however, accelerate the rate by which equilibrium is reached.

Figure 5.4 Effect of an enzyme on the activation energy of a reaction.



B. Chemistry of the active site

The active site is not a passive receptacle for binding the substrate but, rather, is a complex molecular machine employing a diversity of 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.

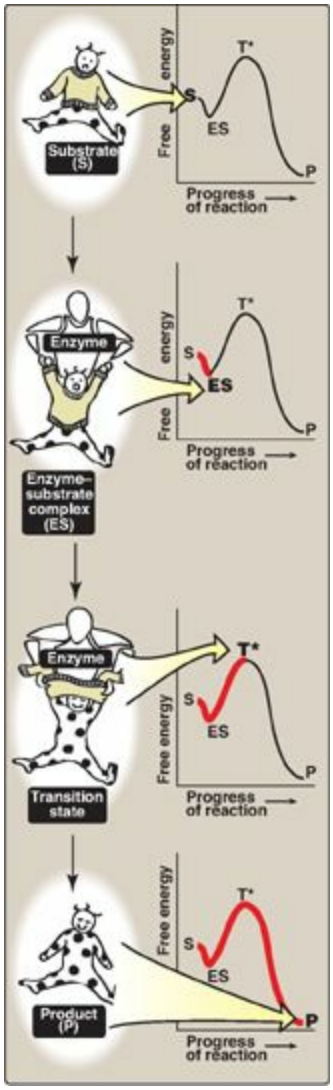
1. **Transition-state stabilization:** The active site often acts as a flexible molecular template that binds the substrate and initiates its conversion to the transition state, a structure in which the bonds are not like those in the substrate or the product (see T* at the top of the curve in Figure 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.]

2. **Other mechanisms:** 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. [Note: 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 covalent link with the substrate.]

3. **Visualization of the transition state:** The enzyme-catalyzed conversion of substrate to product can be visualized as being similar to removing a sweater from an uncooperative infant (Figure 5.5). The process has a high energy of activation because the only reasonable strategy for removing the garment (short of ripping it off) requires that the random flailing of the baby results in both arms being fully

extended over the head, an unlikely posture. However, we can envision a parent acting as an enzyme, first coming in contact with the baby (forming ES), then guiding the baby's arms into an extended, vertical position, analogous to the ES transition state. This posture (conformation) of the baby facilitates the removal of the sweater, forming the disrobed baby, which here 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.]

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



V. FACTORS AFFECTING REACTION VELOCITY

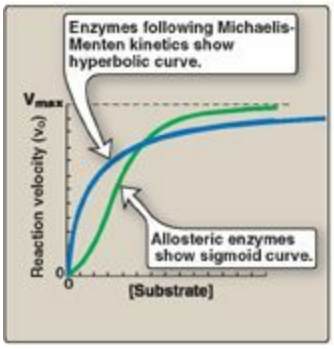
Enzymes can be isolated from cells and their properties studied in a test tube (that is, in vitro). Different enzymes show different responses to changes in substrate concentration, temperature, and pH. This section describes factors that influence the reaction velocity of enzymes. Enzymic responses 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 minute. The rate of an enzyme-catalyzed reaction increases with substrate concentration until a maximal velocity (V_{max}) is reached (Figure 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.

2. **Hyperbolic shape of the enzyme kinetics curve:** Most enzymes show Michaelis-Menten kinetics (see p. 58), in which the plot of initial reaction velocity (v_o) against substrate concentration ([S]), is hyperbolic (similar in shape to that of the oxygen-dissociation curve of myoglobin, see p. 29). In contrast, allosteric enzymes do not follow Michaelis-Menton kinetics and show a sigmoidal curve (see p. 62) that is similar in shape to the oxygen-dissociation curve of hemoglobin (see p. 29).

Figure 5.6 Effect of substrate concentration on reaction velocity.



B. Temperature

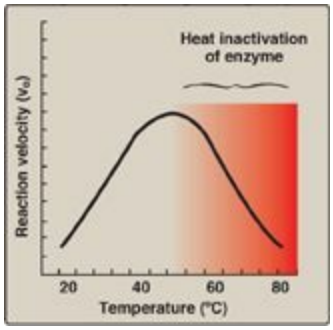
1. **Increase of velocity with temperature:** The reaction velocity increases with temperature until a peak velocity is reached (Figure 5.7). This increase is the result of the increased number of molecules having sufficient energy to pass over the energy barrier and form the products of the reaction.

2. **Decrease of velocity 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 Figure 5.7).

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

Figure 5.7 Effect of temperature on an enzymecatalyzed reaction.



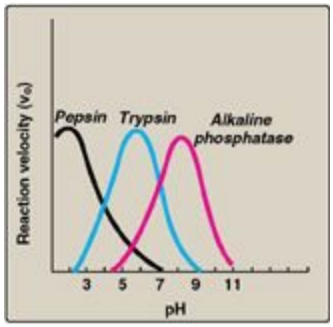
C. pH

1. **Effect of pH on the ionization of the active site:** 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 un-ionized state in order to interact. For example, catalytic activity may require that an amino group of the enzyme be in the protonated form ($–NH_3^+$). At alkaline pH, this group is deprotonated, and the rate of the reaction, therefore, declines.

2. **Effect of pH 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 (Figure 5.8).

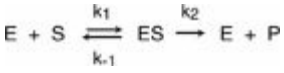
Figure 5.8 Effect of pH on enzyme-catalyzed reactions.


Reaction velocity (v_0)
Pepsin
Trypsin
Alkaline phosphatase
3
5
7
9
11
pH

VI. MICHAELIS-MENTEN KINETICS

A. Reaction model

Leonor Michaelis and Maude Menten proposed a simple model that accounts for most of the features of 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 model, involving one substrate molecule, is represented below:



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 are rate constants

B. Michaelis-Menten equation

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

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

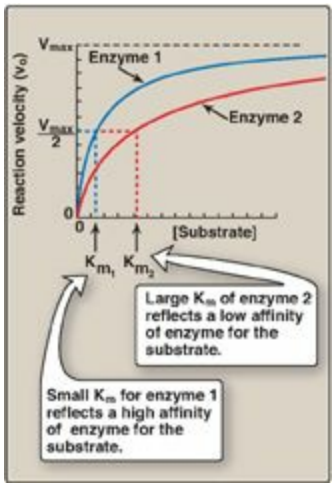
where V_o = initial reaction velocity
V_{max} = maximal velocity
K_m = Michaelis constant = $(k_{-1} + k_2)/k_1$
[S] = substrate concentration

The following assumptions are made in deriving the Michaelis-Menten rate equation:

1. **Relative concentrations of enzyme and substrate:** The concentration of substrate ([S]) is much greater than the concentration of enzyme ([E]), so that the percentage of total substrate bound by the enzyme at any one time is small.

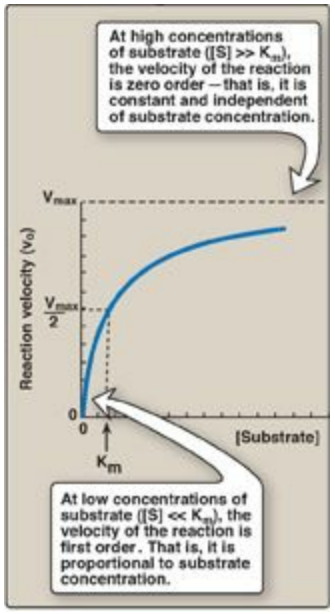
2. **Steady-state assumption:** [ES] does not change with time (the steady-state assumption), that is, the rate of formation of ES is equal to that of the breakdown of ES (to E + S and to E + P). In general, an intermediate in a series of reactions is said to be in steady state when its rate of synthesis is equal to its rate of degradation.

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. **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 back reaction from product to substrate can be ignored.

Figure 5.10 Effect of substrate concentration on reaction velocity for an enzymecatalyzed reaction. V_{max} = maximal velocity; K_m = Michaelis constant.



C. Important conclusions

1. **Characteristics of K_m:** 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 $^1/_2V_{max}$. K_m does not vary with enzyme concentration.

a. Small Km: 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 $^1/_2V_{max}$ (Figure 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. Relationship of velocity to enzyme concentration: The rate of the reaction is directly proportional to the enzyme concentration at all substrate concentrations. For example, if the enzyme concentration is halved, the initial rate of the reaction (v_o), as well as that of V_{max}, are reduced to half that of the original.

3. Order of reaction: When [S] is much less than K_m, the velocity of the reaction is approximately proportional to the substrate concentration (Figure 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 (the enzyme is saturated with substrate) and is said to be zero order with respect to substrate concentration (see Figure 5.10).

D. Lineweaver-Burk plot

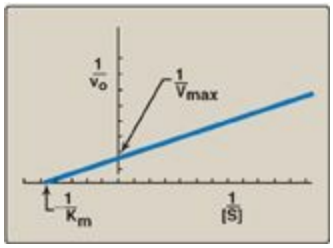
When v_o is plotted against [S], it is not always possible to determine when V_{max} has been achieved because of the gradual upward slope of the hyperbolic curve at high substrate concentrations. However, if $1/v_o$ is plotted versus 1/[S], a straight line is obtained (Figure 5.11). This plot, the Lineweaver-Burk 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.

1. 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 5.11 Lineweaver-Burk plot. v_o = reaction velocity; V_{max} = maximal velocity; K_m = Michaelis constant; [S] = substrate concentration.


1/v_o
1/V_max
-1/K_m
1/[S]

VII. INHIBITION OF ENZYME ACTIVITY

Any substance that can decrease the velocity of an enzyme-catalyzed reaction is called an inhibitor. Inhibitors can be reversible or irreversible. Irreversible inhibitors bind to enzymes through covalent bonds. Lead, for example, forms covalent bonds with the sulfhydryl side chain of cysteine in proteins. Ferrochelatase, an enzyme involved in heme synthesis (see p. 279), is irreversibly inhibited by lead. [Note: An important group of irreversible inhibitors are the mechanism-based inhibitors that are converted by the enzyme itself to a form that covalently links to the enzyme, thereby inhibiting it. They also are referred to as "suicide" inhibitors.] Reversible inhibitors bind to enzymes through noncovalent bonds and, thus, 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

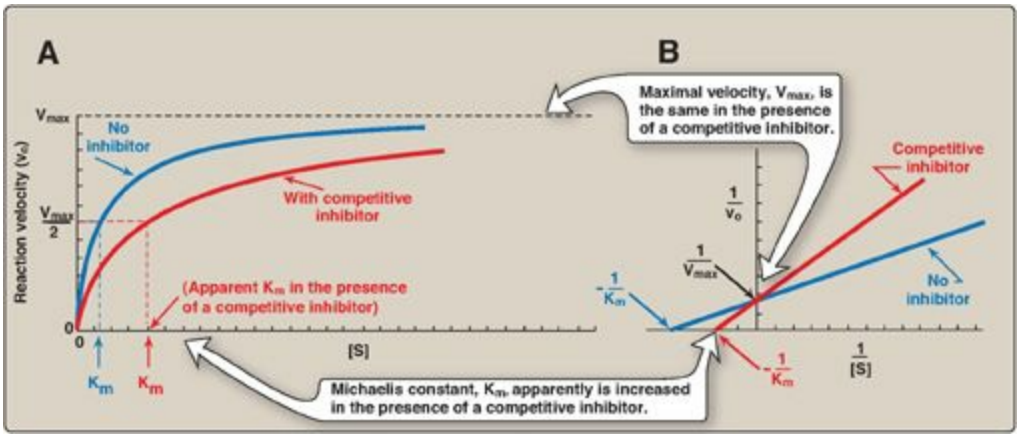
This type of inhibition occurs when the inhibitor binds reversibly to the same site that the substrate would normally occupy and, therefore, competes with the substrate for that site.

1. **Effect on V_{max}:** The effect of a competitive inhibitor is reversed by increasing [S]. At a sufficiently high substrate concentration, the reaction velocity reaches the V_{max} observed in the absence of inhibitor (Figure 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 $^1/2V_{max}$.

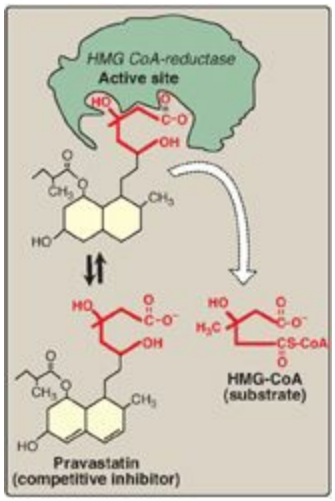
3. **Effect on the Lineweaver-Burk plot:** Competitive inhibition shows a characteristic Lineweaver-Burk plot in which the plots of the inhibited and uninhibited reactions intersect on the y axis at $1/V_{max}$ (V_{max} is unchanged). The inhibited and uninhibited reactions show different x-axis intercepts, 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 Figure 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 with a higher affinity than the substrate.]

Figure 5.12 A. Effect of a competitive inhibitor on the reaction velocity versus substrate ([S]) plot. B. Lineweaver-Burk plot of competitive inhibition of an enzyme.



4. **Statin drugs as examples of competitive inhibitors:** This group of antihyperlipidemic agents competitively inhibits the rate-limiting (slowest) step in cholesterol biosynthesis. This reaction is catalyzed by hydroxymethylglutaryl–CoA reductase (HMG-CoA reductase, see p. 220). Statins, such as atorvastatin (Lipitor) and pravastatin (Pravachol), 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, thereby lowering plasma cholesterol levels (Figure 5.13).

Figure 5.13 Pravastatin competes with HMGCoA for the active site of HMGCoA reductase. HMG-CoA = hydroxymethylglutaryl-coenzyme A.



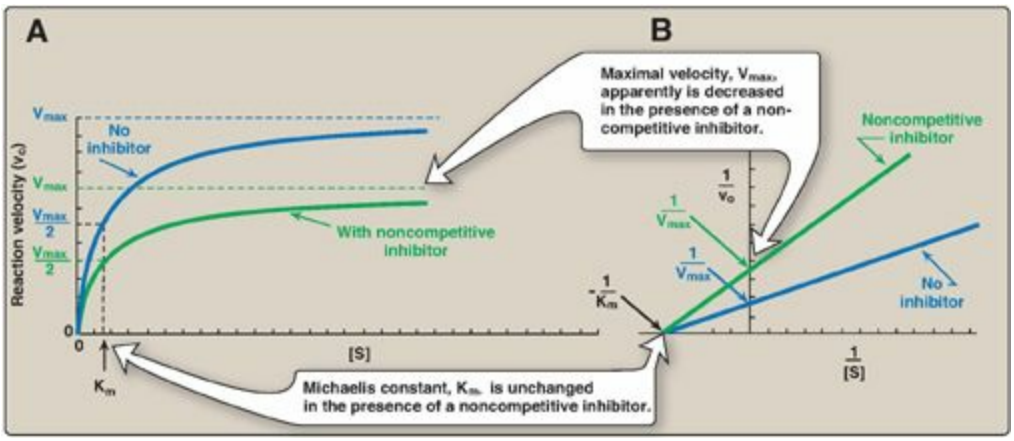
B. Noncompetitive inhibition

This type of inhibition is recognized by its characteristic effect on V_{max} (Figure 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 enzyme-substrate complex, thereby preventing the reaction from occurring (Figure 5.15).

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

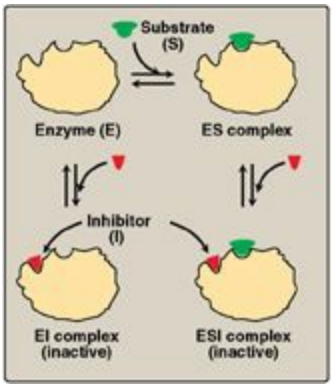
2. **Effect on K_m:** Noncompetitive inhibitors do not interfere with the binding of substrate to enzyme. Therefore, the enzyme shows the same K_m in the presence or absence of the noncompetitive inhibitor.

Figure 5.14 A. Effect of a noncompetitive inhibitor on the reaction velocity versus substrate ([S]) plot. B. Lineweaver-Burk plot of noncompetitive inhibition of an enzyme.



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 Figure 5.14). [Note: Oxypurinol, a metabolite of the drug allopurinol, is a noncompetitive inhibitor of xanthine oxidase, an enzyme of purine degradation (see p. 301).]

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



C. Enzyme inhibitors as drugs

At least half of the ten 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

the enzyme 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 (see p. 214).

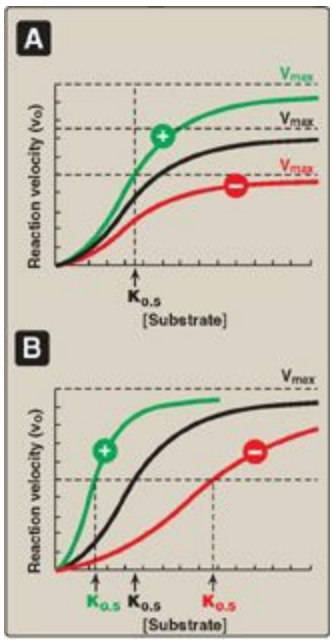
VIII. REGULATION OF ENZYME ACTIVITY

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. Regulation of allosteric enzymes

Allosteric enzymes are regulated by molecules called effectors that bind 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 (Figure 5.16). [Note: Allosteric enzymes frequently catalyze the committed step early in a pathway.]

Figure 5.16 Effects of negative ⊖ or ⊕ positive effectors on an allosteric enzyme. A. Vmax is altered. B. The substrate concentration that gives half-maximal velocity ($K_{0.5}$) is altered.

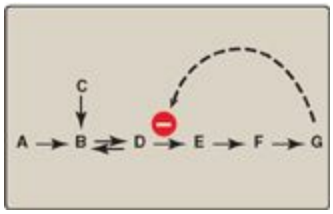


1. **Homotropic effectors:** When the substrate itself serves as an effector, the effect is said to be homotropic. Most often, an allosteric substrate functions as a positive

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 exhibit cooperativity. These enzymes show a sigmoidal curve when reaction velocity (v_o) is plotted against substrate concentration ([S]), 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 p. 29).]

2. **Heterotropic effectors:** The effector may be different from the substrate, in which case the effect 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 endproduct, G. If the concentration of G increases (for example, 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 (see p. 99).

Figure 5.17 Feedback inhibition of a metabolic pathway.



B. Regulation of enzymes by covalent modification

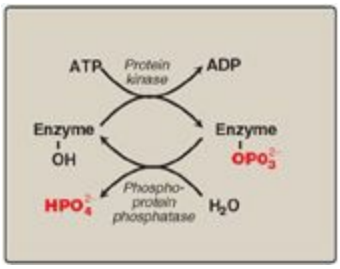
Many enzymes are regulated by covalent modification, most often by the addition or removal of phosphate groups from specific serine, threonine, or tyrosine residues of the enzyme. Protein phosphorylation is recognized as one of the primary ways in which cellular processes are regulated. [Note: Protein phosphorylation is mediated by hormonal signals (see p. 132).]

1. **Phosphorylation and dephosphorylation:** Phosphorylation reactions are catalyzed by a family of enzymes called protein kinases that use ATP as the phosphate donor. Phosphate groups are cleaved from phosphorylated enzymes by the action of phosphoprotein phosphatases (Figure 5.18).

2. **Response of enzyme to phosphorylation:** Depending on the specific enzyme, the phosphorylated form may be more or less active than the unphosphorylated enzyme. For example, phosphorylation of glycogen phosphorylase (an enzyme that

degrades glycogen) increases activity, whereas phosphorylation of glycogen synthase (an enzyme that synthesizes glycogen) decreases activity (p. 132).

Figure 5.18 Covalent modification by the addition and removal of phosphate groups. [Note: HPO_4^{2-} may be represented as P_i.]



C. Induction and repression of enzyme synthesis

The regulatory mechanisms described above 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 p. 105). 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. Figure 5.19 summarizes the common ways that enzyme activity is regulated.

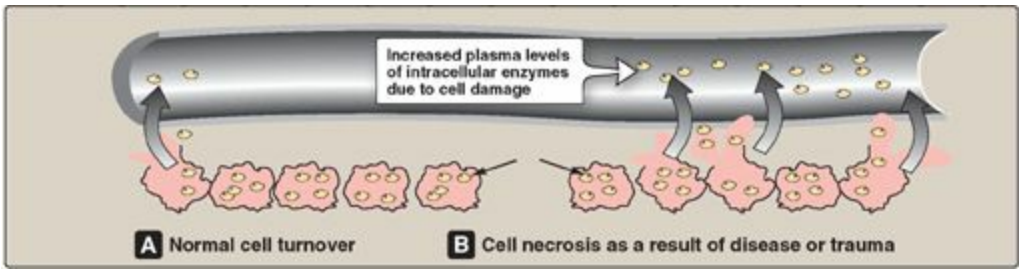
Figure 5.19 Mechanisms for regulating enzyme activity. [Note: Inhibition by pathway end product is also referred to as feedback inhibition.]

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

IX. ENZYMES IN CLINICAL DIAGNOSIS

Plasma enzymes 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 enzymes involved in blood coagulation. Second, a large number of enzyme species are released from cells during normal cell turnover. These enzymes almost always function intracellularly and have no physiologic use in the 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 plasma levels of these enzymes may indicate tissue damage (Figure 5.20).

Figure 5.20 Release of enzymes from normal and diseased or traumatized cells.



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

A. Alteration of plasma enzyme levels in disease states

Many diseases that cause tissue damage result in an increased release of intracellular enzymes into the plasma. The activities of many of these enzymes are routinely determined for diagnostic purposes in diseases of the heart, liver, skeletal muscle, and other tissues. The level of specific enzyme activity in the plasma frequently correlates with the extent of tissue damage. Therefore, determining the degree of elevation of a particular enzyme activity in the plasma is often useful in evaluating the prognosis for the patient.

B. Plasma enzymes as diagnostic tools

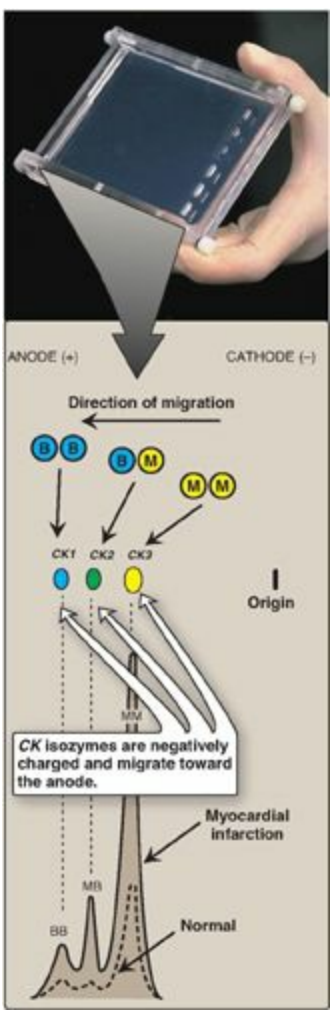
Some enzymes show relatively high activity in only one or a few tissues. The presence of increased levels of these enzymes in plasma thus reflects damage to the corresponding tissue. For example, the enzyme alanine aminotransferase ([ALT] see p. 251) is abundant in the liver. The appearance of elevated levels of ALT in plasma signals possible damage to hepatic tissue. [Note: Measurement of ALT 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 and diseases of the heart

Isoenzymes (also called isozymes) are enzymes that catalyze the same reaction. However, they do not necessarily have the same physical properties because of genetically determined differences in amino acid sequence. For this reason, isoenzymes may contain different numbers of charged amino acids and may, therefore, be separated from each other by electrophoresis (Figure 5.21). Different organs commonly contain characteristic proportions of different isoenzymes. The pattern of isoenzymes found in the plasma may, therefore, serve as a means of identifying the site of tissue damage. For example, the plasma levels of creatine kinase (CK) are commonly determined in the diagnosis of myocardial infarction. They are particularly useful when the electrocardiogram is difficult to interpret such as when there have been previous episodes of heart disease.

Figure 5.21 Subunit composition, electrophoretic mobility, and enzyme activity of creatine kinase (CK) isoenzymes.

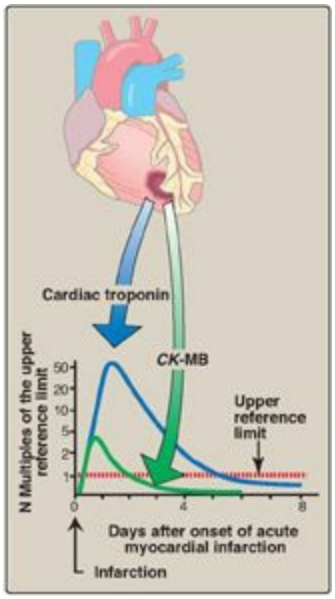


1. **Quaternary structure of isoenzymes:** Many isoenzymes contain different

subunits in various combinations. For example, CK occurs as three isoenzymes. Each isoenzyme is a dimer composed of two polypeptides (called B and M subunits) associated in one of three combinations: CK1 = BB, CK2 = MB, and CK3 = MM. Each CK isoenzyme shows a characteristic electrophoretic mobility (see Figure 5.21). [Note: Virtually all CK in the brain is the BB isoform, whereas in skeletal muscle it is MM. In cardiac muscle, about one third is MB with the rest as MM.]

2. **Diagnosis of myocardial infarction:** Measurement of blood levels of proteins with cardiac specificity (biomarkers) is used in the diagnosis of myocardial infarction (MI). Myocardial muscle is the only tissue that contains more than 5% of the total CK activity as the CK2 (MB) isoenzyme. Appearance of this hybrid isoenzyme in plasma is virtually specific for infarction of the myocardium. Following an acute MI, CK2 appears approximately 4–8 hours following onset of chest pain, reaches a peak of activity at approximately 24 hours, and returns to baseline after 48–72 hours (Figure 5.22). Troponin T and troponin I are regulatory proteins involved in myocardial contractility. They, too, are released into the plasma in response to cardiac damage. Cardiac troponin I (cTnI) is highly sensitive and specific for damage to cardiac tissue. cTnI appears in plasma within 4–6 hours after an MI, peaks in 8–28 hours, and remains elevated for 3–10 days. Elevated cTns, in combination with the clinical presentation and characteristic changes in the electrocardiogram, are currently considered the "gold standard" in the diagnosis of a MI.

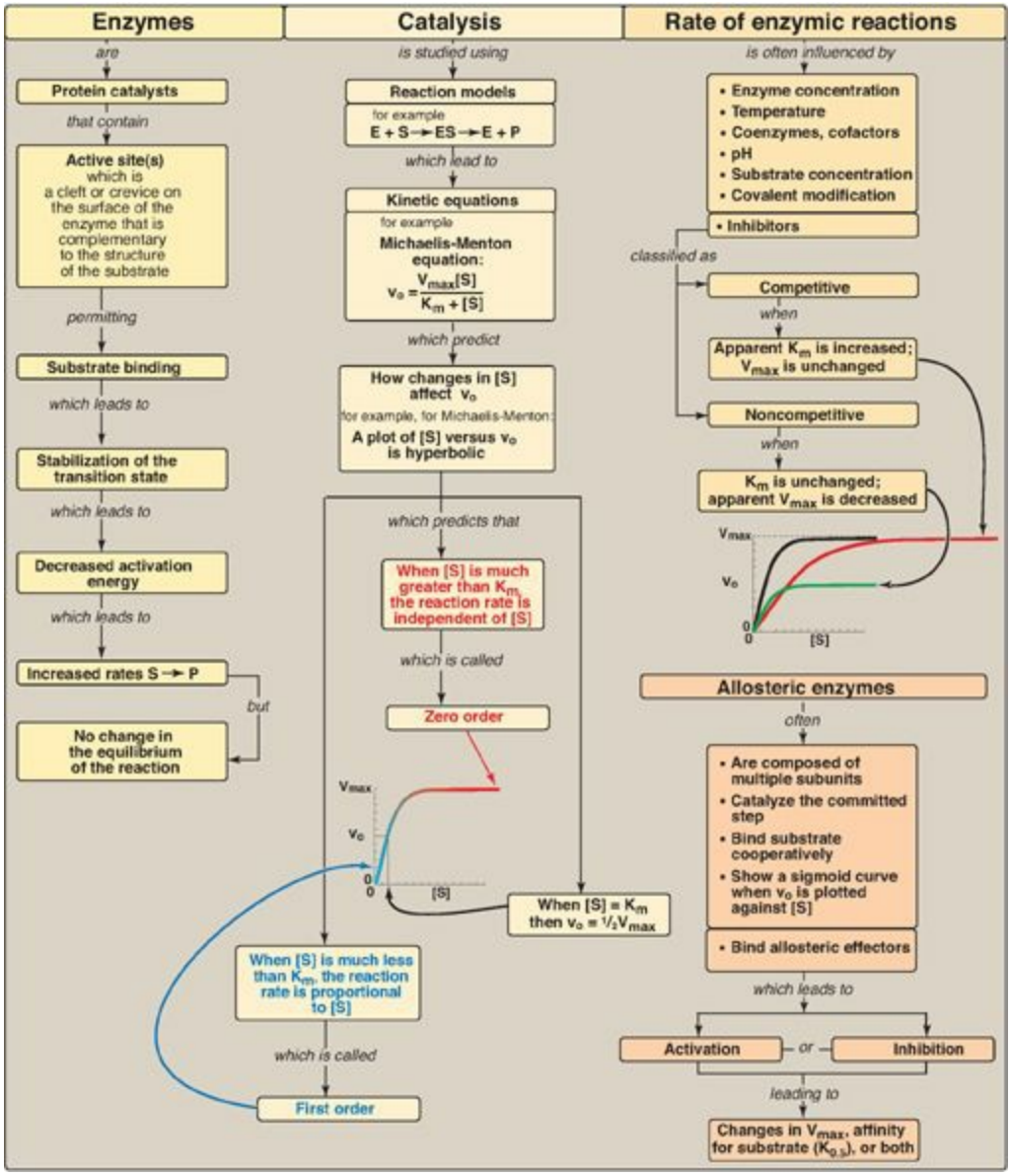
Figure 5.22 Appearance of creatine kinase isozyme CK-MB and cardiac troponin in plasma after a myocardial infarction.



X. CHAPTER SUMMARY

Enzymes are **protein catalysts** that increase the velocity of a chemical reaction by lowering the energy of the transition state (Figure 5.23). Enzymes are not consumed during the reaction they catalyze. Enzyme molecules contain a special pocket or cleft called the **active site**. The active site contains amino acid side chains that participate in substrate binding and catalysis. The active site binds the substrate, forming an **enzyme–substrate (ES) complex**. Binding is thought to cause a conformational change in the enzyme (induced fit) that allows catalysis. ES is converted to enzyme-product (EP), which subsequently dissociates to enzyme and product. An enzyme allows a reaction to proceed rapidly under conditions prevailing in the cell by providing an **alternate reaction pathway** with a **lower free energy of activation**. The enzyme does not change the free energies of the reactants or products and, therefore, does not change the equilibrium of the reaction. Most enzymes show **Michaelis-Menten kinetics**, and a plot of the **initial reaction velocity** ($\mathbf{v_o}$) against **substrate concentration** (**[S]**) has a **hyperbolic** shape similar to the oxygen-dissociation curve of myoglobin. Any substance that can diminish the velocity of such enzyme-catalyzed reactions is called an inhibitor. The two most commonly encountered types of reversible inhibition are **competitive** (which **increases** the **apparent $\mathbf{K_m}$**) and **noncompetitive** (which **decreases** the **apparent $\mathbf{V_{max}}$**). In contrast, the **multisubunit allosteric enzymes** frequently show a **sigmoidal curve** similar in shape to the oxygen-dissociation curve of hemoglobin. They typically catalyze the **rate-limiting** (slowest step) of a pathway. Allosteric enzymes are regulated by molecules called **effectors** that bind noncovalently at a site other than the active site. Effectors can be either **positive** (accelerate the enzyme-catalyzed reaction) or **negative** (slow down the reaction). An allosteric effector can alter the affinity of the enzyme for its substrate, modify the maximal catalytic activity of the enzyme, or both. Enzymes can also be regulated by covalent modification and by changes in the rate of synthesis or degradation. Enzymes have diagnostic and therapeutic value in medicine.

Figure 5.23 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 which gives half maximal velocity.


Enzymes
Catalysis
Rate of enzymic reactions
are
Protein catalysts
that contain
Active site(s) which is a cleft or crevice on the surface of the enzyme that is complementary to the structure of the substrate
permitting
Substrate binding
which leads to
Stabilization of the transition state
which leads to
Decreased activation energy
which leads to
Increased rates S → P
but
No change in the equilibrium of the reaction
is studied using
Reaction models
for example
E + S → ES → E + P
which lead to
Kinetic equations
for example
Michaelis-Menton equation:
$v_o = \frac{V_{max}[S]}{K_m + [S]}$
which predict
How changes in [S] affect v_o
for example, for Michaelis-Menton:
A plot of [S] versus v_o is hyperbolic
which predicts that
When [S] is much greater than K_m, the reaction rate is independent of [S]
which is called
Zero order
V_{max}
v_o
0
0
[S]
When [S] = K_m then $v_o = \frac{1}{2}V_{max}$
When [S] is much less than K_m, the reaction rate is proportional to [S]
which is called
First order
is often influenced by
• Enzyme concentration
• Temperature
• Coenzymes, cofactors
• pH
• Substrate concentration
• Covalent modification
• Inhibitors
classified as
Competitive
when
Apparent K_m is increased; V_{max} is unchanged
Noncompetitive
when
K_m is unchanged; apparent V_{max} is decreased
V_{max}
v_o
0
0
[S]
Allosteric enzymes
often
• Are composed of multiple subunits
• Catalyze the committed step
• Bind substrate cooperatively
• Show a sigmoid curve when v_o is plotted against [S]
• Bind allosteric effectors
which leads to
Activation
or
Inhibition
leading to
Changes in V_{max}, affinity for substrate ($K_{0.5}$), or both

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	Rate of Reaction (mol/L/s)	Substrate Concentration without Ethanol	Rate of Reaction (mol/L/s)
5 mM	3×10^{-7}	5 mM	8×10^{-7}
10 mM	5×10^{-7}	10 mM	1.2×10^{-6}
20 mM	1.0×10^{-6}	20 mM	1.8×10^{-6}
40 mM	1.6×10^{-6}	40 mM	1.9×10^{-6}
80 mM	2.0×10^{-6}	80 mM	2.0×10^{-6}

Correct answer = 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 $^1/2\ V_{max}$. 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 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.

For Questions 5.3 and 5.4, use the graph below which shows the changes in free energy when a reactant is converted to a product in the presence and absence of an

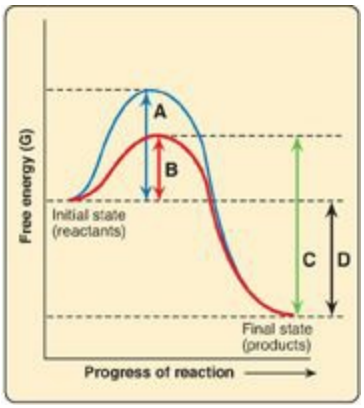
enzyme. Select the letter that best represents:

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.

5.3 The free energy of activation of the catalyzed forward reaction.

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

5.4 The free energy of the reaction.



Bioenergetics and Oxidative Phosphorylation

6

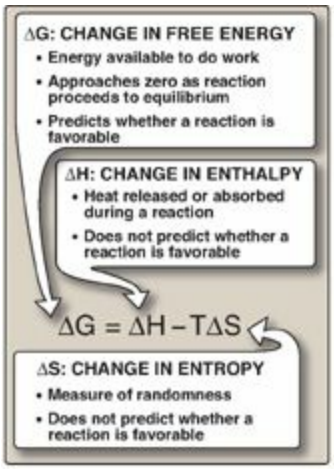
I. OVERVIEW

Bioenergetics describes the transfer and utilization of energy in biologic systems. It makes use of a few basic ideas from the field of thermodynamics, particularly the concept of free energy. Changes in free energy provide a measure of the energetic feasibility of a chemical reaction and can, therefore, allow prediction of whether a reaction or process can take place. Bioenergetics concerns only the initial and final energy states of reaction components, not the mechanism or how much time is needed for the chemical change to take place (the rate). In short, bioenergetics predicts if a process is possible, whereas kinetics measures how fast the reaction occurs (see p. 54).

II. FREE ENERGY

The direction and extent to which a chemical reaction proceeds is determined by the degree to which two factors change during the reaction. These are enthalpy (ΔH, a measure of the change in heat content of the reactants and products) and entropy (ΔS, a measure of the change in randomness or disorder of reactants and products; 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 Figure 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): K = ºC + 273.



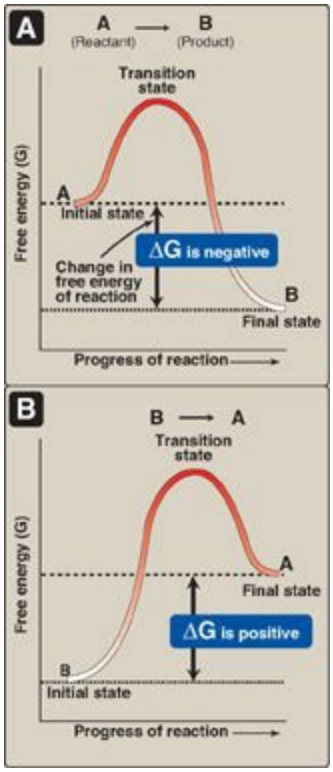
III. FREE ENERGY CHANGE

The change in free energy is represented in two ways, ΔG and ΔG°. The first, ΔG (without the superscript "o"), represents the change in free energy and, thus, the direction of a reaction at any specified concentration of products and reactants. DG, then, is a variable. This contrasts with the standard free energy change, ΔG° (with the superscript "o"), which is the energy change when reactants and products are at a concentration of 1 mol/l. [Note: The concentration of protons is assumed to be 10^{-7} mol/l (that is, pH = 7). This may be shown by a prime sign (**l**), for example, ΔG°**l**.] Although ΔG°, 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, ΔG° can readily be determined from measurement of the equilibrium constant (see p. 72). This section outlines the uses of ΔG, and ΔG° is described on p. 71.

A. Sign of ΔG and the direction of a reaction

ΔG can be used to predict the direction of a reaction at constant temperature and pressure. Consider the reaction:

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.



A ⇄ B

1. **Negative ΔG:** If ΔG is negative, there is a net loss of energy, and the reaction goes spontaneously as written (that is, A is converted into B) as shown in Figure 6.2A. The reaction is said to be exergonic.

2. **Positive ΔG:** If ΔG is positive, there is a net gain of energy, and the reaction does not go spontaneously from B to A (see Figure 6.2B). Energy must be added to the system to make the reaction go from B to A. The reaction is said to be endergonic.

3. **ΔG is zero:** If ΔG = 0, the reactants are in equilibrium. [Note: When a reaction is proceeding spontaneously (that is, free energy is being lost) then the reaction continues until ΔG reaches zero and equilibrium is established.]

B. ΔG of the forward and back reactions

The free energy of the forward reaction (A → B) is equal in magnitude but opposite in sign to that of the back 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).]

C. ΔG and the concentration of reactants and products

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

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

where

where ΔG° is the standard free energy change (see 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

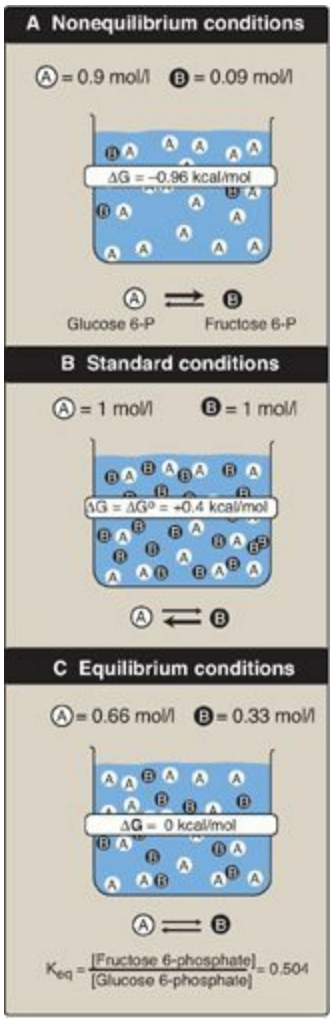
In represents the natural logarithm

A reaction with a positive ΔG° can proceed in the forward direction (have a negative overall ΔG) if the ratio of products to reactants ([B]/[A]) is sufficiently small (that is, the ratio of reactants to products is large). 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° being positive. Thus, the reaction can proceed in the forward direction.

Figure 6.3 Free energy change (Ⓐ) 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.



D. Standard free energy change

The standard free energy change, ΔG^o, is so called because it is equal to the free energy change, ΔG, under standard conditions (that is, when reactants and products are at 1 mol/l concentrations; see Figure 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:

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

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

2. Relationship between ΔG^o and K_{eq}: In a reaction A ⇄ B, a point of equilibrium is reached at which no further net chemical change takes place (that is, when A is being converted to B as fast as B is being converted to A). 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 go to equilibrium at constant temperature and pressure, then, at equilibrium, the overall ΔG is zero. Therefore,

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

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,

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

This equation allows some simple predictions:

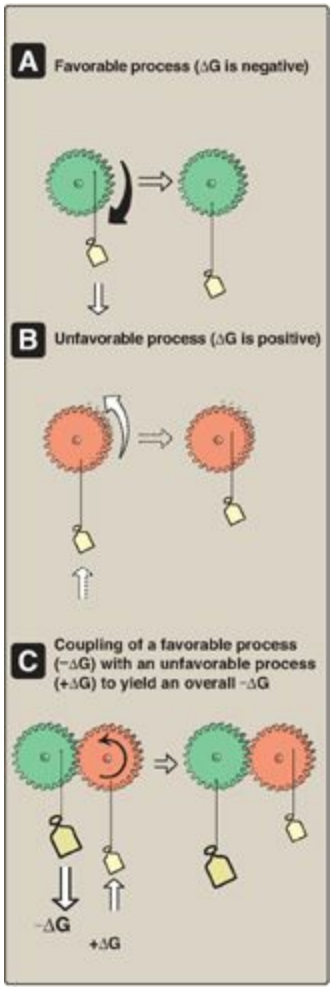
If $K_{eq} = 1$, then $\Delta G^o = 0$	A ⇌ B
If $K_{eq} > 1$, then $\Delta G^o < 0$	A ⇌ B
If $K_{eq} < 1$, then $\Delta G^o > 0$	A ⇌ B

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

Glucose + ATP	→ glucose 6-phosphate + ADP	ΔG^o = –4,000 cal/mol
Glucose 6-phosphate	→ fructose 6-phosphate	ΔG^o = +400 cal/mol
Glucose + ATP	→ fructose 6-phosphate + ADP	ΔG^o = –3,600 cal/mol

4. ΔGs of a pathway: The additive property of free energy changes is very important in biochemical pathways through which substrates must pass in a particular direction (for example, A → B → C → D →...). As long as the sum of the ΔGs of the individual reactions is negative, the pathway can potentially proceed as written, even if some of the individual reactions of the pathway have a positive ΔG. The actual rate of the reactions does, of course, depend on the lowering of activation energies by the enzymes that catalyze the reactions (see p. 55).

Figure 6.4 Mechanical model of 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.



IV. ADENOSINE TRIPHOSPHATE AS AN ENERGY CARRIER

Reactions or processes that have a large positive ΔG, such as moving ions against a concentration gradient across a cell membrane, 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 adenosine triphosphate (ATP). [Note: In the absence of enzymes, ATP is a stable molecule because its hydrolysis has a high activation energy (see p. 55).] 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

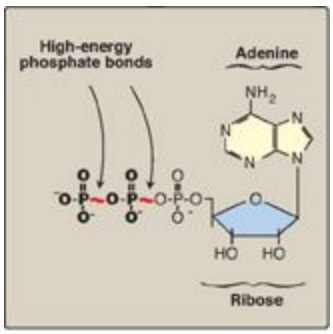
Two chemical reactions have a common intermediate when they occur sequentially so that the product of the first reaction is a substrate for the second. For example, given the reactions

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

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

D is the common intermediate and can serve as a carrier of chemical energy between the two reactions. 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 adenosine diphosphate (ADP), forming ATP.

Figure 6.5 Adenosine triphosphate.



B. Energy carried by adenosine triphosphate

ATP consists of a molecule of adenosine (adenine + ribose) to which three phosphate groups are attached (Figure 6.5). If one phosphate is removed, ADP is produced. If two phosphates are removed, adenosine monophosphate (AMP) results. The standard free energy of hydrolysis of ATP, ΔG^{o}, is approximately −7.3 kcal/mol for each of the two terminal phosphate groups. Because of this large negative ΔG^{o}, ATP is called a high-energy phosphate compound.

V. ELECTRON TRANSPORT CHAIN

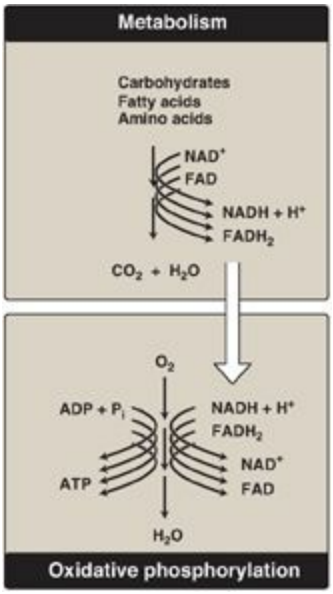
Energy-rich molecules, such as glucose, are metabolized by a series of oxidation reactions ultimately yielding CO_2 and water (Figure 6.6). The metabolic intermediates of these reactions donate electrons to specific coenzymes, nicotinamide adenine dinucleotide (NAD^+) and flavin adenine dinucleotide (FAD), to form the energy-rich reduced forms, NADH and $FADH_2$. 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 protons across the inner mitochondrial membrane, creating a proton gradient that drives the production of ATP from ADP and inorganic phosphate (P_i), described on p. 77. The coupling of electron transport with ATP synthesis is called oxidative phosphorylation, often denoted as OXPHOS. It proceeds continuously in all tissues that contain mitochondria. [Note: The remainder of the free energy not trapped as ATP is used to drive ancillary reactions such as calcium transport into mitochondria (see p. 133) and to generate heat.]

A. The electron transport chain of the mitochondrion

The ETC (except for cytochrome c; see p. 75) 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).

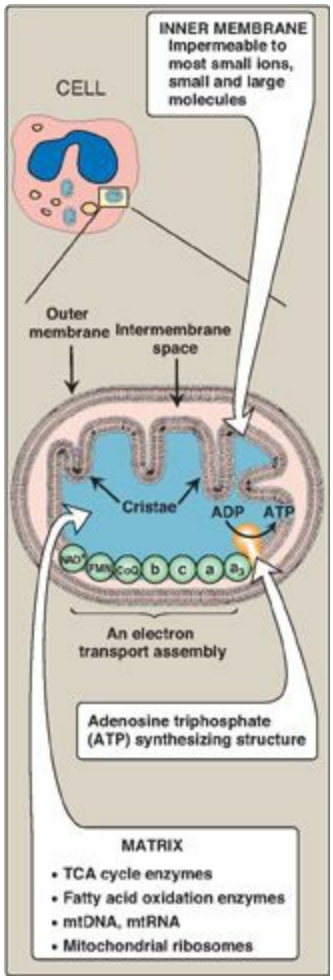
1. **Membranes of the mitochondrion:** The mitochondrion contains an outer and an inner membrane separated by the intermembrane space. Although the outer membrane contains special channels (formed by the protein porin), making it freely permeable to most ions and small molecules, the inner membrane is a specialized structure that is impermeable to most small ions, including protons and small molecules such as ATP, ADP, pyruvate, and other metabolites important to mitochondrial function (Figure 6.7). Specialized carriers or transport systems are required to move ions or molecules across this membrane. The inner mitochondrial membrane is unusually rich in protein, over half of which is directly involved in oxidative phosphorylation. It also is highly convoluted. The convolutions, called cristae, serve to greatly increase the surface area of the inner membrane.

Figure 6.6 The metabolic breakdown of energyyielding molecules. NAD(H) = nicotinamide adenine dinucleotide; FAD(H_2)= flavin adenine dinucleotide; ADP = adenosine diphosphate; ATP = adenosine triphosphate; P_i = inorganic phosphate.



2. **Matrix of the mitochondrion:** This gel-like solution in the interior of mitochondria is also rich in protein. These molecules 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 hydrogen acceptors), and ADP and P_i, which are used to produce ATP. [Note: The matrix also contains mitochondrial DNA (mtDNA) and RNA (mtRNA) and ribosomes.]

Figure 6.7 Structure of a mitochondrion showing schematic representation of the electron transport chain and the ATP synthesizing structure on the inner membrane. [Note: In contrast to the inner membrane, the outer membrane is highly permeable, and the milieu of the intermembrane space is like that of the cytosol.] mtDNA = mitochondrial DNA; mtRNA = mitochondrial RNA; TCA = tricarboxylic acid.



B. Organization of the electron transport chain

The inner mitochondrial membrane contains five separate protein complexes, called Complexes I, II, III, IV, and V. Complexes I–IV each contain part of the ETC (Figure 6.8). These complexes accept or donate electrons to the relatively mobile electron carriers, coenzyme Q 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 protons to form water. 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. Complex V is described on p. 78.

C. Reactions of the electron transport chain

With the exception of coenzyme Q, which is a lipid-soluble quinone, all members of this chain 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 center, may contain iron as part of the porphyrin prosthetic group of heme as in the cytochromes, or may contain copper as does the cytochrome a + a_3 complex.

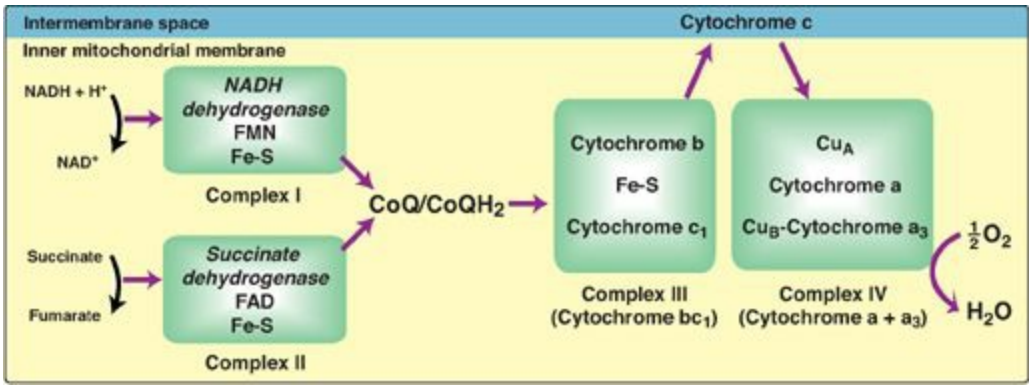
1. **Formation of NADH:** NAD^+ is reduced to NADH by dehydrogenases that remove two hydrogen atoms from their substrate. (For examples of these reactions, see the discussion of the dehydrogenases of the TCA cycle, p. 112.) Both electrons but only

one proton (that is, a hydride ion [:H⁻]) are transferred to the NAD^+, forming NADH plus a free proton.

2. **NADH dehydrogenase:** The free proton 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 (see Figure 28.15, p. 380) that accepts the two hydrogen atoms ($2e^- + 2H^+$), becoming $FMNH_2$. NADH dehydrogenase also contains peptide subunits with iron-sulfur centers (Figure 6.9). At Complex I, electrons move from NADH to FMN to the iron of the iron-sulfur centers and then to coenzyme Q. As electrons flow, they lose energy. This energy is used to pump protons 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 iron-sulfur protein, and then to coenzyme Q. [Note: No energy is lost in this process, and, therefore, no protons are pumped at Complex II.]

Figure 6.8 Electron transport chain. The flow of electrons is shown by the magenta arrows. NAD(H) = nicotinamide adenine dinucleotide; FMN = flavin mononucleotide; FAD = flavin adenine dinucleotide; Fe-S = iron-sulfur center; CoQ = coenzyme Q.



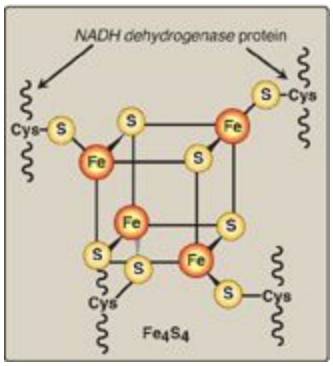
4. **Coenzyme Q:** Coenzyme Q (CoQ) is a quinone derivative with a long, hydrophobic isoprenoid tail. It is also called ubiquinone because it is ubiquitous in biologic systems. CoQ is a mobile electron carrier and can accept hydrogen atoms from NADH dehydrogenase (Complex I), from succinate dehydrogenase (Complex II), and from other mitochondrial dehydrogenases: glycerophosphate dehydrogenase (see p. 79) and acyl CoA dehydrogenase (see p. 192). CoQ transfers electrons to Complex III (cytochrome bc_1). CoQ, then, links the flavoprotein dehydrogenases to the cytochromes.

5. **Cytochromes:** The remaining members of the ETC are cytochrome proteins. Each contains a heme group (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 Figure 6.8). As electrons flow, protons are pumped across the inner mitochondrial membrane at Complexes III and 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 carrier of electrons.]

6. **Cytochrome a + a_3:** 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 and so also is called cytochrome oxidase. At Complex IV, the transported electrons, O_2, and free protons are brought together, and O_2 is reduced to water (see Figure 6.8). [Note: Four electrons are required to reduce one molecule of O_2 to two molecules of water.] Cytochrome oxidase contains copper (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.

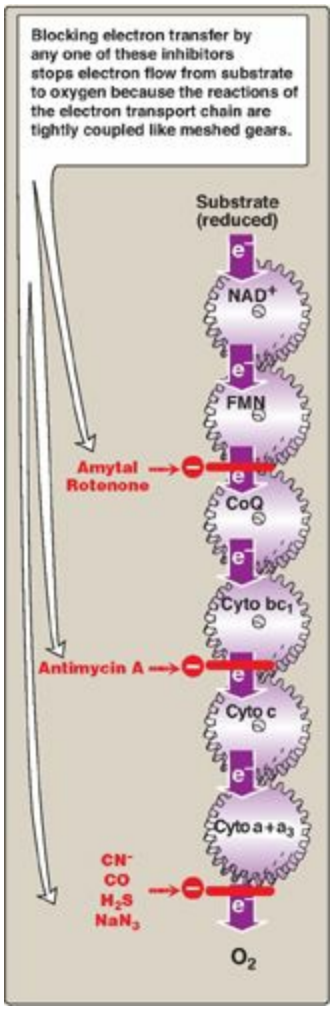
Figure 6.9 Iron-sulfur (Fe-S) center of Complex I. [Note: Complexes II and III also contain iron-sulfur centers.] NADH = nicotinamide adenine dinucleotide;Cys = cysteine.



7. **Site-specific inhibitors:** Site-specific inhibitors of electron transport have been identified and are illustrated in Figure 6.10. These compounds 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 electron transport inhibits ATP synthesis because these processes are tightly coupled (see p. 77).]

Incomplete reduction of oxygen to water produces reactive oxygen species (ROS), such as superoxide ($O_2^{-}\bullet$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH•). ROS damage DNA and proteins and cause lipid peroxidation. Enzymes such as superoxide dismutase (SOD) , catalase, and glutathione peroxidase are cellular defenses against ROS.

Figure 6.10 Site-specific inhibitors of electron transport shown using a mechanical model for the coupling of oxidationreduction reactions. [Note: Figure illustrates normal direction of electron flow.] CN^- = cyanide; CO = carbon monoxide; H_2S = hydrogen sulfide; NaN3 = sodium azide; FMN = flavin mononucleotide; FAD = flavin adenine dinucleotide; CoQ = coenzyme Q; Cyto = cytochrome.



D. Release of free energy during electron transport

The free energy released as electrons are transferred along the ETC from an electron donor (reducing agent or reductant) to an electron acceptor (oxidizing agent or oxidant) is used to pump protons at Complexes I, III, and IV. [Note: The electrons can be transferred as hydride ions (**:**H^-) to NAD^+; as hydrogen atoms (•H) to FMN, CoQ, and FAD; or as electrons (e^-) 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 oxidation-reduction reactions can be written as the sum of two separate half-reactions, one an oxidation and the other a reduction (see Figure 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_o (the standard reduction potential), with units in volts.

2. **Standard reduction potential:** The E_o of various redox pairs can be ordered from the most negative E_o to the most positive. The more negative the E_o of a redox pair, the greater the tendency of the reductant member of that pair to lose electrons. The more positive the E_o, 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_o to that with the more positive E_o. The E_o 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_o values.]

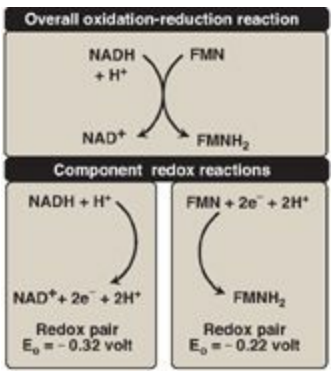
3. **Relationship of ΔG^o to ΔE_{ol}:** The ΔG^o is related directly to the magnitude of the change in E_o:

$$\Delta G^o = - n F \Delta E_o$$

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

4. **ΔG^o of ATP:** The ΔG^o 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 52.58 kcal. Therefore, more than sufficient energy is available to produce 3 ATP from 3 ADP and 3 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.]

Figure 6.11 Oxidation of NADH by FMN, separated into two component half-reactions. NAD(H) = nicotinamide adenine dinucleotide; FMN(H_2) = flavin mononucleotide.



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.

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 the phosphorylation of ADP by the pumping of protons across the inner mitochondrial membrane, from the matrix to the intermembrane space, at Complexes I, III, and IV. This process creates an electrical gradient (with more positive charges on the outside of the membrane than on the inside) and a pH gradient (the outside of the membrane is at a lower pH than the inside) as shown in Figure 6.13. The energy generated by this proton gradient is sufficient to drive ATP synthesis. Thus, the proton gradient serves as the common intermediate that couples oxidation to phosphorylation.

2. **ATP synthase:** The multisubunit enzyme ATP synthase (Complex V; see Figure 6.14) synthesizes ATP using the energy of the proton gradient. It contains a 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 Figure 6.13). The chemiosmotic hypothesis proposes that after protons have been pumped to the cytosolic side of the inner mitochondrial membrane, they reenter the matrix by passing through a proton 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. F_o rotation causes conformational changes in the β subunits of the F_1 domain that allow them to bind ADP + P_i, phosphorylate ADP to ATP, and release ATP. [Note: ATP synthase is also called F_1/F_o-ATPase because the isolated enzyme can catalyze the hydrolysis of ATP to ADP and P_i.]

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

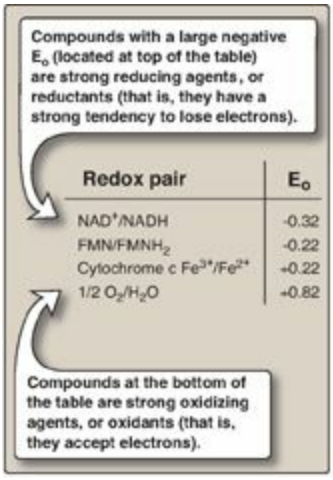
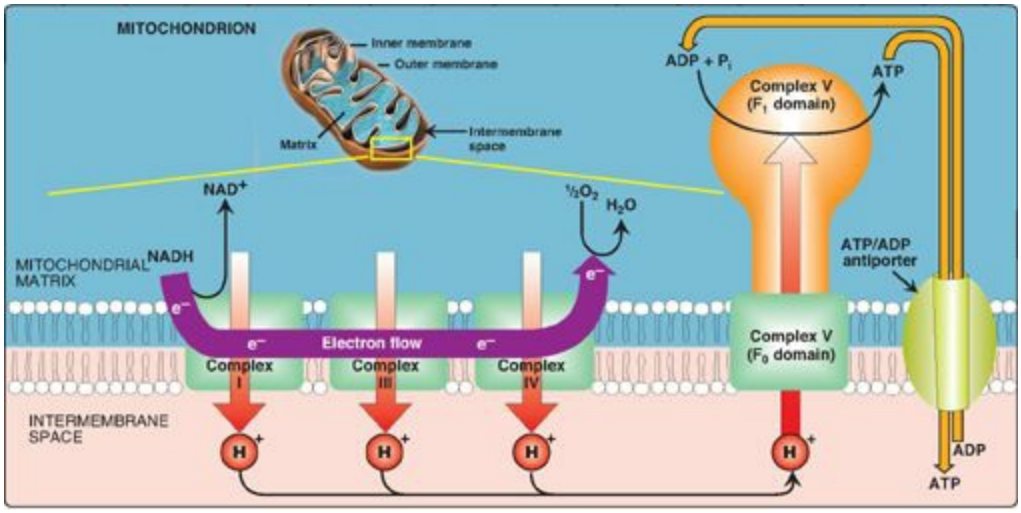


Figure 6.13 Electron transport chain shown in association with the transport of protons (H^+). A total of ten H+ are pumped for each nicotinamide adenine dinucleotide (NADH) oxidized. [Note: H^+ are not pumped at Complex II.]



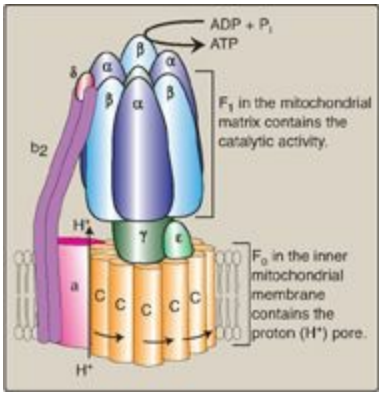
a. Coupling in oxidative phosphorylation: In normal mitochondria, ATP synthesis is coupled to electron transport through the proton gradient. Increasing (or decreasing) one process has the same effect on the other. For example, hydrolysis of ATP to ADP and P_i in energy-requiring reactions increases the availability of substrates for ATP synthase and, thus, increases proton flow through the enzyme. Electron transport and proton pumping by the ETC increase to maintain the proton gradient. [Note: Increased oxidation of NADH at Complex I and, consequently, an increase in NADH-producing pathways of metabolism, such as the TCA cycle, results.]

b. Oligomycin: This drug binds to the F_0 (hence the letter "o") domain of ATP synthase, closing the proton channel and preventing reentry of protons into the matrix, thereby preventing phosphorylation of ADP to ATP. Because the pH and electrical gradients cannot be dissipated in the presence of this drug, electron transport stops because of the difficulty of pumping any more protons against the steep gradients. 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.

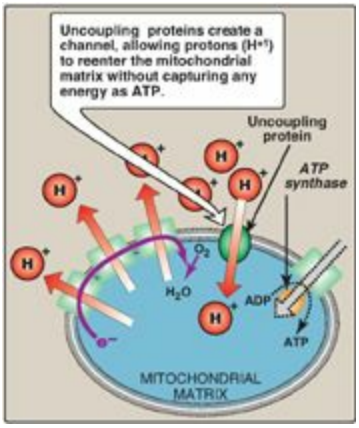
c. Uncoupling proteins: Uncoupling proteins (UCPs) occur in the inner mitochondrial membrane of mammals, including humans. These proteins form channels that allow protons to reenter the mitochondrial matrix without energy being captured as ATP (Figure 6.15). The energy is released as heat, and the process is called nonshivering thermogenesis. UCP1, also called thermogenin, is responsible for heat production in the brown adipocytes of mammals. In brown fat, unlike the more abundant white fat, almost 90% of its respiratory energy is used for thermogenesis in response to cold in the neonate and during arousal in hibernating animals. However, humans appear to have few concentrated deposits of brown fat (except in the newborn), and UCP1 does not appear to play a major role in energy balance. [Note: Uncoupling proteins UCP2–UCP5 have been found in other tissues, but their full significance remains unclear.]

Figure 6.14 ATP synthase (F_1F_o-ATPase). [Note: The rotation of the ring of c subunits in the Fo domain results in conformational changes in the β subunits of the F1 domain that allow phosphorylation of adenosine diphosphate (ADP) to adenosine triphosphate (ATP). P_i = inorganic phosphate.



d. Synthetic uncouplers: Electron transport and phosphorylation can also be uncoupled by compounds that pick up protons in the intermembrane space and release them in the matrix, dissipating the gradient. The classic example is 2,4-dinitrophenol, a lipophilic proton carrier that readily diffuses through the mitochondrial membrane. This uncoupler causes electron transport to proceed at a rapid rate without establishing a proton gradient, much as do the UCPs (see Figure 6.15). Again, energy is released as heat rather than being used to synthesize ATP. [Note: In high doses, aspirin and other salicylates uncouple oxidative phosphorylation. This explains 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; ATP = adenosine triphosphate.



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 specific molecules from the cytosol (or more correctly, the intermembrane space) 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 Figure 6.13). A transporter moves P_i from the cytosol into mitochondria.

2. **Transport of reducing equivalents:** The inner mitochondrial membrane lacks an NADH transporter, and NADH produced in the cytosol (for example, in glycolysis; see p. 101) cannot directly enter the mitochondrial matrix. However, two electrons (reducing equivalents) of NADH are transported from the cytosol into the matrix using substrate shuttles. In the glycerophosphate shuttle (Figure 6.16A), two electrons are transferred from NADH to dihydroxyacetone phosphate by cytosolic glycerophosphate 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 $FADH_2$. The glycerophosphate shuttle, therefore, results in the synthesis of two ATPs for each cytosolic NADH oxidized. This contrasts with the malate-aspartate shuttle (Figure 6.16B), which produces NADH (rather than $FADH_2$) in the mitochondrial matrix and, therefore, yields three ATPs for each cytosolic NADH oxidized by malate dehydrogenase as oxaloacetate is reduced to malate. A transport protein moves malate into the matrix.

C. Inherited defects in oxidative phosphorylation

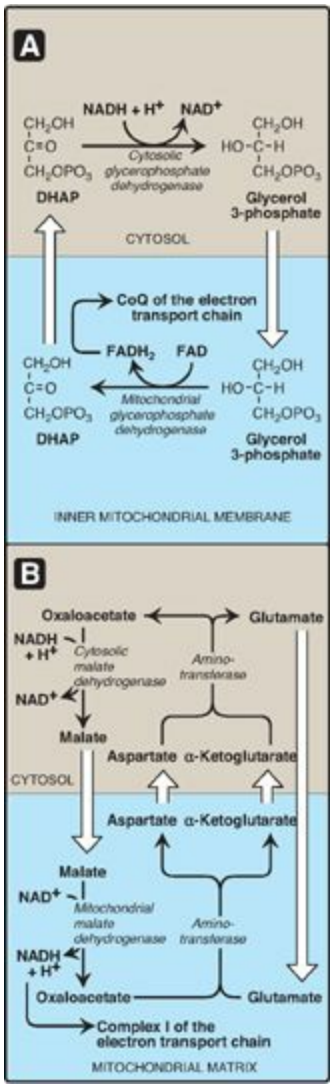
Thirteen of the approximately 90 polypeptides required for oxidative phosphorylation are coded for by mtDNA and synthesized in mitochondria, whereas the remaining proteins are coded for by nuclear DNA, synthesized in the cytosol, and transported into

mitochondria posttranslationally. 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. Tissues with the greatest ATP requirement (for example, central nervous system, skeletal and heart muscle, and liver) are most affected by defects in oxidative phosphorylation. Mutations in mtDNA are responsible for several diseases, including some cases of mitochondrial myopathies, and Leber hereditary optic neuropathy, a disease in which bilateral loss of central vision occurs as a result of neuroretinal degeneration, including damage to the optic nerve. [Note: mtDNA is maternally inherited because mitochondria from the sperm cell do not enter the fertilized egg.]

D. Mitochondria and apoptosis

The process of apoptosis, or programmed cell death, may be initiated through the intrinsic (mitochondrial-mediated) pathway by the formation of pores in the outer mitochondrial membrane. These pores allow cytochrome c to leave the intermembrane space and enter the cytosol. There, cytochrome c, in association with proapoptotic factors, activates a family of proteolytic enzymes (the caspases), causing cleavage of key proteins and resulting in the morphologic and biochemical changes characteristic of apoptosis.

Figure 6.16 Substrate shuttles for the transportof electrons across the inner mitochondrial membrane. A. Glycerophosphate shuttle. B. Malate-aspartate shuttle. DHAP = dihydroxyacetone phosphate; NAD(H) = nicotinamide adenine dinucleotide; FAD(H_2) = flavin adenine dinucleotide; CoQ = coenzyme Q.

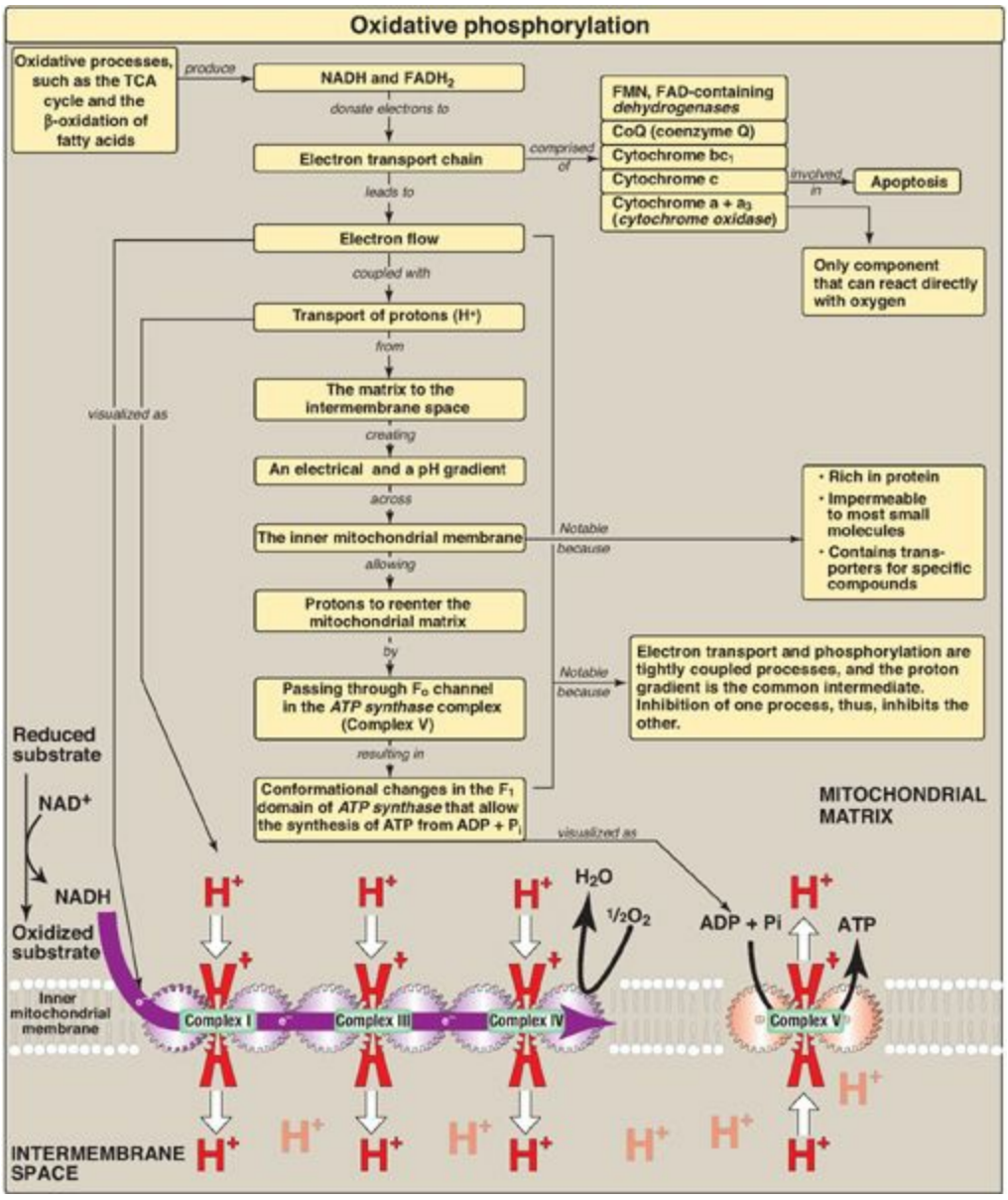

A
NADH + H+
NAD+
CH2OH
C=O
CH2OPO3
DHAP
Cytosolic glycerophosphate dehydrogenase
CH2OH
HO-C-H
CH2OPO3
Glycerol 3-phosphate
CYTOSOL
CoQ of the electron transport chain
FADH2
FAD
Mitochondrial glycerophosphate dehydrogenase
INNER MITOCHONDRIAL MEMBRANE
B
Oxaloacetate
Glutamate
NADH + H+
Cytosolic malate dehydrogenase
Amino-transferase
NAD+
Malate
Aspartate
α-Ketoglutarate
CYTOSOL
Mitochondrial malate dehydrogenase
Complex I of the electron transport chain
MITOCHONDRIAL MATRIX

VII. CHAPTER SUMMARY

The change in **free energy** (**ΔG**) occurring during a reaction predicts the direction in which that reaction will spontaneously proceed. If **ΔG** is **negative** (that is, the product has a lower free energy than the substrate), the **reaction goes spontaneously**. If **ΔG** is **positive**, the reaction **does not go spontaneously**. If **ΔG = 0**, the reactions are in **equilibrium**. The **ΔG** of the forward reaction is equal in magnitude but opposite in sign to that of the back reaction. The **ΔG**s are **additive** in any sequence of consecutive reactions, as are the standard free energy changes (**ΔG°**s). Therefore, reactions or processes that have a large, positive ΔG are made possible by **coupling** with those that have a large, negative ΔG such as hydrolysis of **adenosine triphosphate** (**ATP**). The reduced coenzymes **nicotinamide adenine dinucleotide** (**NADH**) and **flavin adenine dinucleotide** (**$FADH_2$**) each donate a pair of electrons to a specialized set of electron carriers, consisting of **flavin mononucleotide** (**FMN**) , **iron-sulfur centers**, **coenzyme Q**, and a series of **cytochromes**, collectively called the **electron transport chain**. This pathway is present in the **inner mitochondrial membrane** (impermeable to most substances) and is the final common pathway by which electrons derived from different fuels of the body flow to O_2, reducing it to water. The terminal cytochrome, cytochrome oxidase, is the only cytochrome able to bind O_2. **Electron transport** results in the **pumping of protons** across the inner mitochondrial membrane from the matrix to the intermembrane space. This process creates **electrical** and **pH gradients** across the inner mitochondrial membrane. After protons have been transferred to the cytosolic side of the membrane, they reenter the matrix by passing through the F_o proton channel in **ATP synthase** (**Complex V**), dissipating the pH and electrical gradients and causing conformational changes in the β subunits of F_1 that result in the synthesis of ATP from adenosine diphosphate + inorganic phosphate. **Electron transport** and **phosphorylation** are **tightly coupled** in oxidative phosphorylation (OXPHOS, Figure 6.17). Inhibition of one process inhibits the other. These processes can be **uncoupled** by **uncoupling protein-1** of the inner mitochondrial membrane of cells in brown fat and by synthetic compounds such as **2,4-dinitrophenol** and **aspirin**, all of which dissipate the proton gradient. In uncoupled mitochondria, the energy produced by the transport of electrons is released as **heat** rather than being used to synthesize ATP. Mutations in **mitochondrial DNA**, which is maternally inherited, are responsible for some cases of **mitochondrial diseases** such as **Leber hereditary optic neuropathy**. The release of cytochrome c into the cytoplasm and subsequent activation of proteolytic caspases results in apoptotic cell death.

Figure 6.17 Key concept map for oxidative phosphorylation (OXPHOS). [Note: Electron (e^-) flow and ATP synthesis are envisioned as sets of interlocking gears to emphasize the

idea of coupling.] TCA = tricarboxylic acid; NAD(H) = nicotinamide adenine dinucleotide; $FAD(H_2)$ = flavin adenine dinucleotide; FMN = flavin mononucleotide.



Study Questions

Choose the ONE best answer.

6.1 2,4-Dinitrophenol, 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-dinitrophenol?

A. Adenosine triphosphate 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 proton 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 is expected. In an attempt to compensate for this defect in energy capture, metabolism and electron flow to oxygen is 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, NADH can be oxidized to NAD^+ by the cytoplasmic isozyme of malate dehydrogenase as oxaloacetate is reduced to malate. The malate is transported across the inner membrane, and the mitochondrial isozyme of malate dehydrogenase oxidizes it to oxaloacetate 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 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 to adenosine triphosphate?

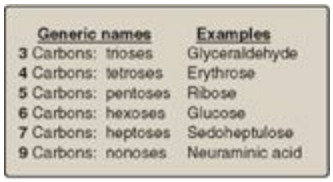
Inhibition of the electron transport chain by respiratory inhibitors such as carbon monoxide results in an inability to maintain the proton gradient. Phosphorylation of ADP to ATP is, therefore, inhibited, as are ancillary reactions such as calcium uptake by mitochondria, because they also require the proton gradient.

Introduction to Carbohydrates 7

I. OVERVIEW

Carbohydrates (saccharides) 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 many 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."

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

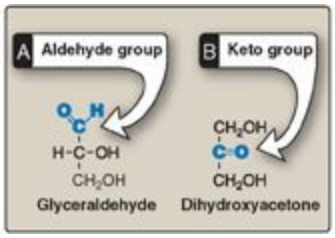


Generic names		Examples
3 Carbons:	trioses	Glyceraldehyde
4 Carbons:	tetroses	Erythrose
5 Carbons:	pentoses	Ribose
6 Carbons:	hexoses	Glucose
7 Carbons:	heptoses	Sedoheptulose
9 Carbons:	nonoses	Neuraminic acid

II. CLASSIFICATION AND STRUCTURE

Monosaccharides (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 (Figure 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 xyulose. There are exceptions, such as fructose, to this rule.] Monosaccharides can be linked by glycosidic bonds to create larger structures (Figure 7.3). Disaccharides contain two monosaccharide units, oligosaccharides contain three to ten monosaccharide units, and polysaccharides contain more than ten monosaccharide units and can be hundreds of sugar units in length.

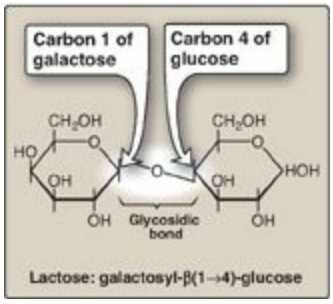
Figure 7.2 Examples of an aldose (A) and a ketose (B) sugar.



A. Isomers and epimers

Compounds that have the same chemical formula but have different structures are called isomers. For example, fructose, glucose, mannose, and galactose are all isomers of each other, having the same chemical formula, $C_6H_{12}O_6$. Carbohydrate isomers that differ in configuration around only one specific carbon atom (with the exception of the carbonyl carbon; see "anomers" 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 group at carbon 4. [Note: The carbons in sugars are numbered beginning at the end that contains the carbonyl carbon (that is, the aldehyde or keto group) as shown in Figure 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 Figure 7.4).

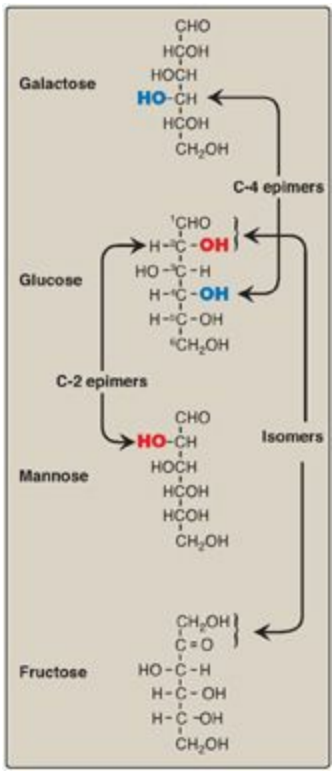
Figure 7.3 A glycosidic bond between two hexoses producing a disaccharide.



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 (Figure 7.5). The vast majority of the sugars in humans are D-sugars. 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 racemases are able to interconvert D- and L-isomers.

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



C. Cyclization of monosaccharides

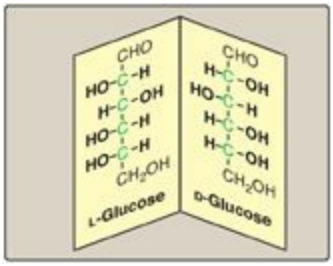
Less than 1% of each of the monosaccharides with five or more carbons exists in the open-chain (acyclic) form in solution. Rather, they are predominantly found in a ring (cyclic) form, in which the aldehyde (or keto) group has reacted with an alcohol group on the same sugar, making the carbonyl carbon (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 (for example, α-D-glucopyranose and β-D-glucopyranose; see 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 (Figure 7.6A) and is trans to the CH_2OH group in a Haworth projection formula (Figure 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 Figure 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, 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 (for example, the Benedict reagent) causing the reagent to be reduced and colored, with the aldehyde group of the acyclic sugar becoming oxidized. All monosaccharides, but not all disaccharides, are reducing sugars. [Note: Glucose can have its terminal hydroxyl group oxidized to a carboxyl group, forming glucuronic acid (see p. 161), or its aldehyde group oxidized to a hydroxyl group, forming a sugar alcohol.]

> 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.

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



D. Joining of monosaccharides

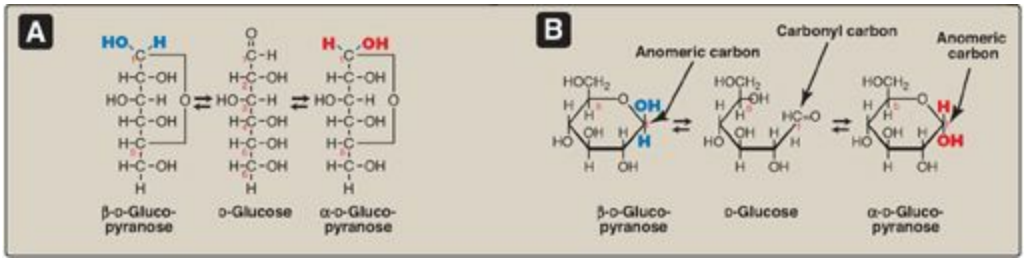
Monosaccharides can be joined to form disaccharides, oligosaccharides, and polysaccharides. Important disaccharides include lactose (galactose + glucose), sucrose (glucose + fructose), and maltose (glucose + glucose). Important polysaccharides include branched glycogen (from animal sources) and starch (plant sources) and unbranched cellulose (plant sources). Each is a polymer of glucose. The bonds that link sugars are called glycosidic bonds. These are formed by enzymes known as glycosyltransferases that use nucleotide sugars such as uridine diphosphate glucose as substrates.

1. **Naming glycosidic bonds:** 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 sugar involved in the bond. If this anomeric hydroxyl is in the α configuration, the linkage is an α-bond. If it is in the β configuration, 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. The linkage is, therefore, a β(1→4) glycosidic bond (see Figure 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.]

E. Complex carbohydrates

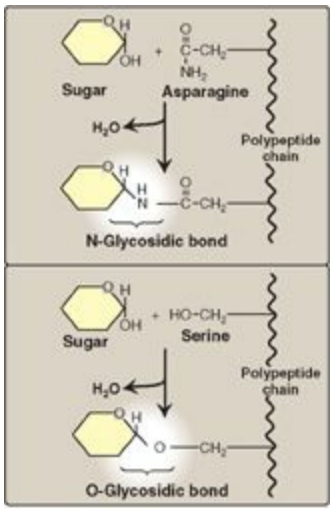
Carbohydrates can be attached by glycosidic bonds to noncarbohydrate structures, including purine and pyrimidine bases (found in nucleic acids), aromatic rings (such as those found in steroids and bilirubin), proteins (found in glycoproteins and proteoglycans), and lipids (found in glycolipids), to form glycosides.

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



1. **N- and O-glycosides:** If the group on the noncarbohydrate molecule to which the sugar is attached is an $–NH_2$ group, the structure is an N-glycoside, and the bond is called an N-glycosidic link. If the group is an –OH, the structure is an O-glycoside, and the bond is an O-glycosidic link (Figure 7.7). [Note: All sugar–sugar glycosidic bonds are O-type linkages.]

Figure 7.7 Glycosides: examples of N- and O-glycosidic bonds.



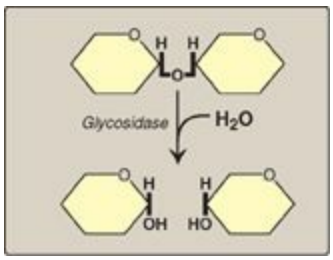
III. DIGESTION OF DIETARY CARBOHYDRATES

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 (Figure 7.8). Because there is little monosaccharide present in diets of mixed animal and plant origin, the enzymes are primarily endoglycosidases that hydrolyze polysaccharides and oliosaccharides, and disaccharidases that hydrolyse 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 of the small intestine.

A. Salivary a-amylase

The major dietary polysaccharides are of plant (starch, composed of amylose and amylopectin) and animal (glycogen) origin. During mastication, 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 (Figure 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.

Figure 7.8 Hydrolysis of a glycosidic bond.



B. Pancreatic a-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.

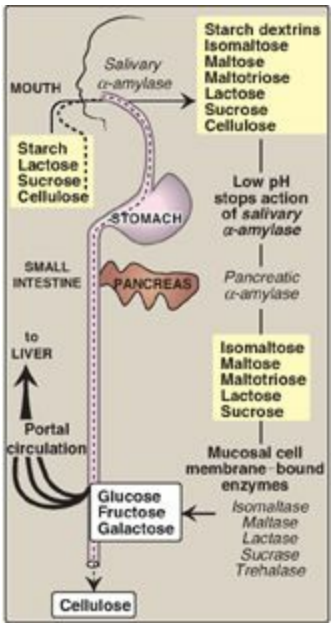
C. Intestinal disaccharidases

The final digestive processes occur primarily at the mucosal lining of the upper

jejunum and include the action of several disaccharidases (see Figure 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 surface of the intestinal mucosal cells.

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

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



D. Intestinal absorption of monosaccharides

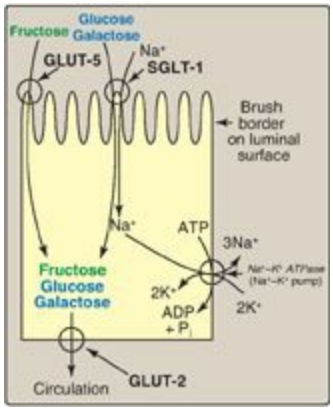
The duodenum and upper jejunum absorb the bulk of the monosaccharide products of digestion. However, different sugars have different mechanisms of absorption (Figure 7.10). For example, galactose and glucose are transported into the mucosal cells by an active, energy-dependent process that requires a concurrent uptake of sodium ions, and the transport protein is the sodium-dependent glucose cotransporter 1 (SGLT-1).

Fructose utilizes an energy- and sodium-independent monosaccharide transporter (GLUT-5) for its absorption. All three monosaccharides are transported from the intestinal mucosal cell into the portal circulation by yet another transporter, GLUT-2. (See p. 97 for a discussion of these transporters.)

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 CO_2 and H_2 gas, causing abdominal cramps, diarrhea, and flatulence.

Figure 7.10 Digestion of carbohydrates. [Note: Indigestible cellulose enters the colon and is excreted.]



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.

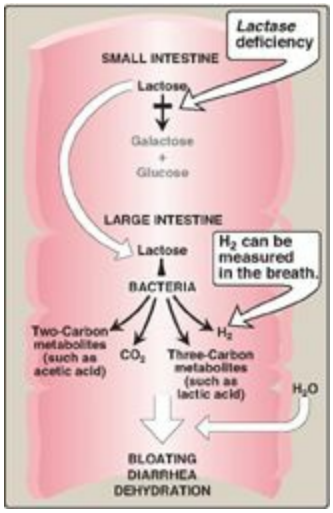
2. **Lactose intolerance:** More than 70% of the world's adults arelactose intolerant (Figure 7.11). This is particularly manifested in certain populations. For example, up to 90% of adults of African or Asian descent are lactase-deficient and, therefore, are less able to metabolize lactose than individuals of Northern European origin. The

age-dependent loss of lactase activity 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 and eat yogurts and some cheeses (bacterial action and aging process decrease lactose content) as well as green vegetables, such as broccoli, to ensure adequate calcium intake; to use lactase-treated products; or to take lactase in pill form prior to eating. [Note: Because the loss of lactase is the norm for most of the world's adults, use of the term "adult hypolactasia" for lactose intolerance is becoming more common.] Rare cases of congenital lactase deficiency are known.

3. **Congenital sucrase-isomaltase deficiency:** This autosomal recessive disorder results in an intolerance of ingested sucrose. Congenital sucrase-isomaltase deficiency has a prevalence of 0.02% in individuals of European descent and appears to be much more common in the Inuit people of Greenland and Canada. Treatment includes the dietary restriction of sucrose and enzyme replacement therapy.

4. **Diagnosis:** Identification of a specific enzyme deficiency can be obtained by performing oral tolerance tests with the individual disaccharides. Measurement of hydrogen gas 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 Figure 7.11).

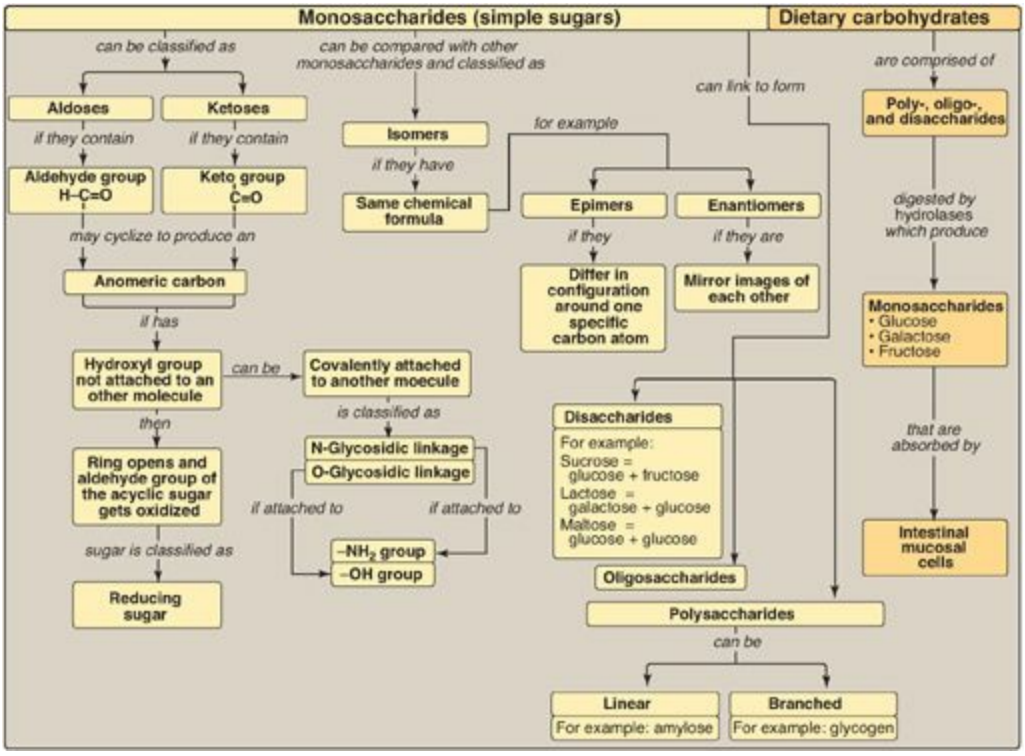
Figure 7.11 Abnormal lactose metabolism. H_2 = hydrogen gas.



IV. CHAPTER SUMMARY

Monosaccharides (Figure 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**. If two monosaccharide isomers differ in configuration around one specific carbon atom (with the exception of the carbonyl carbon), they are defined as **epimers** of each other. If a pair of sugars are mirror images (**enantiomers**), the two members of the pair are designated as **D-** and **L-sugars**. If the aldehyde group on an acyclic sugar gets oxidized as a chromogenic agent gets reduced, that sugar is a reducing sugar. When a sugar cyclizes, an **anomeric carbon** is created from the aldehyde group of an aldose or keto group of a ketose. The sugar can have two configurations, **α** or **β**. A sugar with its anomeric carbon linked to another structure forms a **glycoside**. Sugars can be attached either to an $-NH_2$ or an –OH group, producing **N-** and **O-glycosides**. **Salivary α-amylase** acts o n **dietary polysaccharides** (starch, glycogen), producing oligosaccharides. **Pancreatic α-amylase** continues the process of carbohydrate digestion. The final digestive processes occur at the **mucosal lining** of the **small intestine**. Several disaccharidases (for example, **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**. 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 CO_2 and H_2, causing abdominal cramps, diarrhea, and flatulence. **Lactose intolerance**, primarily caused by the age-dependent loss of **lactase** (**adult 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.


Monosaccharides (simple sugars)
Dietary carbohydrates
can be classified as
Aldoses
Ketoses
if they contain
Aldehyde group H–C=O
if they contain
Keto group C=O
may cyclize to produce an
Anomeric carbon
if has
Hydroxyl group not attached to an other molecule
then
Ring opens and aldehyde group of the acyclic sugar gets oxidized
sugar is classified as
Reducing sugar
can be
Covalently attached to another moecule
is classified as
N-Glycosidic linkage
O-Glycosidic linkage
if attached to
if attached to
–NH2 group
–OH group
can be compared with other monosaccharides and classified as
Isomers
if they have
Same chemical formula
for example
Epimers
Enantiomers
if they
Differ in configuration around one specific carbon atom
if they are
Mirror images of each other
can link to form
Disaccharides
For example:
Sucrose = glucose + fructose
Lactose = galactose + glucose
Maltose = glucose + glucose
Oligosaccharides
Polysaccharides
can be
Linear
For example: amylose
Branched
For example: glycogen
are comprised of
Poly-, oligo-, and disaccharides
digested by hydrolases which produce
Monosaccharides
• Glucose
• Galactose
• Fructose
that are absorbed by
Intestinal mucosal cells

Study Question

Choose the ONE best answer.

7.1 Which of the following statements best describes glucose?

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

> Correct answer = A. Glucose and galactose differ only in configuration around carbon 4 and so 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 most typically the form found in biologic systems, in contrast to amino acids.

7.2 A young man entered his physician's office complaining of bloating and diarrhea. His eyes were sunken, and the physician noted additional signs of dehydration. The patient's temperature was normal. He explained that the episode had occurred following a birthday party at which he had participated in an ice cream–eating contest. The patient reported prior episodes of a similar nature following ingestion of a significant amount of dairy products. This clinical picture is most probably due to 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.

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 in the face of a positive reducing sugar test means that glucose cannot be the reducing sugar in the patient's urine.

7.4 Why are α-glucosidase inhibitors that are taken with meals, such as acarbose and miglitol, used in the treatment of diabetes? 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 diabetics. These drugs have no effect on lactose digestion because the disaccharide lactose contains a β-glycosidic bond, not an α-glycosidic bond.

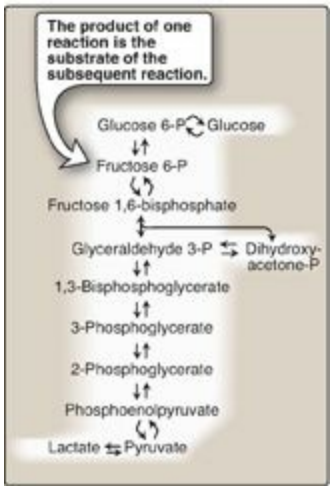
Introduction to Metabolism and Glycolysis

8

I. INTRODUCTION TO METABOLISM

In Chapter 5, individual enzymic reactions were analyzed in an effort to explain the mechanisms of catalysis. However, in cells, these reactions rarely occur in isolation but, rather, are organized into multistep sequences called pathways, such as that of glycolysis (Figure 8.1). In a pathway, the product of one reaction serves as the substrate of the subsequent reaction. Different pathways can also intersect, forming an integrated and purposeful network of chemical reactions. These are collectively called metabolism, which is the sum of all the chemical changes occurring in a cell, a tissue, or the body. Most pathways can be classified as either catabolic (degradative) or anabolic (synthetic). Catabolic reactions break down complex molecules, such as proteins, polysaccharides, and lipids, to a few simple molecules (for example, CO_2, NH_3 [ammonia], and H_2O). Anabolic pathways form complex end products from simple precursors, for example, the synthesis of the polysaccharide, glycogen, from glucose. [Note: Pathways that regenerate a component are called cycles.] In the following chapters, this text focuses on the central metabolic pathways that are involved in synthesizing and degrading carbohydrates, lipids, and amino acids.

Figure 8.1 Glycolysis, an example of ametabolic 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

It is convenient to investigate metabolism by examining its component pathways. Each pathway is composed of multienzyme sequences, and each enzyme, in turn, may exhibit important catalytic or regulatory features. To provide the reader with the "big picture," a metabolic map containing the important central pathways of energy metabolism is presented in Figure 8.2. This map is useful in tracing connections between pathways, visualizing the purposeful "movement" of metabolic intermediates, and depicting the effect on the flow of intermediates if a pathway is 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 adenosine triphosphate (ATP) from the degradation of energy-rich fuel molecules. Catabolism also allows molecules in the diet (or nutrient molecules stored in cells) to be converted into building blocks needed for the synthesis of complex molecules. Energy 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^+).]

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.

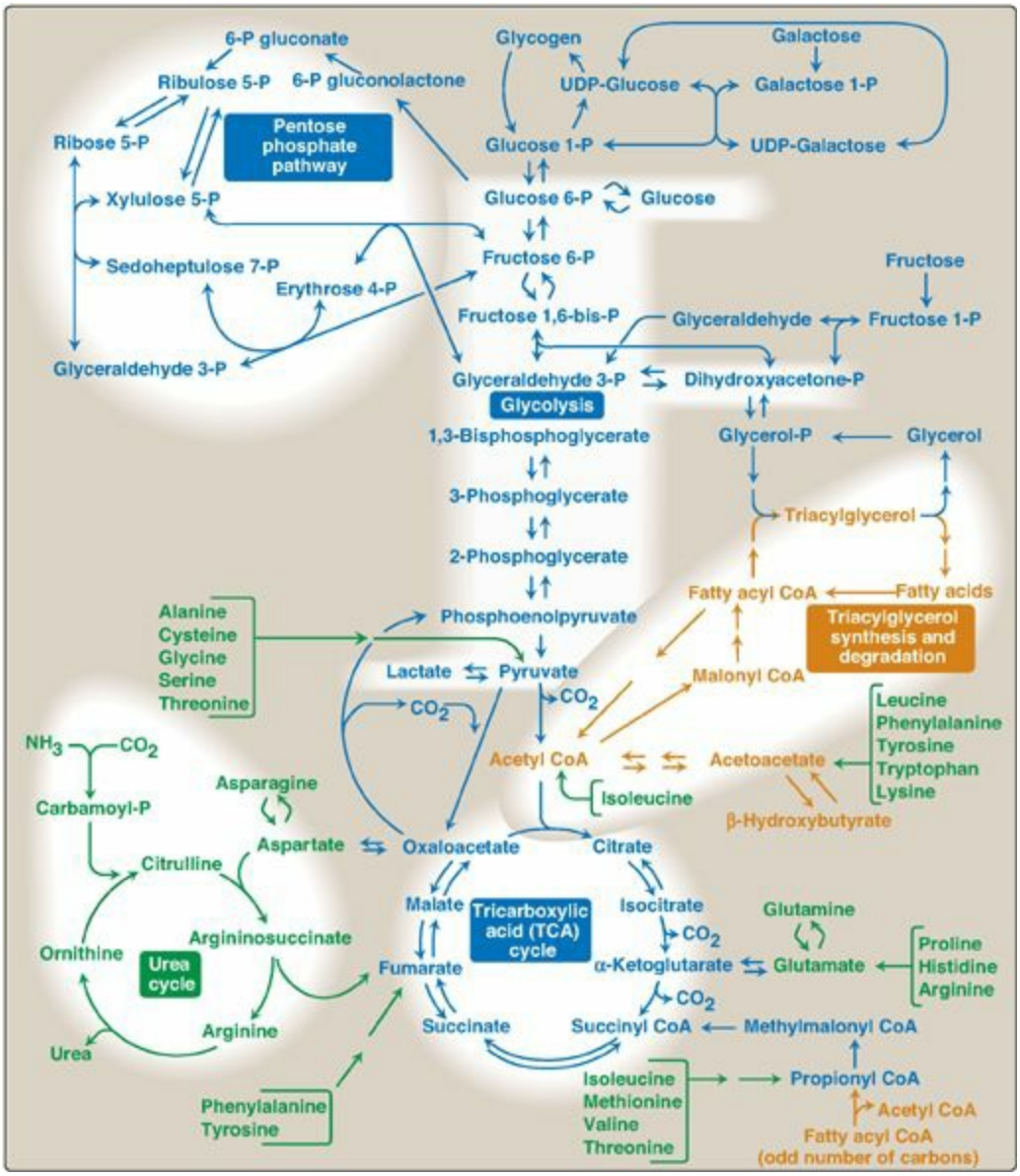
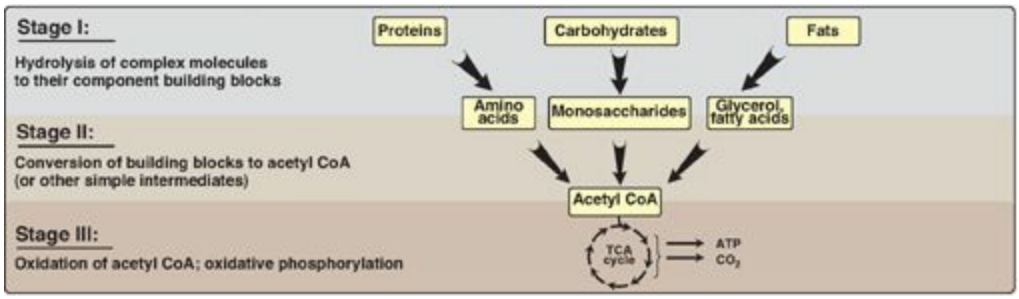


Figure 8.3 Three stages of catabolism. CoA = coenzyme A; TCA = tricarboxylic acid.



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.

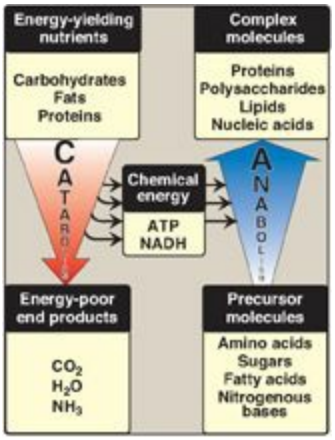
2. **Conversion of building blocks to simple intermediates:** In the second stage, these diverse building blocks are further degraded to acetyl coenzyme A (CoA) and a few other simple molecules. Some energy is captured as ATP, but the amount is small compared with the energy produced during the third stage of catabolism.

3. **Oxidation of acetyl coenzyme A:** The tricarboxylic acid (TCA) cycle (see p. 109) 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 (see p. 73).

C. Anabolic pathways

Anabolic reactions combine small molecules, such as amino acids, to form complex molecules such as proteins (Figure 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). Anabolic reactions often involve chemical reductions in which the reducing power is most frequently provided by the electron donor NADPH (see p. 147). Note that catabolism is a convergent process (that is, a wide variety of molecules are transformed into a few common end products). By contrast, anabolism is a divergent process in which a few biosynthetic precursors form a wide variety of polymeric, or complex, products.

Figure 8.4 Comparison of catabolic and anabolic pathways. ATP = adenosine triphosphate; NADH = nicotinamide adenine dinucleotide.



II. REGULATION OF METABOLISM

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 do not function in isolation but, rather, are part of a community of interacting tissues. 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 (Figure 8.5).

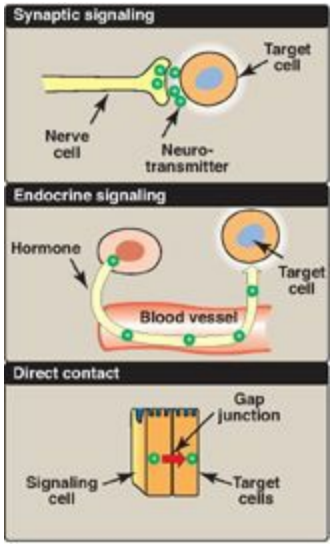
A. Intracellular communication

The rate of a metabolic pathway can respond to regulatory signals that arise from within the cell. For example, the rate of a pathway 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 bloodborne hormones or by neurotransmitters.

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



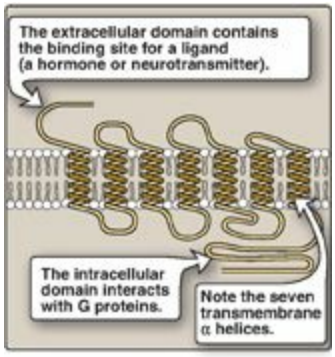
C. Second messenger systems

Hormones or neurotransmitters can be thought of as signals and their receptors as signal detectors. Each component serves as a link in the communication between extracellular events and chemical changes within the cell. Many receptors signal their recognition of a bound ligand by initiating a series of reactions that ultimately result in a specific intracellular response. "Second messenger" molecules, so named because they intervene between the original messenger (the neurotransmitter or hormone) and the ultimate effect on the cell, are part of the cascade of events that translates (transduces) hormone or neurotransmitter binding into a cellular response. Two of the most widely recognized second messenger systems are the calcium/phosphatidylinositol system (see p. 205) and the adenylyl cyclase (adenylate cyclase) system, which is particularly important in regulating the pathways of intermediary metabolism.

D. Adenylyl cyclase

The recognition of a chemical signal by some plasma (cell) membrane receptors, such as the β- and α_2-adrenergic receptors, triggers either an increase or a decrease in the activity of adenylyl cyclase (AC). This is a membrane-bound enzyme that converts ATP to 3**I**,5**I**-adenosine monophosphate (commonly called cyclic AMP, or cAMP). The chemical signals are most often hormones or neurotransmitters, each of which binds to a unique type of membrane receptor. Therefore, tissues that respond to more than one chemical signal must have several different receptors, each of which can be linked t o AC. These receptors, known as G protein–coupled receptors (GPCRs), are characterized by an extracellular ligand-binding domain, seven transmembrane α helices, and an intracellular domain that interacts with G proteins (Figure 8.6).

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



1. **Guanosine triphosphate–dependent regulatory proteins:** The effect of the activated, occupied GPCR on second messenger formation is not direct but, rather, is mediated by specialized trimeric proteins (α, β, and γ subunits) of the cell membrane. These proteins, referred to as G proteins because the α subunit binds guanine nucleotides (GTP and GDP), form a link in the chain of communication between the receptor and AC. In the inactive form of a G protein, the a-subunit is

bound to GDP (Figure 8.7). Binding of ligand 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 AC, which is thereby activated. Many molecules of active Gα protein are formed by one activated receptor. [Note: The ability of a hormone or neurotransmitter to stimulate or inhibit AC depends on the type of Gα protein that is linked to the receptor. One type, designated G_s, stimulates AC, whereas another type, designated G_i, inhibits the enzyme (not shown in Figure 8.7).] 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 the Gα, its dissociation from AC, and 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. With cholera, the GTPase activity of $G\alpha_s$ is inhibited in intestinal cells. With whooping cough, $G\alpha_i$ is inactivated in respiratory-tract cells.

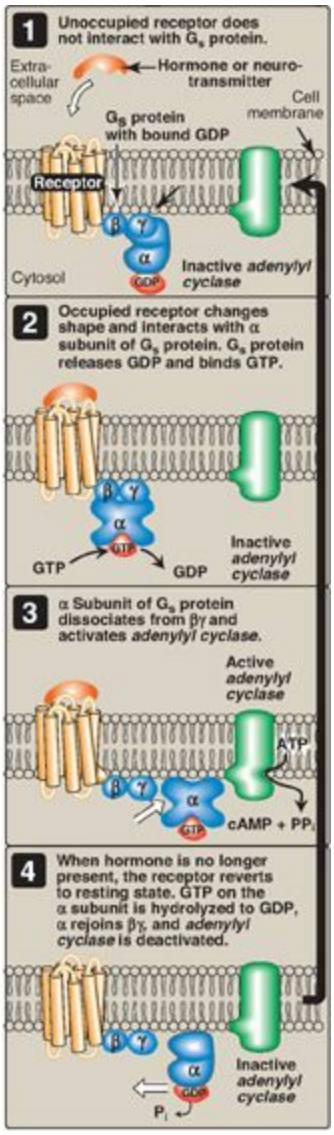
2. **Protein kinases:** The next key link in the cAMP second messenger system is the activation by cAMP of a family of enzymes called cAMP-dependent protein kinases such as protein kinase A (Figure 8.8). cAMP activates protein kinase A by binding to its two regulatory subunits, causing the release of two active, catalytic subunits. The active subunits catalyze the transfer of phosphate from ATP to specific serine or threonine residues of protein substrates. The phosphorylated proteins may act directly on the cell's ion channels or, if enzymes, may become activated or inhibited. Protein kinase A can also phosphorylate proteins that bind to DNA, causing changes in gene expression (see p. 456). [Note: Several types of protein kinases are not cAMP dependent, for example, protein kinase C described on p. 205.]

3. **Dephosphorylation of proteins:** The phosphate groups added to proteins by protein kinases are removed by protein phosphatases, enzymes that hydrolytically cleave phosphate esters (see Figure 8.8). This ensures that changes in protein activity induced by phosphorylation are not permanent.

4. **Hydrolysis of cyclic adenosine monophosphate:** cAMP is rapidly hydrolyzed to 5′-AMP by cAMP phosphodiesterase, one of a family of enzymes that cleave 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: Phosphodiesterase is inhibited by the methylxanthine derivative, caffeine.]

Figure 8.7 The recognition of chemical signals by certain membrane receptors triggers

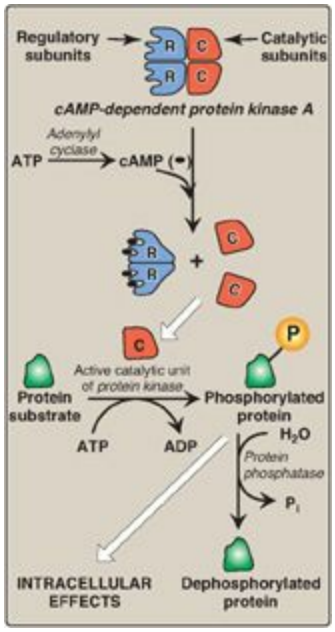
an increase (or, less often, a decrease) in the activity of adenylyl cyclase. GDP = guanosine diphosphate; GTP = guanosine triphosphate; cAMP = cyclic AMP.



III. OVERVIEW OF GLYCOLYSIS

The glycolytic pathway is employed by all tissues for the oxidation of glucose to provide energy (in the form of 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 (Figure 8.9A). Pyruvate is the end product of glycolysis in cells with mitochondria and an adequate supply of oxygen. This series of ten reactions is called aerobic glycolysis because oxygen is required to reoxidize the NADH formed during the oxidation of glyceraldehyde 3-phosphate (Figure 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^+ (Figure 8.9C). This conversion of glucose to lactate is called anaerobic glycolysis because it can occur without the participation of oxygen. Anaerobic glycolysis allows the production of ATP in tissues that lack mitochondria (for example, red blood cells and parts of the eye) or in cells deprived of sufficient oxygen.

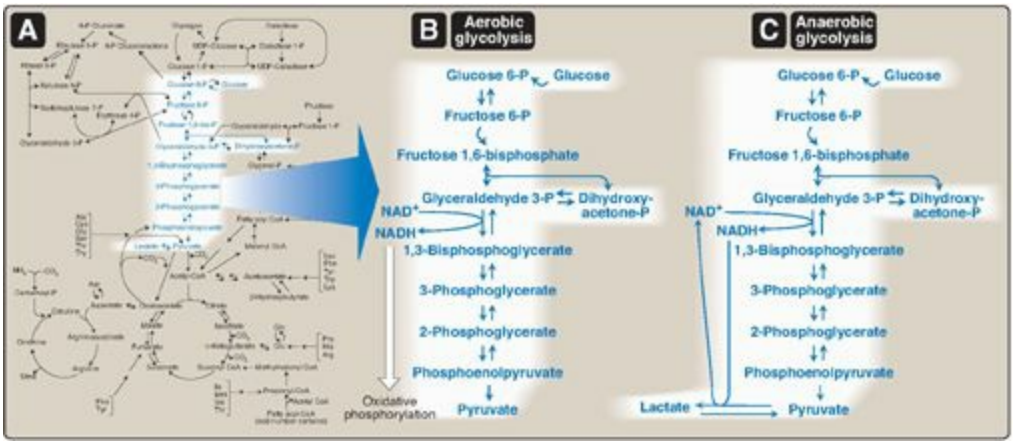
Figure 8.8 Actions of cyclic AMP (cAMP). P_i = inorganic phosphate.



IV. TRANSPORT OF GLUCOSE INTO CELLS

Glucose cannot diffuse directly into cells but enters by one of two transport mechanisms: a Na^+-independent, facilitated diffusion transport system or an ATP-dependent Na^+-monosaccharide 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-independent facilitated diffusion transport system

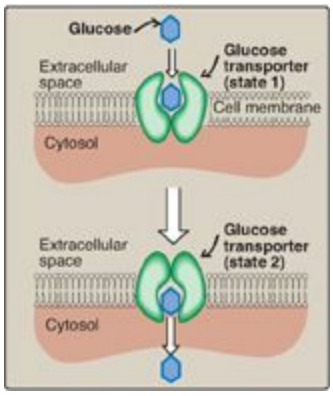
This system is mediated by a family of 14 glucose transporters found in cell membranes. They are designated GLUT-1 to GLUT-14 (glucose transporter isoforms 1–14). These monomeric protein transporters exist in the membrane in two conformational states (Figure 8.10). Extracellular glucose binds to the transporter, which then alters its conformation, transporting glucose across the cell membrane.

1. **Tissue specificity of glucose transporter gene expression:** The GLUTs display a tissue-specific pattern of expression. For example, GLUT-3 is the primary glucose transporter in neurons. GLUT-1 is abundant in erythrocytes and the blood–brain barrier but is low in adult muscle, whereas GLUT-4 is abundant in muscle and adipose tissue. [Note: The number of GLUT-4 transporters active in these tissues is increased by insulin. (See p. 311 for a discussion of insulin and glucose transport.)] GLUT-2 is abundant in liver, kidney, and β cells of the pancreas. The other GLUT isoforms also have tissue-specific distributions.

2. **Specialized functions of glucose transporter isoforms:** In facilitated diffusion, transporter-mediated glucose movement is down a concentration gradient (that is, from a high glucose concentration to a lower one and, therefore, does not require 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 kidney, 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 (for example, during fasting). GLUT-5 is unusual in that it is the primary transporter for fructose (not

glucose) in the small intestine and the testes.

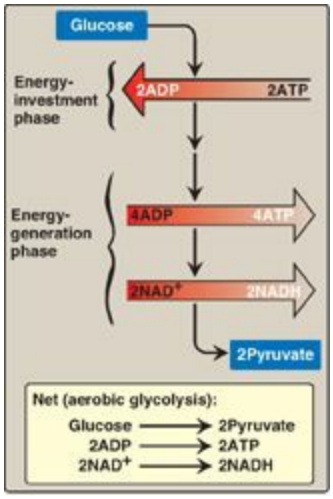
Figure 8.10 Schematic representation of the facilitated transport of glucose through a cell membrane. [Note: Glucose transporter proteins are monomeric and contain 12 transmembrane β helices.]



B. Sodium–monosaccharide cotransport system

This is an energy-requiring process that transports glucose "against" a concentration gradient (that is, from low glucose concentrations outside the cell to higher concentrations within the cell). This system is a transporter-mediated process in which the movement of glucose is coupled to the concentration gradient of Na^+, which is transported into the cell at the same time. The transporter is a sodium-dependent glucose transporter (SGLT). This type of transport occurs in the epithelial cells of the intestine (see p. 87), renal tubules, and choroid plexus. [Note: The choroid plexus, part of the blood–brain barrier, also contains GLUT-1.]

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



V. REACTIONS OF GLYCOLYSIS

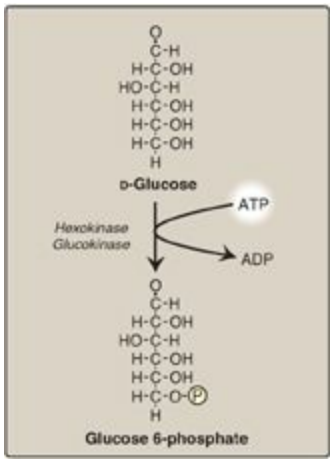
The conversion of glucose to pyruvate occurs in two stages (Figure 8.11). The first five reactions of glycolysis correspond to an energy–investment phase in which the phosphorylated forms of intermediates are synthesized at the expense of ATP. The subsequent reactions of glycolysis constitute an energy–generation phase in which a net of two molecules of ATP are formed by substrate-level phosphorylation (see p. 102) per glucose molecule metabolized.

A. Phosphorylation of glucose

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. The irreversible phosphorylation of glucose (Figure 8.12), therefore, effectively traps the sugar as cytosolic glucose 6-phosphate, thereby committing it to further metabolism in the cell. Mammals have four (I–IV) isozymes of the enzyme hexokinase that catalyze the phosphorylation of glucose to glucose 6-phosphate.

1. **Hexokinases I–III:** In most tissues, phosphorylation of glucose is catalyzed by one of these isozymes of hexokinase, which is one of three regulatory enzymes of glycolysis (see also phosphofructokinase and pyruvate kinase). These isozymes have broad substrate specificity and are able to phosphorylate several hexoses in addition to glucose. 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. 59) for glucose. This permits the efficient phosphorylation and subsequent metabolism of glucose even when tissue concentrations of glucose are low (Figure 8.13). These isozymes, however, have a low maximal velocity ([V_{max}] see p. 59) for glucose and, therefore, do not sequester (trap) cellular phosphate in the form of phosphorylated hexoses, or phosphorylate more sugars than the cell can use.

Figure 8.12 Energy-investment phase: phosphorylation of glucose. [Note: Kinases utilize ATP complexed with a divalent metal ion, most typically Mg^{2+}.]

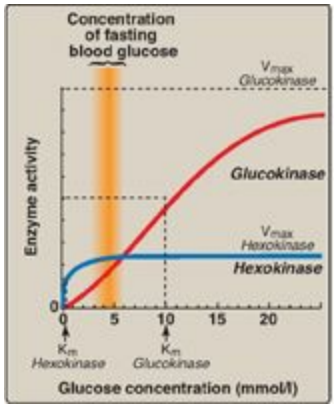


2. **Hexokinase IV (or, glucokinase):** In liver parenchymal cells and b cells of the pancreas, glucokinase (the hexokinase IV isozyme) is the predominant enzyme responsible for the phosphorylation of glucose. In β cells, glucokinase functions as a glucose sensor, determining the threshold for insulin secretion (see p. 309). [Note: Hexokinase IV also serves as a glucose sensor in neurons of the hypothalamus, playing a key role in the adrenergic response to hypoglycemia (see p. 315.] 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 isozymes.

   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 Figure 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 insures that blood glucose equilibrates rapidly across the membrane of the hepatocyte.]

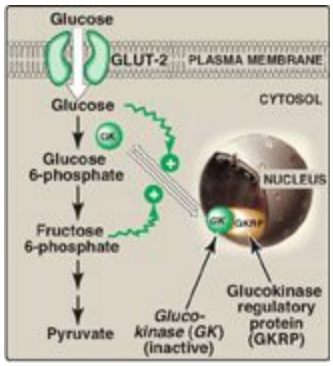
   b. **Regulation by fructose 6-phosphate and glucose:** Glucokinase activity is not directly inhibited by glucose 6-phosphate as are the other hexokinases but, rather, is indirectly inhibited by fructose 6-phosphate (which is in equilibrium with glucose 6-phosphate, a product of glucokinase) and is indirectly stimulated by glucose (a substrate of glucokinase) via the following mechanism. Glucokinase regulatory protein (GKRP) in the liver regulates the activity of glucokinase through reversible binding. In the presence of fructose 6-phosphate, glucokinase is translocated into the nucleus and binds tightly to the regulatory protein, thereby rendering the enzyme inactive (Figure 8.14). When glucose levels in the blood (and also in the hepatocyte, as a result of GLUT-2) increase, glucokinase is released from the regulatory protein, and the enzyme reenters the cytosol where it phosphorylates glucose to glucose 6-phosphate. [Note: Fructose 1-phosphate inhibits formation of the glucokinase–GKRP complex.]

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.



Glucokinase functions as a glucose sensor in the maintenance of 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.

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



B. Isomerization of glucose 6-phosphate

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

C. Phosphorylation of fructose 6-phosphate

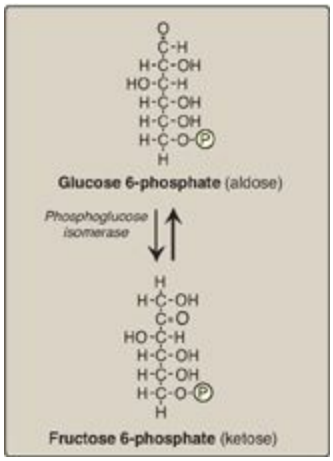
The irreversible phosphorylation reaction catalyzed by phosphofructokinase-1 (PFK-1) is the most important control point and the rate-limiting and committed step of glycolysis (Figure 8.16) . PFK-1 is controlled by the available concentrations of the substrates ATP and fructose 6-phosphate as well as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

1. **Regulation by energy levels within the cell:** 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. 109), also inhibit PFK-1. [Note: Inhibition by citrate favors the use of glucose for glycogen synthesis (see p. 125).] Conversely, PFK-1 is activated allosterically by high concentrations of AMP, which signal that the cell's energy stores are depleted.

2. **Regulation by fructose 2,6-bisphosphate:** Fructose 2,6-bisphosphate is the most potent activator of PFK-1 (see Figure 8.16) and is able to activate the enzyme even when ATP levels are high. Fructose 2,6-bisphosphate is formed from fructose 6-phosphate by phosphofructokinase-2 (PFK-2), an enzyme different than 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 back to fructose 6-phosphate. In the liver, the kinase domain is active if dephosphorylated and is inactive if phosphorylated (Figure 8.17). [Note: Fructose 2,6-bisphosphate is an inhibitor of fructose 1,6-bisphosphatase, an enzyme of gluconeogenesis (see p. 120). 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 in which glucose would be converted to pyruvate followed by resynthesis of glucose from pyruvate.]

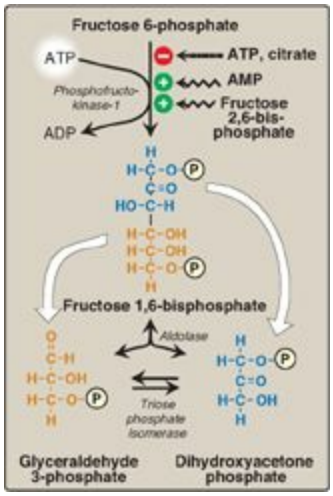
Figure 8.15 Aldose-ketose isomerization of glucose 6-phosphate to fructose 6-phosphate. P = phosphate.



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 fructose 2,6-bisphosphate and, thus, in the rate of glycolysis in the liver (see Figure 8.17). Fructose 2,6-bisphosphate, therefore, acts as an intracellular signal, indicating that glucose is abundant.

b. **During fasting:** Elevated levels of glucagon and low levels of insulin, such as occur during fasting (see p. 327), decrease the intracellular concentration of hepatic fructose 2,6-bisphosphate. This results in inhibition of glycolysis and activation of gluconeogenesis.

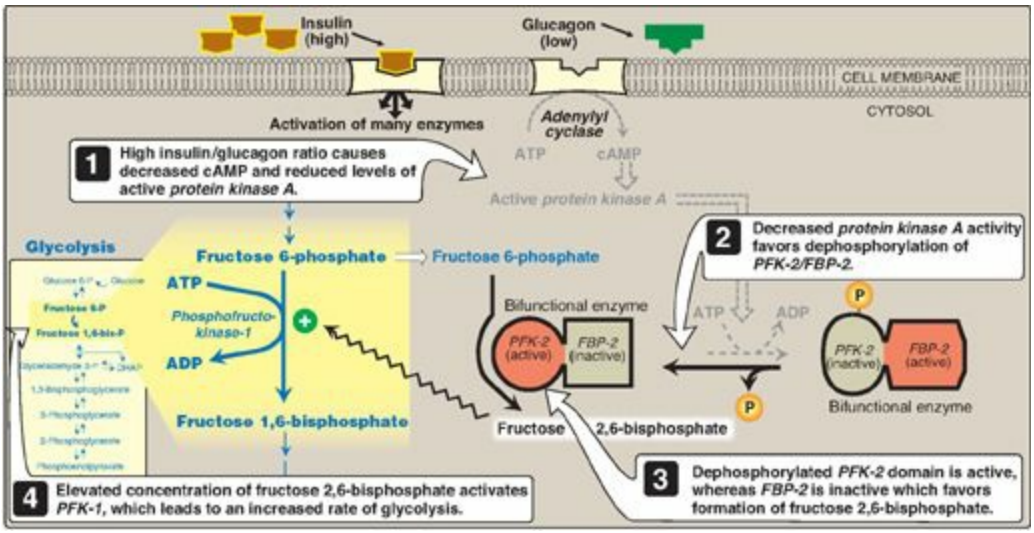
Figure 8.16 Energy-investment phase (continued): Conversion of fructose 6-phosphate to triose phosphates. P = phosphate; AMP = adenosine monophosphate.



D. Cleavage of fructose 1,6-bisphosphate

Aldolase cleaves fructose 1,6-bisphosphate to dihydroxyacetone phosphate and glyceraldehyde 3-phosphate (see Figure 8.16). The reaction is reversible and not regulated. [Note: Aldolase B, the isoform found primarily in the liver, also cleaves fructose 1-phosphate and functions in the metabolism of dietary fructose (see p. 138).]

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



E. Isomerization of dihydroxyacetone phosphate

Triose phosphate isomerase interconverts dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (see Figure 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 (see p. 188).]

F. Oxidation of glyceraldehyde 3-phosphate

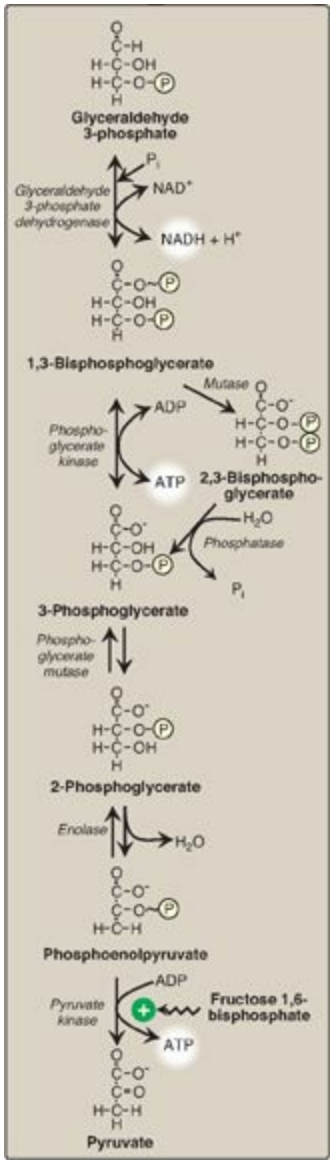
The conversion of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate (1,3-BPG) by glyceraldehyde 3-phosphate dehydrogenase is the first oxidation-reduction reaction of glycolysis (Figure 8.18). [Note: Because there is only a limited amount of NAD^+ in the cell, the NADH formed by this reaction must be reoxidized to NAD^+ for glycolysis to continue. Two major mechanisms for oxidizing NADH are 1) the NADH-linked conversion of pyruvate to lactate (anaerobic; see p. 96) and 2) oxidation of NADH via the respiratory chain (aerobic; see p. 74). The latter requires the malate-aspartate and glycerol 3-phosphate substrate shuttles (see p. 79.]

1. **Synthesis of 1,3-bisphosphoglycerate:** 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. The high-energy phosphate group at carbon 1 of 1,3-BPG conserves much of the free energy produced by the oxidation of glyceraldehyde 3-phosphate. The energy of this high-energy phosphate drives the synthesis of ATP in the next reaction of glycolysis.

2. **Mechanism of arsenic poisoning:** The toxicity of arsenic is due primarily to the inhibition by trivalent arsenic (arsenite) of enzymes such as the pyruvate dehydrogenase complex, which require lipoic acid as a coenzyme (see p. 110). 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 Figure 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 on the F_1 domain of ATP synthase (see p. 77), resulting in formation of ADP-arsenate that is rapidly hydrolyzed.]

3. **Synthesis of 2,3-bisphosphoglycerate in red blood cells:** Some of the 1,3-BPG is converted to 2,3-BPG by the action of bisphosphoglycerate mutase (see Figure 8.18). 2,3-BPG, which is found in only trace amounts in most cells, is present at high concentration in red blood cells (RBCs) and serves to increase O_2 delivery (see p. 31). 2,3-BPG is hydrolyzed by a phosphatase to 3-phosphoglycerate, which is also an intermediate in glycolysis (see Figure 8.18). In the RBC, glycolysis is modified by inclusion of these "shunt" reactions.

Figure 8.18 Energy-generating phase: conversion of glyceraldehyde 3-phosphate to pyruvate. NAD(H) = nicotinamide adenine dinucleotide P = phosphate; P_i = inorganic phosphate.



G. Synthesis of 3-phosphoglycerate, producing ATP

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 (see Figure 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 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 electron transport chain (see J. below and p. 113 for other examples).]

H. Shift of the phosphate group

The shift of the phosphate group from carbon 3 to carbon 2 of phosphoglycerate by phosphoglycerate mutase is freely reversible (see Figure 8.18).

I. Dehydration of 2-phosphoglycerate

The dehydration of 2-phosphoglycerate by enolase redistributes the energy within the substrate, resulting in the formation of phosphoenolpyruvate (PEP), which contains a high-energy enol phosphate (see Figure 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. Formation of pyruvate, producing ATP

The conversion of PEP to pyruvate is catalyzed by pyruvate kinase (PK), 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 (see Figure 8.18).

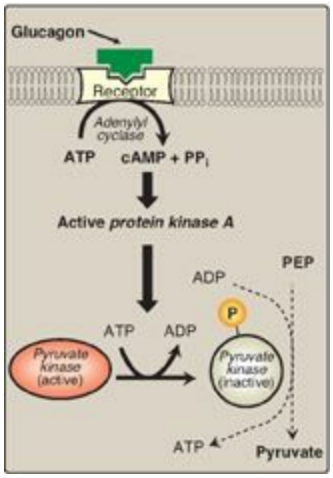
1. **Feedforward regulation:** PK is activated by fructose 1,6-bisphosphate, the product of the phosphofructokinase-1 reaction. This feedforward (instead of the more usual feedback) regulation has the effect of linking the two kinase activities: increased phosphofructokinase activity results in elevated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

2. **Covalent modulation of pyruvate kinase:** Phosphorylation by a cAMP-dependent protein kinase leads to inactivation of the hepatic isozyme of PK (Figure 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, in part, 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:** Mature RBCs lack mitochondria and are, therefore, 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. The resulting alterations in the RBC membrane lead to changes in cell shape and, ultimately, to phagocytosis by cells of the reticuloendothelial system, particularly macrophages of the spleen. The premature death and lysis of RBCs result in hemolytic anemia. Among patients exhibiting the rare genetic defects of glycolytic enzymes, the majority has a deficiency in PK. The effects of PK deficiency are restricted to RBCs and include mild-to-severe nonspherocytic hemolytic anemia, with the severe form requiring regular transfusions. [Note: Hepatic PK is encoded by the same gene as the RBC isozyme. Liver cells show no effect, however, because they have mitochondria and can generate ATP by oxidative phosphorylation.] Severity depends both on the degree of enzyme deficiency (generally 5–35% of normal levels) and on the extent to which RBCs compensate by synthesizing increased levels of 2,3-BPG

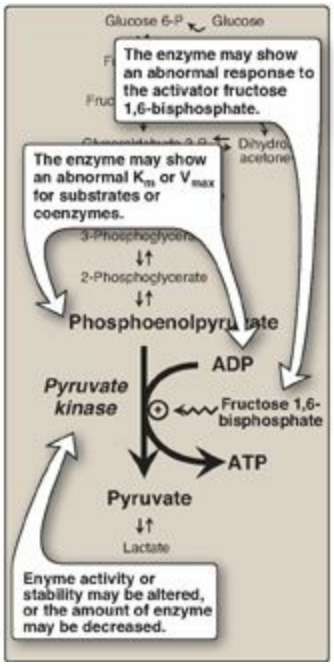
(see p. 31). Almost all individuals with PK deficiency have a mutant enzyme that shows abnormal properties such as altered kinetics (Figure 8.20). Individuals heterozygous for PK deficiency have resistance to the most severe forms of malaria.

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



The tissue-specific expression of PK in RBCs and the liver is the result of differential promoter utilization in transcription (see p. 422) of the gene that encodes both isozymes.

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



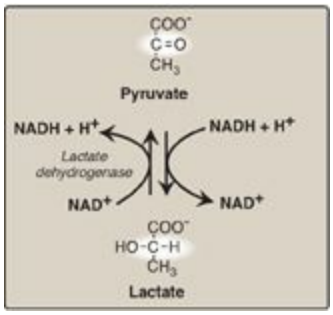
K. Reduction of pyruvate to lactate

Lactate, formed by the action of lactate dehydrogenase, is the final product of anaerobic glycolysis in eukaryotic cells (Figure 8.21). The formation of lactate is the major fate for pyruvate in the lens and cornea of the eye, kidney medulla, testes, leukocytes, and RBCs, because these are all poorly vascularized and/or 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 p. 112) exceeds the oxidative capacity of the respiratory chain. This results in an elevated NADH/NAD^+ ratio, favoring reduction of pyruvate to lactate. 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 (see p. 118).

2. **Lactate utilization:** The direction of the lactate dehydrogenase reaction depends on the relative intracellular concentrations of pyruvate and lactate and on the ratio of NADH/NAD^+ in the cell. For example, in the liver and heart, the ratio of NADH/NAD^+ is lower than in exercising muscle. These tissues oxidize lactate (obtained from the blood) to pyruvate. In the liver, pyruvate is either converted to glucose by gluconeogenesis or oxidized in the TCA cycle. Heart muscle exclusively oxidizes lactate to CO_2 and H_2O via the TCA cycle.

Figure 8.21 Interconversion of pyruvate and lactate. [Note: Lactate produced in muscle enters the circulation, is picked up by liver through facilitated diffusion, and is oxidized to pyruvate. Pyruvate is used by liver to make glucose.] NAD(H) = nicotinamide adenine dinucleotide.

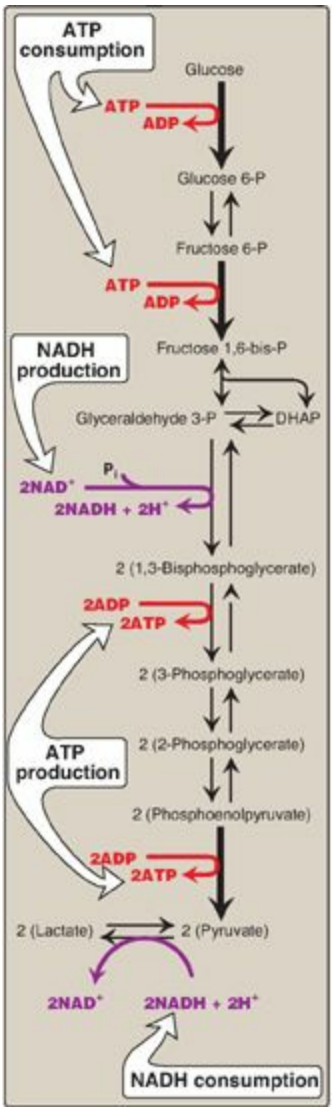


3. **Lactic acidosis:** Elevated concentrations of lactate in the plasma, termed lactic acidosis (a type of metabolic acidosis), occur when there is a collapse of the circulatory system, such as in myocardial infarction, pulmonary embolism, and uncontrolled hemorrhage, or when an individual is in shock. The failure to bring adequate amounts of oxygen 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 life-saving during the period required to reestablish adequate blood flow to the tissues.] The excess oxygen required to recover from a period when the availability of oxygen has been inadequate is termed the "oxygen debt."

> The oxygen 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 oxygen debt in patients and the monitoring of their recovery.

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



L. Energy yield from glycolysis

Despite the production of some ATP 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 (see p. 109).

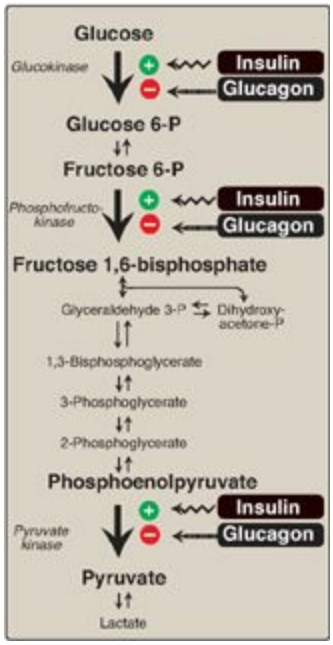
1. **Anaerobic glycolysis:** Two molecules of ATP are generated for each molecule of glucose converted to two molecules of lactate (Figure 8.22). There is no net production or consumption of NADH.

2. **Aerobic glycolysis:** The direct consumption and formation of ATP is the same as in anaerobic glycolysis (that is, 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 electron transport chain, producing approximately three ATP for each NADH molecule entering the chain (see p. 77). [Note: NADH cannot cross the inner mitochondrial membrane, and substrate shuttles are required (see p. 79).]

VI. HORMONAL REGULATION OF GLYCOLYSIS

The regulation of glycolysis by allosteric activation or inhibition, or the covalent phosphorylation/dephosphorylation of rate-limiting enzymes, is short-term (that is, they influence glucose consumption over periods of minutes or hours). Superimposed on these moment-to-moment effects are slower, and often more profound, hormonal influences on gene expression, or the amount of enzyme protein synthesized. These effects can result in 10-fold to 20-fold increases in enzyme activity that typically occur over hours to days. Although the current focus is on glycolysis, reciprocal changes occur in the rate-limiting enzymes of gluconeogenesis, which are described in Chapter 10 (see p. 117). Regular consumption of meals rich in carbohydrate or administration of insulin initiates an increase in the amount of glucokinase, phosphofructokinase, and PK in the liver (Figure 8.23). These changes reflect an increase in gene transcription, resulting in increased enzyme synthesis. High activity of these three enzymes favors the conversion of glucose to pyruvate, a characteristic of the absorptive state (see p. 321). Conversely, gene transcription and synthesis of glucokinase, phosphofructokinase, and PK are decreased when plasma glucagon is high and insulin is low (for example, as seen in fasting or diabetes).

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



VII. ALTERNATE FATES OF PYRUVATE

A. Oxidative decarboxylation of pyruvate

Oxidative decarboxylation of pyruvate by the pyruvate dehydrogenase complex is an important pathway in tissues with a high oxidative capacity such as cardiac muscle (Figure 8.24). Pyruvate dehydrogenase irreversibly converts pyruvate, the end product of glycolysis, into acetyl CoA, a major fuel for the TCA cycle (see p. 109) and the building block for fatty acid synthesis (see p. 183).

B. Carboxylation of pyruvate to oxaloacetate

Carboxylation of pyruvate to oxaloacetate by pyruvate carboxylase is a biotin-dependent reaction (see Figure 8.24). This reaction is important because it replenishes the TCA cycle intermediates and provides substrate for gluconeogenesis (see p. 118).

C. Reduction of pyruvate to ethanol (microorganisms)

The conversion of pyruvate to ethanol occurs by the two reactions summarized in Figure 8.24. The decarboxylation of pyruvate by pyruvate decarboxylase occurs in yeast and certain other microorganisms but not in humans. The enzyme requires thiamine pyrophosphate as a coenzyme and catalyzes a reaction similar to that described for pyruvate dehydrogenase (see p. 110).

VIII. CHAPTER SUMMARY

Most pathways can be classified as either **catabolic** (**degrade** complex molecules to a few simple products) or **anabolic** (**synthesize** complex end products from simple precursors). **Catabolic reactions** also **capture chemical energy** in the form of **ATP** from the degradation of energy-rich molecules. **Anabolic reactions require energy**, which is generally provided by the hydrolysis of ATP. The rate of a metabolic pathway can respond to **regulatory signals** such as **allosteric activators** or **inhibitors** that arise from **within the cell**. Signaling **between cells** provides for the integration of metabolism. The most important route of this communication is **chemical signaling** (for example, by **hormones** or **neurotransmitters)**. **Second messenger molecules** transduce a chemical signal (hormone or neurotransmitter) to appropriate intracellular responders. **Adenylyl cyclase** is a cell membrane enzyme that synthesizes **cyclic AMP** (**cAMP**) in response to chemical signals, such as the hormones **glucagon** and **epinephrine**. Following binding of a hormone to its **cell-surface receptor**, a GTP-dependent regulatory protein (**G protein**) is activated that, in turn, activates **adenylyl cyclase**. The cAMP produced activates a **protein kinase**, which phosphorylates a cadre of enzymes, causing their activation or deactivation. Phosphorylation is reversed by **protein phosphatases**. **Aerobic glycolysis**, in which **pyruvate** is the end product, occurs in cells with mitochondria and an adequate supply of oxygen (Figure 8.25) . **Anaerobic glycolysis**, in which **lactic acid** is the end product, occurs in cells that lack mitochondria and in cells deprived of sufficient oxygen. Glucose is transported across membranes by one of 14 **glucose transporter isoforms** (**GLUTs**). **GLUT-1** is abundant in **erythrocytes** and the **brain**, **GLUT-4** (which is **insulin dependent**) is found in **muscle** and **adipose tissue**, and **GLUT-2** is found in the **liver**, **kidney**, and **β cells** of the pancreas. The conversion of glucose to pyruvate (**glycolysis**; Figure 8.25) occurs in two stages: 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. In the energy-investment phase, glucose is phosphorylated by **hexokinase** (found in **most tissues**) or **glucokinase** (a hexokinase found in **liver cells** and the **β cells** of the pancreas). **Hexokinase** has a **high affinity** (**low K_m**) and a **low 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 indirectly **inhibited** by **fructose 6-phosphate** and **activated** by **glucose**. The **transcription** of the gene for glucokinase is **enhanced by insulin**. Glucose 6-phosphate is isomerized to fructose 6-phosphate, which is phosphorylated to **fructose 1,6-bisphosphate** by **phosphofructokinase-1** (**PFK-1**). This enzyme is **allosterically inhibited** by **ATP** and **citrate** and **activated** by **AMP**. **Fructose 2,6-bisphosphate**, whose synthesis by **phosphofructokinase-2** (**PFK-2**) is **activated** by **insulin**, is the most potent allosteric activator of PFK-1. A total of **two ATP 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 these reactions, **four ATP** and **two NADH are produced** from ADP and NAD^+. The final step in pyruvate synthesis from phosphoenolpyruvate is catalyzed by **pyruvate kinase (PK)**. This enzyme is **allosterically activated** by **fructose 1,6-bisphosphate** and **hormonally activated** by **insulin** and **inhibited** in the liver by **glucagon** via the **cAMP pathway**. **PK deficiency** accounts for the majority of all inherited defects in glycolytic enzymes. Effects are restricted to **erythrocytes** and present as mild to severe **chronic, nonspherocytic hemolytic anemia**. In **anaerobic glycolysis**, NADH is reoxidized to NAD^+ by the **conversion of pyruvate to lactate**. This occurs in cells, such as **erythrocytes**, that have few or no mitochondria, and in tissues, such as **exercising muscle**, where production of NADH exceeds the oxidative capacity of the respiratory chain. Elevated concentrations of lactate in the plasma (**lactic acidosis**) occur when there is a **collapse of the circulatory system** or when an individual is in **shock**. Pyruvate can be 1) **oxidatively decarboxylated** by **pyruvate dehydrogenase**, producing **acetyl coenzyme A**; 2) **carboxylated** to **oxaloacetate** (a tricarboxylic acid cycle intermediate) by **pyruvate carboxylase**; or 3) **reduced** by microorganisms to **ethanol** by **pyruvate decarboxylase**.

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

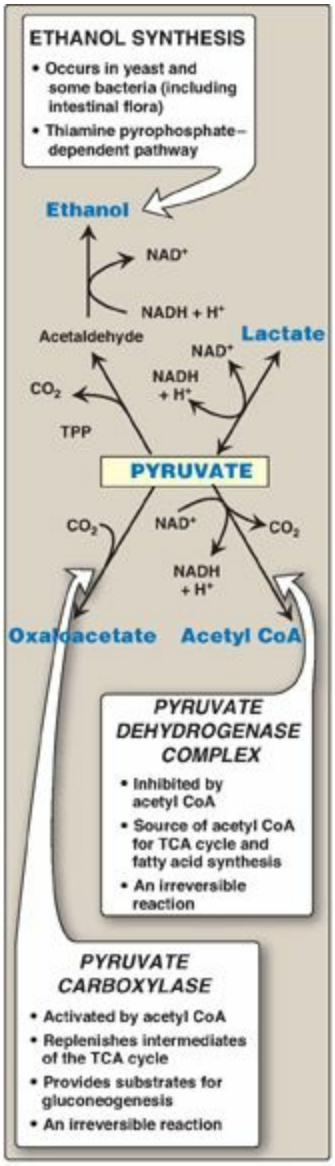
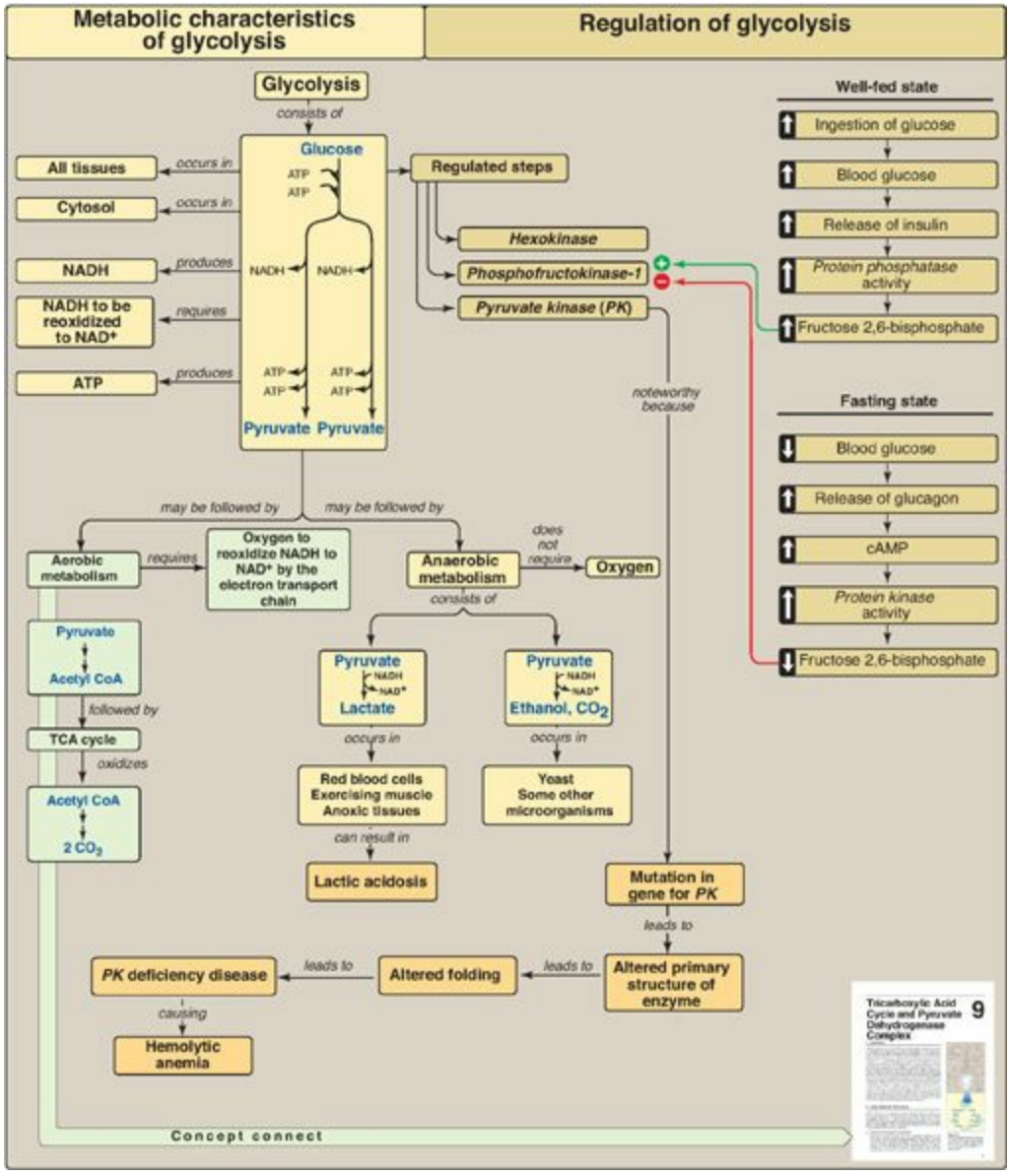


Figure 8.25 Key concept map for glycolysis. NAD(H) = nicotinamide adenine dinucleotide; cAMP = cyclic adenosine monophosphate; CoA = coenzyme A; TCA = tricarboxylic acid.


Metabolic characteristics of glycolysis
Regulation of glycolysis
Glycolysis
consists of
Glucose
ATP
ATP
Pyruvate Pyruvate
NADH NADH
ATP ATP ATP ATP
All tissues
occurs in
Cytosol
occurs in
NADH
produces
NADH to be reoxidized to NAD+
requires
ATP
produces
Regulated steps
Hexokinase
Phosphofructokinase-1
Pyruvate kinase (PK)
noteworthy because
Well-fed state
Ingestion of glucose
Blood glucose
Release of insulin
Protein phosphatase activity
Fructose 2,6-bisphosphate
Fasting state
Blood glucose
Release of glucagon
cAMP
Protein kinase activity
Fructose 2,6-bisphosphate
may be followed by
may be followed by
Aerobic metabolism
requires
Oxygen to reoxidize NADH to NAD+ by the electron transport chain
Anaerobic metabolism
does not require
Oxygen
consists of
Pyruvate
Acetyl CoA
followed by
TCA cycle
oxidizes
Acetyl CoA
2 CO2
Pyruvate
NADH
NAD+
Lactate
occurs in
Red blood cells
Exercising muscle
Anoxic tissues
can result in
Lactic acidosis
Pyruvate
NADH
NAD+
Ethanol, CO2
occurs in
Yeast
Some other microorganisms
Mutation in gene for PK
leads to
Altered primary structure of enzyme
leads to
Altered folding
leads to
PK deficiency disease
causing
Hemolytic anemia
Concept connect
Tricarboxylic Acid Cycle and Pyruvate Dehydrogenase Complex
9

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; P = phosphorylated.

Choice	PFK-1		PFK-2		Pyruvate Kinase	
	Activity	P	Activity	P	Activity	P
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. In the period 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-to-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.

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 muscle shows an increase in the reduction of pyruvate to lactate compared with resting skeletal muscle. The levels of adenosine monophosphate (AMP) and reduced nicotinamide adenine dinucleotide (NADH) increase, whereas change in the concentration of fructose 2,6-bisphosphate is not a key regulatory factor in skeletal muscle. The rise in the NADH to NAD^+ ratio exceeds the oxidative capacity of the respiratory chain.

8.4 Glucose uptake by:

A. liver cells is through facilitated diffusion involving a glucose transporter.
B. intestinal mucosal cells requires insulin.
C. brain cells is through energy-requiring (active) transport.
D. most cells is through simple diffusion up a concentration gradient.

Correct answer = A. Glucose uptake in the liver, brain, muscle, and adipose tissue is down a concentration gradient, and the diffusion is facilitated by tissue-specific glucose transporters (GLUTs). In adipose and muscle, insulin is required for glucose uptake. Moving glucose against a concentration gradient requires energy, and is seen with sodium-dependent glucose transporter-1 (SGLT-1) of intestinal mucosal cells.

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 is that substrate concentration that gives $^1/_2$ V_{max}. When blood glucose concentration is 5 mM, hexo-kinase (K_m = 0.1 mM) will be saturated, but glucokinase (K_m = 10 mM) will not.

8.6 In patients with whooping cough, $G\alpha_i$ is inhibited. How does this lead to a rise in cyclic AMP?

Liganded G proteins of the $G\alpha_i$ type inhibit adenylyl cyclase. If $G\alpha_i$ is inhibited by toxin, adenylyl cyclase production of cyclic adenosine monophosphate (cAMP) is inappropriately activated.

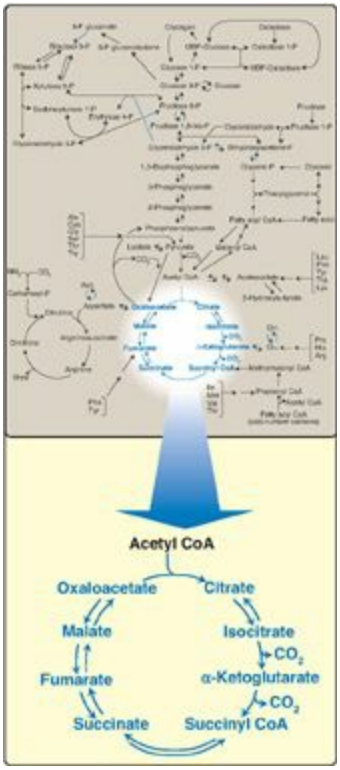
Tricarboxylic Acid Cycle and Pyruvate Dehydrogenase Complex

9

I. OVERVIEW

The tricarboxylic acid cycle ([TCA cycle] also called the citric acid cycle or the Krebs cycle) 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 CO_2 (Figure 9.1). This oxidation provides energy for the production of the majority of adenosine triphosphate (ATP) in most animals, including humans. The TCA cycle occurs totally in the mitochondria and is, therefore, in close proximity to the reactions of electron transport (see p. 73), which oxidize the reduced coenzymes (NADH and $FADH_2$) produced by the cycle. The TCA cycle is an aerobic pathway, because 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 ("filling up") reactions. The TCA cycle also supplies intermediates for a number of important synthetic reactions. For example, the cycle functions in the formation of glucose from the carbon skeletons of some amino acids, and it provides building blocks for the synthesis of some amino acids (see p. 267) and heme (see p. 278). Therefore, this cycle should not be viewed as a closed circle but, instead, as a traffic circle 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. (See Figure 8.2, p. 92 for a more detailed view of the metabolic map.) CoA = coenzyme A.



II. REACTIONS OF THE CYCLE

In the TCA cycle, oxaloacetate is first condensed with an acetyl group from acetyl coenzyme A (CoA) and then is regenerated as the cycle is completed (Figure 9.1). 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. [Note: Two carbons entering the cycle as acetyl CoA are balanced by two CO_2 exiting.]

A. Oxidative decarboxylation of pyruvate

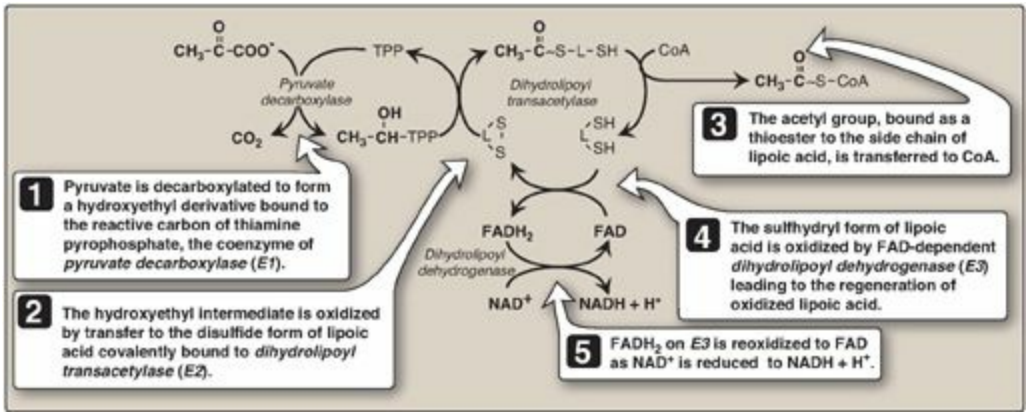
The major source of acetyl CoA, the two-carbon substrate for the TCA cycle, is the oxidative decarboxylation of pyruvate. Pyruvate, the end product of aerobic glycolysis, must be transported from the cytosol into the mitochondrion. This is accomplished by a specific transporter that facilitates movement of pyruvate across the inner mitochondrial membrane. Once in the mitochondrial matrix, pyruvate is converted to acetyl CoA by the pyruvate dehydrogenase complex (PDH complex), which is a multienzyme complex. [Note: Strictly speaking, the PDH complex is not part of the TCA cycle, but it supplies substrate for the cycle.]

1. **Component enzymes:** The PDH complex is a protein aggregate of multiple copies of three enzymes, pyruvate carboxylase (E1, sometimes called pyruvate dehydrogenase), dihydrolipoyl transacetylase (E2), and dihydrolipoyl dehydrogenase (E3). Each catalyzes a part of the overall reaction (Figure 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 complex also contains two tightly bound regulatory enzymes, pyruvate dehydrogenase kinase (PDH kinase) and pyruvate dehydrogenase phosphatase (PDH phosphatase).

2. **Coenzymes:** The PDH complex 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 flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide (NAD^+). [Note: TPP, lipoic acid, and FAD are tightly bound to the enzymes and function as coenzymes-prosthetic groups (see p. 54).]

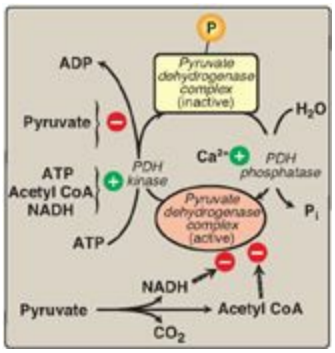
> Deficiencies of thiamine or niacin can cause serious central nervous system problems. This is because brain cells are unable to produce sufficient ATP (via the TCA cycle) if the PDH complex is inactive. Wernicke-Korsakoff, an encephalopathy-psychosis syndrome due to thiamine deficiency, may be seen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

Figure 9.2 Mechanism of action 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.] TPP = thiamine pyrophosphate; L = lipoic acid; CoA = coenzyme A; FAD(H_2) = flavin adenine dinucleotide; NAD(H) = nicotinamide adenine dinucleotide.



3. **Regulation of the pyruvate dehydrogenase complex:** Covalent modifications by the two regulatory enzymes that are part of the complex alternately activate and inactivate E1. The cyclic AMP–independent PDH kinase phosphorylates and, thereby, inactivates E1, whereas PDH phosphatase dephosphorylates and activates E1 (Figure 9.3). The kinase itself is allosterically activated by ATP, acetyl CoA, and NADH. Therefore, in the presence of these high-energy signals, the PDH complex is turned off. [Note: It is actually the rise in the ATP/ADP, NADH/NAD^+, or acetyl CoA/CoA ratios that affects enzymic activity.] Pyruvate is a potent inhibitor of PDH kinase. Thus, 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 release of Ca^{2+} during contraction stimulates the PDH complex and, thereby, energy production. [Note: Although covalent regulation by the kinase and phosphatase is primary, the complex is also subject to product (NADH and acetyl CoA) inhibition.]

Figure 9.3 Regulation of pyruvate dehydrogenase (PDH) complex. [⇢ denotes product inhibition.]



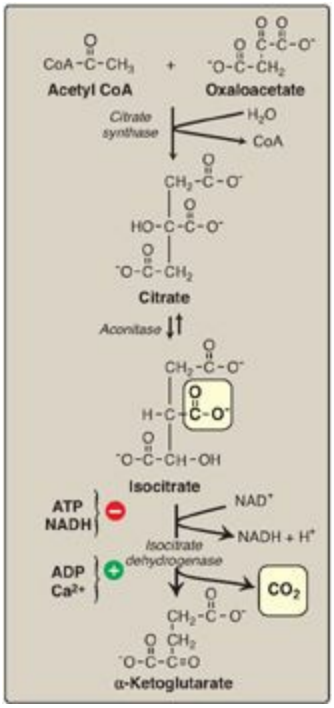
4. **Pyruvate dehydrogenase complex deficiency:** A deficiency in the activity of the

α subunit of the dimeric E1 component of the PDH complex, although rare, is the most common biochemical cause of congenital lactic acidosis. This enzyme deficiency results in an inability to convert pyruvate to acetyl CoA, causing pyruvate to be shunted to lactate via lactate dehydrogenase (see p. 103). 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 X linked, and, because both males and females may be affected, the deficiency is classified as X-linked dominant. Although there is no proven treatment for PDH complex 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 code for proteins of the PDH complex, the electron transport chain, or ATP synthase. Both nuclear and mitochondrial DNA can be affected.

5. Mechanism of arsenic poisoning: As previously described (see p. 101), pentavalent arsenic (arsenate) can interfere with glycolysis at the glyceraldehyde 3-phosphate step, thereby decreasing ATP production. "Arsenic poisoning" is, however, due primarily to inhibition of enzymes that require lipoic acid as a coenzyme, including E2 of the PDH complex, α-ketoglutarate dehydrogenase (see below), and branched-chain α-keto acid dehydrogenase (see p. 266). 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 PDH complex, pyruvate (and, consequently, lactate) accumulates. As with PDH complex deficiency, this particularly affects the brain, causing neurologic disturbances and death.

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



B. Synthesis of citrate from acetyl coenzyme A and oxaloacetate

The condensation of acetyl CoA and oxaloacetate (OAA) to form citrate (a tricarboxylic acid) is catalyzed by citrate synthase (Figure 9.4). This aldol condensation has an equilibrium far in the direction of citrate synthesis. In humans, citrate synthase is not an allosteric enzyme. It is inhibited by its product, citrate. Substrate availability is another means of regulation for citrate synthase. The binding of OAA causes a conformational change in the enzyme that generates a binding site for acetyl CoA. [Note: Citrate, in addition to being an intermediate in the TCA cycle, provides a source of acetyl CoA for the cytosolic synthesis of fatty acids (see p. 183). Citrate also inhibits phosphofructokinase-1 (PFK-1), the rate-limiting enzyme of glycolysis (see p. 99), and activates acetyl CoA carboxylase (the rate-limiting enzyme of fatty acid synthesis; see p. 183).]

C. Isomerization of citrate

Citrate is isomerized to isocitrate by aconitase (aconitate hydratase), an Fe-S protein (see Figure 9.4). [Note: Aconitase is inhibited by fluoroacetate, a plant toxin that is used as a pesticide. Fluoroacetate is converted to fluoroacetyl CoA, which condenses with OAA to form fluorocitrate (a potent inhibitor of aconitase), resulting in citrate accumulation.]

D. Oxidative decarboxylation of isocitrate

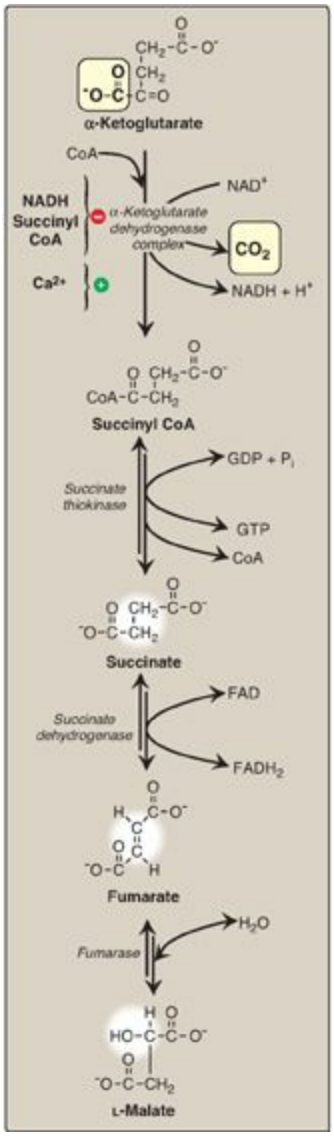
Isocitrate dehydrogenase catalyzes the irreversible oxidative decarboxylation of isocitrate, yielding the first of three NADH molecules produced by the cycle and the first release of CO_2 (see Figure 9.4). This is one of the rate-limiting steps of the TCA cycle. The enzyme is allosterically activated 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 conversion of α-ketoglutarate to succinyl CoA is catalyzed by the α-ketoglutarate dehydrogenase complex, a protein aggregate of multiple copies of three enzymes (Figure 9.5). The mechanism of this oxidative decarboxylation is very similar to that used for the conversion of pyruvate to acetyl CoA by the PDH complex. 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 PDH complex (see p. 110). The equilibrium of the reaction is far in the direction of succinyl CoA, a high-energy thioester similar to acetyl CoA. 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 PDH complex. [Note: α-Ketoglutarate is also produced by the oxidative deamination (see p. 252) and transamination of the amino acid glutamate (see p. 250).]

Figure 9.5 Formation of malate from α-ketoglutarate. NAD(H) = nicotinamide adenine dinucleotide; GDP = guanosine diphosphate; P = phosphate; CoA = coenzyme A; $FAD(H_2)$ = flavin adenine dinucleotide.



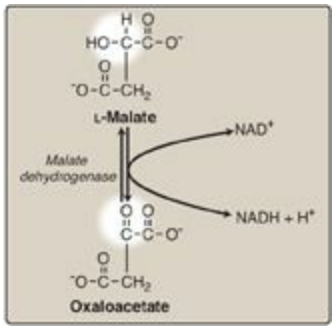
F. Cleavage of succinyl coenzyme A

Succinate thiokinase (also called succinyl CoA synthetase, named for the reverse reaction) cleaves the high-energy thioester bond of succinyl CoA (see Figure 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:

$$\text{GTP} + \text{ADP} \quad \rightleftarrows \quad \text{GDP} + \text{ATP}$$

The generation of GTP by succinate thiokinase is another example of substrate-level phosphorylation (see p. 102). [Note: Succinyl CoA is also produced from propionyl CoA derived from the metabolism of fatty acids with an odd number of carbon atoms (see p. 193), and from the metabolism of several amino acids (see pp. 265–266).]

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



G. Oxidation of succinate

Succinate is oxidized to fumarate by succinate dehydrogenase, as FAD (its coenzyme) is reduced to $FADH_2$ (see Figure 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 electron transport chain (see p. 75). [Note: FAD, rather than NAD^+, is the electron acceptor because the reducing power of succinate is not sufficient to reduce NAD^+.]

H. Hydration of fumarate

Fumarate is hydrated to malate in a freely reversible reaction catalyzed by fumarase (fumarate hydratase; see Figure 9.5). [Note: Fumarate is also produced by the urea cycle (see p. 255), in purine synthesis (see p. 294), and during catabolism of the amino acids phenylalanine and tyrosine (see p. 263).]

I. Oxidation of malate

Malate is oxidized to oxaloacetate by malate dehydrogenase (Figure 9.6). This reaction produces the third and final NADH of the cycle. The standard free energy change (ΔG^0; see p. 70) 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 (see p. 250).]

III. ENERGY PRODUCED BY THE CYCLE

Two carbon atoms enter the cycle as acetyl CoA and leave as CO_2. The cycle does not involve net consumption or production of OAA or of any other intermediate. Four pairs of electrons are transferred during one turn of the cycle: three pairs of electrons reducing three NAD^+ to NADH and one pair reducing FAD to $FADH_2$. Oxidation of one NADH by the electron transport chain leads to formation of approximately three ATP, whereas oxidation of $FADH_2$ yields approximately two ATP (see p. 77). 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.

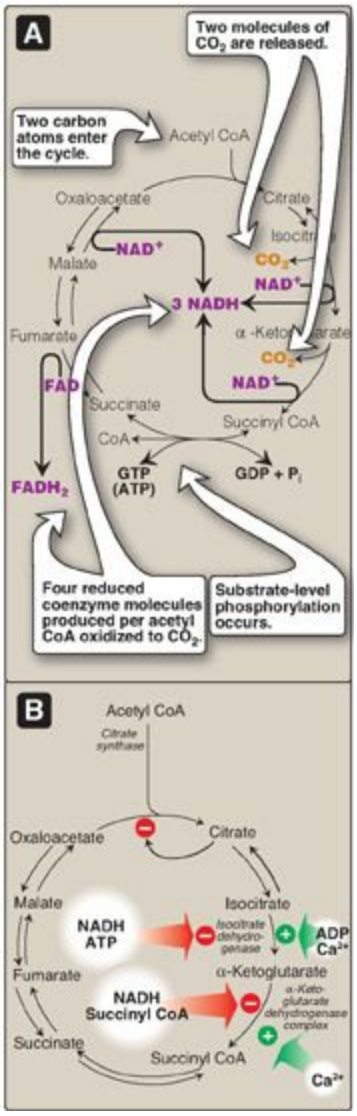
Figure 9.7 Number of ATP molecules produced from the oxidation of one molecule of acetyl coenzyme A (CoA) using both substrate-level and oxidative phosphorylation.

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

IV. REGULATION OF THE CYCLE

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

Figure 9.8 A. [Note: GTP and ATP are interconverted by nucleoside diphosphate kinase.] Production of reduced coenzymes, ATP, and CO_2 in the citric acid cycle. B. Inhibitors and activators of the cycle.

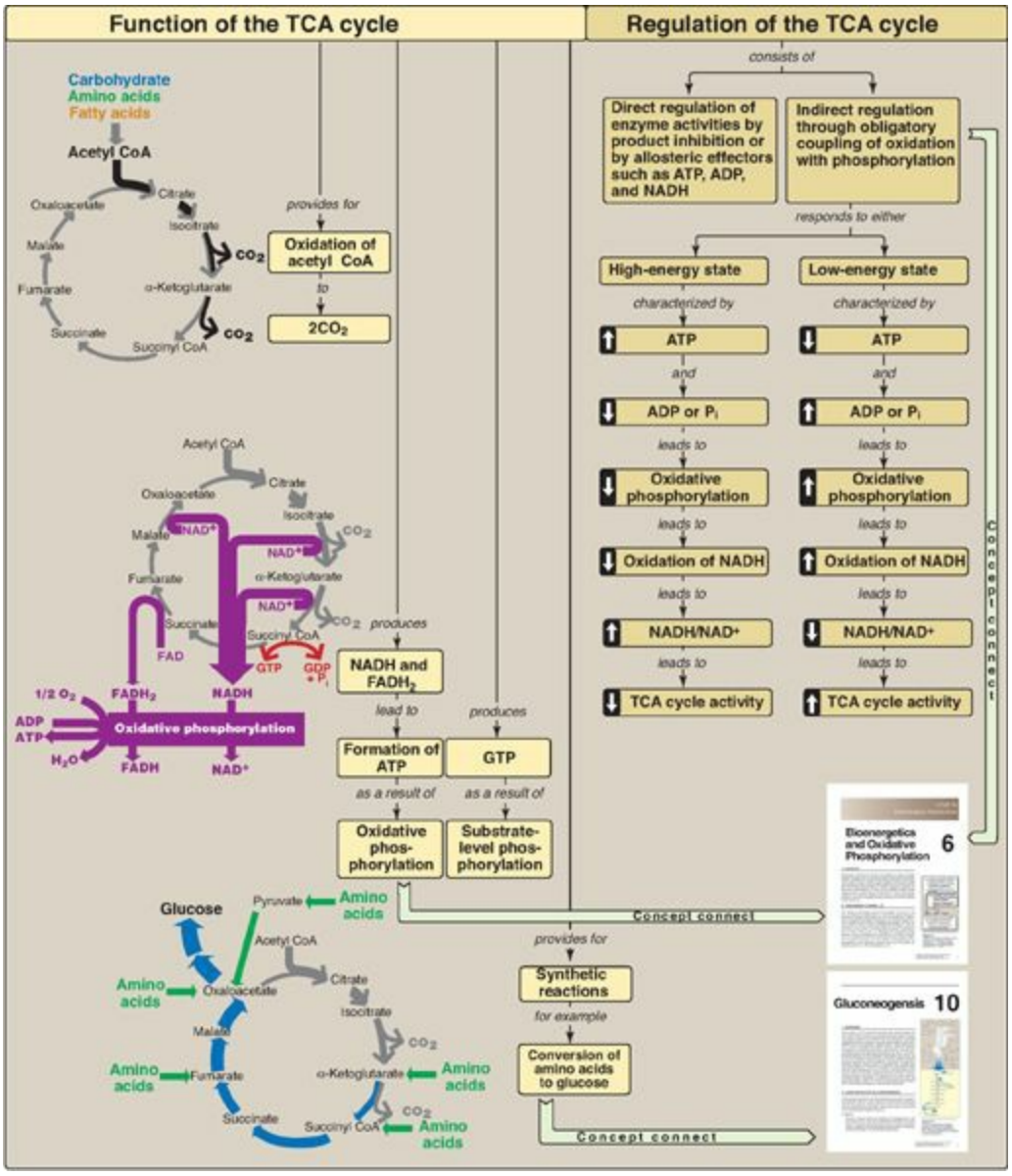


V. CHAPTER SUMMARY

Pyruvate is **oxidatively decarboxylated** by **pyruvate dehydrogenase (PDH) complex**, producing **acetyl coenzyme A (CoA)**, which is the major fuel for the tricarboxylic acid cycle ([TCA cycle] Figure 9.9). This multienzyme complex requires five coenzymes: **thiamine pyrophosphate**, **lipoic acid**, **flavin adenine dinucleotide** (**FAD)**, **nicotinamide adenine dinucleotide** (**NAD^+**), and **CoA**. PDH complex is regulated by covalent modification of E1 (pyruvate decarboxylase) 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 Ca^{2+}. **PDH complex deficiency** is the most common biochemical cause of **congenital lactic acidosis**. The central nervous system is particularly affected in this **X-linked dominant** disorder. **Arsenic poisoning** causes inactivation of the PDH complex by binding to lipoic acid. **Citrate** is synthesized from **oxaloacetate** and **acetyl CoA** by **citrate synthase**. This enzyme is subject to product inhibition by citrate. 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 PDH complex and uses the same coenzymes. 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** (also called **succinyl CoA synthetase**), 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 **oxaloacetate** by **malate dehydrogenase**, producing **NADH**. **Three NADH**, **one $FADH_2$**, and **one GTP** (whose terminal phosphate can be transferred to ADP by nucleoside diphosphate kinase, producing ATP) are produced by one round of the TCA cycle. The generation of acetyl CoA by the oxidation of pyruvate via the PDH complex also produces an NADH. Oxidation of the NADH and $FADH_2$ by the electron transport chain yields 14 ATP. An additional ATP (GTP) comes from substrate level phosphorylation in the TCA cycle. 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. CoA = coenzyme A; NAD(H) = nicotinamide adenine dinucleotide; FAD(H_2) = flavin adenine dinucleotide; GDP = guanosine diphosphate; GTP = guanosine triphosphate; 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 CO_2:

A. involves the participation of lipoic acid.
B. is activated when pyruvate decarboxylase of the pyruvate dehydrogenase (PDH) complex 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. Pyruvate dehydrogenase complex catalyzes an irreversible reaction that is inhibited when the decarboxylase component is phosphorylated. The enzyme complex 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 ratio limits the rates of the NAD^+-requiring dehydrogenases. High availability of calcium and substrate (acetyl CoA), and a low ATP/ADP ratio stimulates 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	Reactant 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 + $H_2O \rightarrow$ oxaloacetate + NADH + $FADH_2$

9.4 A 1-month-old male shows neurologic problems and lactic acidosis. Enzyme assay for pyruvate dehydrogenase (PDH) complex activity 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 PDH complex, is expected to be active.

Correct answer = A. The patient appears to have a thiamine-responsive pyruvate dehydrogenase (PDH) complex deficiency. The pyruvate decarboxylase (E1) component of the PDH complex 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 of the enzyme for the coenzyme, is present in some, but not all, cases of PDH complex deficiency. Because the PDH complex 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 PDH complex. Decreased activity of the complex decreases production of acetyl coenzyme A, a substrate for citrate synthase. PDH kinase is allosterically inhibited by pyruvate and, therefore, is inactive.

9.5 Which coenzyme-cosubstrate is used by the dehydrogenases of both glycolysis and the tricarboxylic acid cycle?

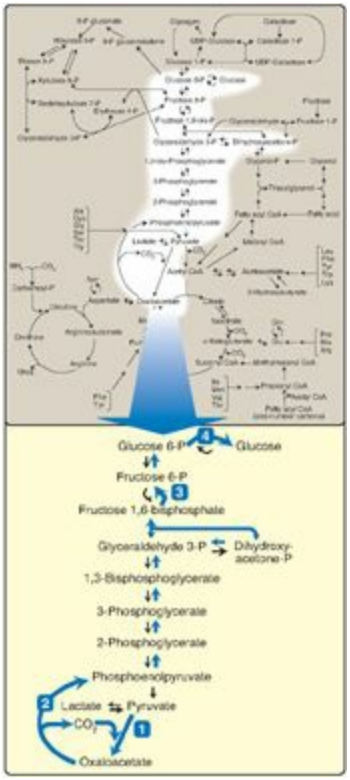
Oxidized nicotinamide adenine dinucleotide (NAD^+) is used by glyceraldehyde 3-phosphate dehydrogenase of glycolysis and by isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and malate dehydrogenase of the tricarboxylic acid cycle.

Gluconeogenesis 10

I. OVERVIEW

Some tissues, such as the brain, red blood cells (RBCs), kidney medulla, lens and cornea of the eye, testes, and exercising muscle, require a continuous supply of glucose as a metabolic fuel. Liver glycogen, an essential postprandial source of glucose, can meet these needs for only 10–18 hours in the absence of dietary intake of carbohydrate (see p. 329). During a prolonged fast, however, hepatic glycogen stores are depleted, and glucose is formed from noncarbohydrate precursors such as lactate, pyruvate, glycerol (derived from the backbone of triacylglycerols; see p. 190), and α-keto acids (derived from the catabolism of glucogenic amino acids; see p. 261). 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 by a special pathway, gluconeogenesis, which requires both mitochondrial and cytosolic enzymes. During an overnight fast, approximately 90% of gluconeogenesis occurs in the liver, with the remaining 10% occurring in the kidneys. However, during prolonged fasting, the kidneys become major glucose-producing organs, contributing an estimated 40% of the total glucose production. Figure 10.1 shows the relationship of gluconeogenesis to other essential pathways of energy metabolism.

Figure 10.1 The gluconeogenesis pathway shown as one of the essential pathways of energy metabolism. The numbered reactions are unique to gluconeogenesis. (See Figure 8.2, p. 92, for a more detailed map of metabolism.) P = phosphate.



II. SUBSTRATES FOR GLUCONEOGENESIS

Gluconeogenic precursors are molecules that can be used to produce a net synthesis of glucose. The most important gluconeogenic prescurors are glycerol, lactate, and the α-keto acids obtained from the metabolism of glucogenic amino acids. [Note: Alanine, which directly gives rise to pyruvate, is an important example of a glucogenic amino acid.]

A. Glycerol

Glycerol is released during the hydrolysis of triacylglycerols in adipose tissue (see p. 190) and is delivered by the blood to the liver. Glycerol is phosphorylated by glycerol kinase to glycerol phosphate, which is oxidized by glycerol phosphate dehydrogenase to dihydroxyacetone phosphate, an intermediate of glycolysis. [Note: Adipocytes cannot phosphorylate glycerol because they essentially lack glycerol kinase.]

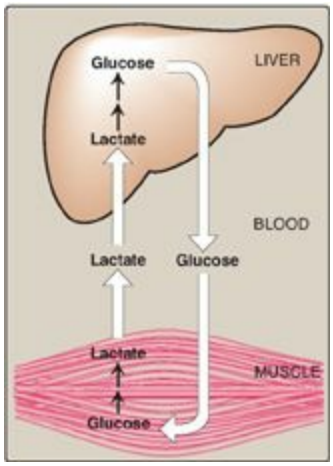
B. Lactate

Lactate is released into the blood by exercising skeletal muscle and by cells that lack mitochondria such as RBCs. In the Cori cycle, bloodborne glucose is converted by exercising muscle to lactate, which diffuses into the blood. This lactate is taken up by the liver and reconverted to glucose, which is released back into the circulation (Figure 10.2).

C. Amino acids

Amino acids derived from hydrolysis of tissue proteins are the major sources of glucose during a fast. The metabolism of the glucogenic amino acids generates α-keto acids. α-Keto acids, such as α-ketoglutarate 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 (for example, acetoacetate and amino acids such as lysine and leucine) cannot give rise to a net synthesis of glucose. This is due to the irreversible nature of the pyruvate dehydrogenase (PDH) reaction, which converts pyruvate to acetyl CoA (see p. 109). These compounds give rise instead to ketone bodies (see p. 195) and are, therefore, termed ketogenic.]

Figure 10.2 The intertissue Cori cycle. [Note: Diffusion of lactate across membranes is facilitated by a transport protein.]


Glucose
LIVER
Lactate
BLOOD
Lactate
Glucose
Lactate
MUSCLE
Glucose

III. REACTIONS UNIQUE TO GLUCONEOGENESIS

Seven glycolytic reactions are reversible and are used in the synthesis of glucose from lactate or pyruvate. However, three of the reactions are irreversible and must be circumvented by four alternate reactions that energetically favor the synthesis of glucose. These reactions, unique to gluconeogenesis, are described below.

A. Carboxylation of pyruvate

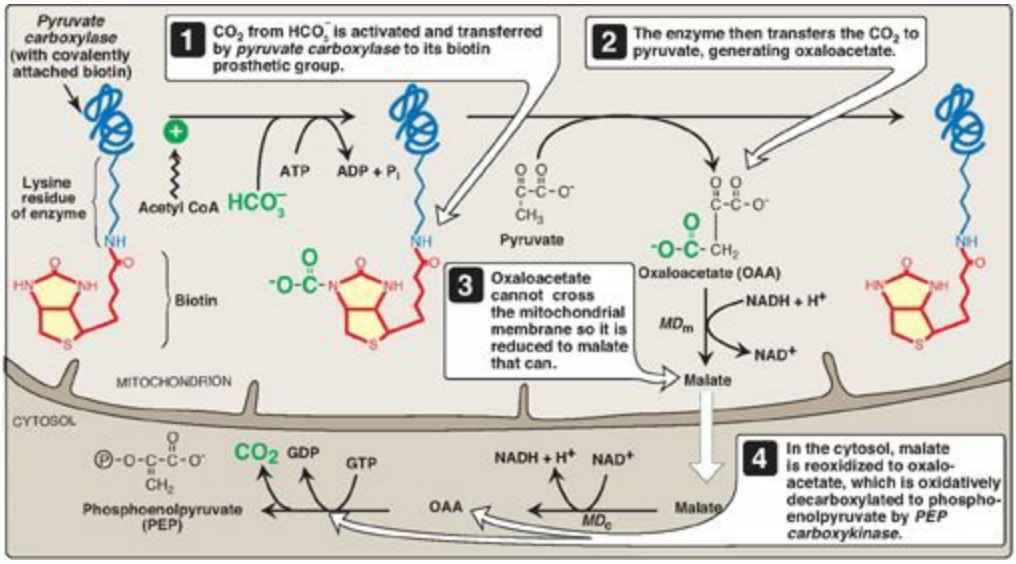
The first "roadblock" to overcome in the synthesis of glucose from pyruvate is the irreversible conversion in glycolysis of PEP to pyruvate by pyruvate kinase (PK). In gluconeogenesis, pyruvate is first carboxylated by pyruvate carboxylase to OAA, which is then converted to PEP by the action of PEP-carboxykinase (Figure 10.3).

1. **Biotin, a coenzyme:** Pyruvate carboxylase requires biotin (see p. 381) covalently bound to the ε-amino group of a lysine residue in the enzyme (see Figure 10.3). Hydrolysis of ATP drives the formation of an enzyme–biotin–CO_2 intermediate, which subsequently carboxylates pyruvate to form OAA. [Note: HCO_3^- is the source of the CO_2.] The pyruvate carboxylase reaction occurs in the mitochondria of liver and kidney cells and has two purposes: to provide an important substrate for gluconeogenesis and to provide OAA that can replenish the TCA cycle intermediates that may become depleted, depending on the synthetic needs of the cell. Muscle cells also contain pyruvate carboxylase but use the OAA produced only for the replenishment (anaplerotic) purpose and do not synthesize glucose.

> Pyruvate carboxylase is one of several carboxylases that require biotin. Others include acetyl CoA carboxylase (p. 183), propionyl CoA carboxylase (p. 193), and methylcrotonyl CoA carboxylase (p. 266).

2. **Allosteric regulation:** Pyruvate carboxylase is allosterically activated by acetyl CoA. Elevated levels of acetyl CoA in mitochondria signal a metabolic state in which the increased synthesis of OAA is required. For example, this occurs during fasting, when OAA is used for the synthesis of glucose by gluconeogenesis in the liver and kidney. Conversely, at low levels of acetyl CoA, pyruvate carboxylase is largely inactive, and pyruvate is primarily oxidized by the PDH complex to produce acetyl CoA that can be further oxidized by the TCA cycle (see p. 109).

Figure 10.3 Carboxylation of pyruvate to OAA, followed by reduction of OAA to malate for transfer to the cystol and subsequent decarboxylation to PEP. [Note: OAA can also be converted to PEP or aspartate for transfer to the cytosol.] MD_m = mitochondrial malate dehydrogenase; MD_c = cytosolic malate dehydrogenase.



B. Transport of oxaloacetate to the cytosol

OAA must be converted to PEP for gluconeogenesis to continue. The enzyme that catalyzes this reaction is found in both the mitochondria and the cytosol in humans. The PEP generated in the mitochondria is transported to the cytosol by a specific transporter, whereas that generated in the cytosol requires the transport of OAA from the mitochondria to the cytosol. However, OAA is unable to be transported across the inner mitochondrial membrane, so it must first be reduced to malate by mitochondrial malate dehydrogenase (MD). Malate can be transported from the mitochondria to the cytosol, where it is reoxidized to OAA by cytosolic MD as nicotinamide adenine dinucleotide (NAD^+) is reduced (see Figure 10.3). The NADH produced is used in the reduction of 1,3-bisphosphoglycerate to glyceraldehyde 3-phosphate (see p. 101), a step common to both glycolysis and gluconeogenesis. [Note: OAA also can be converted to aspartate, which is transported out of the mitochondria.]

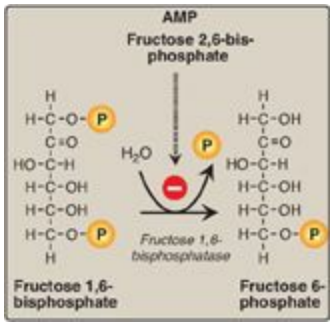
C. Decarboxylation of cytosolic oxaloacetate

OAA is decarboxylated and phosphorylated to PEP in the cytosol by PEP-carboxykinase (also referred to as PEPCK). The reaction is driven by hydrolysis of guanosine triphosphate ([GTP] see Figure 10.3). The combined actions of pyruvate carboxylase and PEP-carboxykinase 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, as seen in gluconeogenesis, drives reactions that would otherwise be energetically unfavorable. A similar strategy is used in fatty acid synthesis (see pp. 183–184).

Figure 10.4 Dephosphorylation of fructose 1,6- bisphosphate. AMP = adenosine

monophosphate; P = phosphate.

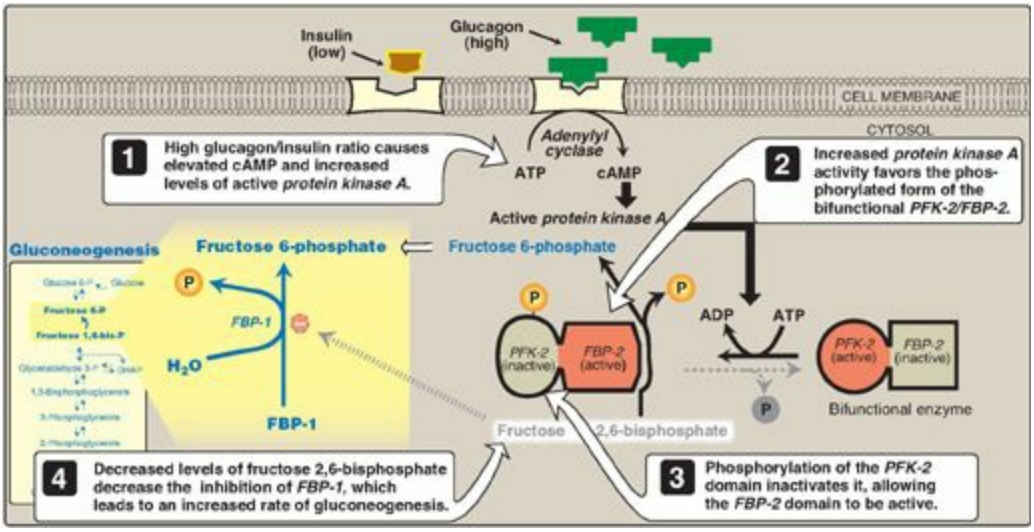


D. Dephosphorylation of fructose 1,6-bisphosphate

Hydrolysis of fructose 1,6-bisphosphate by fructose 1,6-bisphos-phatase, found in liver and kidney, bypasses the irreversible phosphofructokinase-1 (PFK-1) reaction, and provides an energetically favorable pathway for the formation of fructose 6-phosphate (Figure 10.4). This reaction is an important regulatory site of gluconeogenesis.

1. **Regulation by energy levels within the cell:** Fructose 1,6-bisphosphatase is inhibited by elevated levels of adenosine monophosphate (AMP), which signal an "energy-poor" state in the cell. Conversely, high levels of ATP and low concentrations of AMP stimulate gluconeogenesis, an energy-requiring pathway.

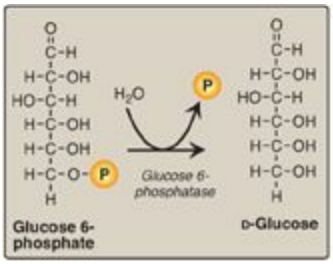
Figure 10.5 Effect of elevated glucagon on the intracellular concentration of fructose 2,6-bisphosphate in the liver. cAMP = cyclic AMP; PFK-2 = phosphofructokinase-2; FBP-2 = fructose 2,6-bisphosphatase; FBP-1 = fructose 1,6-bisphosphatase; P = phosphate.



2. **Regulation by fructose 2,6-bisphosphate:** Fructose 1,6-bisphos-phatase is inhibited by fructose 2,6-bisphosphate, an allosteric effector whose concentration is influenced by the insulin to glucagon ratio: when glucagon is high, the effector is not made and, thus, the phosphatase is active. (Figure 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 (see

p. 100).]

Figure 10.6 Dephosphorylation of glucose 6-phosphate allows release of free glucose from the liver and kidney into blood. P = phosphate.



E. Dephosphorylation of glucose 6-phosphate

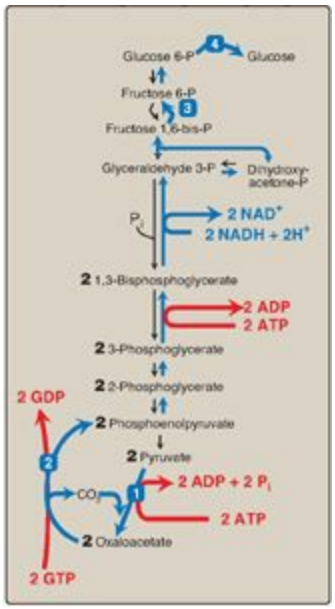
Hydrolysis of glucose 6-phosphate by glucose 6-phosphatase bypasses the irreversible hexokinase/glucokinase reaction and provides an energetically favorable pathway for the formation of free glucose (Figure 10.6). Liver and kidney are the only organs that release free glucose from glucose 6-phosphate. This process actually requires a complex of two proteins: glucose 6-phosphate translocase, which transports glucose 6-phosphate across the endoplasmic reticular (ER) membrane, and the enzyme glucose 6-phosphatase (found only in gluconeogenic cells), which removes the phosphate, producing free glucose (see Figure 10.6). [Note: These ER-membrane proteins are also required for the final step of glycogen degradation (see p. 130). Type Ia and Ib glycogen storage disease, caused by deficiencies in the phosphatase and the transferase, respectively, are characterized by severe fasting hypoglycemia, because free glucose is unable to be produced from either gluconeogenesis or glycogenolysis.] Specific glucose transporters (GLUTs) are responsible for moving free glucose into the cytosol and then into blood. [Note: Glucose 6-phosphate translocase moves inorganic phosphate out of the ER as it moves glucose 6-phosphate in.]

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 (Figure 10.7). The irreversible reactions of glycolysis catalyzed by hexokinase/glucokinase, PFK-1, and PK are circumvented by glucose 6-phosphatase, fructose 1,6-bisphosphatase, and pyruvate carboxylase/PEP-carboxykinase. In gluconeogenesis, the equilibria of the 7 reversible reactions of glycolysis are pushed in favor of glucose synthesis as a result of the essentially irreversible formation of PEP, fructose 6-phosphate, and glucose catalyzed by the gluconeogenic enzymes. [Note: The stoichiometry of gluconeogenesis from pyruvate couples the cleavage of six high-energy phosphate bonds and the oxidation of two NADH with the formation of each molecule of glucose (see Figure 10.7).]

Figure 10.7 Summary of the reactions of glycolysis and gluconeogenesis, showing the energy requirements of gluconeogenesis. The numbered reactions are unique to

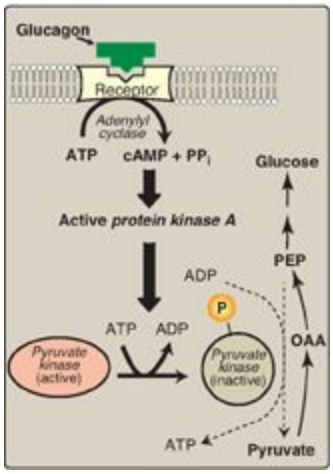
gluconeogenesis. P = phosphate; GDP = guanosine diphosphate; GTP = guanosine triphosphate; NAD(H) = nicotinamide adenine dinucleotide.



IV. REGULATION OF GLUCONEOGENESIS

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 activity 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.]

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; cAMP = cyclic AMP; PP_i = pyrophosphate; P = phosphate.



A. Glucagon

This peptide hormone from the a cells of pancreatic islets (see p. 313) stimulates gluconeogenesis by three mechanisms.

1. **Changes in allosteric effectors:** Glucagon lowers the level of fructose 2,6-bisphosphate, resulting in activation of fructose 1,6-bisphosphatase and inhibition of PFK-1, thus favoring gluconeogenesis over glycolysis (see Figure 10.5). [Note: See p. 99 for the role of fructose 2,6-bisphosphate in the regulation of glycolysis.]

2. **Covalent modification of enzyme activity:** Glucagon binds its G protein–coupled receptor (see p. 95) and, via an elevation in cyclic AMP (cAMP) level and cAMP-dependent protein kinase activity, stimulates the conversion of hepatic PK to its inactive (phosphorylated) form. This decreases the conversion of PEP to pyruvate, which has the effect of diverting PEP to the synthesis of glucose (Figure 10.8).

3. **Induction of enzyme synthesis:** Glucagon increases the transcription of the gene for PEP-carboxykinase, thereby increasing the availability of this enzyme as levels of its substrate rise during fasting. [Note: Glucocorticoids also increase expression of the gene, whereas insulin decreases expression.]

B. Substrate availability

The availability of gluconeogenic precursors, particularly glucogenic amino acids, significantly influences the rate of glucose synthesis. Decreased levels of insulin favor mobilization of amino acids from muscle protein and provide the carbon skeletons for gluconeogenesis. The ATP and NADH coenzymes-cosubstrates required for gluconeogenesis are primarily provided by the catabolism of fatty acids.

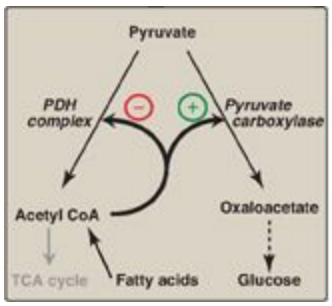
C. Allosteric activation by acetyl coenzyme A

Allosteric activation of hepatic pyruvate carboxylase by acetyl CoA occurs during fasting. As a result of increased lipolysis in adipose tissue, the liver is flooded with fatty acids (see p. 330). The rate of formation of acetyl CoA by β-oxidation of these fatty acids exceeds the capacity of the liver to oxidize it to CO_2 and H_2O. As a result, acetyl CoA accumulates and activates pyruvate carboxylase. [Note: Acetyl CoA inhibits the PDH complex (by activating PDH kinase; see p. 111). Thus, this single compound can divert pyruvate toward gluconeogenesis and away from the TCA cycle (Figure 10.9).]

D. Allosteric inhibition by adenosine monophosphate

Fructose 1,6-bisphosphatase is inhibited by AMP—a compound that activates PFK-1. This results in a reciprocal regulation of glycolysis and gluconeogenesis seen previously with fructose 2,6-bisphosphate (see p. 121). [Note: Elevated AMP, thus, stimulates pathways that oxidize nutrients to provide energy for the cell.]

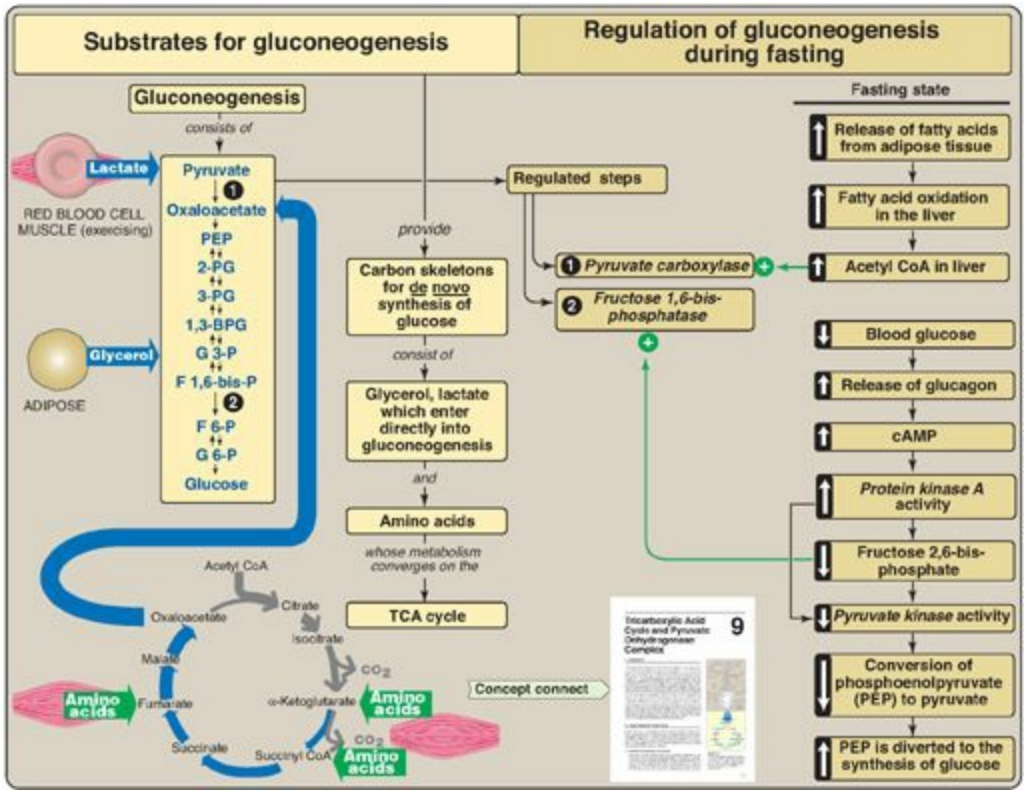
Figure 10.9 Acetyl coenzyme A (CoA) diverts pyruvate away from oxidation and toward gluconeogenesis. PDH = pyruvate dehydrogenase; TCA = tricarboxylic acid.



V. CHAPTER SUMMARY

Gluconeogenic precursors include the **intermediates of glycolysis** and the **tricarboxylic acid cycle**, **glycerol** released during the hydrolysis of triacylglycerols in adipose tissue, **lactate** released by cells that lack mitochondria and by exercising skeletal muscle, and **α-keto acids** derived from the metabolism of glucogenic amino acids (Figure 10.10). Seven of the reactions of glycolysis are reversible and are used for gluconeogenesis in the liver and kidneys. Three reactions are **physiologically irreversible** and must be circumvented. These reactions are catalyzed by the glycolytic enzymes **pyruvate kinase**, **phosphofructokinase**, and **hexokinase**. **Pyruvate** is converted to oxaloacetate and then to **phosphoenolpyruvate** (**PEP**) by **pyruvate carboxylase** and **PEP-carboxykinase**. The carboxylase requires **biotin** and **ATP** and is allosterically activated by **acetyl coenzyme A**. PEP-carboxykinase requires **GTP**. The transcription of its gene is increased by glucagon and the glucocorticoids and decreased by insulin. **Fructose 1,6-bisphosphate** is converted to **fructose 6-phosphate** by **fructose 1,6-bisphosphatase**. This enzyme is **inhibited** by elevated levels of **AMP** and **activated** when **ATP** levels are elevated. The enzyme is also **inhibited** by **fructose 2,6-bisphosphate**, the primary allosteric activator of glycolysis. **Glucose 6-phosphate** is converted to **glucose** by **glucose 6-phosphatase**. This enzyme of the endoplasmic reticular membrane is required for the final step in gluconeogenesis as well as hepatic and renal 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; PG = phosphoglycerate; BPG = bisphosphoglycerate.


Substrates for gluconeogenesis
Regulation of gluconeogenesis during fasting
Gluconeogenesis
consists of
Lactate
RED BLOOD CELL
MUSCLE (exercising)
Pyruvate
Oxaloacetate
PEP
2-PG
3-PG
1,3-BPG
G 3-P
F 1,6-bis-P
F 6-P
G 6-P
Glucose
Glycerol
ADIPOSE
provide
Carbon skeletons for de novo synthesis of glucose
consist of
Glycerol, lactate which enter directly into gluconeogenesis
and
Amino acids
whose metabolism converges on the
TCA cycle
Regulated steps
Pyruvate carboxylase
Fructose 1,6-bis-phosphatase
Fasting state
Release of fatty acids from adipose tissue
Fatty acid oxidation in the liver
Acetyl CoA in liver
Blood glucose
Release of glucagon
cAMP
Protein kinase A activity
Fructose 2,6-bis-phosphate
Pyruvate kinase activity
Conversion of phosphoenolpyruvate (PEP) to pyruvate
PEP is diverted to the synthesis of glucose
Acetyl CoA
Citrate
Oxaloacetate
Isocitrate
Malate
Fumarate
α-Ketoglutarate
Succinate
Succinyl CoA
CO2
CO2
Amino acids
Amino acids
Amino acids
Concept connect
9

Study Questions

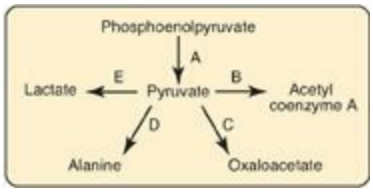
Choose the ONE best answer.

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 fast.
C. It is inhibited by a fall in the insulin-to-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 fast, glycogen stores are depleted, and gluconeogenesis maintains blood glucose. Gluconeogenesis is an energy-requiring (endergonic) pathway (both ATP and GTP get hydrolyzed) that occurs in liver, with kidney becoming a major glucose-producing organ in prolonged fasting. It utilizes both mitochondrial and cytosolic enzymes. Gluconeogenesis 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 reaction is physiologically irreversible. It is the carbon skeletons of most amino acids that are gluconeogenic.

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 (PDH complex) requires thiamine pyrophosphate, lipoic acid, FAD, coenzyme A, NAD; 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 downregulate gluconeogenesis through inhibition of fructose 1,6-bisphosphatase and upregulate glycolysis through activation of phosphofructokinase-1. This results in reciprocal regulation of the two pathways.

10.5 The metabolism of ethanol by alcohol dehydrogenase produces reduced nicotinamide adenine dinucleotide (NADH). What effect is the change in the NAD^+/NADH ratio expected to have on gluconeogenesis? Explain.

The increase in NADH as ethanol is oxidized will decrease 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 the high availability of NADH. Additionally, the reversible reduction of pyruvate to lactate by lactate dehydrogenase of glycolysis is driven in the forward direction by NADH. Thus, two important gluconeogenic substrates, OAA and pyruvate, are decreased as a result of the increase in NADH during ethanol metabolism. This results in a decrease in gluconeogenesis.

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

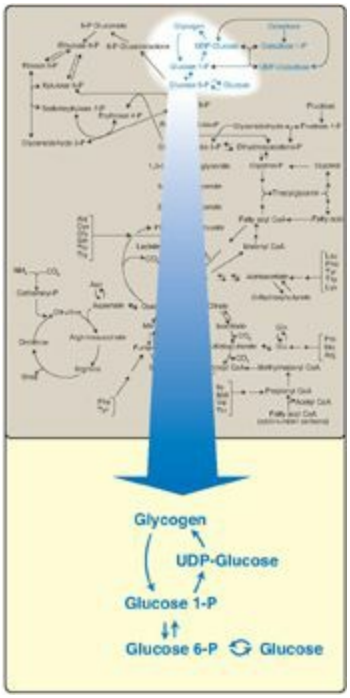
activates pyruvate carboxylase, pushing pyruvate to gluconeogenesis and away from oxidation.

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, degradation of glycogen, 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 (see p. 117) 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 mobilizable form, namely, glycogen. In the absence of a dietary source of glucose, this sugar is rapidly released from liver and kidney 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 amino acids from the body's proteins as a primary source of carbons for the gluconeogenic pathway. 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 (see Figure 8.2, p. 92, for a more detailed view of the overall reactions of metabolism). P = phosphate; UDP = uridine diphosphate.



II. STRUCTURE AND FUNCTION OF GLYCOGEN

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 adenosine triphosphate (ATP) during muscle contraction. That of liver glycogen is to maintain the blood glucose concentration, particularly during the early stages of a fast (Figure 11.2; also see p. 329). [Note: Liver glycogen can maintain blood glucose for 10–18 hours.]

A. Amounts of liver and muscle glycogen

Approximately 400 g of glycogen make up 1%–2% of the fresh weight of resting muscle, and approximately 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 Figure 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 muscle.]

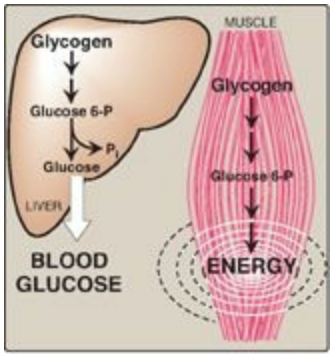
B. Structure of glycogen

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 eight to ten glucosyl residues, there is a branch containing an α(1→6) linkage (Figure 11.3). A single glycogen molecule can have a molecular weight of up to 10^8 Da. These polymers of glucose exist in discrete cytoplasmic granules that also contain most of the enzymes necessary for glycogen synthesis and degradation.

C. Fluctuation of glycogen stores

Liver glycogen stores increase during the well-fed state (see p. 323) and are depleted during a fast (see p. 329). 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 differences between the rates of these two processes determine the levels of stored glycogen during specific physiologic states.]

Figure 11.2 Functions of muscle and liver glycogen. P = phosphate; P_i = inorganic phosphate.


Glycogen
Glucose 6-P
P_i
Glucose
LIVER
BLOOD GLUCOSE
MUSCLE
Glycogen
Glucose 6-P
ENERGY

III. SYNTHESIS OF GLYCOGEN (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).

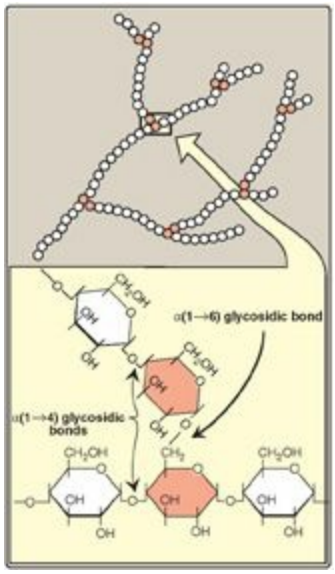
A. Synthesis of uridine diphosphate glucose

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 (Figure 11.4) is synthesized from glucose 1-phosphate and UTP by UDP-glucose pyrophosphorylase (Figure 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, ensuring 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 (Figure 11.6).]

B. Synthesis of a primer to initiate glycogen synthesis

Glycogen synthase makes 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 cells whose glycogen stores are not totally depleted. In the absence of a glycogen fragment, a protein called glycogenin can serve as an acceptor of glucose residues from UDP-glucose (see Figure 11.5). The side-chain hydroxyl group of a specific tyrosine in the protein serves as 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 the next few 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 as described below [Note: Glycogenin stays associated with and forms the core of a glycogen granule.]

Figure 11.3 Branched structure of glycogen, showing α(1→4) and α(1→6) glycosidic bonds.



C. Elongation of glycogen chains 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 Figure 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 compound, making the terminal sugar nonreducing (see p. 84).] 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; see p. 296).]

Figure 11.4 The structure of UDP-glucose, a nucleotide sugar.

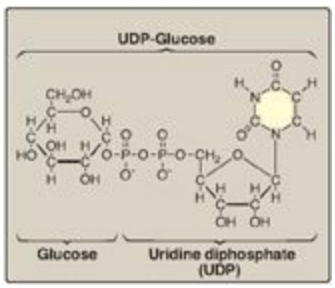
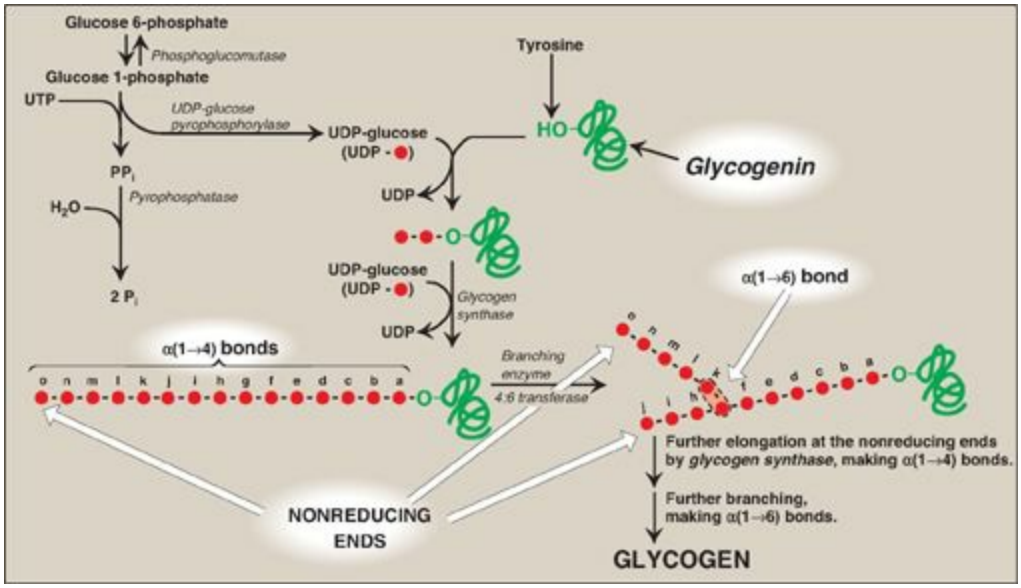


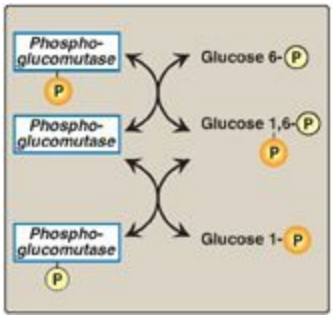
Figure 11.5 Glycogen synthesis. UTP = uridine triphosphate; UDP = uridine diphosphate; PP_i = pyrophosphate; P_i = inorganic phosphate.



D. Formation of branches in glycogen

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 Figure 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 later, 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.

Figure 11.6 Interconversion of glucose 6-phosphate and glucose 1-phosphate by phosphoglucomutase. P = phosphate.



1. **Synthesis of branches:** Branches are made by the action of the branching enzyme, amylo-α(1→4)→α(1→6)-transglucosidase. 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 non-terminal glucosyl residue by an α(1→6) linkage, thus functioning as a 4:6 transferase. The resulting new, nonreducing end (see "j" in Figure 11.5), as well as the old nonreducing end from which the six to eight residues were removed (see "o"

in Figure 11.5), can now be further elongated by glycogen synthase.

2. **Synthesis of additional branches:** 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 OF GLYCOGEN (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. Shortening of chains

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 before a branch point (Figure 11.7). [Note: Phosphorylase contains a molecule of covalently bound pyridoxal phosphate that is required as a coenzyme.] The resulting structure is called a limit dextrin, and phosphorylase cannot degrade it any further (Figure 11.8).

B. Removal of branches

Branches are removed by the two enzymic activities of a single bifunctional protein, the debranching enzyme (see Figure 11.8). First, oligo-α(1→4)→α(1→4)-glucantransferase activity removes the outer three of the four glucosyl residues attached 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 glucose. The glucosyl chain is now available again for degradation by glycogen phosphorylase until four glucosyl units in the next branch are reached.

Figure 11.7 Cleavage of an α(1→4)-glycosidic bond. PLP= pyridoxal phosphate; P_i = inorganic phosphate; P = phosphate.

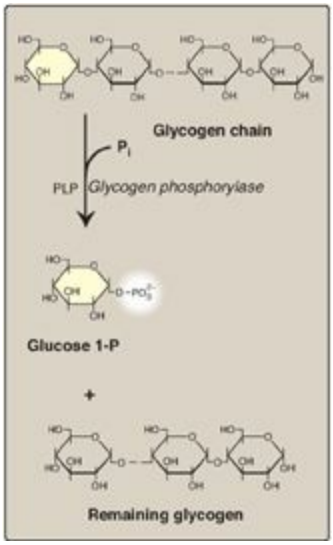
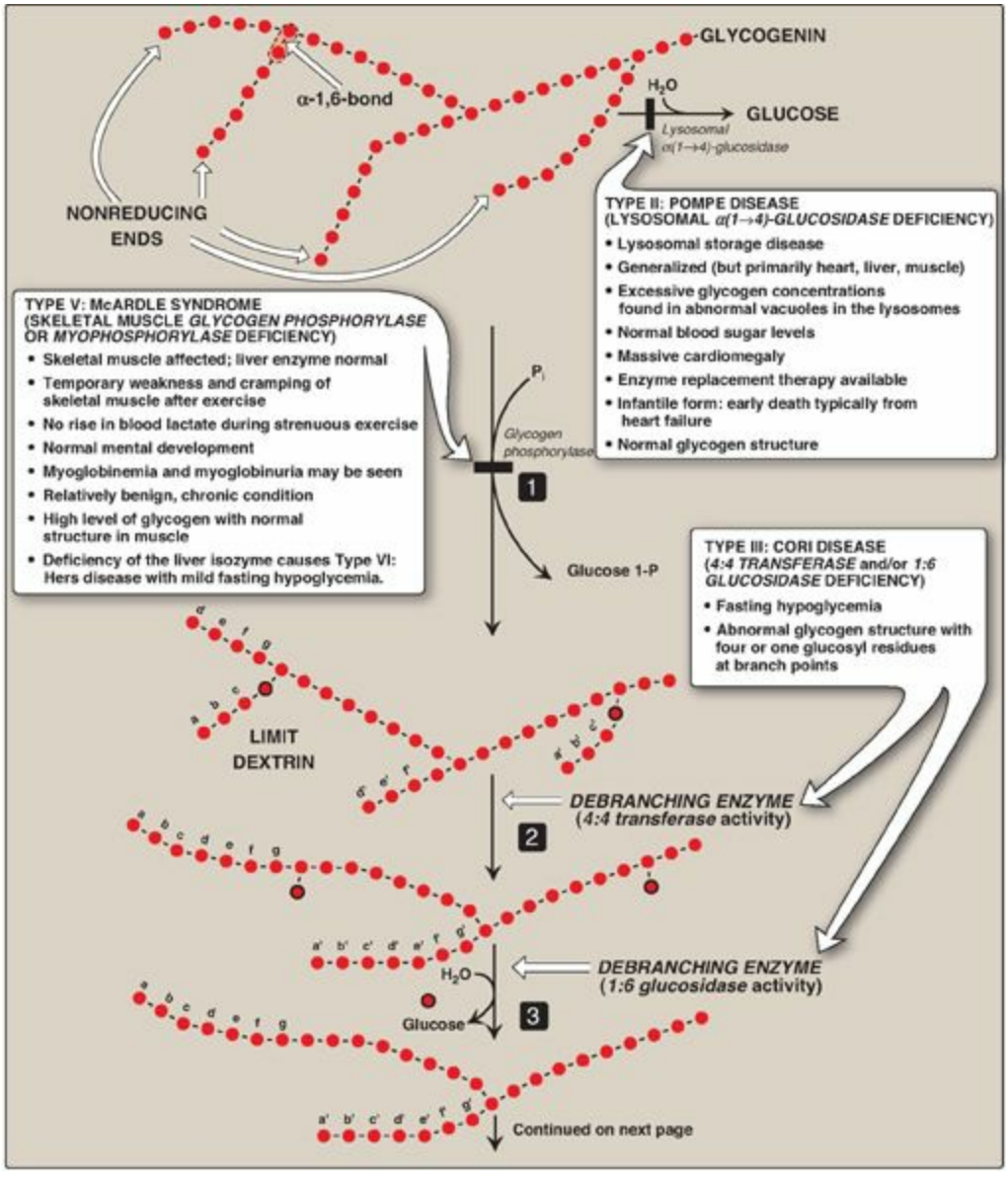
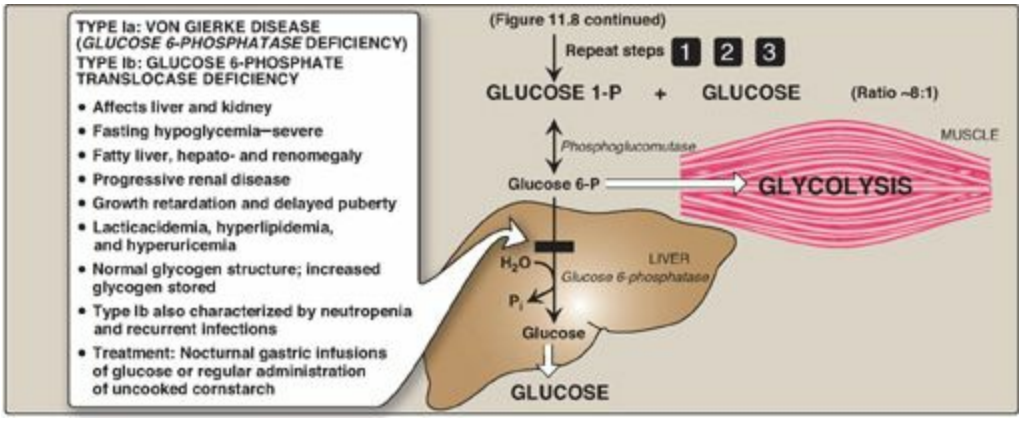


Figure 11.8 Glycogen degradation, showing some of the glycogen storage diseases (GSDs). [Note: A GSD can also be caused by defects in branching enzyme, an enzyme of synthesis, resulting in Type IV: Andersen disease and causing death in early childhood from liver cirrhosis.] P_i = inorganic phosphate; P = phosphate. Glycogen degradation, showing some of the glycogen storage diseases (GSDs).





C. Conversion of glucose 1-phosphate to glucose 6-phosphate

Glucose 1-phosphate, produced by glycogen phosphorylase, is converted in the cytosol to glucose 6-phosphate by phosphoglucomutase (see Figure 11.6). In the liver, glucose 6-phosphate is transported into the endoplasmic reticulum (ER) by glucose 6-phosphate translocase. There it is converted to glucose by glucose 6-phosphatase (the same enzyme used in the last step of gluconeogenesis; see p. 121). The glucose then is 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: In the muscle, glucose 6-phosphate cannot be dephosphorylated and sent into the blood because of a lack of glucose 6-phosphatase. Instead, it enters glycolysis, providing energy needed for muscle contraction.]

D. Lysosomal degradation of glycogen

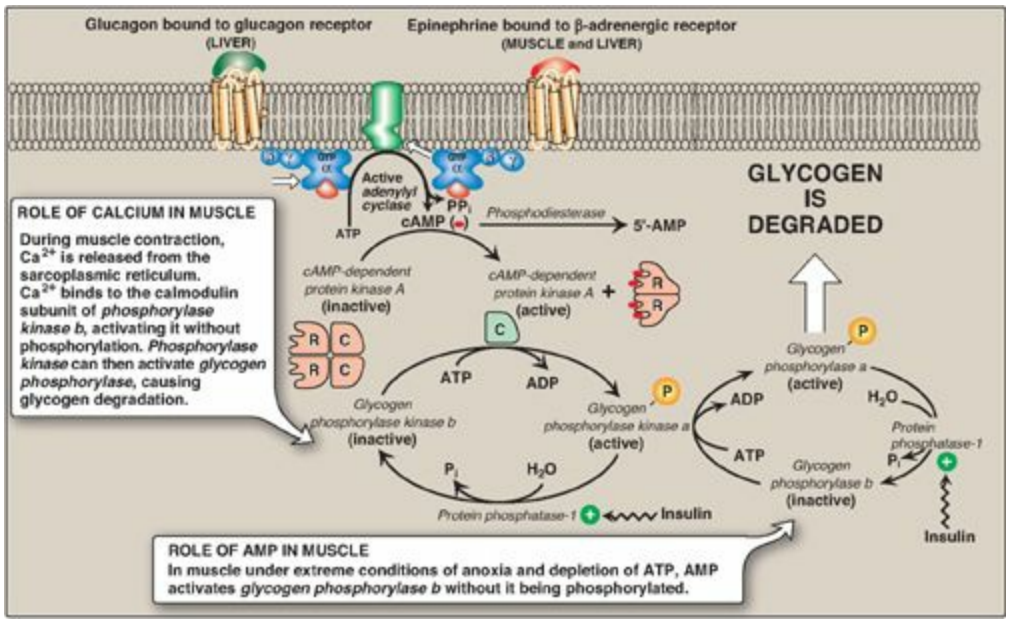
A small amount (1%–3%) of glycogen is continuously degraded by the lysosomal enzyme, α(1→4)-glucosidase (acid maltase). The purpose of this pathway is unknown. However, a deficiency of this enzyme causes accumulation of glycogen in vacuoles in the lysosomes, resulting in the serious glycogen storage disease (GSD) Type II: Pompe disease (see Figure 11.8). [Note: Type II: Pompe disease is the only GSD that is a lysosomal storage disease.]

> Lysosomal storage diseases are genetic disorders characterized by the accumulation of abnormal amounts of carbohydrates or lipids primarily due to their decreased lysosomal degradation.

V. REGULATION OF GLYCOGENESIS AND GLYCOGENOLYSIS

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 glycogen synthesis and degradation is accomplished on two levels. First, glycogen synthase and glycogen phosphorylase are hormonally regulated (by phosphorylation/dephosphorylation) to meet the needs of the body as a whole. [Note: Phosphorylation of glycogen phosphorylase is catalyzed by glycogen phosphorylase kinase (see p. 132).] Second, these same enzymes are allosterically regulated (by effector molecules) to meet the needs of a particular tissue.

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.



A. Activation of glycogen degradation

The binding of hormones, such as glucagon or epinephrine, to plasma membrane G protein–coupled receptors (GPCRs) signals the need for glycogen to be degraded, either to elevate blood glucose levels or to provide energy for exercising muscle.

1. **Activation of protein kinase A:** 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), as described on page 95. PKA is a tetramer, having two regulatory subunits (R) and two catalytic subunits (C). cAMP binds to the

regulatory subunits, releasing individual catalytic subunits that are active (Figure 11.9). PKA then phosphorylates several enzymes of glycogen metabolism, affecting their activity. [Note: When cAMP is removed, the inactive tetramer, R_2C_2, is again formed.]

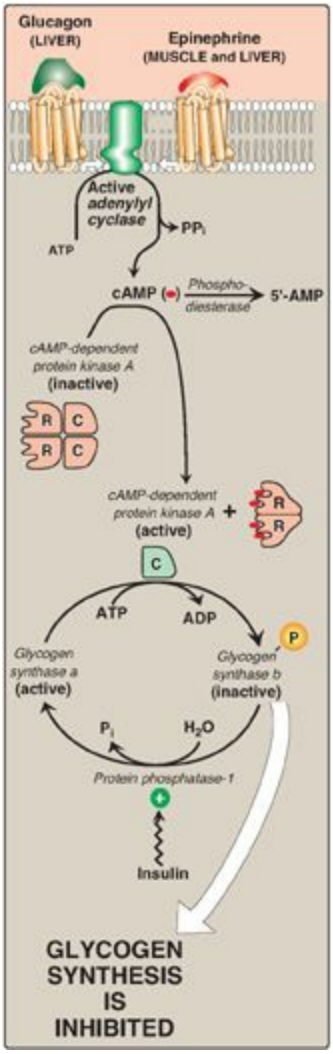
2. **Activation of phosphorylase kinase:** Phosphorylase kinase exists in two forms: an inactive "b" form and an active "a" form. Active PKA phosphorylates the inactive "b" form of phosphorylase kinase, producing the active "a" form (see Figure 11.9).

3. **Activation of glycogen phosphorylase:** Glycogen phosphorylase also exists in two forms: the dephosphorylated, inactive "b" form and the phosphorylated, active "a" form. Active phosphorylase kinase is the only enzyme that phosphorylates glycogen phosphorylase b to its active "a" form, which then begins glycogenolysis (see Figure 11.9).

4. **Summary of the regulation of glycogen degradation:** The cascade of reactions listed above results in 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 receptors results in a number of PKA molecules being activated that can each activate many phosphorylase kinase molecules). This causes the production of many active glycogen phosphorylase a molecules that can degrade glycogen.

5. **Maintenance of the phosphorylated state:** 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 Figure 11.9). [Note: PP1 is activated by a signal cascade initiated by insulin (see p. 311). Insulin also activates the phosphodiesterase that degrades cAMP, and, thus, insulin opposes the effects of glucagon and epinephrine.]

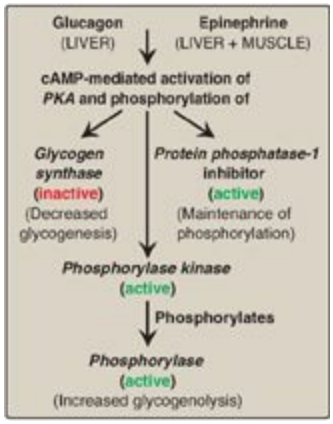
Figure 11.10 Hormonal regulation of glycogen synthesis. [Note: In contrast to glycogen phosphorylase, glycogen synthase is inactivated by phosphorylation.] cAMP = cyclic adenosine monophosphate; P = phosphate; PP_i = pyrophosphate; R = regulatory subunit; C = catalytic subunit.



B. Inhibition of glycogen synthesis

The regulated enzyme in glycogenesis is glycogen synthase. It also exists in two forms, the active "a" form and the inactive "b" form. However, for glycogen synthase, in contrast to phosphorylase kinase and phosphorylase, the active form is dephosphorylated, whereas the inactive form is phosphorylated (Figure 11.10). Glycogen synthase a is converted to the inactive "b" form by phosphorylation at several sites on the enzyme, with the level of inactivation proportional to its degree of phosphorylation. Phosphorylation is catalyzed by several different protein kinases that are regulated by cAMP 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 11.11 Summary of the hormone-mediated covalent regulation of glycogen metabolism. cAMP = cyclic AMP; PKA = protein kinase A.



C. Allosteric regulation of glycogen synthesis and degradation

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 substrate availability 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.

1. Regulation of glycogen synthesis and degradation in the well- fed state:

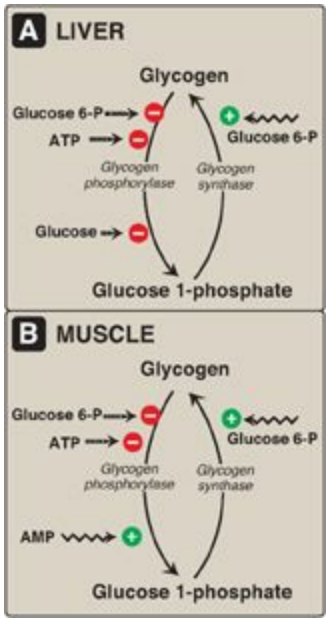
In the well-fed state, glycogen synthase b in both liver and muscle is allosterically activated by glucose 6-phosphate, which is present in elevated concentrations (Figure 11.12). In contrast, glycogen phosphorylase a is allosterically inhibited by glucose 6-phosphate, as well as by ATP, a high-energy signal in the cell. [Note: In liver, but not muscle, nonphosphorylated glucose is also an allosteric inhibitor of glycogen phosphorylase a, making it a better substrate for PP1.]

2. **Activation of glycogen degradation by calcium:** Ca^{2+} is released into the cytoplasm in muscle in response to neural stimulation and in 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, calcium-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 (see Figure 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 Ca^{2+} (see p. 131).]

   a. **Calcium activation of muscle phosphorylase kinase:** During muscle contraction, there is a rapid and urgent need for ATP. This energy is supplied by the degradation of muscle glycogen to glucose, which can then enter 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 Figure 11.9).

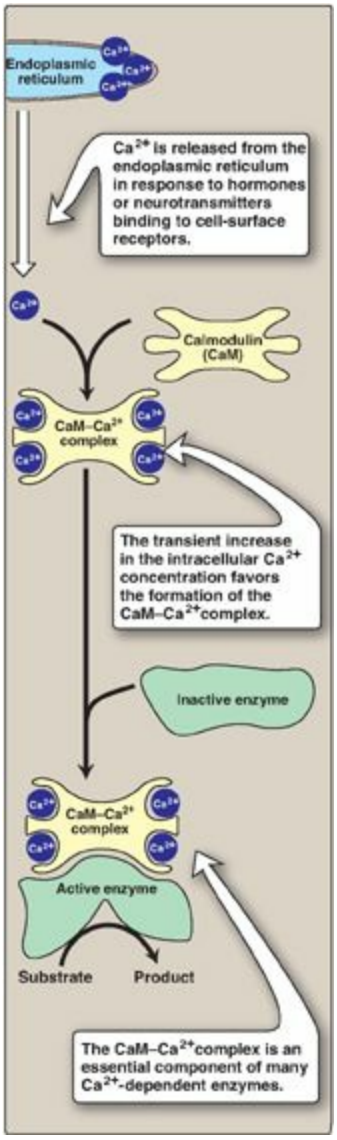
Figure 11.12 Allosteric regulation of glycogen synthesis and degradation. A. Liver. B. Muscle. P = phosphate.



b. Calcium activation of liver phosphorylase kinase: 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-adrenergic GPCRs activates a phospholipid-dependent cascade (see p. 205) 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: The released Ca^{2+} also helps to activate protein kinase C that can phosphorylate (therefore, inactivate) glycogen synthase a.]

3. Activation of glycogen degradation in muscle: Muscle glycogen phosphorylase is active in the presence of the high adenosine monophosphate (AMP) concentrations that occur under extreme conditions of anoxia and ATP depletion. AMP binds to glycogen phosphorylase b, causing its activation without phosphorylation (see Figure 11.9). [Note: Recall that AMP also activates phosphofructokinase-1 of glycolysis (see p. 99), allowing glucose from glycogenolysis to be oxidized.]

Figure 11.13 Calmodulin mediates many effects of intracellular Ca^{2+}.


Endoplasmic reticulum
Ca^{2+} is released from the endoplasmic reticulum in response to hormones or neurotransmitters binding to cell-surface receptors.
Calmodulin (CaM)
CaM–Ca^{2+} complex
The transient increase in the intracellular Ca^{2+} concentration favors the formation of the CaM–Ca^{2+} complex.
Inactive enzyme
CaM–Ca^{2+} complex
Active enzyme
Substrate
Product
The CaM–Ca^{2+} complex is an essential component of many Ca^{2+}-dependent enzymes.

VI. GLYCOGEN STORAGE DISEASES

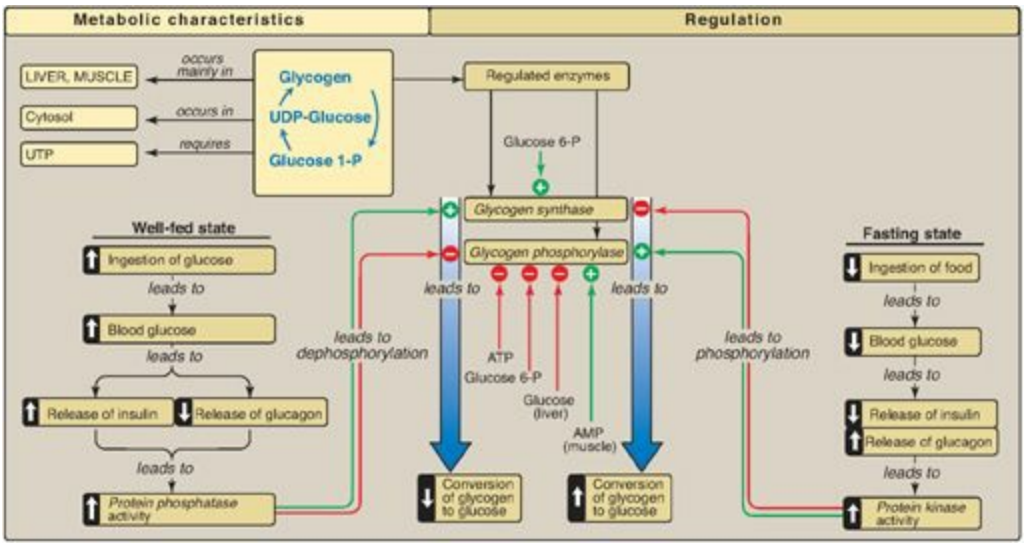
These are a group of genetic diseases that are caused by defects in enzymes required for glycogen degradation or, more rarely, glycogen synthesis. They result either in formation of glycogen that has an abnormal structure or in the accumulation of excessive amounts of normal glycogen in specific tissues as a result of impaired degradation. A particular enzyme may be defective in a single tissue, such as liver (resulting in hypoglycemia) or muscle (causing muscle weakness), or the defect may be more generalized, affecting a variety of tissues. The severity of the GSDs ranges from fatal in early childhood to mild disorders that are not life threatening. Some of the more prevalent GSDs are illustrated in Figure 11.8. [Note: Only one GSD is lysosomal because glycogen metabolism occurs primarily in the cytosol.]

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**. The primary glycosidic bond is an **α(1→4) linkage**. After about eight to ten glucosyl residues, there is a **branch** containing an **α(1→6) linkage**. **Uridine diphosphate (UDP)-glucose**, the **building block** of glycogen, is synthesized from **glucose 1-phosphate** and **UTP** by **UDP-glucose pyrophosphorylase** (Figure 11.14). Glucose from UDP-glucose is transferred to the nonreducing ends of glycogen chains by primer-requiring **glycogen synthase**, which makes **α(1→4) linkages**. The primer is made by glycogenin. **Branches** are formed by **amylo-α(1→4)→α(1→6)-transglucosidase** (common name, **glucosyl 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 attaches it with an **α(1→6) linkage** to another residue in the chain. Pyridoxal phosphate–requiring **glycogen phosphorylase** cleaves the **α(1→4) bonds** between glucosyl residues at the **nonreducing ends** of the glycogen chains, producing **glucose 1-phosphate**. This sequential degradation continues until four glucosyl units remain before a branch point. The resulting structure is called a **limit dextrin** that is degraded by the bifunctional debranching enzyme. **Oligo-α(1→4)→α(1→4)-glucantransferase** (common name, **glucosyl 4:4 transferase**) removes the outer three of the four glucosyl residues at a branch and transfers them to the nonreducing end of another chain, where they can be converted to glucose 1-phosphate by glycogen phosphorylase. The remaining single glucose residue attached in an **α(1→6) linkage** is removed hydrolytically by the **amylo-(1→6) glucosidase** activity of debranching enzyme, releasing **free glucose**. **Glucose 1-phosphate** is converted to **glucose 6-phosphate** by **phosphoglucomutase**. In the **muscle**, glucose 6-phosphate enters glycolysis. In the **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 **glycogen storage disease Type 1a** (**Von Gierke disease**). This disease 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 **increased glycogenolysis** and **decreased glycogenesis)**. Key enzymes are phosphorylated by a family of **protein kinases**, some of which are **cyclic adenosine monophosphate dependent** (a compound increased by glucagon and epinephrine). Phosphate groups are removed by protein phosphatase-1 (active when

its inhibitor is inactive in response to elevated insulin levels). **Glycogen synthase, phosphorylase kinase,** and **phosphorylase** are also **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, glucose also serves an an allosteric inhibitor of glycogen phosphorylase. The **Ca^{2+}** released from the endoplasmic reticulum in muscle during exercise and in liver in response to epinephrine **activates phosphorylase kinase** by binding to the enzyme's **calmodulin** subunit. This allows the enzyme to activate **glycogen phosphorylase**, thereby causing glycogen degradation. **AMP** activates glycogen phosphorylase in muscle.

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 = uridine diphosphate; UTP = uridine triphosphate; P = phosphate.



Study Questions

Choose the ONE best answer.

For Questions 11.1–11.4, match the deficient enzyme to the clinical finding in selected glycogen storage diseases (GSDs).

CHOICE	GSD	DEFICIENT ENZYME
A	Type Ia	Glucose 6-phosphatase
B	Type II	Acid maltase
C	Type III	4:4 Transferase
D	Type IV	4:6 Transferase
E	Type V	Myophosphorylase
F	Type VI	Liver phosphorylase

11.1 Exercise intolerance, with no rise in blood lactate during exercise

Correct answer = E. Myophosphorylase deficiency prevents glycogen degradation in muscle, depriving muscle of glycogen-derived glucose, resulting in decreased glycolysis and its anaerobic product, lactate.

Correct Answer = D. 4:6 Transferase (branching enzyme) deficiency, a defect in glycogen synthesis, results in formation of glycogen with fewer branches and decreased solubility.

Correct answer = B. Acid maltase [a(1→4)-glucosidase] deficiency prevents degradation of any glycogen brought into lysosomes. A variety of tissues are affected, with the most severe pathology resulting from heart damage.

Correct answer = A. Glucose 6-phosphatase deficiency prevents the liver from releasing free glucose into the blood, causing severe fasting hypoglycemia, lacticacidemia, hyperuricemia, and hyperlipidemia.

11.2 Fatal, progressive cirrhosis and glycogen with longer-than-normal outer chains

11.3 Generalized accumulation of glycogen, severe hypotonia, and death from heart failure

11.4 Severe fasting hypoglycemia, lacticacidemia, hyperuricemia, and hyperlipidemia

11.5 Epinephrine and glucagon have which one of the following effects on hepatic glycogen metabolism?

A. Both glycogen phosphorylase and glycogen synthase are activated by phosphorylation but at significantly different rates.
B. Glycogen phosphorylase is inactivated by the resuting rise in calcium, whereas

glycogen synthase is activated.

C. Glycogen phosphorylase is phosphorylated and active, whereas glycogen synthase is phosphorylated and inactive.

D. The net synthesis of glycogen is increased.

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. Ca^{2+} released from the sarcoplasmic reticulum during exercise binds to the calmodulin subunit of phosphorylase kinase, thereby allosterically activating the "b" form of this enzyme. The other choices are not caused by an elevation of cytosolic calcium.

11.7 Explain why the hypoglycemia seen with Type Ia glycogen storage disease (glucose 6-phosphatase deficiency) is severe, whereas that seen with Type VI (liver phosphorylase deficiency) is mild.

With Type Ia, the liver is unable to generate free glucose either from glycogenolysis or gluconeogenesis because both processes produce glucose 6-phosphate. With Type VI, the liver is still able to produce free glucose from gluconeogenesis, but glycogenolysis is inhibited.

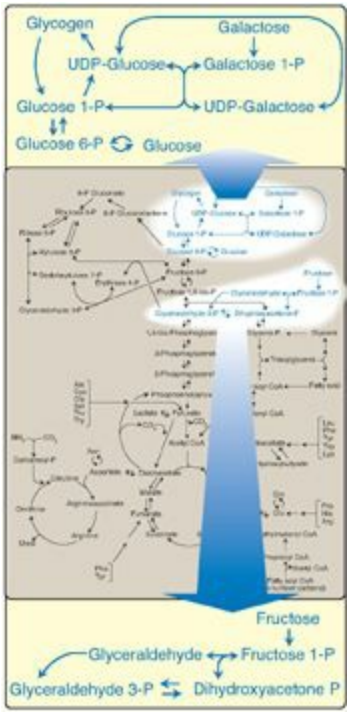
Metabolism of Monosaccharides and Disaccharides

12

I. OVERVIEW

Glucose is the most common monosaccharide consumed by humans, and its metabolism has already been discussed. However, two other monosaccharides, fructose and galactose, 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 structural carbohydrates. Figure 12.1 shows the metabolism of fructose and galactose as part of the essential pathways of energy metabolism.

Figure 12.1 Galactose and fructose metabolism as part of the essential pathways of energy metabolism (see Figure 8.2, p. 92, for a more detailed view of the overall reactions of metabolism). UDP = uridine diphosphate; P = phosphate.



II. FRUCTOSE METABOLISM

About 10% of the calories comprising the Western diet are supplied by fructose (approximately 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/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; see p. 97), and, in contrast to glucose, fructose does not promote the secretion of insulin.

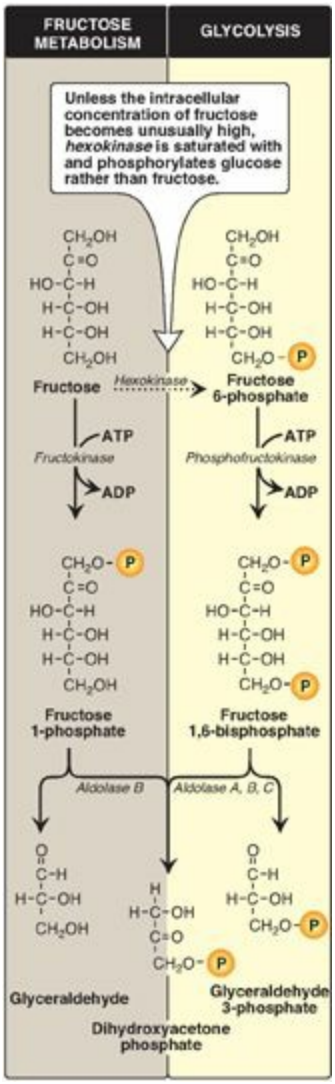
A. Phosphorylation of fructose

For fructose to enter the pathways of intermediary metabolism, it must first be phosphorylated (Figure 12.2). This can be accomplished by either hexokinase or fructokinase. Hexokinase phosphorylates glucose in most cells of the body (see p. 98), and several additional hexoses can serve as substrates for this enzyme. However, it has a low affinity (that is, a high Michaelis constant [K_m]; see p. 59) 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 Figure 12.2). The enzyme has a low K_m for fructose and a high V_{max} (or, maximal velocity; see p. 59). It is found in the liver (which processes most of the dietary fructose), kidney, and the small intestinal mucosa and converts fructose to fructose 1-phosphate, using adenosine triphosphate (ATP) as the phosphate donor. [Note: These three tissues also contain aldolase B, discussed in section B.]

B. Cleavage of fructose 1-phosphate

Fructose 1-phosphate is not phosphorylated to fructose 1,6-bisphos-phate as is fructose 6-phosphate (see p. 99) but is cleaved by aldolase B (also called fructose 1-phosphate aldolase) to dihydroxyacetone phosphate (DHAP) and glyceraldehyde. [Note: Humans express three aldolases, A, B and C, the products of three different genes. Aldolase A (found in most tissues), aldolase B (in liver, kidney, and small intestine), and aldolase C (in brain) all cleave fructose 1,6-bisphosphate produced during glycolysis to DHAP and glyceraldehyde 3-phosphate (see p. 100), but only aldolase B cleaves fructose 1-phosphate.] DHAP can directly enter glycolysis or gluconeogenesis, whereas glyceraldehyde can be metabolized by a number of pathways, as illustrated in Figure 12.3.

Figure 12.2 Phosphorylation products of fructose and their cleavage. P = phosphate; ADP = adenosine diphosphate.



C. Kinetics of fructose metabolism

The rate of fructose metabolism is more rapid than that of glucose because the trioses formed from fructose 1-phosphate bypass phosphofructokinase-1, the major rate-limiting step in glycolysis (see p. 99).

D. Disorders of fructose metabolism

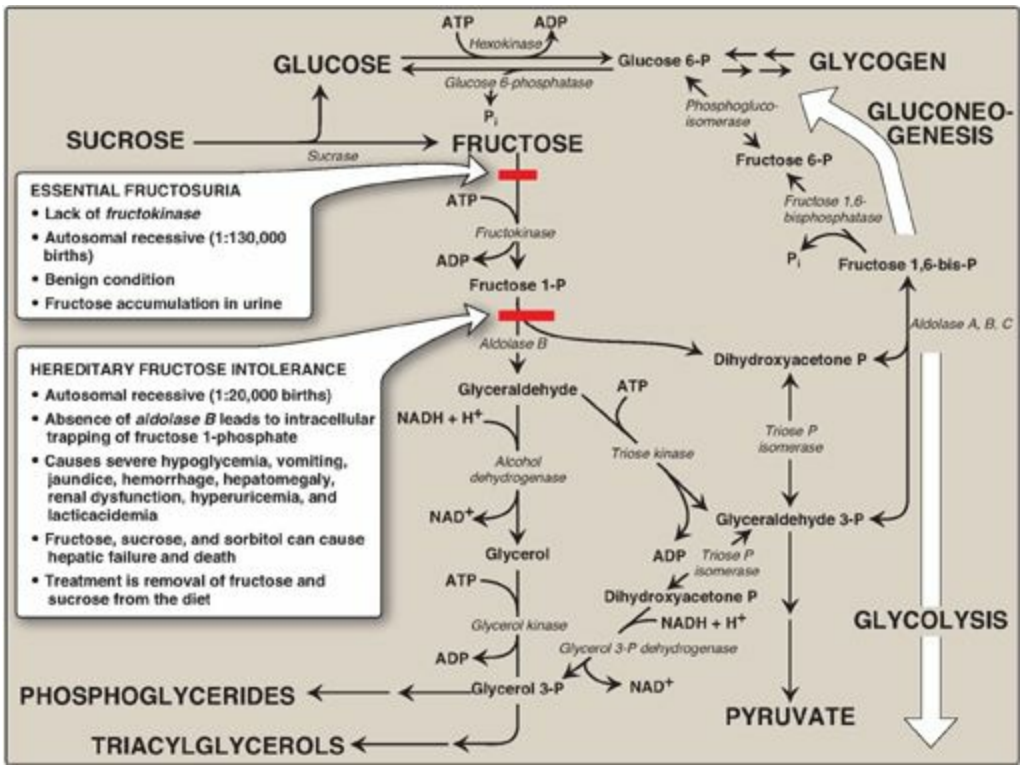
A deficiency of one of the key enzymes required for the entry of fructose into metabolic pathways can result in either a benign condition as a result of fructokinase deficiency (essential fructosuria) or a severe disturbance of liver and kidney metabolism as a result of aldolase B deficiency (hereditary fructose intolerance [HFI]), which is estimated to occur in 1:20,000 live births (see Figure 12.3). The first symptoms of HFI appear when a baby is weaned from milk (see p. 142) and begins to be fed 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 acidosis; see p. 299). The decreased availability of hepatic ATP affects gluconeogenesis (causing hypoglycemia with vomiting) and protein synthesis (causing a decrease in blood clotting factors and other essential proteins). Kidney function may

also be affected. [Note: The drop in P_i also inhibits glycogenolysis (see p. 128).] 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 33). Aldolase B deficiency is part of the newborn screening panel. With HFI, sucrose, as well as fructose, must be removed from the diet to prevent liver failure and possible death. Individuals with HFI display an aversion to sweets and, consequently, have an absence of dental caries.

E. Conversion of mannose to fructose 6-phosphate

Mannose, the C-2 epimer of glucose (see p. 84), is an important component of glycoproteins (see p. 166). Hexokinase phosphorylates mannose, producing mannose 6-phosphate, which, in turn, is reversibly isomerized to fructose 6-phosphate by phosphomannose isomerase. [Note: There is little mannose in dietary carbohydrates. Most intracellular mannose is synthesized from fructose or is preexisting mannose produced by the degradation of structural carbohydrates and salvaged by hexokinase.]

Figure 12.3 Summary of fructose metabolism. P = phosphate; P_i = inorganic phosphate; NAD(H) = nicotinamide adenine dinucleotide; ADP = adenosine diphosphate.



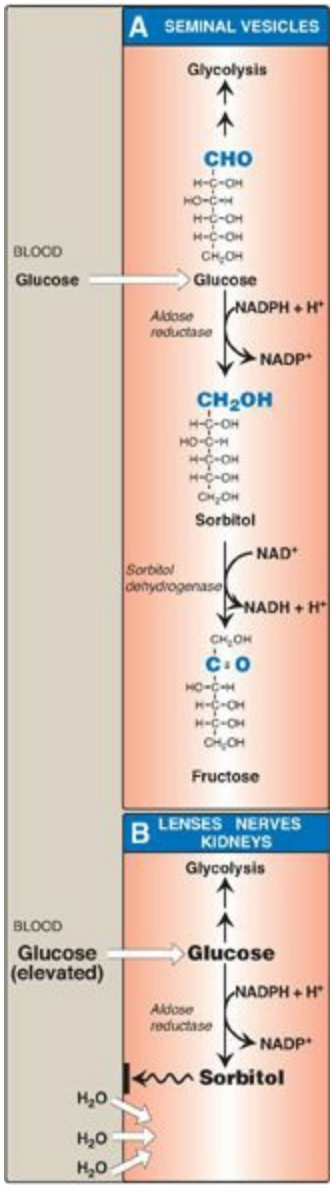
F. Conversion of glucose to fructose via sorbitol

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. **Synthesis of sorbitol:** Aldose reductase reduces glucose, producing sorbitol (glucitol; Figure 12.4). This enzyme is found in many tissues, including the lens, retina, Schwann cells of peripheral nerves, liver, kidney, placenta, red blood cells, and cells of the ovaries and seminal vesicles. In cells of the liver, ovaries, and seminal vesicles, there is a second enzyme, sorbitol dehydrogenase, which can oxidize the sorbitol to produce fructose (see Figure 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 or gluconeogenesis.

2. **Effect of hyperglycemia on sorbitol metabolism:** Because insulin is not required for the entry of glucose into the cells listed in the previous paragraph, large amounts of glucose may enter these cells during times of hyperglycemia (for example, in uncontrolled diabetes). Elevated intracellular glucose concentrations and an adequate supply of reduced nicotinamide adenine dinucleotide phosphate (NADPH) cause aldose reductase to produce a significant increase in the amount of sorbitol, which cannot pass efficiently through cell membranes and, in turn, remains trapped inside the cell (see Figure 12.4). This is exacerbated when sorbitol dehydrogenase is low or absent (for example, in retina, lens, kidney, and nerve cells). As a result, sorbitol accumulates in these cells, causing strong osmotic effects and, therefore, cell swelling as a result of water retention. Some of the pathologic alterations associated with diabetes can be attributed, in part, to this phenomenon, including cataract formation, peripheral neuropathy, and microvascular problems leading to nephropathy and retinopathy. (See p. 344 for a discussion of the complications of diabetes.) [Note: Use of NADPH in the aldose reductase reaction decreases the generation of reduced glutathione, an important antioxidant (see p. 148), and may be related to diabetic complications.]

Figure 12.4 Sorbitol metabolism. NAD(H) = nicotinamide adenine dinucleotide; NADP(H) = nicotinamide adenine dinucleotide phosphate.


A SEMINAL VESICLES
Glycolysis
CHO
BLOCD
Glucose
Glucose
Aldose reductase
NADPH + H+
NADP+
CH2OH
Sorbitol
Sorbitol dehydrogenase
NAD+
NADH + H+
C = O
Fructose
B LENSES NERVES KIDNEYS
Glycolysis
BLOCD
Glucose (elevated)
Glucose
Aldose reductase
NADPH + H+
NADP+
Sorbitol
H2O
H2O
H2O

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 (lactase) of the intestinal mucosal cell membrane was discussed on p. 87.] Some galactose can also be obtained by lysosomal degradation of complex carbohydrates, such as glycoproteins and glycolipids, which are important membrane components. Like fructose, the transport of galactose into cells is not insulin dependent.

A. Phosphorylation of galactose

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 (Figure 12.5). As with other kinases, ATP is the phosphate donor.

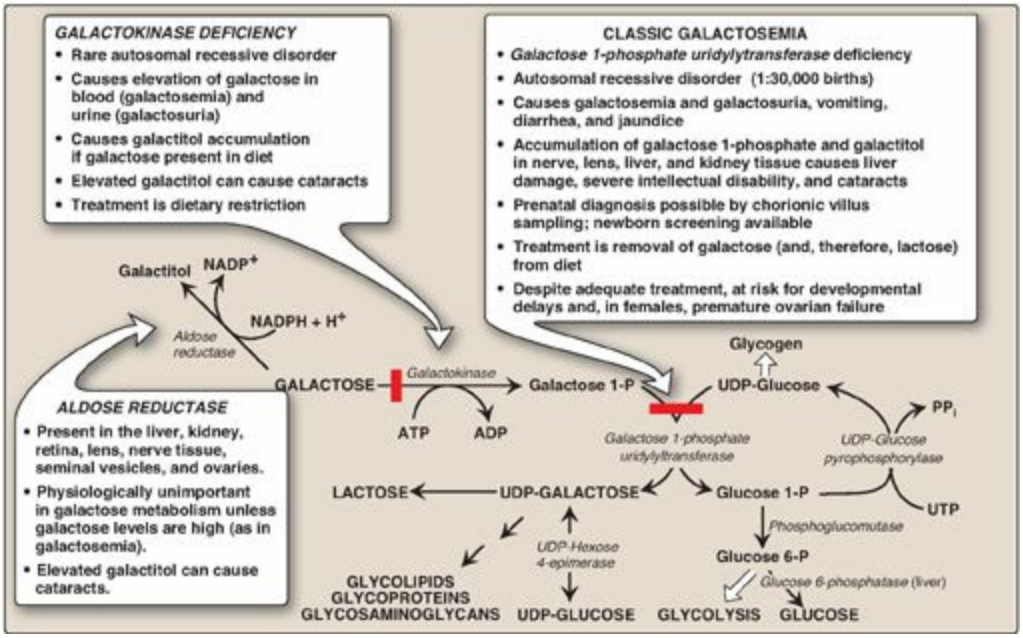
B. Formation of uridine diphosphate-galactose

Galactose 1-phosphate cannot enter the glycolytic pathway unless it is first converted to uridine diphosphate (UDP)-galactose (Figure 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 Figure 12.5). The enzyme that catalyzes this reaction is galactose 1-phosphate uridylyltransferase (GALT).

C. Use of uridine diphosphate-galactose as a carbon source for glycolysis or gluconeogenesis

For UDP-galactose to enter the mainstream of glucose metabolism, it must first be converted to its C-4 epimer, UDP-glucose, by UDP-hexose 4-epimerase. This "new" UDP-glucose (produced from the original UDP-galactose) can then participate in many biosynthetic reactions as well as being used in the GALT reaction described above. (See Figure 12.5 for a summary of this interconversion.)

Figure 12.5 Metabolism of galactose. UDP = uridine diphosphate; UTP = uridine triphosphate; P = phosphate; PP_i = pyrophosphate; NADP(H) = nicotinamide adenine dinucleotide phosphate; ADP = adenosine diphosphate.



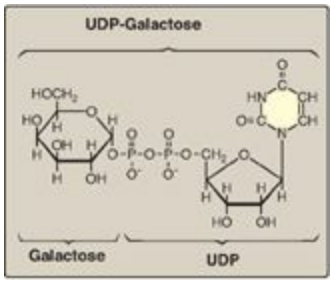
D. Role of uridine diphosphate-galactose in biosynthetic reactions

UDP-galactose can serve as the donor of galactose units in a number of synthetic pathways, including synthesis of lactose (see below), glycoproteins (see p. 166), glycolipids (see p. 210), and glycosaminoglycans (see p. 158). [Note: If galactose is not provided by the diet (for example, when it cannot be released from lactose as a result of 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 (see Figure 12.5).]

E. Disorders of galactose metabolism

GALT is deficient in individuals with classic galactosemia (see Figure 12.5). In this disorder, galactose 1-phosphate and, therefore, galactose accumulate. Physiologic consequences are similar to those found in hereditary fructose intolerance (see p. 138), 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 converts glucose to sorbitol (see p. 139). Treatment requires removal of galactose and lactose from the diet. GALT deficiency is part of the newborn screening panel. [Note: A deficiency in galactokinase results in a less severe disorder of galactosemia metabolism, although cataracts are common (see Figure 12.5).]

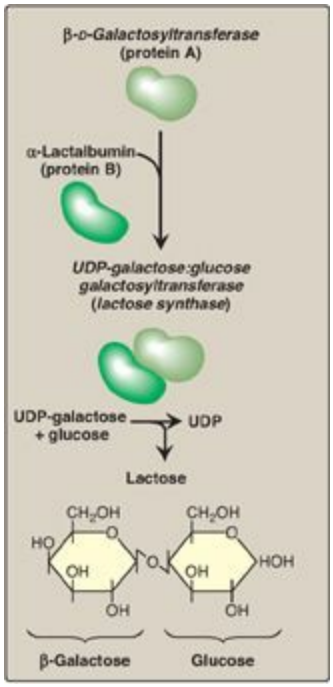
Figure 12.6 Structure of UDP-galactose. UDP = uridine diphosphate.


UDP-Galactose
Galactose
UDP

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. Lactose, known as "milk sugar," is made by lactating (milk-producing) mammary glands. Therefore, milk and other dairy products are the dietary sources of lactose. Lactose is synthesized in the Golgi b y lactose synthase (UDP-galactose:glucose galactosyltransferase), which transfers galactose from UDP-galactose to glucose, releasing UDP (Figure 12.7). This enzyme is composed of two proteins, A and B. 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. 167). 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 so that lactose, rather than N-acetyllactosamine, is produced (see Figure 12.7).

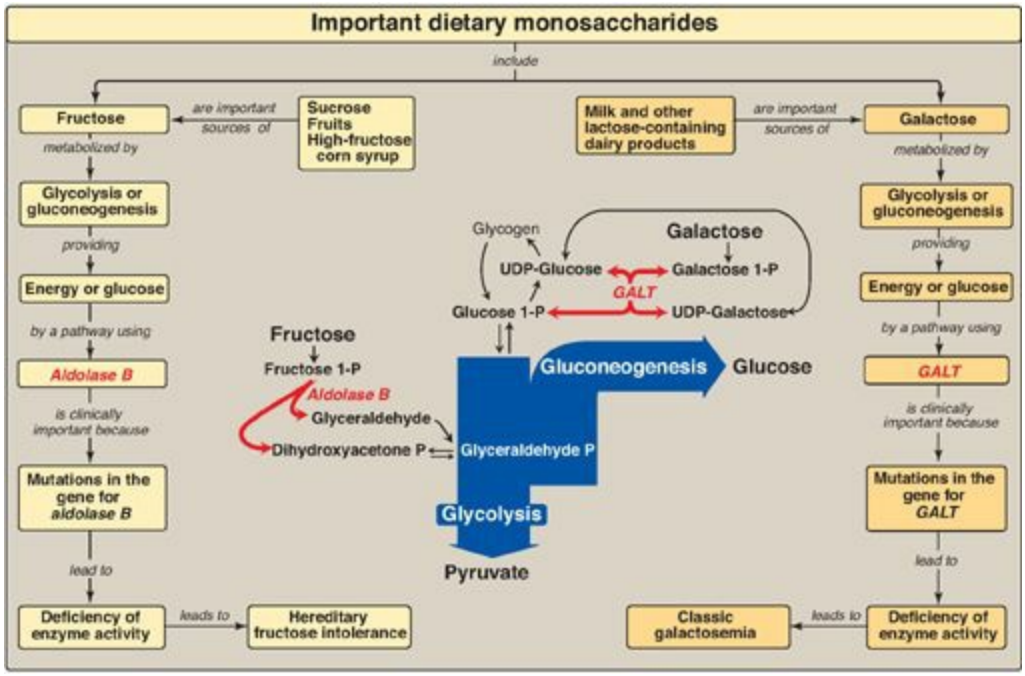
Figure 12.7 Lactose synthesis. UDP = uridine diphosphate.



V. CHAPTER SUMMARY

The major source of fructose is **sucrose**, which, when cleaved, releases equimolar amounts of fructose and glucose (Figure 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 **dihydroxyacetone phosphate** and **glyceraldehyde**. These enzymes are found in the **liver**, **kidney**, and **small intestinal mucosa**. A **deficiency** of **fructokinase** causes a benign condition (**essential fructosuria**), but a **deficiency** of **aldolase B** causes **hereditary fructose intolerance (HFI)**, in which **severe hypoglycemia** and **liver failure** lead to **death** if fructose (and sucrose) in the diet is not eliminated. **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**, **Schwann cells**, **liver**, **kidney**, **ovaries**, and **seminal vesicles**. In cells of 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 not insulin dependent. Galactose is first phosphorylated by **galactokinase** (a deficiency results in cataracts) to **galactose 1-phosphate**. This compound is converted to **uridine diphosphate (UDP)-galactose** by **galactose 1-phosphate uridyltransferase (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**, **severe intellectual disability**, 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 converted 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 the synthesis of structural carbohydrates. **Lactose** is a disaccharide that consists of **galactose** and **glucose**. Milk and other dairy 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 the 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 nursing female with classic galactosemia is on a galactose-free diet. She is able to produce lactose in 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 hormone produced in the mammary gland.

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 deficiency.

12.2 A 5-month-old boy is brought to his physician because of vomiting, night sweats, and tremors. History revealed 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 the baby's urine were positive for reducing sugar but negative for glucose. The infant most likely suffers from 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 hypolactasia) presents at a later age.

12.3 Lactose synthesis is essential in the production of milk by mammary glands. In lactose synthesis:

A. galactose from galactose 1-phosphate is transferred to glucose by galactosyltransferase (protein A), generating lactose.
B. protein A is used exclusively in the synthesis of lactose.
C. α-lactalbumin (protein B) regulates the specificity of protein A by increasing its K_m for glucose.
D. protein B expression is stimulated by prolactin.

Correct answer = D. The expression of α-lactalbumin (protein B) 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 increases the affinity of protein A for glucose and, so, decreases the K_m.

12.4 A 3-month-old girl is developing cataracts. Other than not having a social smile or being able to track objects visually, all other aspects of the girl's examination are normal. Tests on the baby's urine are positive for reducing sugar but negative for glucose. Which enzyme is most likely deficient in this girl?

A. Aldolase B
B. Fructokinase
C. Galactokinase
D. Galactose 1-phosphate uridylyltransferase

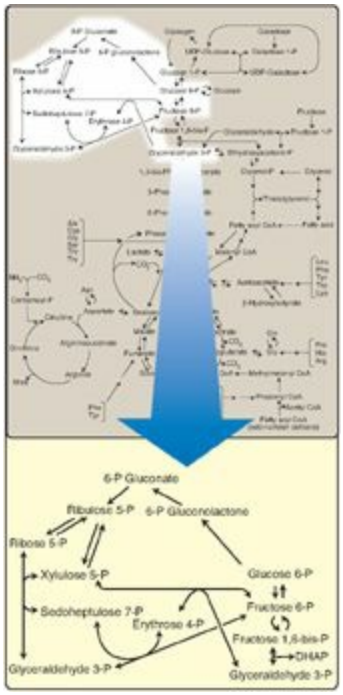
Correct answer = C. The girl is deficient in galactokinase and is unable to appropriately phosphorylate galactose. Galactose accumulates in the blood (and urine). In the lens of the eye, galactose is reduced by aldose reductase to galactitol, a sugar alcohol, which causes osmotic effects that result 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 affects on several tissues. Cataracts are not typically seen.

Pentose Phosphate Pathway and Nicotinamide Adenine Dinucleotide Phosphate 13

I. OVERVIEW

The pentose phosphate pathway (also called the hexose monophosphate shunt) occurs in the cytosol of the cell. It includes two irreversible oxidative reactions, followed by a series of reversible sugar–phosphate interconversions (Figure 13.1). No adenosine triphosphate (ATP) is directly consumed or produced in the cycle. Carbon 1 of glucose 6-phosphate is released as CO_2, and two reduced nicotinamide adenine dinucleotide phosphates (NADPHs) are produced for each glucose 6-phosphate molecule entering the oxidative part of the pathway. The rate and direction of the reversible reactions of the pentose phosphate pathway are determined by the supply of and demand for intermediates of the cycle. The pathway provides a major portion of the body's NADPH, which functions as a biochemical reductant. It also produces ribose 5-phosphate, required for the biosynthesis of nucleotides (see p. 293), and provides a mechanism for the metabolic use of five-carbon sugars obtained from the diet or the degradation of structural carbohydrates.

Figure 13.1 Pentose phosphate pathway shown as a component of the metabolic map (see Figure 8.2, p. 92 for a more detailed view of the metabolic pathways). P = phosphate; DHAP = dihydroxyacetone phosphate.



II. IRREVERSIBLE OXIDATIVE REACTIONS

The oxidative portion of the pentose phosphate pathway consists of three 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 (Figure 13.2). This portion of the pathway is particularly important in the liver, lactating mammary glands, and adipose tissue, which are active in the NADPH-dependent biosynthesis of fatty acids (see p. 186); in the testes, ovaries, placenta, and adrenal cortex, which are active in the NADPH-dependent biosynthesis of steroid hormones (see p. 237); and in red blood cells (RBCs), which require NADPH to keep glutathione reduced (see p. 152).

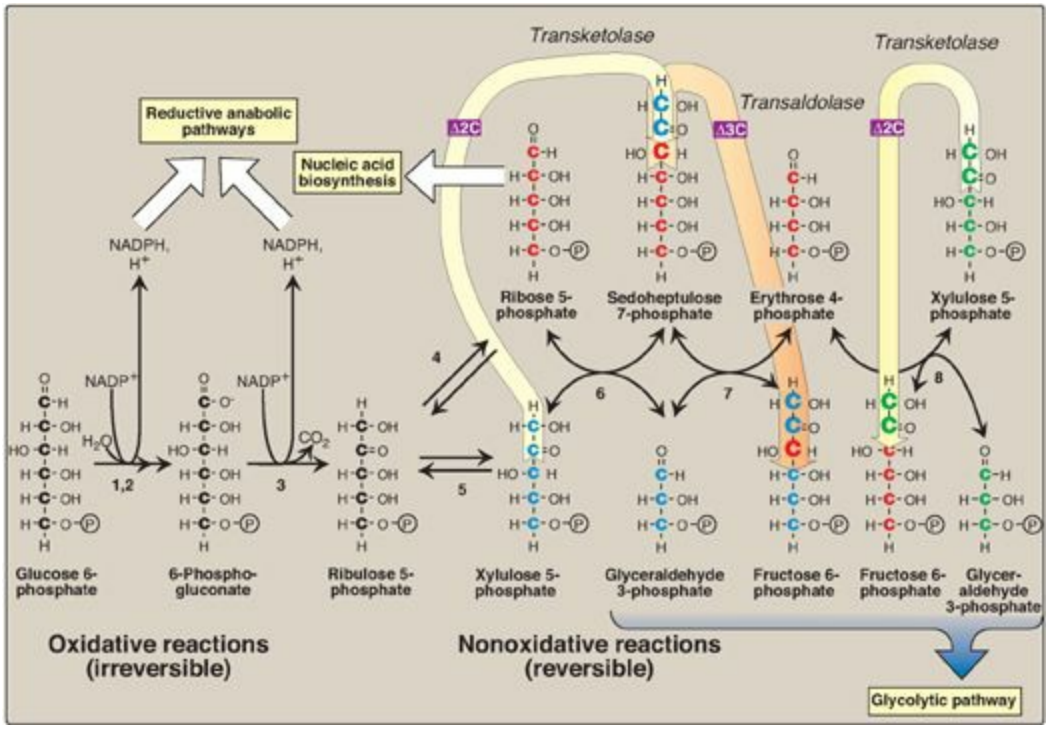
A. Dehydrogenation of glucose 6-phosphate

Glucose 6-phosphate dehydrogenase (G6PD) catalyzes an irreversible oxidation of glucose 6-phosphate to 6-phosphogluconolactone in a reaction that is specific for oxidized NADP ($NADP^+$) as the coenzyme. The pentose phosphate pathway is regulated primarily at the G6PD reaction. NADPH is a potent competitive inhibitor of the enzyme, and, under most metabolic conditions, the ratio of NADPH/$NADP^+$ is sufficiently high to substantially inhibit enzyme activity. However, with increased demand for NADPH, the ratio of NADPH/$NADP^+$ decreases, and flux through the cycle increases in response to the enhanced activity of G6PD. Insulin upregulates expression of the gene for G6PD, and flux through the pathway increases in the absorptive state (see p. 323).

B. Formation of ribulose 5-phosphate

6-Phosphogluconolactone is hydrolyzed by 6-phosphogluconolactone hydrolase. The reaction is irreversible and not rate limiting. The oxidative decarboxylation of the product, 6-phosphogluconate, is catalyzed by 6-phosphogluconate dehydrogenase. This irreversible reaction produces a pentose sugar–phosphate (ribulose 5-phosphate), CO_2 (from carbon 1 of glucose), and a second molecule of NADPH (see Figure 13.2).

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 and 8) transketolase (coenzyme: thiamine pyrophosphate), and 7) transaldolase. $\Delta 2C$ = two carbons are transferred in transketolase reactions; $\Delta 3C$ = three carbons are transferred in the transaldolase reaction. This can be represented as: 5C sugar + 5C sugar $\xrightarrow{\Delta 2C}$ 7C sugar + 3C sugar $\xrightarrow{\Delta 3C}$ 4C sugar + 6C sugar. NADP(H) = nicotinamide adenine dinucleotide phosphate; P = phosphate.

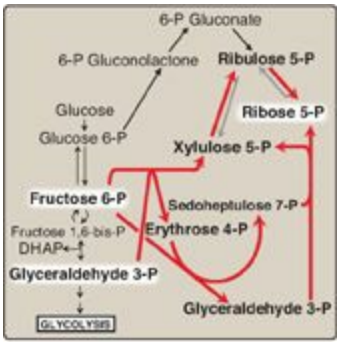

Transketolase
Transketolase
Transaldolase
Reductive anabolic pathways
Nucleic acid biosynthesis
NADPH, H+
NADPH, H+
NADP+
NADP+
H2O
CO2
1,2
3
4
5
6
7
8
Δ2C
Δ3C
Δ2C
Ribose 5-phosphate
Sedoheptulose 7-phosphate
Erythrose 4-phosphate
Xylulose 5-phosphate
Glucose 6-phosphate
6-Phospho-gluconate
Ribulose 5-phosphate
Xylulose 5-phosphate
Glyceraldehyde 3-phosphate
Fructose 6-phosphate
Fructose 6-phosphate
Glycer-aldehyde 3-phosphate
Oxidative reactions (irreversible)
Nonoxidative reactions (reversible)
Glycolytic pathway

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 Figure 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 p. 293) or to intermediates of glycolysis (that is, fructose 6-phosphate and glyceraldehyde 3-phosphate). For example, 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 reactions to glyceraldehyde 3-phosphate and fructose 6-phosphate, which are glycolytic intermediates. 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 (Figure 13.3).

> In addition to transketolases, TPP is required by the enzyme complexes pyruvate dehydrogenase (see p. 110), α-ketoglutarate dehydrogenase of the citric acid cycle (see p. 112), and branched-chain α-keto acid dehydrogenase of branched-chain amino acid catabolism (see p. 266).

Figure 13.3 Formation of ribose 5-phosphate from intermediates of glycolysis. P = phosphate; DHAP = dihydroxyacetone phosphate.



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 (Figure 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 ratio of $NADP^+$/NADPH is approximately 0.1, which favors the use of NADPH in reductive biosynthetic reactions. This contrasts with the high ratio of NAD^+/NADH (approximately 1000), which favors an oxidative role for NAD^+. This section summarizes some important $NADP^+$ and NADPH-specific functions in reductive biosynthesis and detoxification reactions.

A. Reductive biosynthesis

NADPH can be thought of as a high-energy molecule, much in the same way as NADH. However, the electrons of NADPH are destined for use in reductive biosynthesis, rather than for transfer to oxygen as is the case with NADH (see p. 74). Thus, 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 p. 77), that, therefore, can be used in reactions requiring an electron donor, such as fatty acid (see p. 186) and steroid (see p. 237) synthesis.

Figure 13.4 Structure of reduced nicotinamide adenine dinucleotide phosphate (NADPH).

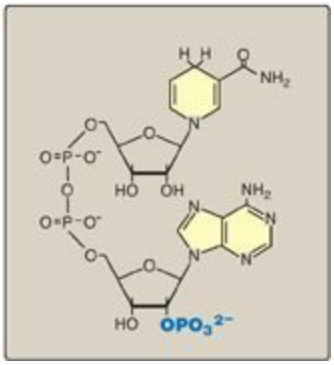
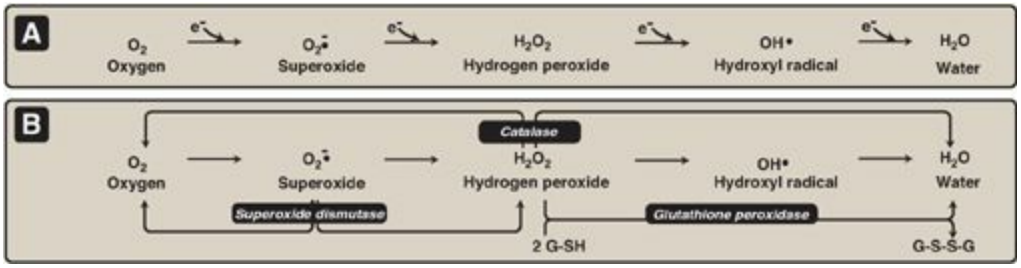


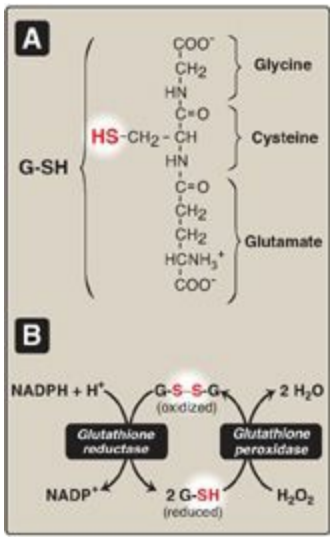
Figure 13.5 A. Formation of reactive intermediates from molecular oxygen. e^- = electrons. B. Actions of antioxidant enzymes. G-SH = reduced glutathione; G-S-S-G = oxidized glutathione. (See Figure 13.6B for the regeneration of G-SH.)



B. Reduction of hydrogen peroxide

Hydrogen peroxide (H_2O_2) is one of a family of reactive oxygen species (ROS) that are formed from the partial reduction of molecular oxygen (Figure 13.5A). These compounds are formed 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. The 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.

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. Glutathione-mediated reduction of hydrogen peroxide (H_2O_2) by reduced nicotinamide adenine dinucleotide phosphate (NADPH). G-S-S-G = oxidized glutathione.



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 (Figure 13.5B). This reaction, catalyzed by the selenium-containing 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 (Figure 13.6). [Note: RBCs are totally dependent on the pentose phosphate pathway for their supply of NADPH because, unlike other cell types, RBCs do not have an alternate source for this essential coenzyme.] Additional enzymes, such as superoxide dismutase and catalase, catalyze the conversion of other reactive oxygen intermediates to harmless products (see Figure 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 (see p. 377), vitamin E (see p. 391), and β-carotene (see p. 382), are able to reduce and, thereby, detoxify reactive oxygen intermediates 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.

C. Cytochrome P450 monooxygenase system

Monooxygenases (mixed-function oxidases) incorporate one atom from molecular oxygen into a substrate (creating a hydroxyl group), with the other atom being reduced to water. In the cytochrome P450 monooxygenase system, NADPH provides the reducing equivalents required by this series of reactions (Figure 13.7). This system performs different functions in two separate locations in cells. The overall reaction catalyzed by a cytochrome P450 enzyme is:

$$R\text{-}H + O_2 + NADPH + H^+ \rightarrow R\text{-}OH + H_2O + NADP^+$$

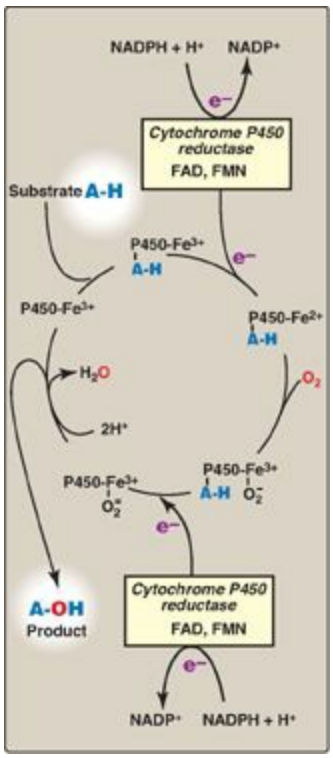
where R may be a steroid, drug, or other chemical. [Note: Cyto-chrome P450 (CYP) enzymes are actually a superfamily of related, heme-containing monooxygenases that participate in a broad variety of reactions. The P450 in the name reflects the absorbance at 450 nm by the protein.]

1. **Mitochondrial system:** An important function of the cytochrome P450 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 p. 237). The liver uses this same system in bile acid synthesis (see p. 224) and the hydroxylation of cholecalciferol to 25-hydroxycholecalciferol (vitamin D_3; see p. 386), and the kidney uses it to hydroxylate vitamin D_3 to its biologically active 1,25-dihydroxylated form.

2. **Microsomal system:** An extremely important function of the microsomal cytochrome P450 monooxygenase system found associated with the membrane of the smooth endoplasmic reticulum (particularly in the liver) is the detoxification of foreign compounds (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. The purpose of these modifications is two-fold. 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 p. 161), which will significantly increase the compound's solubility. [Note: Polymorphisms (see p.473) in the genes for CYP enzymes can lead to differences in drug metabolism.]

Figure 13.7 Cytochrome P450 monooxygenase cycle (simplified). Electrons (e^-) move from NADPH to FAD to FMN of the reductase and then to the heme iron (Fe) of the P450 enzyme. [Note: In the mitochondrial system, electrons move from FAD to an ironsulfur protein and then to the P450 enzyme.] FAD = flavin adenine dinucleotide; FMN = flavin mononucleotide; NADPH = reduced nicotinamide adenine dinucleotide phosphate.



D. Phagocytosis by white blood cells

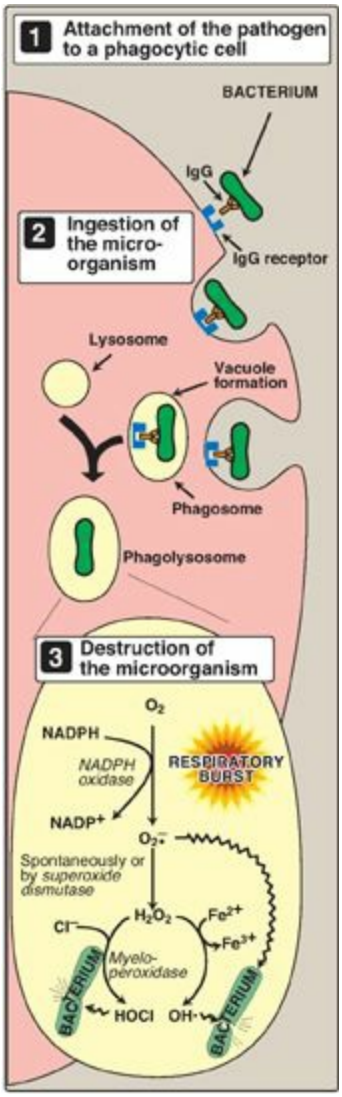
Phagocytosis is the ingestion by receptor-mediated endocytosis of microorganisms, foreign particles, and cellular debris by cells such as neutrophils and macrophages (monocytes). It is an important defense mechanism, particularly in bacterial infections. Neutrophils and monocytes are armed with both oxygen-independent and oxygen-dependent mechanisms for killing bacteria.

1. **Oxygen-independent mechanism:** Oxygen-independent mechanisms use pH changes in phagolysosomes and lysosomal enzymes to destroy pathogens.

2. **Oxygen-dependent system:** Oxygen-dependent mechanisms include the enzymes

NADPH oxidase and myeloperoxidase (MPO) that work together in killing bacteria (Figure 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 ($O_2^{\bullet-}$), a free radical, as NADPH is oxidized. The rapid consumption of O_2 that accompanies formation of $O_2^{\bullet-}$ 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 $O_2^{\bullet-}$. 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, $O_2^{\bullet-}$ is converted to H_2O_2 (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•), a ROS, or be fully reduced to water by catalase or glutathione peroxidase. [Note: Deficiencies in MPO do not confer increased susceptibility to infection because peroxide from NADPH oxidase is bactericidal.]

Figure 13.8 Phagocytosis and the oxygendependent pathway of microbial killing. IgG = the antibody immunoglobulin G; NADPH = reduced nicotinamide adenine dinucleotide phosphate; $O_2^{\bullet-}$ = superoxide; HOCl = hypochlorous acid; OH• = hydroxyl radical.



E. Synthesis of nitric oxide

Nitric oxide (NO) is recognized as a mediator in a broad array of biologic systems. NO is the endothelium-derived relaxing factor, which causes vasodilation by relaxing vascular smooth muscle. NO also acts as a neurotransmitter, prevents platelet aggregation, and plays an essential role in macrophage function. NO has a very short half-life in tissues (3–10 seconds) because it reacts with oxygen and superoxide and then is converted into nitrates and nitrites including peroxynitrite ($O{=}NOO^-$), a reactive nitrogen species (RNS). [Note: 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.]

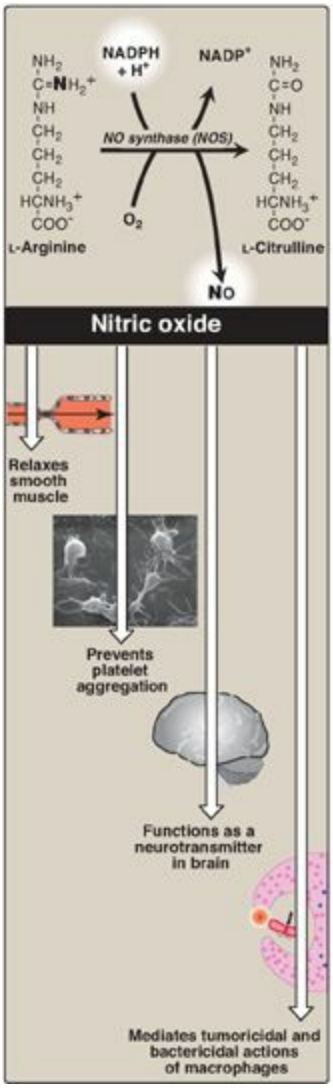
1. **Nitric oxide synthase:** Arginine, O_2, and NADPH are substrates for cytosolic NO synthase ([NOS] Figure 13.9). Flavin mononucleotide (FMN), FAD, heme, and tetrahydrobiopterin (see p. 268) are coenzymes, and NO and citrulline are products of the reaction. Three NOS, each the product of a different gene, have been identified. Two are constitutive (synthesized at a constant rate), Ca^{2+}–calmodulin-dependent enzymes (see p. 133). 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. **Actions of nitric oxide on 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 guanylate cyclase (also known as guanylyl cyclase) to form cyclic guanosine monophosphate (cGMP). [Note: This reaction is analogous to the formation of cyclic AMP by adenylate cyclase (see p. 94), except that this guanylate cyclase is not membrane associated.] 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 calcium–calmodulin 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: NO is involved in penile erection. Sildenafil citrate, used in the treatment of erectile dysfunction, inhibits the phosphodiesterase that inactivates cGMP.]

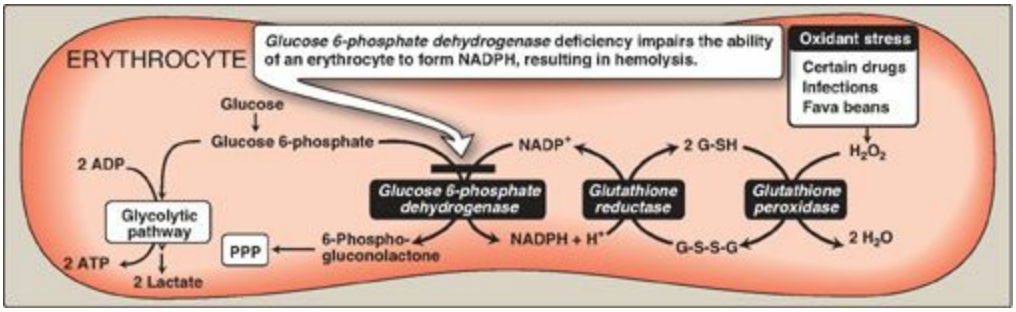
3. **Role of nitric oxide in macrophage bactericidal activity:** In macrophages, iNOS activity is normally low, but synthesis of the enzyme is significantly stimulated by bacterial LPS and by release of IFN-γ and TNF-α in response to the infection. Activated macrophages form $O_2^{-\bullet}$ radicals (see p. 150) that combine with NO to form intermediates that decompose, producing the highly bactericidal OH• radical.

Figure 13.9 Synthesis and some of the actions of nitric oxide (NO). NADPH = reduced nicotinamide adenine dinucleotide phosphate. [Note: Flavin mononucleotide, flavin adenine dinucleotide, heme, and tetrahydrobiopterin are additional coenzymes required by NOS.]



4. **Other functions of nitric oxide:** 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.

Figure 13.10 Pathways of glucose 6-phosphate metabolism in the erythrocyte. NADP(H) = nicotinamide adenine dinucleotide phosphate; G-SH = reduced glutathionine; G-S-S-G = oxidized glutathionine; PPP = pentose phosphate pathway.



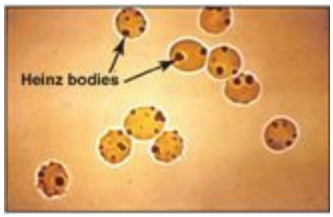
V. GLUCOSE 6-PHOSPHATE DEHYDROGENASE DEFICIENCY

G6PD deficiency is a hereditary disease characterized by hemolytic anemia caused by the inability to detoxify oxidizing agents. G6PD deficiency is the most common disease-producing enzyme abnormality in humans, affecting more than 400 million individuals worldwide. This deficiency has the highest prevalence in the Middle East, tropical Africa and Asia, and parts of the Mediterranean. G6PD deficiency is X linked and is, in fact, a family of deficiencies caused by a number of different mutations in the gene coding for G6PD. Only some of the resulting protein variants cause clinical symptoms. [Note: In addition to hemolytic anemia, a clinical manifestation of G6PD deficiency is neonatal jaundice appearing 1–4 days after birth. The jaundice, which may be severe, typically results from increased production of unconjugated bilirubin (see p. 285).] The life span 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 advantage in survival—an increased resistance to Plasmodium falciparum malaria. [Note: Sickle cell trait and β-thalassemia minor also confer resistance to malaria.]

A. Role of glucose 6-phosphate dehydrogenase in red blood cells

Diminished G6PD activity impairs the ability of the cell to form the NADPH that is essential for the maintenance of the G-SH pool. This results in a decrease in the cellular detoxification of free radicals and peroxides formed within the cell (Figure 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 RBC membranes (Figure 13.11). Additional oxidation of membrane proteins causes RBCs to be rigid (less deformable), and they are removed from the circulation by macrophages in the spleen and liver. Although G6PD deficiency occurs in all cells of the affected individual, it is most severe in RBCs, where the pentose phosphate pathway provides the only means of generating NADPH. Other tissues have alternative sources for NADPH production (such as $NADP^+$-dependent malate dehydrogenase [malic enzyme]; see p. 186) that can keep G-SH reduced. The RBC has no nucleus or ribosomes and cannot renew its supply of the enzyme. Thus, RBCs are particularly vulnerable to enzyme variants with diminished stability.

Figure 13.11 Heinz bodies in erythrocytes of a patient with glucose 6-phosphate dehydrogenase deficiency.



B. Precipitating factors in glucose 6-phosphate dehydrogenase deficiency

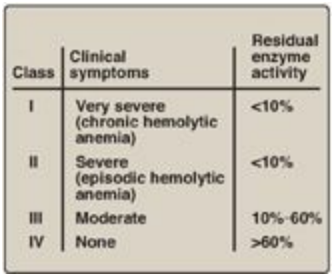
Most individuals who have inherited one of the G6PD mutations do not show clinical manifestations (that is, they are asymptomatic). However, some patients with G6PD deficiency develop hemolytic anemia if they are treated with an oxidant drug, ingest fava beans, or contract a severe infection.

1. **Oxidant drugs:** Commonly used drugs that produce hemolytic anemia in patients with G6PD deficiency are best remembered from the mnemonic AAA: antibiotics (for example, sulfamethoxazole and chloramphenicol), antimalarials (for example, primaquine but not chloroquine or quinine), and antipyretics (for example, acetanilid but not acetaminophen).

2. **Favism:** Some forms of G6PD deficiency, for example the Mediterranean variant, are particularly susceptible to the hemolytic effect of the fava (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 have G6PD deficiency.

3. **Infection:** Infection is the most common precipitating factor of hemolysis in G6PD deficiency. The inflammatory response to infection results in the generation of free radicals in macrophages, which can diffuse into the RBC and cause oxidative damage.

Figure 13.12 Classification of glucose 6-phosphate dehydrogenase (G6PD) deficiency variants. Note: Class V variants (not shown in table) result in overproduction of G6PD.



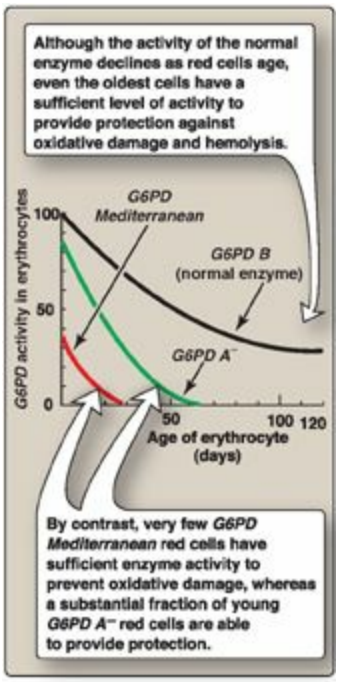
Class	Clinical symptoms	Residual enzyme activity
I	Very severe (chronic hemolytic anemia)	<10%
II	Severe (episodic hemolytic anemia)	<10%
III	Moderate	10%-60%
IV	None	>60%

C. Properties of the variant enzymes

Almost all G6PD variants are caused by point mutations (see p. 433) in the gene for G6PD. Some mutations do not disrupt the structure of the enzyme's active site and, therefore, do not affect enzymic activity. However, many mutant enzymes show altered kinetic properties. For example, variant enzymes may show decreased catalytic activity, decreased stability, or an alteration of binding affinity for $NADP^+$, NADPH, or glucose 6-phosphate. The severity of the disease usually correlates with the amount of residual enzyme activity in the patient's RBC. For example, variants can be classified as shown in Figure 13.12. G6PD A^- is the prototype of the moderate (Class III) form of the disease. The RBCs contain an unstable but kinetically normal G6PD, with most of the enzyme activity present in the reticulocytes and younger RBCs (Figure 13.13). The

oldest RBCs, therefore, have the lowest level of enzyme activity and are preferentially removed in a hemolytic episode. G6PD Mediterranean is the prototype of a more severe (Class II) deficiency in which the enzyme has decreased stability resulting in decreased enzymic activity. Class I mutations (rare) are the most severe and are associated with chronic nonspherocytic hemolytic anemia, which occurs even in the absence of oxidative stress.

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.



D. Molecular biology of glucose 6-phosphate dehydrogenase

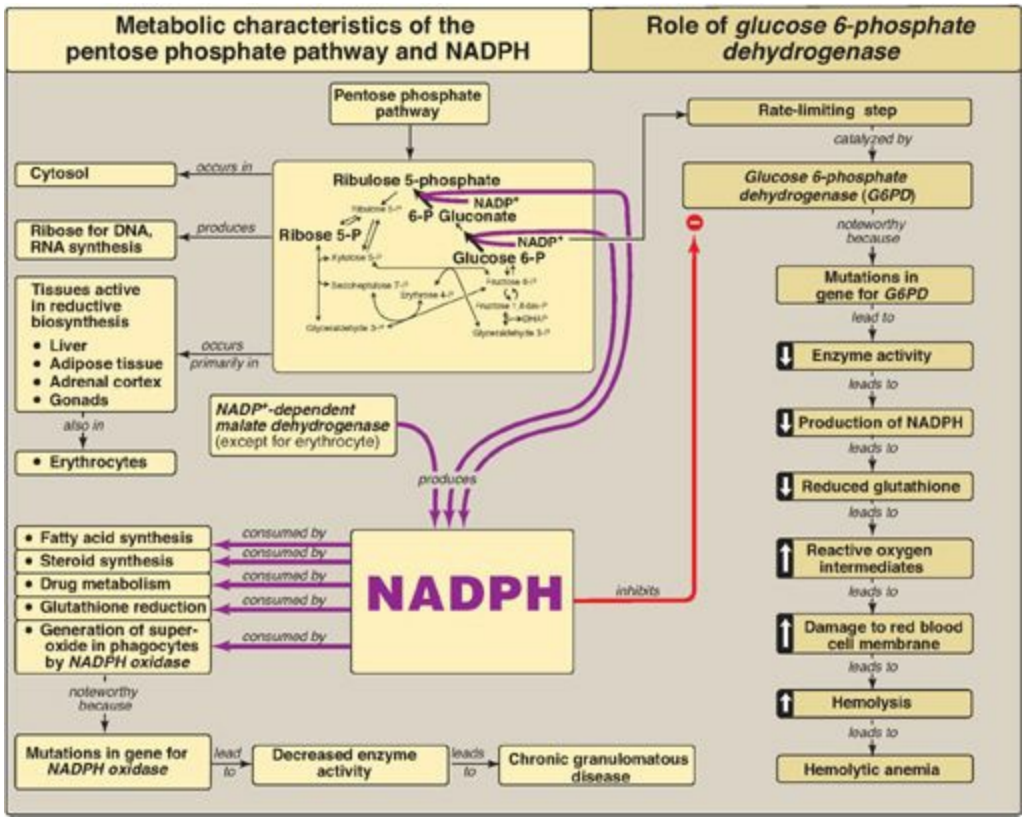
The cloning of the gene for G6PD and the sequencing of its DNA (see p. 467) have permitted the identification of mutations that cause G6PD deficiency. More than 400 different G6PD variants have been identified, a finding that explains the numerous biochemical and clinical phenotypes that have been described. Most mutations that result in enzymic deficiency are missense mutations (see p. 433) in the coding region. Both G6PD A^- and G6PD Mediterranean 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 activity is probably lethal.

VI. CHAPTER SUMMARY

The pentose phosphate pathway includes two irreversible oxidative reactions followed by a series of reversible sugar–phosphate interconversions (Figure 13.14). No ATP is directly consumed or produced in the cycle. The reduced nicotinamide adenine dinucleotide phosphate (NADPH)-producing **oxidative portion** of the pentose phosphate pathway is important in providing 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 NADPH** are produced. The regulated step is catalyzed by **glucose 6-phosphate dehydrogenase** (**G6PD**), which is strongly **inhibited by NADPH**. **Reversible nonoxidative reactions** interconvert sugars. This part of the pathway is the **source of ribose 5-phosphate**, required for nucleotide and nucleic acid synthesis. Because the reactions are reversible, they can be entered from fructose 6-phosphate and glyceraldehyde 3-phosphate (glycolytic intermediates) if ribose is needed and G6PD is inhibited. **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, and steroid hormones in the placenta, ovaries, testes, and adrenal cortex. It is also required by red blood cells (RBCs) for the **reduction of hydrogen peroxide**, providing the reducing equivalents required by **glutathione** (**GSH**). GSH is used by **glutathione peroxidase** to reduce peroxide to water. The oxidized glutathione (GSSH) 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 liver, and **vitamin D activation in the liver and kidney**. The microsomal system uses NADPH to **detoxify** foreign compounds (xenobiotics), such as drugs and a variety of pollutants. NADPH provides the reducing equivalents for phagocytes in the process of eliminating invading microorganisms. **NADPH oxidase** uses molecular oxygen and electrons from NADPH to produce **superoxide radicals**, which, in turn, can be converted to peroxide by superoxide dismutase. **Myeloperoxidase** catalyzes the formation of bactericidal hypochlorous acid from peroxide and chloride ions. Rare genetic defects in NADPH oxidase cause **chronic granulomatous disease** characterized by severe, persistent, infections and formation of granulomas. NADPH is required for the synthesis of **nitric oxide** (**NO**), an important free radical gas that causes **vasodilation** by relaxing vascular smooth muscle, acts as a **neurotransmitter**, prevents **platelet aggregation**, and helps mediate **macrophage bactericidal activity**. NO is made from arginine and O_2 by three different NADPH-dependent NO synthases (NOS). The endothelial (eNOS), and neuronal (nNOS) isozymes constantly produce very low levels of NO for vasodilation and neurotransmission, respectively. The inducible isozyme (iNOS) produces large amounts of NO for defense against pathogens. **G6PD deficiency** impairs the ability of the cell to form the NADPH that is essential for the maintenance of the GSH pool.

The cells most affected are the **RBCs** because they do not have additional sources of NADPH. G6PD deficiency is an **X-linked disease** characterized by **hemolytic anemia** caused by the production of free radicals and peroxides following administration of **oxidant drugs**, ingestion of **fava beans**, or severe **infections.** The extent of the anemia depends on the amount of residual enzyme. Class I variants, the most severe (and least common), are associated with **chronic nonspherocytic hemolytic anemia**. Babies with G6PD deficiency may experience **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 where chloroquine-resistant malaria is endemic, a young man is given primaquine prophylactically. Soon thereafter, he develops a hemolytic condition due to a deficiency in glucose 6-phosphate dehydrogenase. A less-than-normal level of which of the following is a consequence of the enzyme deficiency and the underlying cause of the hemolysis?

A. Glucose 6-phosphate
B. Oxidized form of nicotinamide adenine dinucleotide
C. Reduced form of glutathione
D. Ribose 5-phosphate

Correct answer = C. Glutathione (GSH) is essential for red cell integrity and is maintained in its reduced (functional) form by nicotinamide adenine dinucleotide phosphate (NADPH)-dependent glutathione reductase. The NADPH is generated by the oxidative portion of the pentose phosphate pathway. Individuals with a deficiency of the initiating and regulated enzyme of this pathway, glucose 6-phosphate dehydrogenase (G6PD), have a decreased ability to generate NADPH and, therefore, a decreased ability to keep GSH functional. When treated with an oxidant drug such as primaquine, some patients with G6PD deficiency develop a hemolytic anemia. Primaquine does not affect glucose 6-phosphate levels. Nicotinamide adenine dinucleotide is neither produced by the pentose phosphate pathway nor used as a coenzyme by GSH reductase.

13.2 Septic shock, a state of acute circulatory failure characterized by persistent arterial hypotension (low blood pressure) and inadequate organ perfusion refractory to fluid resuscitation, results from a severe inflammatory response to bacterial infection. It has a high mortality rate and is associated with changes in the level of nitric oxide. Which statement concerning septic shock is most likely correct?

A. Activation of endothelial nitric oxide synthase causes an increase in nitric oxide.
B. High mortality is the result of the long half-life of nitric oxide.
C. Lysine, the nitrogen source for nitric oxide synthesis, is deaminated by bacteria.
D. Overproduction of nitric oxide by a calcium-independent enzyme is the cause of the hypotension.

Correct answer = D. Overproduction of short-lived (not long-lived) nitric

oxide (NO) by calcium-independent, inducible nitric oxide synthase (iNOS) results in excessive vasodilation leading to hypotension. NOS uses arginine, not lysine, as the source of the nitrogen. The endothelial enzyme (eNOS) is constitutive and produces low levels of NO at a consistent rate.

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. Which statement concerning P450 enzymes is most likely correct?

A. They accept electrons from reduced nicotinamide adenine dinucleotide (NADH).
B. They catalyze the hydroxylation of hydrophobic molecules.
C. They differ from nitric oxide synthase in that they contain heme.
D. They function in association with an oxidase.

Correct answer = B. The P450 enzymes hydroxylate hydrophobic compounds, making them more water soluble. Reduced nicotinamide adenine dinucleotide phosphate (NADPH) from the pentose phosphate pathway is the electron donor. The electrons are first transferred to the coenzymes of cytochrome P450 reductase and then to the P450 enzyme. Both the P450 enzymes and the nitric oxide synthase enzymes contain heme.

13.4 In male patients who are hemizygous for X-linked glucose 6-phosphate dehydrogenase deficiency, pathophysiologic consequences are more apparent in red blood cells (RBC) than in other cells such as in the liver. Which one of the following provides the most reasonable explanation for this different response?

A. Excess glucose 6-phosphate in the liver, but not in RBC, can be channeled to glycogen, thereby averting cellular damage.
B. Liver cells, in contrast to RBC, have alternative mechanisms for supplying the reduced nicotinamide adenine dinucleotide phosphate required for maintaining cell integrity.
C. Because RBC do not have mitochondria, production of ATP required to maintain cell integrity depends exclusively on the shunting of glucose 6-phosphate to the pentose phosphate pathway.
D. In RBC, in contrast to liver cells, 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 (RBCs) 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. RBCs do not have glucose 6-phosphatase. The pentose phosphate pathway does not generate ATP.

13.5 An essential prosthetic group for several enzymes of metabolism is derived from the vitamin thiamine. Measurement of the activity of what enzyme in red blood cells could be used to determine thiamine status in the body?

Red blood cells do not have mitochondria and, so, do not contain mitochondrial thiamine pyrophosphate (TPP)-requiring enzymes such as pyruvate dehydrogenase. However, they do contain the cytosolic TPP-requiring transketolase, whose activity can be used to assess thiamine status.

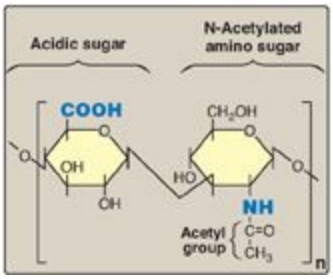
Glycosaminoglycans, Proteoglycans, and Glycoproteins

14

I. OVERVIEW OF GLYCOSAMINOGLYCANS

Glycosaminoglycans (GAGs) are large complexes of negatively charged heteropolysaccharide chains. They are generally associated with a small amount of protein ("core protein"), forming proteoglycans, which typically consist of up to 95% carbohydrate. [Note: This is in comparison to the glycoproteins, which consist primarily of protein with a variable (but typically small) amount of carbohydrate (see p. 165).] GAGs have the special ability to bind large amounts of water, thereby 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, make up the extracellular matrix (ECM). The 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.

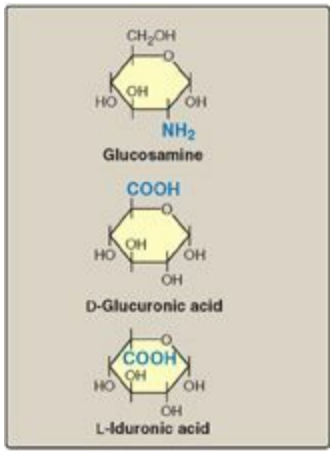
Figure 14.1 Repeating disaccharide unit.



II. STRUCTURE OF GLYCOSAMINOGLYCANS

GAGs are long, unbranched, heteropolysaccharide chains composed of a repeating disaccharide unit [acidic sugar–amino sugar]$_n$ (Figure 14.1). [Note: 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, thus 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 (Figure 14.2). These acidic sugars contain carboxyl groups that are negatively charged at physiologic pH and, together with the sulfate groups, give GAGs their strongly negative nature.

Figure 14.2 Some monosaccharide units found in glycosaminoglycans.



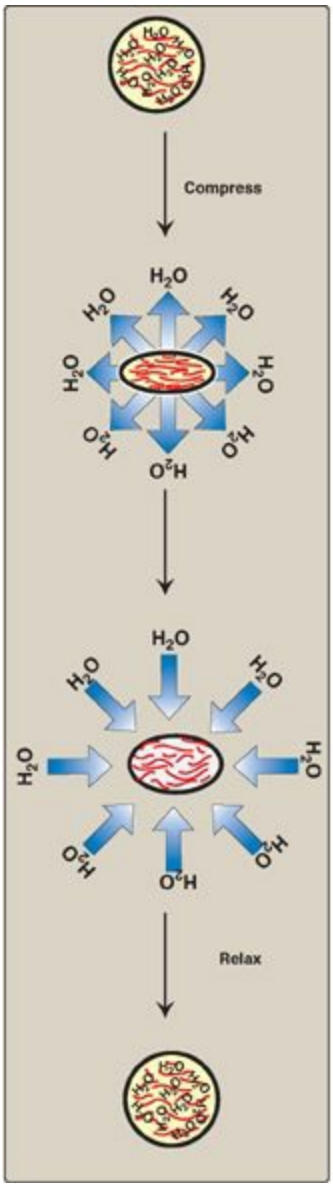
A. Relationship between glycosaminoglycan structure and function

Because of their large number of negative charges, these heteropolysaccharide 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 of 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 synovial fluid and the vitreous humor of the eye (Figure 14.3).

B. Classification of the glycosaminoglycans

The six major types of glycosaminoglycans 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. **Structure of proteoglycan monomers:** 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" (Figure 14.5). In cartilage proteoglycan, the species of GAGs include chondroitin sulfate and keratan sulfate. [Note: Proteoglycans are grouped into gene families that encode core proteins with common structural features. The aggrecan family (aggrecan, versecan, neurocan, and brevican), abundant in cartilage, is an example.]

2. **Linkage between the carbohydrate chain and the protein:** This linkage is most commonly through a trihexoside (galactose-galactose-xylose) and a serine

residue, respectively. An O-glycosidic bond is formed between the xylose and the hydroxyl group of the serine (Figure 14.6).

3. **Proteoglycan aggregates:** The proteoglycan monomers associate with a molecule of hyaluronic acid to form proteoglycan aggregates. The association is not covalent but occurs primarily through ionic interactions between the core protein and the hyaluronic acid. The association is stabilized by additional small proteins called link proteins (Figure 14.7).

III. SYNTHESIS OF GLYCOSAMINOGLYCANS

The heteropolysaccharide chains are elongated by the sequential addition of alternating acidic and amino sugars donated by their uridine diphosphate (UDP)-derivatives. The reactions are catalyzed by a family of specific glycosyltransferases. The synthesis of GAGs is analogous to that of glycogen (see p. 126) except that GAGs are produced for export from the cell. Their synthesis occurs, therefore, primarily in the Golgi, rather than in the cytosol.

Figure 14.4 Structure and distribution of glycosaminoglycans (GAGs). Sulfate groups (Ⓢ) are shown in all possible positions. GlcUA = glucuronic acid; IdUA = iduronic acid; GalNAc = N-acetylgalactosamine; GlcNAc = N-acetylglucosamine; GlcN = glucosamine; Gal = galactose.

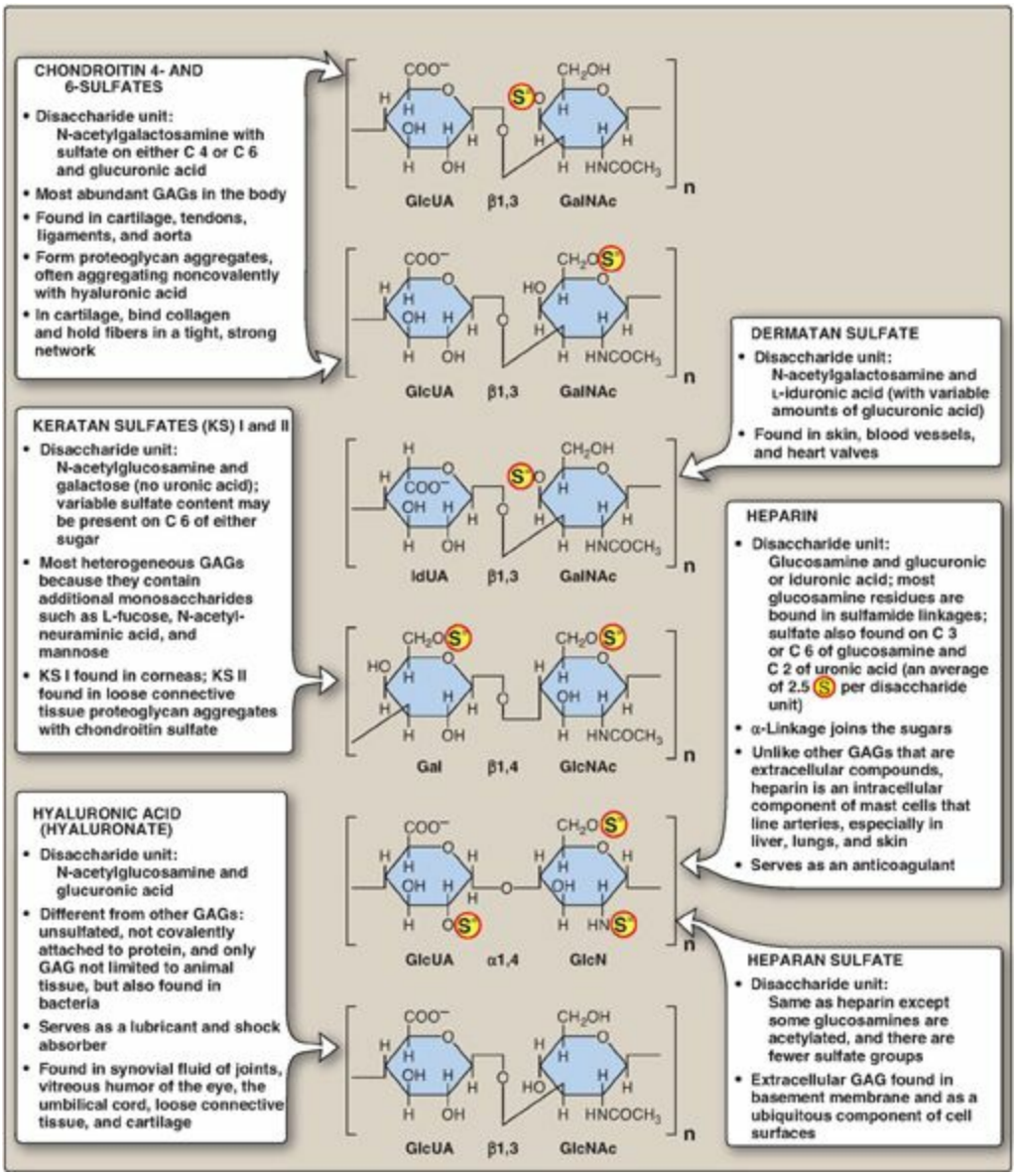
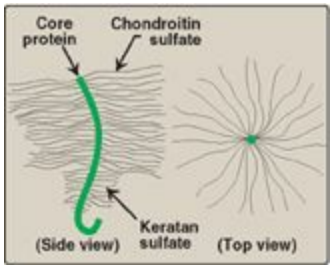


Figure 14.5 "Bottle-brush" model of a cartilage proteoglycan monomer.



A. Synthesis of amino sugars

Amino sugars are essential components of GAGs, glycoproteins, and glycolipids and are also found in some antibiotics. The synthetic pathway of amino sugars 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 N-acetyl-glucosamine (GlcNAc), N-acetylgalactosamine (GalNAc), and the sialic acids, including N-acetylneuraminic acid (NANA). In each of these sugars, a hydroxyl group of the precursor is replaced by an amino group donated by glutamine (Figure 14.8). [Note: The amino groups are then almost always acetylated.] The UDP-derivatives of GlcNAc and GalNAc are synthesized by reactions analogous to those described for UDP-glucose synthesis (see p. 126). These nucleotide sugars are the activated forms of the monosaccharides that can be used to elongate the carbohydrate chains.

2. **N-Acetylneuraminic acid:** NANA, a nine-carbon, acidic monosaccharide, 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; glycolipids; or, less frequently, of GAGs. The carbons and nitrogens in NANA come from N-acetylmannosamine and phosphoenolpyruvate (an intermediate in the glycolytic pathway; see p. 102). [Note: Before NANA can be added to a growing oligosaccharide, it must be converted into its active form by reacting with cytidine triphosphate (CTP). The enzyme CMP-NANA synthetase catalyzes the reaction. This is the only nucleotide sugar in human metabolism in which the carrier nucleotide is a monophosphate.]

Figure 14.6 Linkage region of glycosaminoglycans.

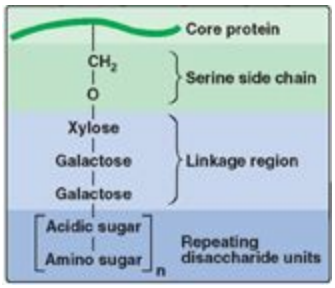


Figure 14.7 Proteoglycan aggregate.

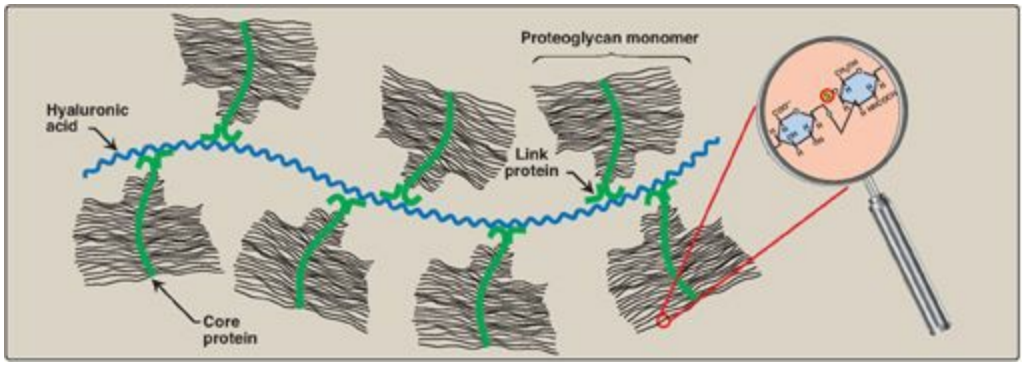
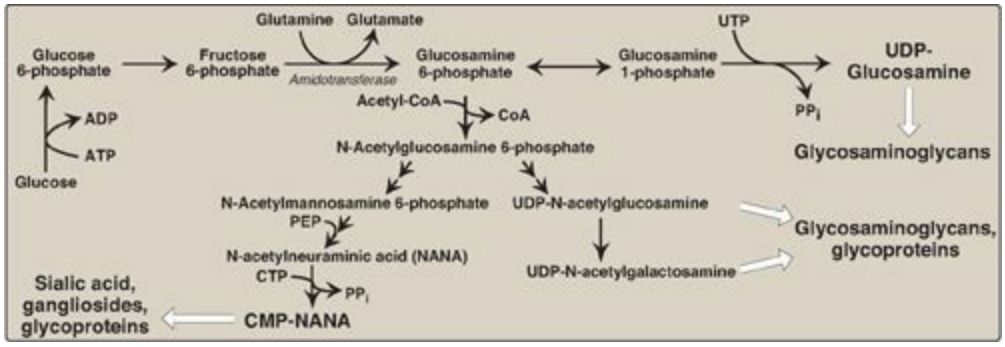


Figure 14.8 Synthesis of the amino sugars. UTP = uridine triphosphate; UDP = uridine diphosphate; CoA = coenzyme A; PEP = phosphoenoylpyruvate; CTP = cytidine triphosphate; CMP = cytidine monophosphate; PP_i = pyrophosphate.



B. Synthesis of acidic sugars

D-Glucuronic acid, whose structure is that of glucose with an oxidized carbon 6 (–CH_2OH →**I**–COOH) and its C-5 epimer, L-iduronic acid, are essential components of GAGs. Glucuronic acid is also required in detoxification reactions of a number of insoluble compounds, such as bilirubin (see p. 282), steroids, and many drugs, including the statins (see p. 224). In plants and mammals (other than guinea pigs and primates, including humans), glucuronic acid serves as a precursor of ascorbic acid (vitamin C). The 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. It can also be obtained from the lysosomal degradation of GAGs, or from glucose 6-phosphate via the uronic acid pathway. 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 (Figure 14.9; see also Figure 13.2, p. 146). The active form of glucuronic acid that donates the sugar in GAG synthesis and other glucuronidation reactions is UDP-glucuronic acid, which is produced by oxidation of UDP-glucose (Figure 14.10).

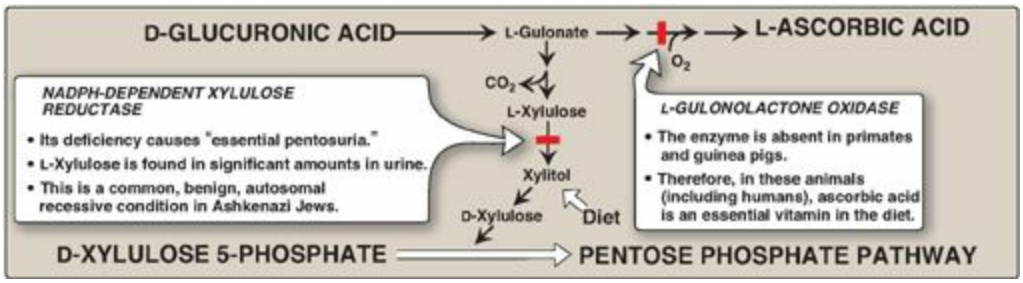
2. **L-Iduronic acid synthesis:** Synthesis of L-iduronic acid residues occurs after D-glucuronic acid has been incorporated into the carbohydrate chain. Uronosyl 5-

epimerase causes epimerization of the D- to the L-sugar.

C. Synthesis of the core protein

The core protein is synthesized by ribosomes on the rough endoplasmic reticulum (RER) and enters the RER lumen. The protein is then glycosylated by membrane-bound glycosyltransferases located in the Golgi.

Figure 14.9 Metabolism of glucuronic acid. NADPH = reduced nicotinamide adenine dinucleotide phosphate.



D. Synthesis of the carbohydrate chain

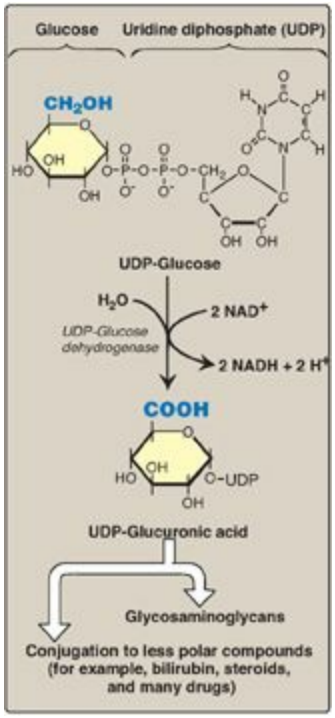
Carbohydrate chain formation begins by synthesis of a short linkage region on the core protein on which carbohydrate chain synthesis will be initiated. The most common linkage region is formed by the transfer of a xylose from UDP-xylose to the hydroxyl group of a serine (or threonine) catalyzed by xylosyltransferase. Two galactose molecules are then added, completing the trihexoside. This is followed by sequential addition of alternating acidic and amino sugars (Figure 14.11) and epimerization of some D-glucuronyl to L-iduronyl residues.

E. Addition of sulfate groups

Sulfation of a GAG occurs after the particular 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). The sulfation reaction is catalyzed by sulfotransferases. [Note: An example of the synthesis of a sulfated GAG, chondroitin sulfate, is shown in Figure 14.11.] PAPS is also the sulfur donor in glycosphingolipid synthesis (see p. 210).

> A defect in the sulfation of the growing glycosaminoglycan chains results in one of several autosomal recessive disorders, the chondrodystrophies, that affect the proper development and maintenance of the skeletal system.

Figure 14.10 Oxidation of UDP-glucose to UDPglucuronic acid. NAD(H) = nicotinamide adenine dinucleotide.



IV. DEGRADATION OF GLYCOSAMINOGLYCANS

GAGs are degraded in lysosomes, which contain hydrolytic enzymes that are most active at a pH of approximately 5. Therefore, as a group, these enzymes are called acid hydrolases. [Note: The low pH optimum is a protective mechanism that prevents the enzymes from destroying the cell should leakage occur into the cytosol where the pH is neutral.] 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. Phagocytosis of extracellular glycosaminoglycans

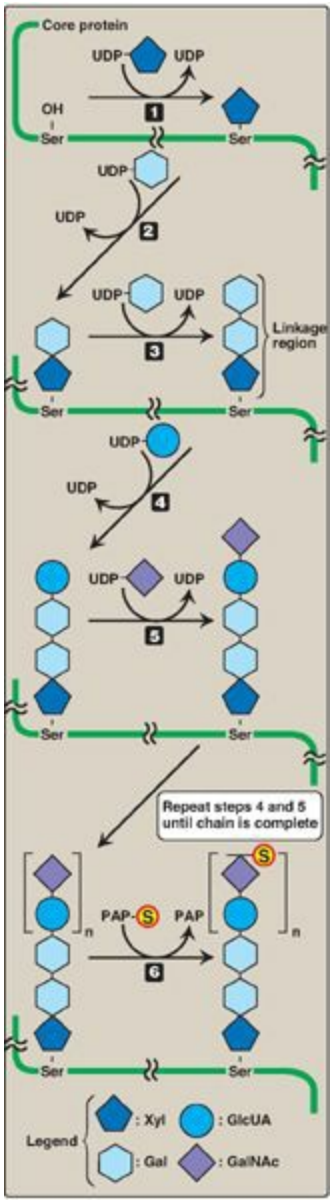
Because GAGs are extracellular or cell-surface compounds, they must first be engulfed by an invagination of the cell membrane (phagocytosis), forming a vesicle inside of which the GAGs are to be degraded. This vesicle then fuses with a lysosome, forming a single digestive vesicle in which the GAGs are efficiently degraded (see p. 150 for a discussion of phagocytosis).

B. Lysosomal degradation of glycosaminoglycans

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 (see p. 127), the last group (sulfate or sugar) added during synthesis being the first group removed (by sulfatases o r exoglycosidases). Examples of some of these enzymes and the bonds they hydrolyze are shown in Figure 14.12. [Note: Endo- and exoglycosidases are also involved in the lysosomal degradation of glycoproteins (see p. 170) and glycolipids (see p. 210). Deficiencies in these enzymes result in the accumulation of partially degraded carbohydrates, resulting in tissue damage.]

> Multiple sulfatase deficiency is a rare lysosomal storage disease in which all sulfatases are nonfunctional due to a defect in the formation of formylglycine, an amino acid derivative required at the active site for enzymic activity to occur.

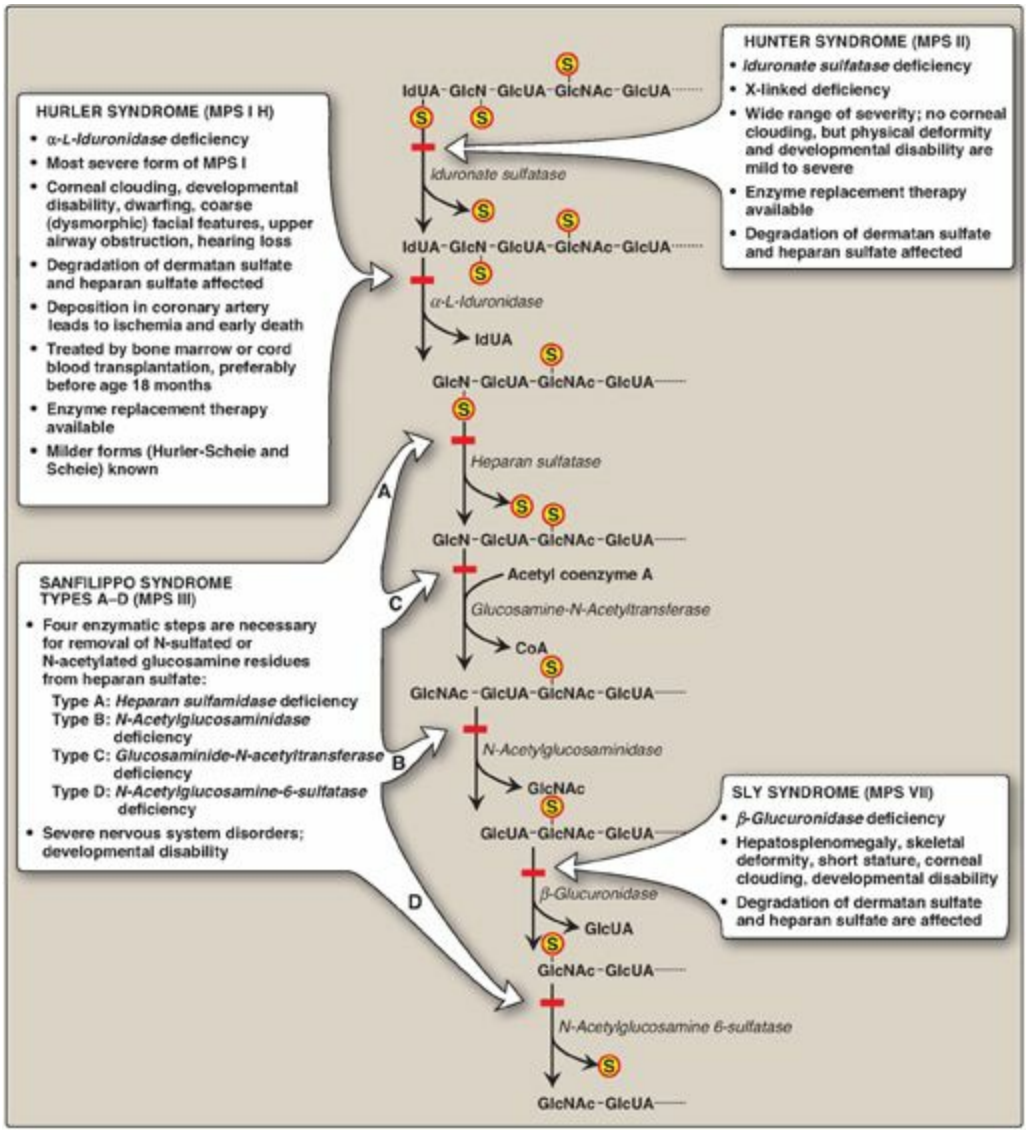
Figure 14.11 Synthesis of chondroitin sulfate.


Core protein
UDP
UDP
OH
Ser
Ser
1
UDP
UDP
2
UDP
UDP
3
Linkage region
Ser
Ser
UDP
UDP
4
UDP
UDP
5
Ser
Ser
Repeat steps 4 and 5 until chain is complete
S
PAP
S
PAP
n
n
6
Ser
Ser
Legend
: Xyl
: GlcUA
: Gal
: GalNAc

V. MUCOPOLYSACCHARIDOSES

The mucopolysaccharidoses are hereditary diseases (1:25,000 live births) caused by a deficiency of any one of the lysosomal hydrolases normally involved in the degradation of heparan sulfate and/or dermatan sulfate (see Figure 14.12). They are progressive disorders characterized by lysosomal accumulation of GAGs in various tissues, causing a range of symptoms, such as skeletal and extracellular matrix deformities, and intellectual disability. All are autosomal recessive disorders except Hunter syndrome, which is X linked. Children who are homozygous for any one of these diseases are apparently normal at birth and then gradually deteriorate. In severe cases, 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.

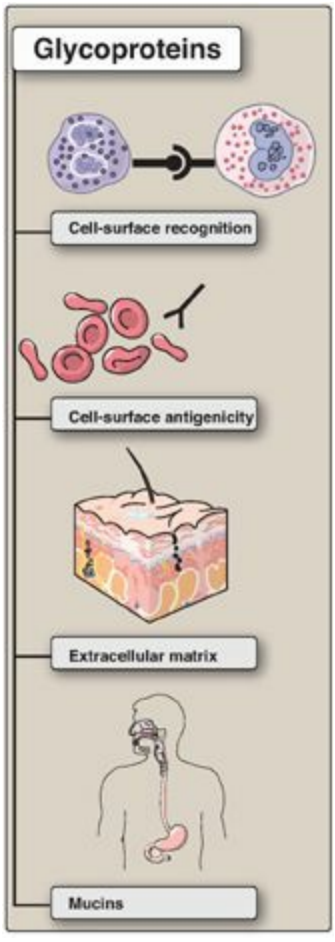
Figure 14.12 Degradation of the glycosaminoglycan heparan sulfate by lysosomal enzymes, indicating sites of enzyme deficiencies in some representative mucopolysaccharidoses (MPSs). [Note: Deficiencies in the degradation of keratan sulfate result in Morquio syndrome, A and B. Deficiencies in the degradation of dermatan sulfate result in Maroteaux-Lamy syndrome.] GlcUA = glucuronic acid; IdUA = iduronic acid; GalNAc = N-acetylgalactosamine; GlcNAc = N-acetylglucosamine; GlcN = glucosamine; S = sulfate.


HURLER SYNDROME (MPS I H)
• α-L-Iduronidase deficiency
• Most severe form of MPS I
• Corneal clouding, developmental disability, dwarfing, coarse (dysmorphic) facial features, upper airway obstruction, hearing loss
• Degradation of dermatan sulfate and heparan sulfate affected
• Deposition in coronary artery leads to ischemia and early death
• Treated by bone marrow or cord blood transplantation, preferably before age 18 months
• Enzyme replacement therapy available
• Milder forms (Hurler-Scheie and Scheie) known
HUNTER SYNDROME (MPS II)
• Iduronate sulfatase deficiency
• X-linked deficiency
• Wide range of severity; no corneal clouding, but physical deformity and developmental disability are mild to severe
• Enzyme replacement therapy available
• Degradation of dermatan sulfate and heparan sulfate affected
SANFILIPPO SYNDROME TYPES A–D (MPS III)
• Four enzymatic steps are necessary for removal of N-sulfated or N-acetylated glucosamine residues from heparan sulfate:
Type A: Heparan sulfamidase deficiency
Type B: N-Acetylglucosaminidase deficiency
Type C: Glucosaminide-N-acetyltransferase deficiency
Type D: N-Acetylglucosamine-6-sultatase deficiency
• Severe nervous system disorders; developmental disability
SLY SYNDROME (MPS VII)
• β-Glucuronidase deficiency
• Hepatosplenomegaly, skeletal deformity, short stature, corneal clouding, developmental disability
• Degradation of dermatan sulfate and heparan sulfate are affected
IdUA-GlcN-GlcUA-GlcNAc-GlcUA
Iduronate sulfatase
IdUA-GlcN-GlcUA-GlcNAc-GlcUA
α-L-Iduronidase
IdUA
GlcN-GlcUA-GlcNAc-GlcUA
Heparan sulfatase
GlcN-GlcUA-GlcNAc-GlcUA
Acetyl coenzyme A
Glucosamine-N-Acetyltransferase
CoA
GlcNAc-GlcUA-GlcNAc-GlcUA
N-Acetylglucosaminidase
GlcNAc
GlcUA-GlcNAc-GlcUA
β-Glucuronidase
GlcUA
GlcNAc-GlcUA
N-Acetylglucosamine 6-sulfatase
GlcNAc-GlcUA
A
B
C
D

VI. OVERVIEW OF GLYCOPROTEINS

Glycoproteins are proteins to which oligosaccharides are covalently attached. They differ from the proteoglycans in that the length of the carbohydrate chain in glycoproteins is relatively short (usually two to ten sugar residues in length, although they can be longer), whereas it can be very long in the GAGs of proteoglycans (see p. 157). In addition, whereas GAGs have repeating disaccharide units, the carbohydrates of glycoproteins do not have serial repeats. The glycoprotein carbohydrate chains are often branched instead of linear and may or may not be negatively charged. Glycoproteins contain highly variable amounts of carbohydrate but typically less than that in GAGS. For example, immunoglobulin IgG contains less than 4% of its mass as carbohydrate, whereas human gastric glycoprotein (mucin) contains more than 80% carbohydrate. 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. (See Figure 14.13 for a summary of some of the functions of glycoproteins.) [Note: Glycosylation is the most common posttranslational modification of proteins.]

Figure 14.13 Functions of glycoproteins.



VII. OLIGOSACCHARIDE STRUCTURE

The oligosaccharide 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. Structure of the linkage between carbohydrate and protein

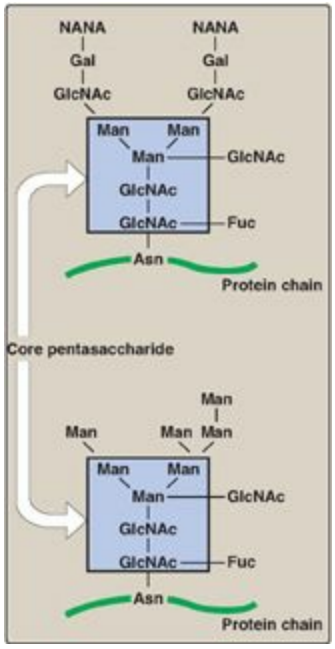
The oligosaccharide may be attached to the protein through an N- or an O-glycosidic link (see p. 86). In the former case, the sugar chain is attached to the amide group of an asparagine side chain and, in the latter case, to the hydroxyl group of either a serine or threonine side chain. [Note: In the case of collagen, there is an O-glycosidic linkage between galactose or glucose and the hydroxyl group of hydroxylysine (see p. 47).]

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 oligosaccharides:** The O-linked oligosaccharides may have one or more of a wide variety of sugars arranged in either a linear or a branched pattern. Many O-linked oligosaccharides 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.
2. **N-linked oligosaccharides:** The N-linked oligosaccharides 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.] NANA = N-acetylneuraminic acid; Gal = galactose; GlcNAc = N-acetylglucosamine; Man = mannose; Fuc = fucose; Asn = asparagine.


NANA
NANA
Gal
Gal
GlcNAc
GlcNAc
Man
Man
Man
GlcNAc
GlcNAc
GlcNAc
Fuc
Asn
Protein chain
Core pentasaccharide
Man
Man
Man
Man
Man
Man
GlcNAc
GlcNAc
GlcNAc
Fuc
Asn
Protein chain

VIII. SYNTHESIS OF GLYCOPROTEINS

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 RER. These proteins contain specific signal sequences that act as molecular "address labels," targeting the proteins to their proper destinations. An N-terminal hydrophobic sequence initially directs these proteins to the RER, allowing the growing polypeptide to be extruded into the lumen. The proteins are then transported via secretory vesicles to the Golgi complex, which acts as a sorting center (Figure 14.15). In the Golgi, those glycoproteins that are to be secreted from the cell (or are targeted for lysosomes) are packaged into vesicles that fuse with the cell (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. [Note: The membrane glycoproteins are, thus, oriented with the carbohydrate portion on the outside of the cell (see Figure 14.15).]

A. Carbohydrate components of glycoproteins

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 (which is synthesized from GDP-mannose), and CMP-NANA may donate sugars to the growing chain. [Note: When the acidic NANA is present, the oligosaccharide has a negative charge at physiologic pH.] The oligosaccharides are covalently attached to the R groups 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. Synthesis of O-linked glycosides

The synthesis of the O-linked glycosides is very similar to that of the GAGs (see p. 158). First, the protein to which the oligosaccharides are to be attached is synthesized on the RER and extruded into its lumen. Glycosylation begins with the transfer of GalNAc (from UDP-GalNAc) onto the R-group of a specific serine or threonine.

1. **Role of glycosyltransferases:** The glycosyltransferases responsible for the stepwise synthesis of the oligosaccharides are bound to the membranes of the Golgi apparatus. They act in a specific order, without using a template as is required for DNA, RNA, and protein synthesis (see Unit VI) but, rather by recognizing the actual structure of the growing oligosaccharide as the appropriate substrate.

C. Synthesis of the N-linked glycosides

The synthesis of N-linked glycosides occurs in the lumen of the RER and requires the

participation of the phosphorylated form of dolichol (dolichol pyrophosphate), a lipid of the ER membrane (Figure 14.16). The initial product is processed in the ER and Golgi.

1. **Synthesis of dolichol-linked oligosaccharide:** First, as with the O-linked glycosides, the protein is synthesized on the RER and enters its lumen. However, the protein does not become glycosylated with individual sugars. Instead, a lipid-linked oligosaccharide is first constructed. This consists of dolichol (an ER membrane lipid 80–100 carbons long) attached through a pyrophosphate linkage to an oligosaccharide containing N-GlcNAc, mannose, and glucose. The sugars to be added sequentially to the dolichol by the membrane-bound glycosyltransferases are first N-GlcNAc, followed by mannose and glucose (see Figure 14.16). The entire 14-sugar oligosaccharide is then transferred from the dolichol to the amide nitrogen (N) of an asparagine in the protein to be glycosylated by a protein-oligosaccharide transferase present in the ER. [Note: Tunicamycin inhibits N-linked glycosylation.]

> Congenital disorders of glycosylation (CDGs) are syndromes caused primarily by defects in the N-linked glycosylation of proteins, either oligosaccharide assembly (Type I) or processing (Type II).

Figure 14.15 Transport of glycoproteins through the Golgi apparatus and their subsequent release or incorporation into a lysosome or the cell membrane.

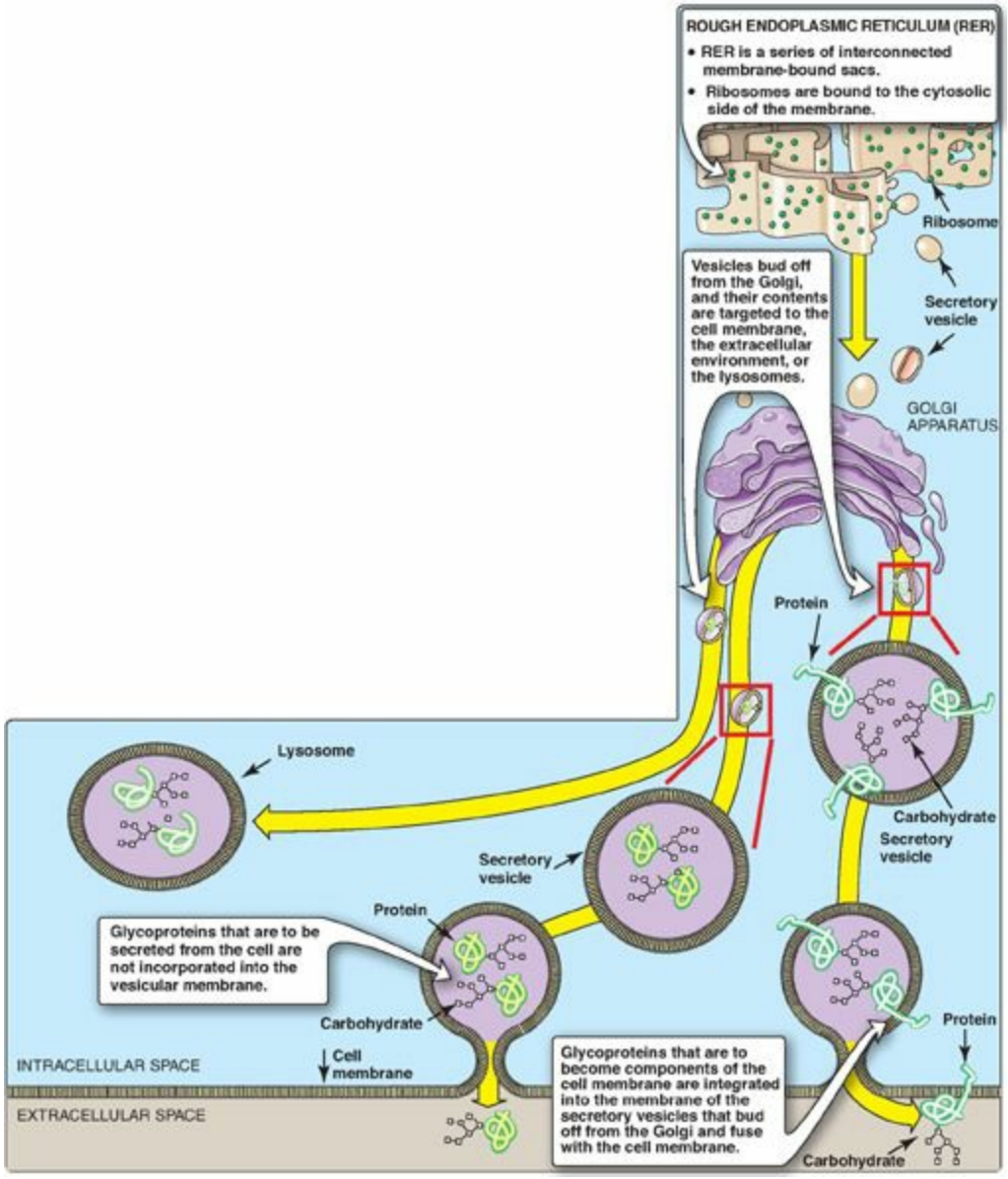
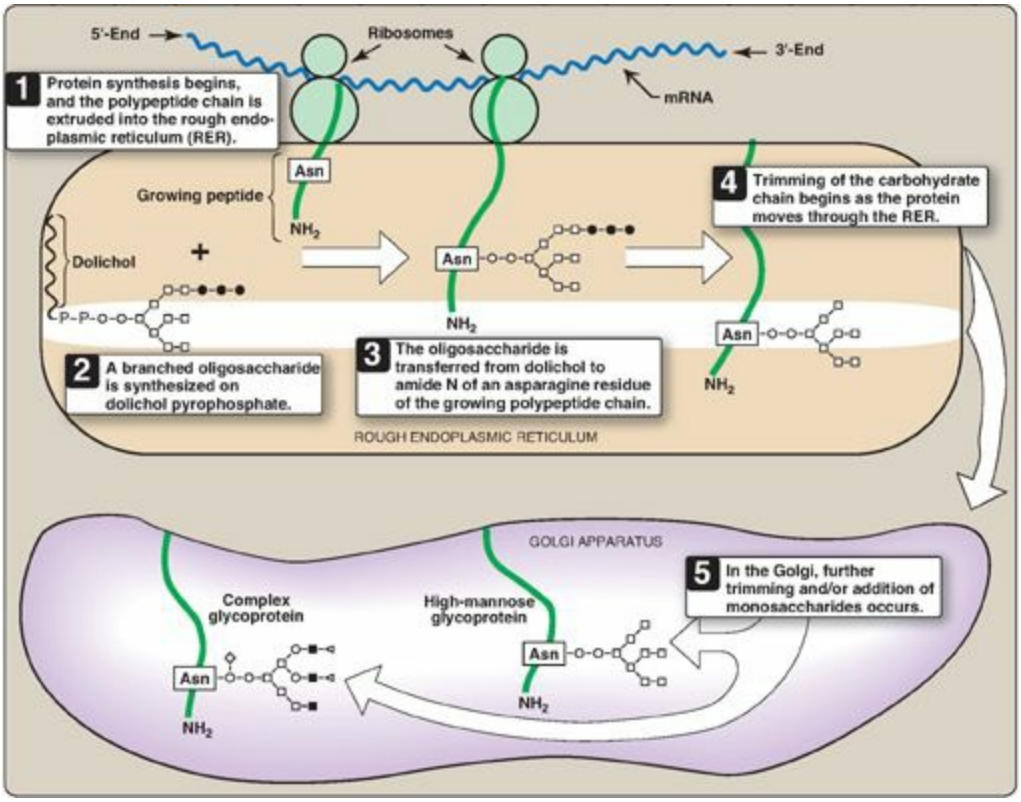
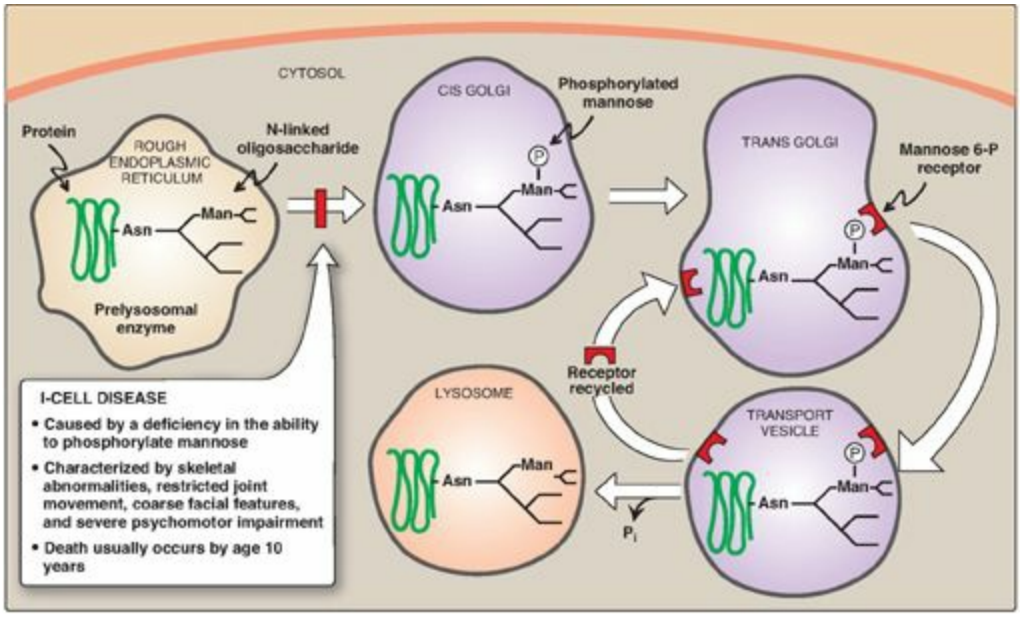


Figure 14.16 Synthesis of N-linked glycoproteins.
○ = N-acetylglucosamine; □ = mannose; ● = glucose; ■ = N-acetylgalactosamine; ◇ or ◁ = terminal group (fucose or N-acetylneuraminic acid); mRNA = messenger RNA; Asn = asparagine.



2. **Final processing of N-linked oligosaccharides:** After incorporation into 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 (for example, N-GlcNAc, N-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 Figure 14.16). The ultimate fate of N-linked glycoproteins is the same as that of the O-glycoproteins linked (for example, 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. [Note: Nonenzymatic glycosylation of proteins is known as glycation (see p. 345).]

Figure 14.17 Mechanism for transport of N-linked glycoproteins to the lysosomes. Asn = asparagine; Man = mannose; P = phosphate; P_i = inorganic phosphate.



3. **Enzymes destined for lysosomes:** N-linked glycoproteins being processed through the Golgi can be phosphorylated on carbon 6 of one or more specific mannosyl residues. Mannose 6-phosphate receptors, located in the Golgi apparatus, bind the mannose 6-phosphate residues of these targeted enzymes, which are then packaged into vesicles and sent to the lysosomes. I-cell disease is a rare lysosomal storage disease in which the acid hydrolases normally found in lysosomes are absent, resulting in an accumulation of substrates normally degraded by these enzymes. [Note: I-cell disease is so-named because of the large inclusion bodies seen in cells of patients with this disease.] In addition, high amounts of lysosomal enzymes are found in the patient's plasma and urine, indicating that the targeting process to lysosomes (rather than the synthetic pathway of these enzymes) is deficient. Individuals with I-cell disease are lacking the phosphotransferase needed to phosphorylate the mannose residues of potential lysosomal enzymes, causing the enzymes to be secreted (by default), rather than being targeted to lysosomal vesicles (Figure 14.17). I-cell disease is characterized by skeletal abnormalities, restricted joint movement, coarse (dysmorphic) facial features, and severe psychomotor impairment. [Note: Because I-cell disease has features in common with the mucopolysaccharidoses and sphingolipidoses (see p. 211), it is termed a mucolipidosis.] Currently, there is no cure, and death from cardiopulmonary complications usually occurs by age 10 years.

IX. LYSOSOMAL DEGRADATION OF GLYCOPROTEINS

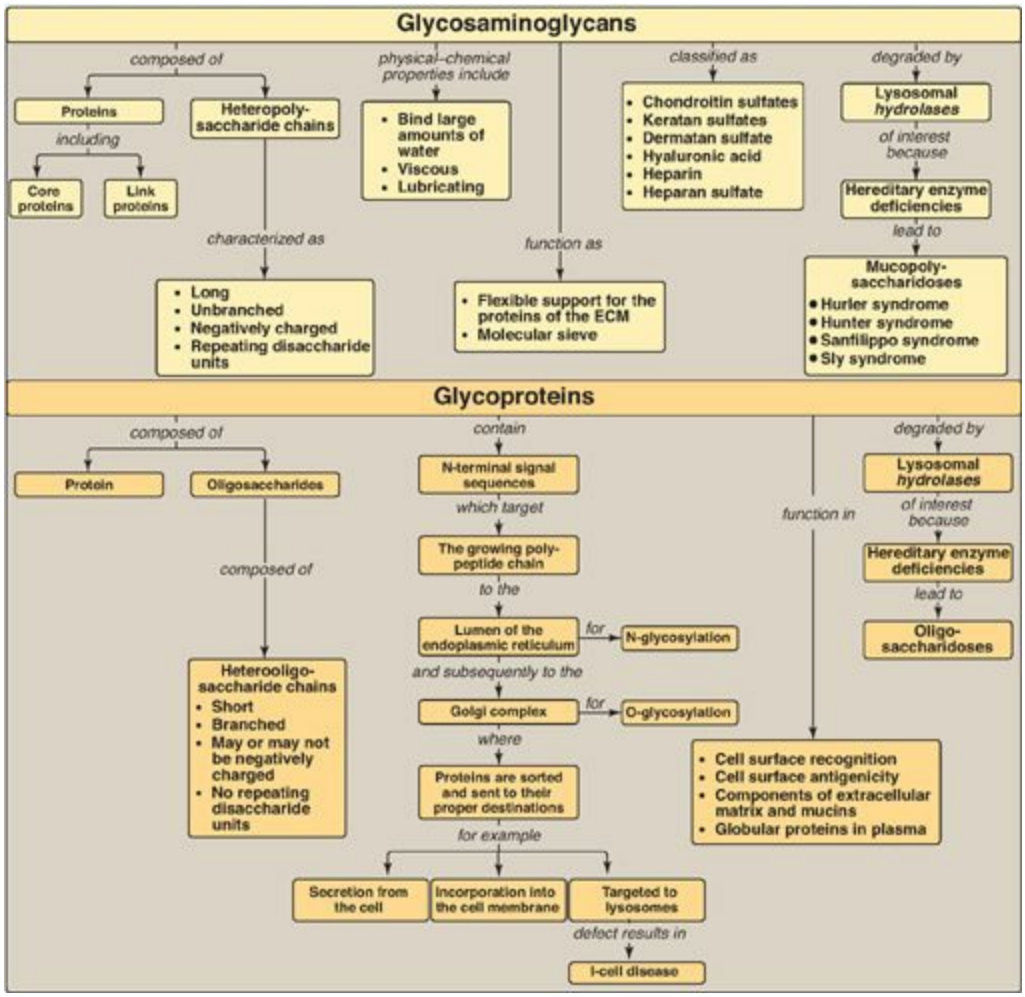
Degradation of glycoproteins is similar to that of the GAGs (see p. 162). The lysosomal acid hydrolases are each generally specific for the removal of one component of the glycoprotein. They are primarily exoenzymes that remove their respective groups in the reverse order of their incorporation ("last on, first off"). If any one 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 1 is a 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 assay.

X. CHAPTER SUMMARY

Glycosaminoglycans (**GAGs**) are **long**, **negatively charged**, **unbranched**, **heteropolysaccharide chains** generally composed of a **repeating disaccharide unit** [acidic sugar–amino sugar]$_n$ (Figure 14.18). The **amino sugar** is either **D-glucosamine** or **D-galactosamine** in which the amino group is usually acetylated, thus 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**. GAGs **bind large amounts of water**, thereby producing the gel-like matrix that forms the basis of the body's **ground substance.** The viscous, lubricating properties of **mucous secretions** are also caused by the presence of GAGs, which led to the original naming of these compounds as **mucopolysaccharides.** There are **six major types** of GAGs, including **chondroitin 4- and 6-sulfates**, **keratan sulfate**, **dermatan sulfate**, **heparin**, **heparan sulfate**, and **hyaluronic acid**. All of the GAGs, except hyaluronic acid, are found covalently attached to protein, forming **proteoglycan monomers**, which consist of a **core protein** to which the linear GAG chains are covalently attached. The proteoglycan monomers associate with a molecule of **hyaluronic acid** to form **proteoglycan aggregates**. GAGs are synthesized in the **Golgi**. The polysaccharide chains are elongated by the sequential addition of alternating acidic and amino sugars, donated by their **UDP-derivatives**. D-glucuronate may be epimerized to L-iduronate. The last step in synthesis is sulfation of some of the amino sugars. The source of the sulfate is **3'-phosphoadenosyl-5'-phosphosulfate**. The completed proteoglycans are secreted into the extracellular matrix or remain associated with the outer surface of cells. GAGs are **degraded** by **lysosomal acid hydrolases**. They are first broken down to oligosaccharides, which are degraded sequentially from the nonreducing end of each chain. A **deficiency** of any one of the hydrolases results in a **mucopolysaccharidosis**. These are hereditary disorders in which GAGs accumulate in tissues, causing symptoms such as **skeletal** and **extracellular matrix deformities** and **intellectual disability**. Examples of these genetic diseases include **Hunter** and **Hurler syndromes**. **Glycoproteins** are proteins to which **oligosaccharides** are covalently attached. They differ from the proteoglycans in that the length of the glycoprotein's carbohydrate chain is relatively short (usually two to ten sugar residues long, although they can be longer), may be branched, and does not contain serial disaccharide units. **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 **extracellular matrix** 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. Glycoproteins are **synthesized** in the **rough endoplasmic reticulum (RER)** and the **Golgi**. The precursors of the carbohydrate components of glycoproteins are

nucleotide sugars. **O-linked glycoproteins** are synthesized in the Golgi by the sequential transfer of sugars from their nucleotide carriers to the hydroxyl group of a serine or threonine residue in the protein. **N-linked glycoproteins** contain varying amounts of **mannose**. They are synthesized by the transfer of a preformed oligosaccharide from its ER membrane lipid carrier, **dolichol pyrophosphate**, to the amide N of an asparagine residue in the protein. A deficiency in the phosphorylation of mannose residues in N-linked glycoprotein enzymes destined for the lysosomes results in **I-cell disease**. Glycoproteins are degraded in lysosomes by acid hydrolases. A deficiency of any one of these enzymes results in a lysosomal **glycoprotein storage disease** (**oligosaccharidosis**), resulting in accumulation of partially degraded structures in the lysosome.

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. They are caused by:

A. defects in the degradation of glycosaminoglycans.
B. defects in the targeting of 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.
F. the synthesis of heteropolysaccharides with an altered structure.

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, both in terms of 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 boy with coarse facial features, skeletal abnormalities, and delays in both growth and development is diagnosed with I-cell disease based on his

presentation and on histologic and biochemical testing. I-cell disease is characterized by:

A. decreased production of cell-surface O-linked glycoproteins.
B. elevated levels of acid hydrolases in the blood.
C. an 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 a protein essential for synthesis of the mannose 6-phosphate signal that targets acid hydrolases to the lysosome. This results in secretion of these enzymes from the cell and accumulation of materials within the lysosome due to impaired degradation. None of the other choices relate in any way to I-cell disease or lysosomal function. Oligosaccharides in the urine are characteristic of the muco- and polysaccharidoses but not I-cell disease (a mucolipidosis).

14.4 An infant with corneal clouding has dermatan sulfate and heparin 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. β-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 GAG synthesis.

14.5 Distinguish between glycoproteins and proteoglycans.

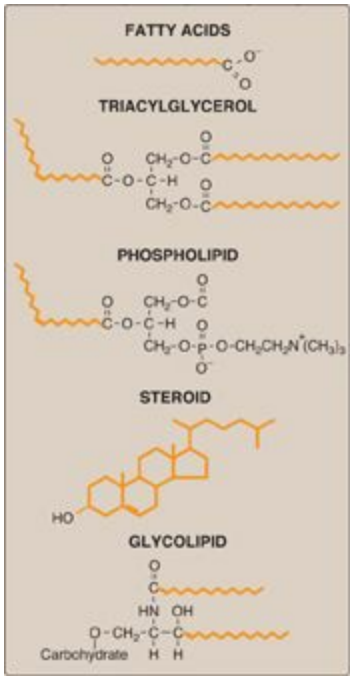
Glycoproteins are proteins to which short, branched, oligosaccharide chains are attached. Proteoglycans consist of a core protein to which long, unbranched, glycosaminoglycan (GAG) chains are attached. GAGs are large complexes of negatively charged heteropolysaccharides composed of repeating [acidic sugar–amino sugar]$_n$ disaccharide units.

Dietary Lipid Metabolism 15

I. OVERVIEW

Lipids are a heterogeneous group of water-insoluble (hydrophobic) organic molecules (Figure 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 in adipocytes, or transported in plasma in association with protein, as in lipoprotein particles (see p. 227), 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 (for example, 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). Not surprisingly, 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.



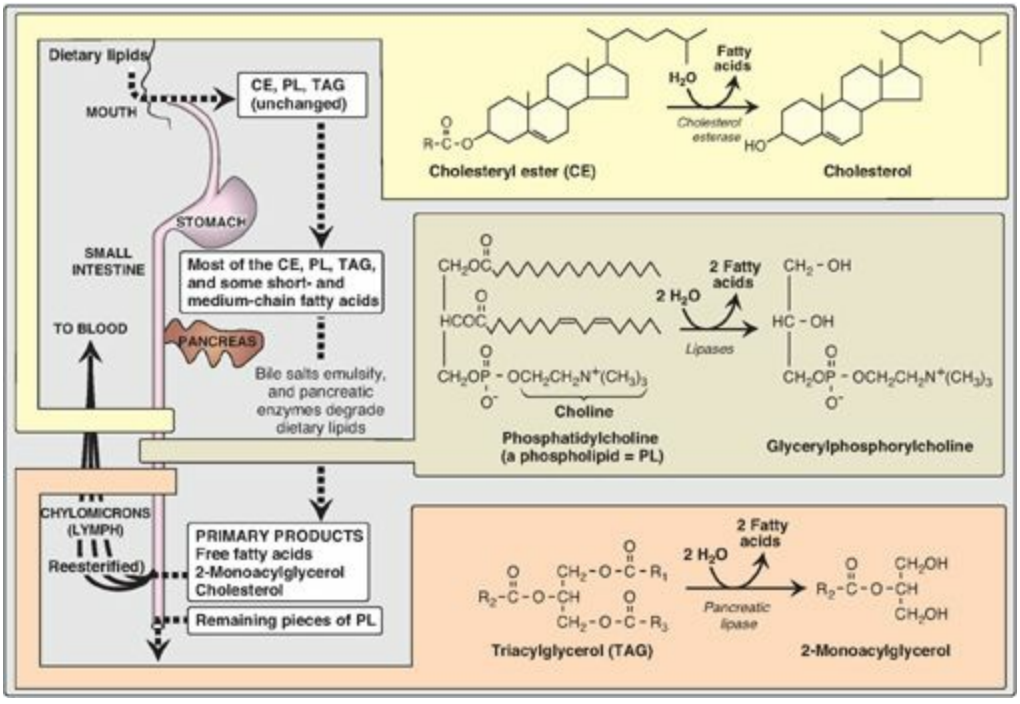
II. DIGESTION, ABSORPTION, SECRETION, AND UTILIZATION OF DIETARY LIPIDS

The average daily intake of lipids by U.S. adults is about 81 g, of which more than 90% is normally triacylglycerol ([TAG], formerly called triglyceride). The remainder of the dietary lipids consists primarily of cholesterol, cholesteryl esters, phospholipids, and unesterified ("free") fatty acids. The digestion of dietary lipids is summarized in Figure 15.2.

A. Processing of dietary lipid in the stomach

The digestion of lipids begins in the stomach, catalyzed by a lipase (lingual lipase) that originates from glands at the back of the tongue. TAG molecules, particularly those containing fatty acids of short- or medium-chain length (fewer than 12 carbons such as are found in milk fat), are the primary target of this enzyme. These same TAGs are also degraded by a separate gastric lipase, secreted by the gastric mucosa. Both enzymes are relatively acid stable, with pH optimums of pH 4 to pH 6. These "acid lipases" play a particularly important role in lipid digestion in neonates, 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 length fatty acids) despite a near or complete absence of pancreatic lipase (see below).

Figure 15.2 Overview of lipid digestion.



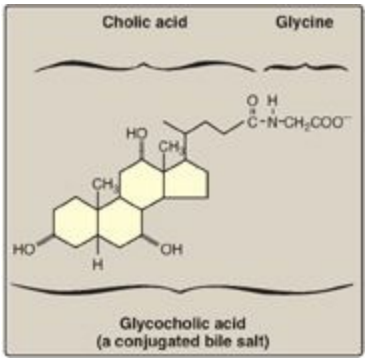
1. **Cystic fibrosis:** CF is the most common lethal genetic disease in Caucasians of Northern European ancestry and has a prevalence of about 1:3,300 births in the United States. CF is an autosomal recessive disorder caused by mutations to the

gene for the CF transmembrane conductance regulator (CFTR) protein that functions as a chloride channel on epithelium in the pancreas, lungs, testes, and sweat gands. 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 secretions that clog 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.]

B. Emulsification of dietary lipid in the small intestine

The critical process of emulsification of dietary lipids 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 (see p. 224). 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 (Figure 15.3). These emulsifying agents interact with the dietary lipid particles and the aqueous duodenal contents, thereby stabilizing the particles as they become smaller from peristalsis and preventing them from coalescing. A more complete discussion of bile salt metabolism is given on p. 225.

Figure 15.3 Structure of glycocholic acid.



C. Degradation of dietary lipids by pancreatic enzymes

The dietary TAG, cholesteryl esters, and phospholipids are enzymically degraded ("digested") by pancreatic enzymes, whose secretion is hormonally controlled.

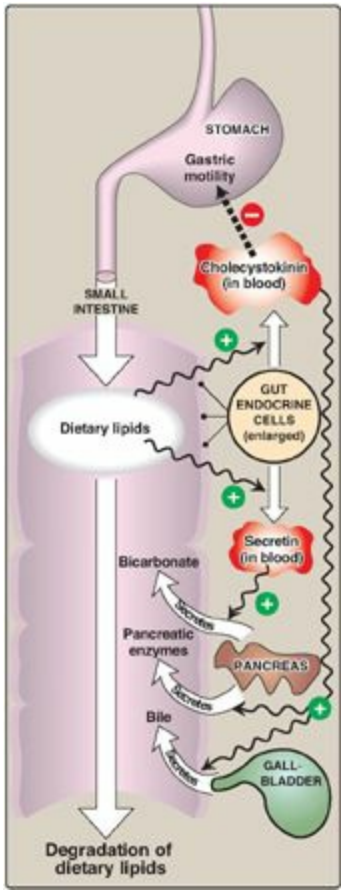
1. **Triacylglycerol degradation:** TAG molecules are too large to be taken up efficiently by the mucosal cells of the intestinal villi. They are, therefore, acted upon by an esterase, pancreatic lipase, which preferentially removes the fatty acids at

carbons 1 and 3. The primary products of hydrolysis are, thus, a mixture of 2-monoacylglycerol and free fatty acids (see Figure 15.2). [Note: This enzyme is found in high concentrations in pancreatic secretions (2%–3% of the total protein present), and it is highly efficient catalytically, thus insuring 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 t o 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%–15% present in the esterified form. Cholesteryl esters are hydrolyzed by pancreatic cholesteryl ester hydrolase (cholesterol esterase), which produces cholesterol plus free fatty acids (see Figure 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 fatty acid from carbon 2 of a phospholipid, leaving a lysophospholipid. For example, phosphatidylcholine (the predominant phospholipid of digestion) becomes lysophosphatidylcholine. The remaining fatty acid at carbon 1 can be removed by lysophospholipase, leaving a glycerylphosphoryl base (for example, glycerylphosphorylcholine, see Figure 15.2) that may be excreted in the feces, further degraded, or absorbed.

Figure 15.4 Hormonal control of lipid digestion in the small intestine.



4. **Control of lipid digestion:** Pancreatic secretion of the hydrolytic enzymes that degrade dietary lipids in the small intestine is hormonally controlled (Figure 15.4). Cells in the mucosa of the lower duodenum and jejunum produce a small peptide hormone, cholecystokinin (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 (see p. 353). Other intestinal cells produce another small peptide hormone, secretin, in response to the low pH of the chyme entering the intestine. 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.

D. Absorption of lipids by intestinal mucosal cells, or enterocytes

Free fatty acids, free cholesterol, and 2-monoacylglycerol 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 (that is, 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). Mixed micelles are, therefore, soluble in the aqueous environment of the intestinal lumen (Figure 15.5). These particles approach the primary site of lipid absorption, the brush border membrane of the enterocytes

(mucosal cell). This 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 less than 5% being lost in the feces. [Note: Relative to other dietary lipids, cholesterol is only poorly absorbed by the enterocytes. Drug therapy (for example, with ezetimibe) can further reduce cholesterol absorption in the small intestine.] Short- and medium-chain length fatty acids are water soluble and, thus, do not require the assistance of mixed micelles for absorption by the intestinal mucosa.

E. Resynthesis of triacylglycerols and cholesteryl esters

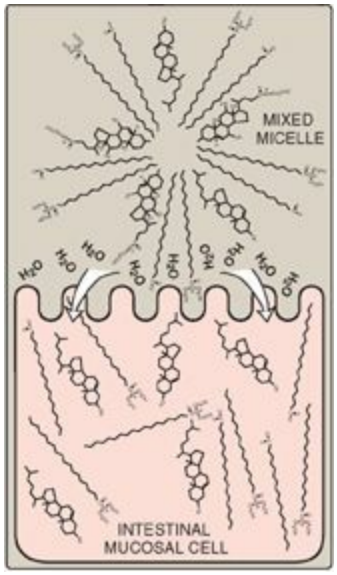
The mixture of lipids absorbed by the enterocytes migrates to the endoplasmic reticulum where biosynthesis of complex lipids takes place. The long-chain length fatty acids are first converted into their activated form by fatty acyl-coenzyme A (CoA) synthetase (thiokinase) as shown in Figure 15.6. Using the fatty acyl CoA derivatives, the 2-monoacylglycerols absorbed by the enterocytes are converted to TAGs through sequential reacylations by two acyltransferases, acyl CoA:monoacylglycerol acyltransferase and acyl CoA:diacylglycerol acyltransferase. Lysophospholipids are reacylated to form phospholipids by a family of acyltransferases, and cholesterol is esterified with a fatty acid primarily by acyl CoA:cholesterol acyltransferase (see p. 232). [Note: Virtually all long-chain fatty acids entering the enterocytes are used in this fashion to form TAGs, phospholipids, and cholesteryl esters. Short- and medium-chain length fatty acids are not converted to their CoA derivatives and are not reesterified to 2-monoacylglycerol. Instead, they are released into the portal circulation, where they are carried by serum albumin to the liver.]

F. Lipid malabsorption

Lipid malabsorption, resulting in increased lipid (including the fat-soluble vitamins and essential fatty acids, see p. 182) in the feces, a condition known as steatorrhea, can be caused by disturbances in lipid digestion and/or absorption (Figure 15.7). Such disturbances can result from several conditions, including CF (causing poor digestion) and short bowel syndrome (causing decreased absorption).

> The ability of short- and medium-chain length fatty acids to be taken up by enterocytes without the aid of mixed micelles has made them important in dietary therapy for individuals with malabsorption disorders.

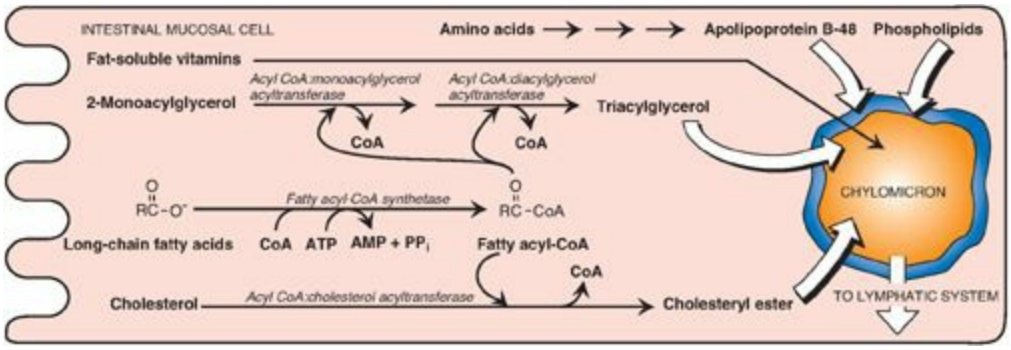
Figure 15.5 Absorption of lipids contained in a mixed micelle by an intestinal mucosal cell. [Note: The micelle itself is not taken up.]



G. Secretion of lipids from enterocytes

The newly resynthesized TAGs and cholesteryl esters are very hydrophobic and aggregate in an aqueous environment. It is, therefore, necessary that they be packaged as particles of lipid droplets surrounded by a thin layer composed of phospholipids, unesterified cholesterol, and a molecule of the protein apolipoprotein B-48 (see p. 228). This layer stabilizes the particle and increases its solubility, thereby preventing multiple particles from coalescing. [Note: Microsomal triglyceride transfer protein is essential for the assembly of these (and other) TAG-rich apolipoprotein B–containing particles in the endoplasmic reticulum.] The lipoprotein particles are released by exocytosis from enterocytes into the lacteals (lymphatic vessels originating 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, chylomicrons pick up apolipoproteins E and C-II.] (For a more detailed description of chylomicron structure and metabolism, see p. 228.)

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 micelles or chylomicrons and directly enter into the blood.] CoA = coenzyme A; AMP = adenosine monophosphate; PP_i = pyrophosphate.



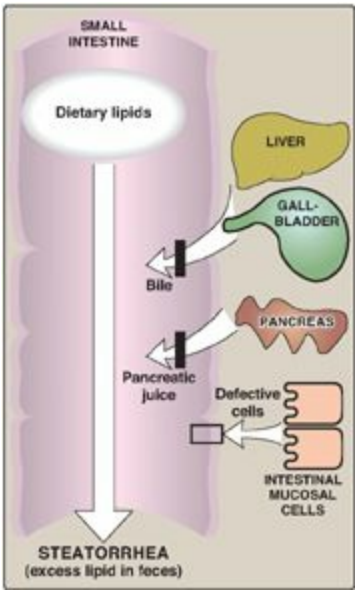
H. Use of dietary lipids by the tissues

TAG contained in chylomicrons is broken down primarily in the capillaries of skeletal and cardiac muscle and adipose tissues. TAG in chylomicrons is degraded to free fatty acids and glycerol by lipoprotein lipase (LPL). This enzyme is synthesized primarily by adipocytes and muscle cells. It is secreted and becomes associated with the luminal surface of endothelial cells in the capillary beds of the peripheral tissues. [Note: Familial LPL deficiency (type I hyperlipoproteinemia) is a rare, autosomal recessive disorder caused by a deficiency of LPL or its coenzyme apolipoprotein C-II (see p. 228). The result is fasting chylomicronemia and hypertriacylglycerolemia.]

1. **Fate of free fatty acids:** The free fatty acids derived from the hydrolysis of TAG may either directly enter adjacent muscle cells or adipocytes or be transported in the blood in association with serum albumin until they are taken up by cells. [Note: Serum albumin is a large glycoprotein secreted by the liver. It transports a number of primarily hydrophobic compounds in the circulation, including free fatty acids and some drugs.] Most cells can oxidize fatty acids to produce energy (see p. 190). Adipocytes can also reesterify free fatty acids to produce TAG molecules, which are stored until the fatty acids are needed by the body (see p. 188).

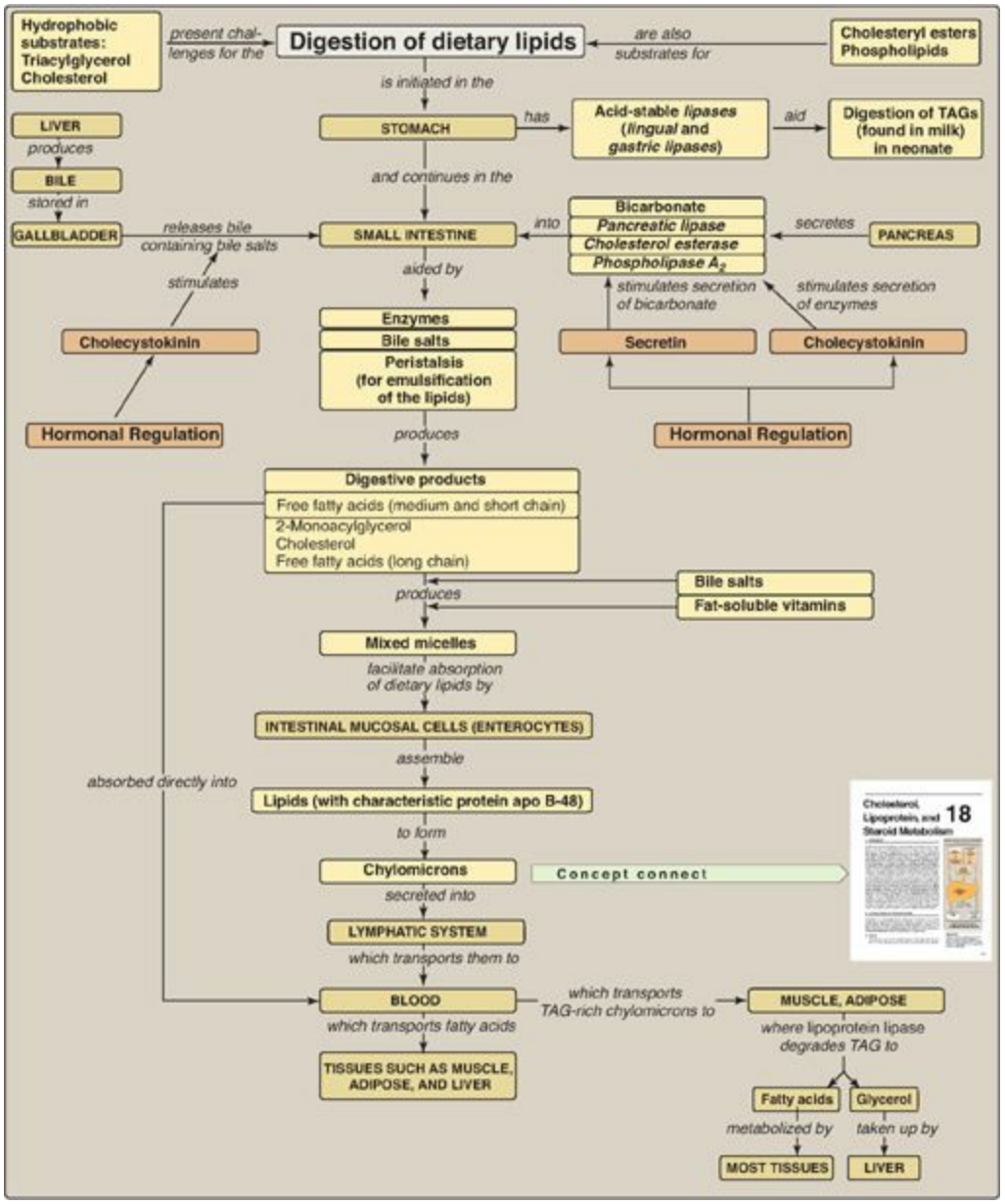
2. **Fate of glycerol:** Glycerol released from TAG is taken up from the blood and phosphorylated by hepatic glycerol kinase to produce glycerol 3-phosphate, which can enter either glycolysis or gluconeogenesis by oxidation to dihydroxyacetone phosphate (see p. 190).

Figure 15.7 Possible causes of steatorrhea.



3. **Fate of the remaining chylomicron components:** 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 (apolipoprotein E is the ligand; see p. 230) and are endocytosed. The intracellular remnants are hydrolyzed to their component parts. Cholesterol and the nitrogenous bases of phospholipids (for example, choline) can be recycled by the body. [Note: If removal of remnants by the liver is decreased due to impaired binding to their receptor, they accumulate in the plasma. This is seen in the rare type III hyperlipoproteinemia (also called familial dysbetalipoproteinemia, see p. 231).]

Figure 15.8 Key concept map for metabolism of dietary lipids. apo = apolipoprotein; TAGs = triacylglycerols.


Hydrophobic substrates: Triacylglycerol Cholesterol
present challenges for the
Digestion of dietary lipids
are also substrates for
Cholesteryl esters Phospholipids
is initiated in the
LIVER
produces
BILE
stored in
GALLBLADDER
releases bile containing bile salts
stimulates
STOMACH
has
Acid-stable lipases (lingual and gastric lipases)
aid
Digestion of TAGs (found in milk) in neonate
and continues in the
SMALL INTESTINE
into
Bicarbonate
Pancreatic lipase
Cholesterol esterase
Phospholipase A2
secretes
PANCREAS
aided by
Enzymes
Bile salts
Peristalsis (for emulsification of the lipids)
stimulates secretion of bicarbonate
stimulates secretion of enzymes
Secretin
Cholecystokinin
Cholecystokinin
Hormonal Regulation
Hormonal Regulation
produces
Digestive products
Free fatty acids (medium and short chain)
2-Monoacylglycerol
Cholesterol
Free fatty acids (long chain)
Bile salts
Fat-soluble vitamins
produces
Mixed micelles
facilitate absorption of dietary lipids by
INTESTINAL MUCOSAL CELLS (ENTEROCYTES)
assemble
absorbed directly into
Lipids (with characteristic protein apo B-48)
to form
Chylomicrons
Concept connect
Cholesterol, Lipoprotein, and Steroid Metabolism 18
secreted into
LYMPHATIC SYSTEM
which transports them to
BLOOD
which transports fatty acids
TISSUES SUCH AS MUSCLE, ADIPOSE, AND LIVER
which transports TAG-rich chylomicrons to
MUSCLE, ADIPOSE
where lipoprotein lipase degrades TAG to
Fatty acids
Glycerol
metabolized by
taken up by
MOST TISSUES
LIVER

III. CHAPTER SUMMARY

The digestion of **dietary lipids** begins in the **stomach** and continues in the **small intestine** (Figure 15.8). The **hydrophobic nature** of lipids requires that the dietary lipids, particularly those that contain long-chain length fatty acids (LCFAs), be **emulsified** for efficient degradation. Triacylglycerols (TAG) obtained from milk contain **short-** to **medium-chain length fatty acids** that can be degraded in the **stomach** by the acid lipases (**lingual lipase** and **gastric lipase**). Cholesteryl esters (CEs), phospholipids (PLs), and TAG containing LCFAs are degraded in the **small intestine** by enzymes secreted by the **pancreas**. The most important of these enzymes are **pancreatic lipase**, **phospholipase A_2**, and **cholesterol esterase**. The dietary lipids are **emulsified** in the small intestine using **peristaltic action** and **bile salts**, which serve as detergents. The primary products resulting from enzymatic degradation of dietary lipid are **2-monoacylglycerol**, unesterified **cholesterol**, and **free fatty acids**. These compounds, plus the fat-soluble vitamins, form **mixed micelles** that facilitate the absorption of dietary lipids by **intestinal mucosal cells** (**enterocytes**). These cells resynthesize TAG, CE, and PL using LCFAs and also synthesize protein (**apolipoprotein B-48**), all of which are then assembled with the fat-soluble vitamins into lipoprotein particles called **chylomicrons**. Short- and medium-chain fatty acids enter blood directly. Chylomicrons are released into the **lymph**, which carries them to the **blood**, where their lipid core is degraded by **lipoprotein lipase** (with apolipoprotein C-II as the coenzyme) in muscle and adipose tissues. Thus, dietary lipids are made available to the peripheral tissues. Problems with fat absorption cause **steatorrhea**. A deficiency in the ability to degrade chylomicron components, or remove their remnants after TAG has been removed, results in accumulation of these particles in blood.

Study Questions

Choose the ONE best answer.

15.1 Which one of the following statements about the digestion of lipids 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 thickened 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 due to a deficiency of a functional chloride transporter, have thickened secretions that impede 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 the absorption of lipids 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 to 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 fewer than 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 (TAGs) in chylomicrons are degraded to fatty acids and glycerol by lipoprotein lipase on the endothelial surface of capillaries in muscle and adipose, thus providing a source of fatty acids to these tissues for degradation or storage and providing glycerol for hepatic metabolism. In the duodenum, TAG are degraded to one 2-monoacyl-glycerol + two free fatty acids that get absorbed. Medium- and short-chain fatty acids 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.

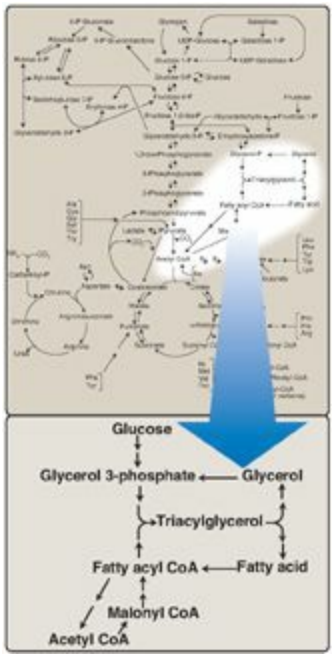
Fatty Acid, Ketone Body, and Triacylglycerol Metabolism

16

I. OVERVIEW

Fatty acids exist "free" in the body (that is, they are unesterified) and as fatty acyl esters in more complex molecules such as triacylglycerols (TAGs). Low levels of free fatty acids (FFAs) occur in all tissues, but substantial amounts can sometimes be found in the plasma, particularly during fasting. Plasma FFAs (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). FFAs can be oxidized by many tissues, particularly liver and muscle, to provide energy and, in liver, to provide the substrate for ketone body synthesis. Fatty acids are also structural components of membrane lipids, such as phospholipids and glycolipids (see p. 201). Fatty acids attached to certain proteins enhance the ability of those proteins to associate with membranes (see p. 206). Fatty acids are also precursors of the hormone-like prostaglandins (see p. 213). Esterified fatty acids, in the form of TAGs 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.

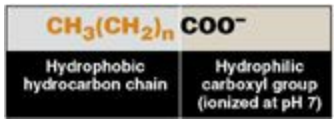
Figure 16.1 Triacylglycerol synthesis and degradation. CoA = coenzyme A.



II. STRUCTURE OF FATTY ACIDS

A fatty acid consists of a hydrophobic hydrocarbon chain with a terminal carboxyl group that has a pK_a of about 4.8 (Figure 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 fatty acids (LCFAs), 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 p. 227). Unesterified (free) fatty acids are transported in the circulation in association with albumin, the most abundant protein in serum.

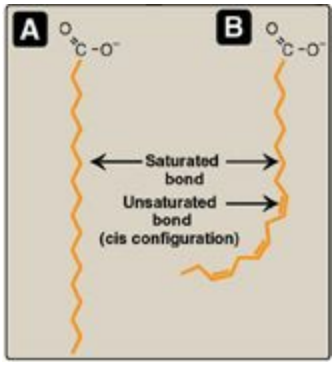
Figure 16.2 Structure of a fatty acid.



A. Saturation of fatty acids

Fatty acid chains may contain no double bonds (that is, be saturated) or contain one or more double bonds (that is, 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. (See p. 363 for a discussion of the dietary occurrence of cis and trans unsaturated fatty acids.) The introduction of a cis double bond causes the fatty acid to bend or "kink" at that position (Figure 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 LCFAs, the presence of double bonds in some fatty acids helps maintain the fluid nature of those lipids.]

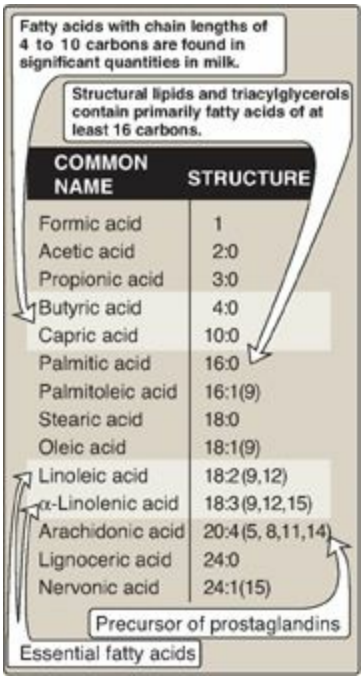
Figure 16.3 A saturated (A) and an unsaturated (B) fatty acid. Orange denotes hydrophobic portions of the molecules. [Note: Cis double bonds cause a fatty acid to "kink."].



B. Chain lengths of fatty acids and positions of double bonds

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 (over 22 carbons) being found in the brain. The carbon atoms are numbered, beginning with the carboxyl 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 4 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 denoted relative to the ω (or methyl) end of the chain. Arachidonic acid is referred to as an ω-6 fatty acid acid (also an n-6 fatty acid, Figure 16.5A) because the terminal double bond is six bonds in from the ω end (Figure 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. (See p. 363 for a discussion of the nutritional significance of ω-3 and ω-6 fatty acids.)

Figure 16.4 Some fatty acids of physiologic importance. [Note: If a fatty acid has 2–4 carbons, it is considered short; if 6–12, medium; if 14–20, long; and if 22 or more, very long.].



C. Essential fatty acids

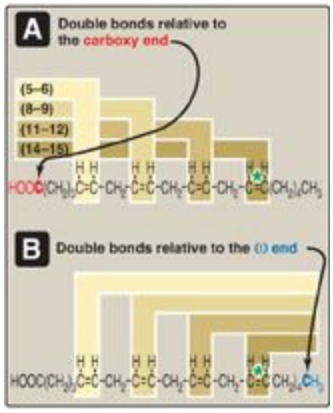
Linoleic acid, the precursor of ω-6 arachidonic acid, which is the substrate for prostaglandin synthesis (see p. 213), and α-linolenic acid, the precursor of ω-3 fatty acids that are important for growth and development, are dietary essentials in humans because we lack the enzymes needed to synthesize them. Plants provide us with these essential fatty acids. [Note: Arachidonic acid becomes essential if linoleic acid is deficient in the diet.]

> 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. 206).

III. DE NOVO SYNTHESIS OF FATTY ACIDS

A large proportion of the fatty acids used by the body is supplied by the diet. Carbohydrates and protein obtained from the diet in excess of the body's needs for these compounds can be converted to fatty acids, which are stored as TAGs. (See p. 321 for a discussion of the metabolism of dietary nutrients in the well-fed state.) In adult humans, fatty acid synthesis occurs primarily in the liver and lactating mammary glands and, to a lesser extent, in adipose tissue. This cytosolic process incorporates carbons from acetyl coenzyme A (CoA) into the growing fatty acid chain, using adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide phosphate (NADPH).

Figure 16.5 Arachidonic acid, illustrating the position of the double bonds. Arachidonic acid, 20:4(5,8,11,14) is an n-6 fatty acid because the double bond furthest from the carboxy end (carbon 1) is 14 carbons from that end: 20 - 14 = 6. It is also referred to as an ω-6 fatty acid because the terminal double bond is six bonds in from the ω end. Thus, the "ω" and "n" designations are equivalent (see *).



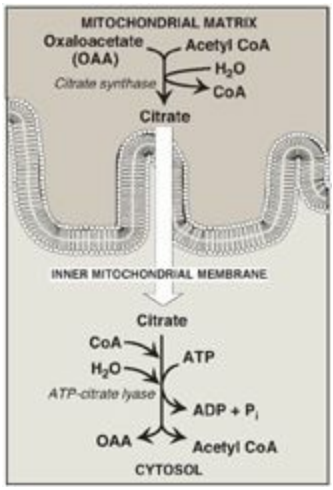
A. Production of cytosolic acetyl coenzyme A

The first step in de novo 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 p. 109) and by the catabolism of certain amino acids (see p. 266). The CoA portion of acetyl CoA, however, 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 (Figure 16.6). [Note: The translocation of citrate to the cytosol occurs when the mitochondrial citrate concentration is high. This is observed when isocitrate dehydrogenase of the citric acid cycle is inhibited by the presence of large amounts of ATP, causing citrate and isocitrate to accumulate (see p. 112). 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.]

B. Carboxylation of acetyl coenzyme A to malonyl coenzyme A

The energy for the carbon-to-carbon condensations in fatty acid synthesis is supplied by the process of carboxylation followed by decarboxylation of acyl groups in the cytosol. The carboxylation of acetyl CoA to form malonyl CoA is catalyzed by acetyl CoA carboxylase (ACC) (Figure 16.7), and requires CO_2 and ATP. The coenzyme is the vitamin biotin, which is covalently bound to a lysyl residue of the carboxylase (see Figure 28.16, p. 381). ACC carboxylates the bound biotin, which transfers the activated carboxyl group to acetyl CoA.

Figure 16.6 Production of cytosolic acetyl coenzyme A (CoA). Citrate is transported by the tricarboxylate transporter system. ADP = adenosine monophosphate; P_i = inorganic phosphate.

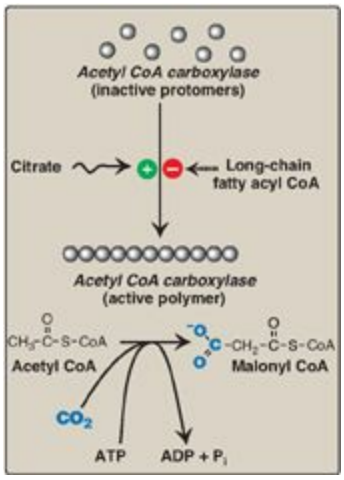


1. **Short-term regulation of acetyl coenzyme A carboxylase:** This carboxylation is both the rate-limiting and the regulated step in fatty acid synthesis (see Figure 16.7). The inactive form of ACC is a protomer. The enzyme undergoes allosteric activation by citrate, which causes protomers to polymerize, and allosteric inactivation by long-chain fatty acyl 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 allosterically activated by AMP and covalently activated by phosphorylation via several kinases. At least one of these AMPK kinases is activated by cAMP-dependent protein kinase A (PKA). Thus, in the presence of counterregulatory hormones, such as epinephrine and glucagon, ACC is phosphorylated and, thereby, inactivated (Figure 16.8). In the presence of insulin, ACC is dephosphorylated and, thereby, activated. [Note: This is analogous to the regulation of glycogen synthase (see p. 131).]

2. **Long-term regulation of acetyl coenzyme A carboxylase:** Prolonged consumption of a diet containing excess calories (particularly high-calorie, high-carbohydrate diets) causes an increase in ACC synthesis, thereby increasing fatty acid synthesis. Conversely, a low-calorie or a high-fat diet causes a reduction in fatty acid synthesis by decreasing ACC synthesis. [Note: Synthesis of the carboxylase is

upregulated by insulin via a sterol regulatory element–binding protein, SREBP-1. The function and regulation of SREBPs are described on p. 222. Fatty acid synthase (see below) is similarly regulated by diet and SREBP-1.] Metformin, used in the treatment of type 2 diabetes, lowers serum TAG through activation of AMPK, resulting in inhibition of ACC activity (by phosphorylation) and inhibition of ACC and fatty acid synthase expression (by decreasing SREBP-1). Metformin also lowers blood glucose by increasing AMPK-mediated uptake of glucose by muscle.

Figure 16.7 Allosteric regulation of malonyl coenzyme A (CoA) synthesis by acetyl CoA carboxylase. The carboxyl group contributed by dissolved CO_2 is shown in blue. P_i = inorganic phosphate; ADP = adenosine diphosphate.



C. Fatty acid synthase: a multifunctional enzyme in eukaryotes

The remaining series of reactions of fatty acid synthesis in eukaryotes is catalyzed by the multifunctional, dimeric enzyme, fatty acid synthase (FAS). Each FAS monomer is a multicatalytic polypeptide with seven different enzymic domains plus a domain that covalently binds a molecule of 4′-phosphopantetheine. [Note: 4′-Phosphopantetheine, a derivative of the vitamin pantothenic acid (see p. 381), carries acyl units on its terminal thiol (–SH) group during fatty acid synthesis. It also is a component of CoA.] In prokaryotes, FAS is a multienzyme complex, and the 4′-phosphopantetheine domain is a separate protein, referred to as the acyl carrier protein (ACP). ACP is used to refer to the phosphopantetheine-containing domain of eukaryotic FAS. The reaction numbers in brackets below refer to Figure 16.9.

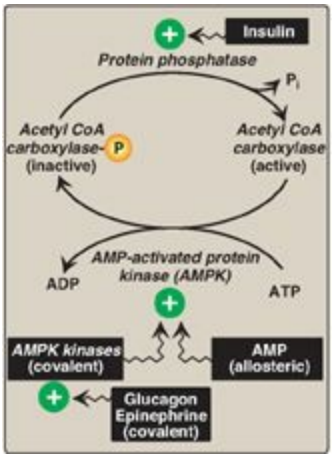
[1] An acetyl group is transferred from acetyl CoA to the –SH group of the ACP. Domain: Acetyl CoA-ACP acetyltransacylase.

[2] Next, this two-carbon fragment is transferred to a temporary holding site, the thiol group of a cysteine residue on the enzyme.

[3] The now-vacant ACP accepts a three-carbon malonyl group from malonyl CoA. Domain: Malonyl CoA-ACP transacylase.

[4] The acetyl group on the cysteine residue condenses with the malonyl group on ACP as the CO_2 originally added by acetyl CoA carboxylase is released. The result is a four-carbon unit attached to the ACP domain. The loss of free energy from the decarboxylation drives the reaction. Domain: 3-Ketoacyl-ACP synthase, also known as "condensing enzyme."

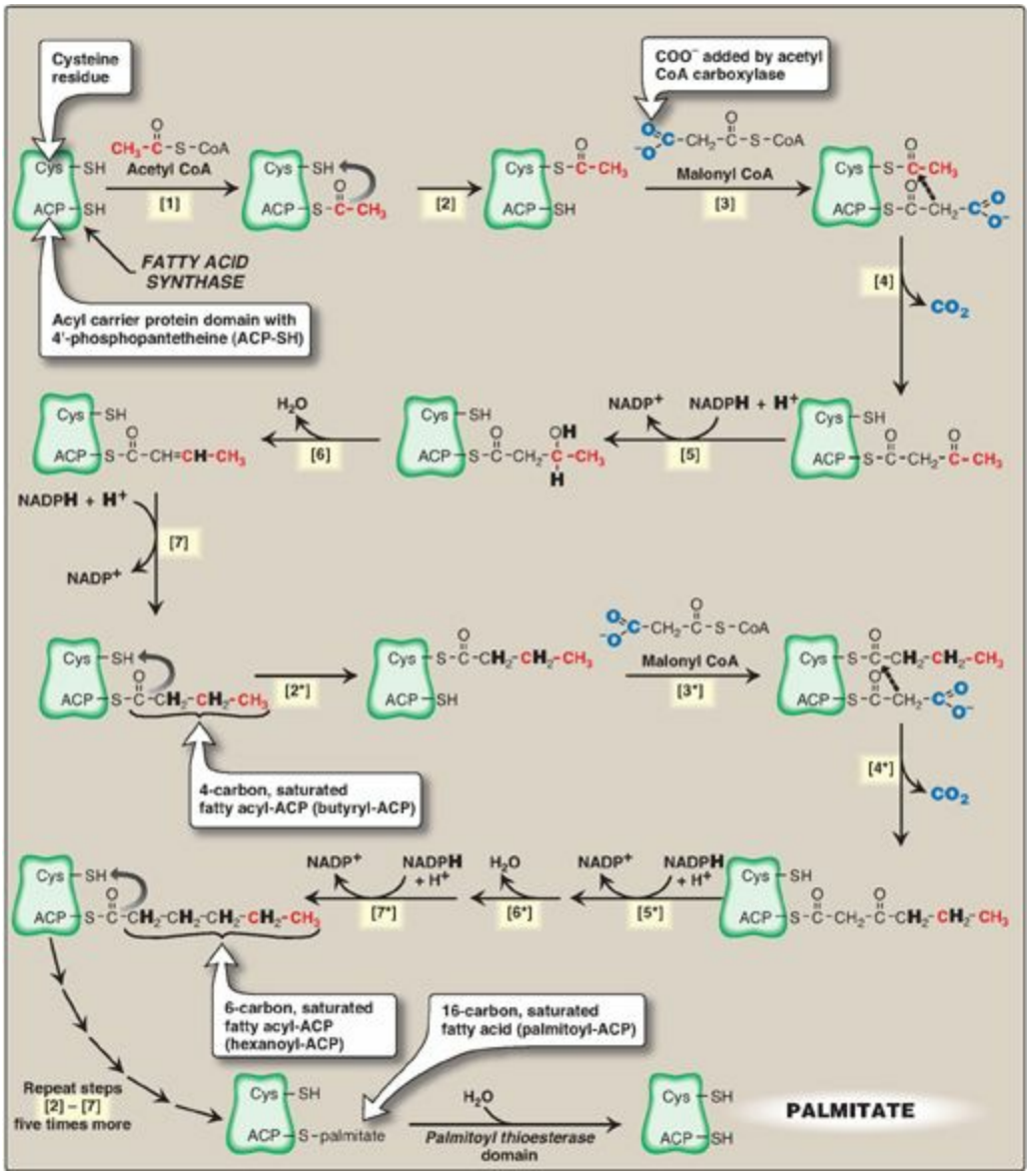
Figure 16.8 Covalent regulation (phosphorylation) of acetyl CoA carboxylase by AMPK, which itself is regulated both covalently and allosterically. CoA = coenzyme A; ADP = adenosine diphosphate; P = phosphate; P_i = inorganic phosphate; AMP = adenosine monophosphate.



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. Domain: 3-Ketoacyl-ACP reductase.

Figure 16.9 Synthesis of palmitate (16:0) by multifunctional fatty acid synthase (FAS). [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.] Cys = cysteine; ACP = acyl carrier protein domain; NADP(H) = nicotinamide adenine dinucleotide phosphate.



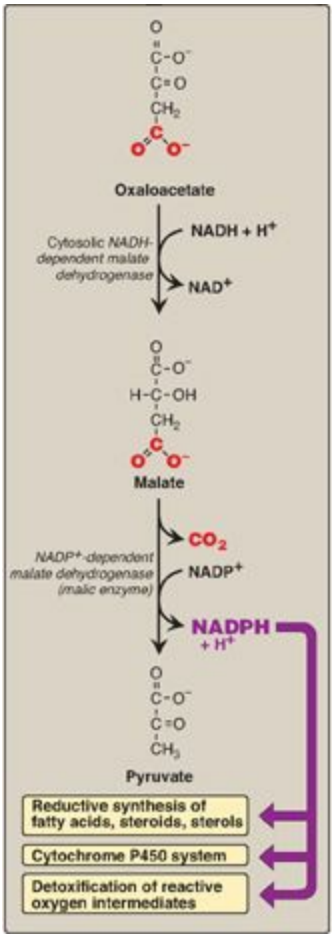
[6] A molecule of water is removed, creating a double bond between carbons 2 and 3 (the α- and β-carbons). Domain: 3-Hydroxyacyl-ACP dehydratase.

[7] The double bond is reduced. Domain: Enoyl-ACP reductase.

The result of these seven steps is production of a four-carbon compound (butyryl) whose three terminal carbons are fully saturated, and which remains attached to the ACP domain. These seven steps are repeated, beginning with the transfer of the butyryl chain from the ACP to the cysteine residue [2*], the attachment of a molecule of malonate to the ACP [3*], and the condensation of the two molecules 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 five more times, each time incorporating a two-carbon unit (derived from malonyl CoA) into the growing fatty acid chain at the carboxyl end. When the fatty acid reaches a length of 16 carbons, the synthetic process is terminated with palmitoyl-S-ACP. [Note: Shorter-length fatty acids are important end products in the lactating mammary gland.] Palmitoyl thioesterase, 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, which are found at the methyl (ω) end of the fatty acid. This underscores the rate-limiting nature of the ACC reaction.]

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.



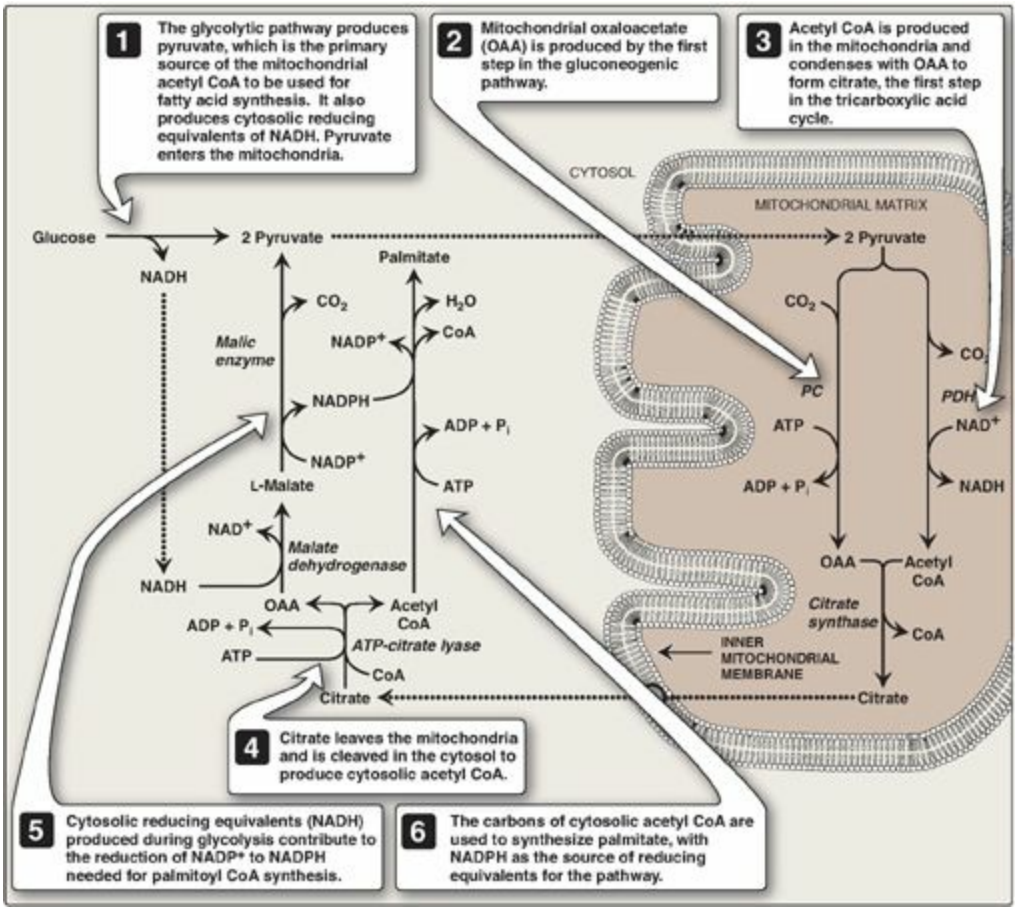
D. Major sources of the reductant required for fatty acid synthesis

The pentose phosphate pathway (see p. 145) is a major supplier of NADPH, the reducatant required for fatty acid synthesis. Two NADPH are produced for each molecule of glucose 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 Figure 16.10). One source of the cytosolic NADH required for this reaction is that produced during glycolysis (see p. 101). OAA, in turn, can arise from citrate. Recall from Figure 16.6 that citrate, formed from OAA and acetyl CoA by citrate synthase, was shown to move from the mitochondria into the cytosol, where it is cleaved into acetyl CoA and OAA by ATP-citrate lyase.] A summary of the interrelationship between glucose metabolism and palmitate synthesis is shown in Figure 16.11.

E. Further elongation of fatty acid chains

Although palmitate, a 16-carbon, fully saturated LCFA (16:0), is the primary end product of fatty acid synthase activity, it can be further elongated by the addition of two-carbon units to the carboxylate end 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 ([VLCFAs] 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; PC = pyruvate carboxylase; PDH = pyruvate dehydrogenase.



F. Desaturation of fatty acid chains

Enzymes (desaturases) also present in the SER are responsible for desaturating LCFAs (that is, adding cis double bonds). The desaturation reactions require O_2, NADH, cytochrome b_5, and its 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.

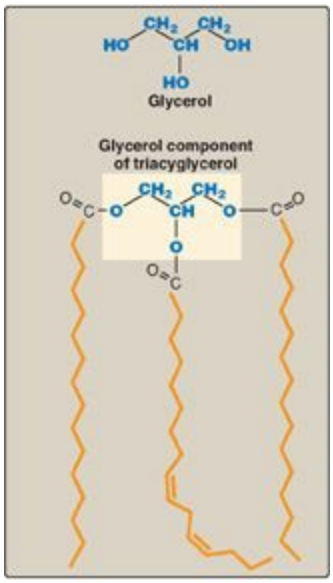
Humans have carbon 9, 6, 5, and 4 desaturases but lack the ability to introduce double bonds from carbon 10 to the ω end of the chain. This is the basis for the nutritional essentiality of the polyunsaturated acids ω-6 linoleic and ω-3 linolenic.

G. Storage of fatty acids as components of triacylglycerols

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: If a species of acylglycerol is solid at room temperature, it is called a fat, whereas if it is liquid, it is called an oil.]

1. **Structure:** 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.

Figure 16.12 A triacylglycerol with an unsaturated fatty acid on carbon 2. Orange denotes the hydrophobic portions of the molecule.

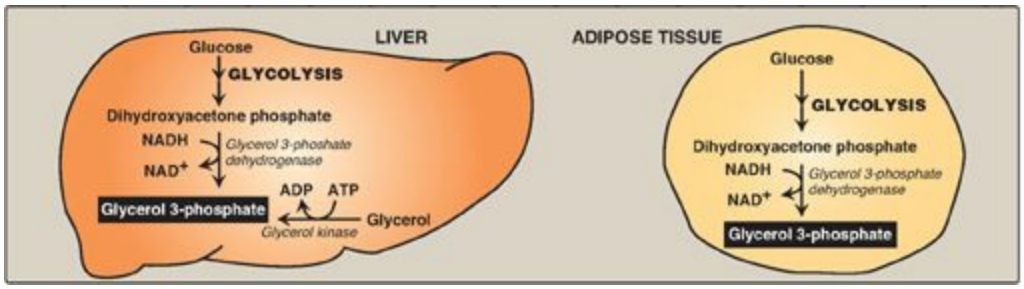


2. **Storage:** Because TAGs are only slightly soluble in water and cannot form stable micelles by themselves, they coalesce within white adipocytes to form large oily droplets that are nearly anhydrous. These cytosolic lipid droplets are the major energy reserve of the body. [Note: TAGs stored in brown adipocytes serve as a source of heat through nonshivering thermogenesis (see p. 78).]

3. **Synthesis of glycerol 3-phosphate:** Glycerol 3-phosphate is the initial acceptor of fatty acids during TAG synthesis. There are two pathways for its production

(Figure 16.13). In both liver (the primary site of TAG synthesis) and adipose tissue, glycerol 3-phosphate can be produced from glucose, using first the reactions of the glycolytic pathway to produce dihydroxyacetone phosphate ([DHAP], see p. 101). 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 phosphate (see Figure 16.13). [Note: The glucose transporter in adipocytes (GLUT-4) is insulin dependent (see p. 312). Thus, when plasma glucose (and, therefore, plasma insulin) 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.



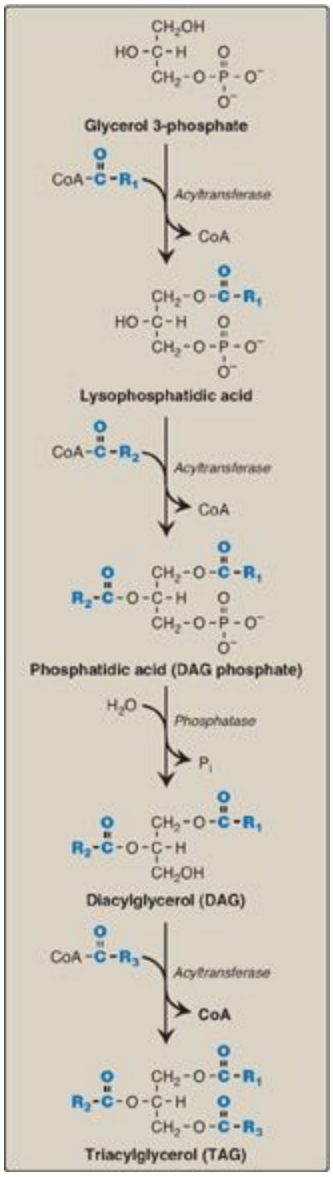
4. **Activation of a free fatty acid:** A fatty acid must be converted to its activated form (bound to CoA) before it can participate in metabolic processes such as TAG synthesis. This reaction, illustrated in Figure 15.6, is catalyzed by a family of fatty acyl CoA synthetases (thiokinases).

5. **Synthesis of triacylglycerol from glycerol 3-phosphate and fatty acyl coenzyme As:** This pathway involves four reactions, shown in Figure 16.14. These include the sequential addition of two fatty acids from fatty acyl CoAs, the removal of phosphate, and the addition of the third fatty acid.

H. Different fates of triacylglycerol in liver and adipose tissue

In WAT, TAG is stored in a nearly anhydrous form as fat droplets in the cytosol of the cells. It serves as "depot fat," ready for mobilization when the body requires it for fuel. 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 (VLDLs). Nascent VLDLs 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.

Figure 16.14 Synthesis of TAG. R_1-R_3 = activated fatty acids. CoA = coenzyme A; P_i = inorganic phosphate.


Glycerol 3-phosphate
Acyltransferase
CoA
Lysophosphatidic acid
Acyltransferase
CoA
Phosphatidic acid (DAG phosphate)
Phosphatase
Diacylglycerol (DAG)
Acyltransferase
CoA
Triacylglycerol (TAG)

IV. MOBILIZATION OF STORED FATS AND OXIDATION OF FATTY ACIDS

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 Figure 27.5 on p. 359).

A. Release of fatty acids from fat

The mobilization of stored fat requires the hydrolytic release of fatty acids and glycerol from their TAG form. This process of lipolysis is achieved by 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.

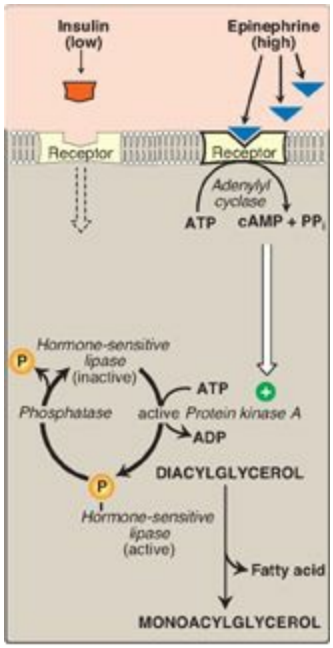
1. **Regulation of hormone-sensitive lipase:** HSL is active when phosphorylated by PKA, a 3′,5′-cyclic AMP(cAMP)–dependent protein kinase. cAMP is produced in the adipocyte when catecholamines (such as epinephrine) bind to cell membrane β-adrenergic receptors and activate adenylyl cyclase (Figure 16.15). The process is similar to that of the activation of glycogen phosphorylase (see Figure 11.9). [Note: Because ACC is inhibited by hormone-directed phosphorylation, when the cAMP-mediated cascade is activated (see Figure 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. [Note: Fat droplets are coated by a protein (perilipin) that limits access of HSL. Phosphorylation of perilipin by PKA allows translocation and binding of HSL to the droplet.]

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, where it can be phosphorylated. 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 free (unesterified) fatty acids move through the cell membrane of the adipocyte and bind to plasma albumin. They are transported to the tissues, enter cells, get activated to their CoA derivatives, and are oxidized for energy in mitochondria. Regardless of their levels, plasma FFAs cannot be used for fuel by red blood cells (RBCs), which have no mitochondria. Brain, too, does not use fatty acids for energy, 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 phosphorylated glycerol 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 reduces plasma FFAs, molecules associated with insulin resistance in type 2 diabetes and obesity (see p. 343).]

Figure 16.15 Hormonal regulation of fat 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.



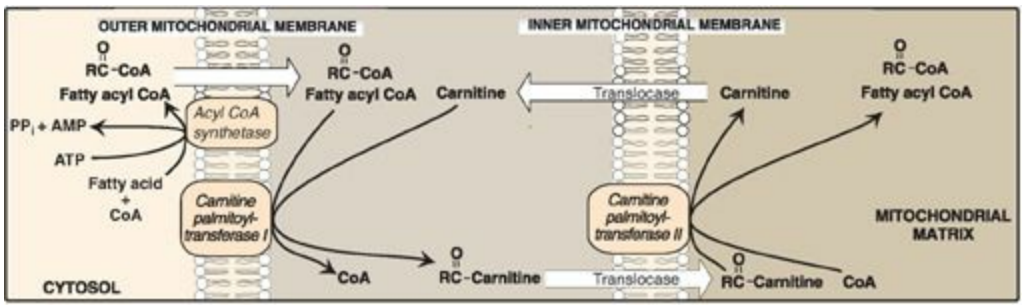
B. β-Oxidation of fatty acids

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 flavin adenine dinucleotide ($FADH_2$).

1. **Transport of long-chain fatty acids into mitochondria:** 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" (Figure 16.16).

Figure 16.16 Carnitine shuttle. The net effect is that a long-chain fatty acyl coenzyme A

(CoA) is transported from the outside to the inside of mitochondria. AMP = adenosine monophosphate; PP_i = pyrophosphate.



a. Steps in translocation: 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 II (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. Inhibitor of the carnitine shuttle: 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, although it does not synthesize fatty acids, contains the mitochondrial isoform of ACC (ACC2), allowing muscle to regulate β-oxidation.] Fatty acid oxidation is also regulated by the acetyl CoA to CoA ratio: as the ratio increases, the CoA-requiring thiolase reaction decreases (Figure 16.17).

c. Sources of carnitine: Carnitine can be obtained from the diet, where it is found primarily in meat products. Carnitine can also be synthesized from the amino acids lysine and methionine by an enzymatic pathway found in the liver and kidney but not in skeletal or heart 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 about 97% of all carnitine in the body.]

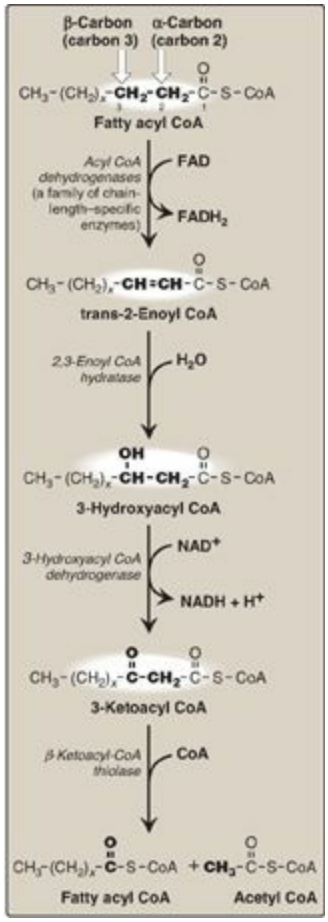
d. Carnitine deficiencies: Such deficiencies result in a decreased ability of tissues to use LCFAs as a fuel. Primary carnitine deficiency is caused by defects in a membrane transporter that prevent uptake of carnitine by cardiac and skeletal muscle and kidney. Treatment includes carnitine supplementation. 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 LCFAs 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 TAGs.]

2. **Entry of short- and medium-chain fatty acids into the mitochondria:** Fatty acids shorter than 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, it is not subject to inhibition by malonyl CoA.]

3. **Reactions of β-oxidation:** 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 chain by two carbons at the carboxylate end. The steps include an oxidation that produces $FADH_2$, a hydration step, a second oxidation that produces NADH, and a thiolytic cleavage that releases a molecule of acetyl CoA. Each step is catalyzed by enzymes with chain-length specificity. 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 acetyl CoA can be oxidized or used in hepatic ketogenesis (see below). The reduced coenzymes are oxidized by the electron transport chain. The final thiolytic cleavage produces two acetyl groups. [Note: Acetyl CoA is a positive allosteric effector of pyruvate carboxylase (see p. 119), thus linking fatty acid oxidation and gluconeogenesis.]

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.

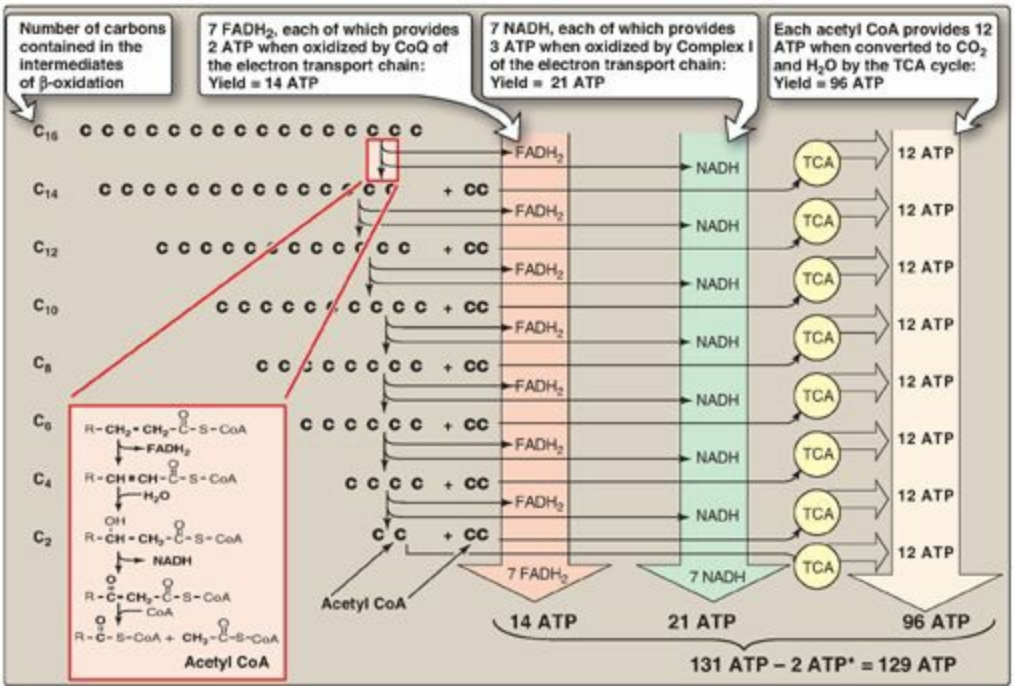


4. **Energy yield from fatty acid oxidation:** The energy yield from the β-oxidation pathway is high. For example, the oxidation of a molecule of palmitoyl CoA to CO_2 and H_2O produces 8 acetyl CoA, 7 NADH, and 7 $FADH_2$, from which 131 ATP can be generated. However, activation of the fatty acid requires 2 ATP. Therefore, the net yield from palmitate is 129 ATP (Figure 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.

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 VLCFAs. Medium-chain fatty acyl CoA dehydrogenase (MCAD) deficiency, an autosomal-recessive disorder, is one of the most common inborn errors of metabolism and the most common inborn error of fatty acid oxidation, being found in 1:14,000 births worldwide, with a higher incidence in Caucasians of Northern European descent. It results in decreased ability to oxidize fatty acids with six to ten carbons (which accumulate and can be measured in urine), severe hypoglycemia (because the tissues must increase their reliance on glucose), and hypoketonemia (because of decreased production of acetyl CoA). See below. Treatment includes avoidance of fasting. MCAD deficiency has been identified as the cause of some cases originally reported as sudden infant death syndrome or Reye syndrome.

Figure 16.18 Summary of the energy yield from the oxidation of palmitoyl coenzyme A

(CoA) (16 carbons). *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.



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 compound, propionyl CoA, is metabolized by a three-step pathway (Figure 16.20). [Note: Propionyl CoA is also produced during the metabolism of certain amino acids (see Figure 20.10).]

   a. **Synthesis of D-methylmalonyl coenzyme A:** First, propionyl CoA is carboxylated, forming D-methylmalonyl coenzyme A. The enzyme propionyl CoA carboxylase has an absolute requirement for the coenzyme biotin, as do ACC and most other carboxylases.

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.

	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	Long-chain fatty acyl 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

b. Formation of L-methylmalonyl coenzyme A: Next, the D-isomer is converted to the L-form by the enzyme, methylmalonyl CoA racemase.

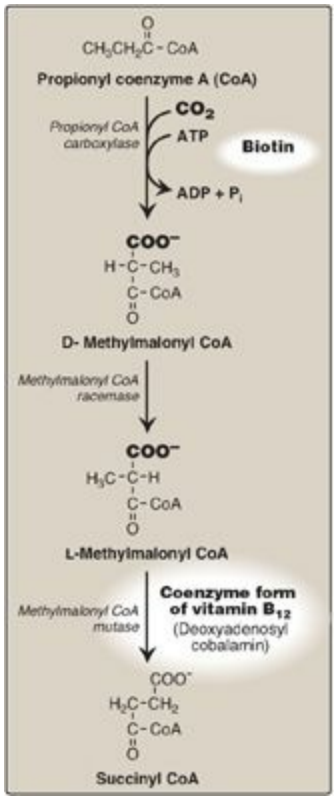
c. Synthesis of succinyl coenzyme A: Finally, the carbons of L-methylmalonyl CoA are rearranged, forming succinyl CoA, which can enter the tricarboxylic acid (TCA) cycle (see p. 109). [Note: This is the only example of a glucogenic precursor generated from fatty acid oxidation.] The enzyme methylmalonyl 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 p. 375). [Note: In patients with vitamin B_{12} deficiency, both propionate and methylmalonate are excreted in the urine. Two types of heritable methylmalonic acidemia and aciduria have been described: one in which the mutase is missing or deficient (or has reduced affinity for the coenzyme), and one in which the patient is unable to convert vitamin B_{12} into its coenzyme form. Either type results in metabolic acidosis and neurologic manifestations.]

7. Oxidation of unsaturated fatty acids: The oxidation of unsaturated fatty acids provides less energy than that of saturated fatty acids because unsaturated fatty acids are less highly reduced, and, therefore, fewer reducing equivalents can be produced from these structures. Oxidation of monounsaturated fatty acids, 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 as a substrate by the enoyl CoA hydratase. Oxidation of polyunsaturated fatty acids, such as 18:2(9,12) (linoleic acid), requires an NADPH-dependent 2,4-dienoyl CoA reductase in addition to the isomerase.

8. β-Oxidation in the peroxisome: VLCFAs of more than 22 carbons undergo a preliminary β-oxidation in peroxisomes, because peroxisomes 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 molecular oxygen, which is reduced to H_2O_2. Therefore, no ATP is generated by this step. The H_2O_2 is reduced to H_2O by catalase (see p. 148). [Note: Genetic defects in the ability either to target matrix proteins to peroxisomes (resulting in Zellweger syndrome, a peroxisomal biogenesis disorder) or to transport VLCFAs across the peroxisomal membrane (resulting in X-linked adrenoleukodystrophy), lead to accumulation of VLCFAs in the blood and tissues.]

Figure 16.20 Metabolism of propionyl CoA. ADP = adenosine diphosphate; P_i = inorganic phosphate.

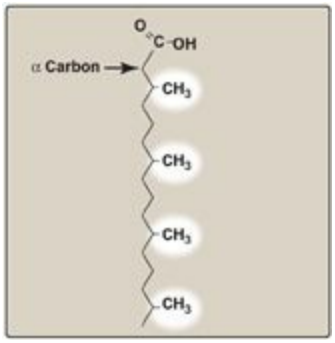


C. Peroxisomal α-oxidation of fatty acids

Branched-chain phytanic acid: This product of chlorophyll metabolism is not a substrate for acyl CoA dehydrogenase because of the methyl group on its β-carbon (Figure 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, 19-carbon 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 ER, its upregulation is seen with conditions such as MCAD deficiency that limit fatty acid β-oxidation.]

Figure 16.21 Phytanic acid, a branched-chain fatty acid.



V. KETONE BODIES: AN ALTERNATE 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, Figure 16.22). [Note: The two functional ketone bodies are actually 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 1) they 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) they are produced in the liver during periods when the amount of acetyl CoA present exceeds the oxidative capacity of the liver; and 3) they are used in proportion to their concentration in the blood by extrahepatic tissues, such as the skeletal and cardiac muscle, intestinal mucosa, and 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 p. 332). [Note: Disorders of fatty acid oxidation present with the general picture of hypoketosis (due to decreased availability of acetyl CoA) and hypoglycemia (due to increased reliance on glucose for energy.]

A. Synthesis of ketone bodies by the liver: ketogenesis

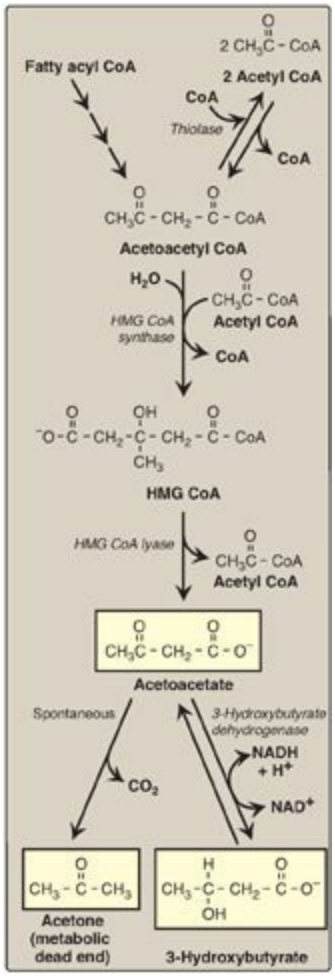
During a fast, the liver is flooded with fatty acids mobilized from adipose tissue. The resulting elevated hepatic acetyl CoA produced by fatty acid oxidation inhibits pyruvate dehydrogenase (see p. 111), and activates pyruvate carboxylase (see p. 119). The OAA produced is used by the liver for gluconeogenesis rather than for the TCA cycle. Therefore, acetyl CoA is channeled into ketone body synthesis. Additionally, fatty acid oxidation decreases the NAD^+ to NADH ratio, and the rise in NADH shifts OAA to malate (see p. 113). This also pushes acetyl CoA into ketogenesis (Figure 16.24).] [Note: Acetyl CoA for ketogenesis is also generated by the catabolism of ketogenic amino acids (see p. 262).]

1. **Synthesis of 3-hydroxy-3-methylglutaryl coenzyme A:** The first step, formation of acetoacetyl CoA, occurs by reversal of the thiolase reaction of fatty acid oxidation (see Figure 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 (see p. 220). The two pathways are separated by location in, and conditions of, the cell.]

2. **Synthesis of the ketone bodies:** 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 hydrogen donor.

Acetoacetate can also spontaneously decarboxylate in the blood to form acetone, a volatile, biologically nonmetabolized compound that can be released 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. [Note: The generation of free CoA during ketogenesis allows fatty acid oxidation to continue.]

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.

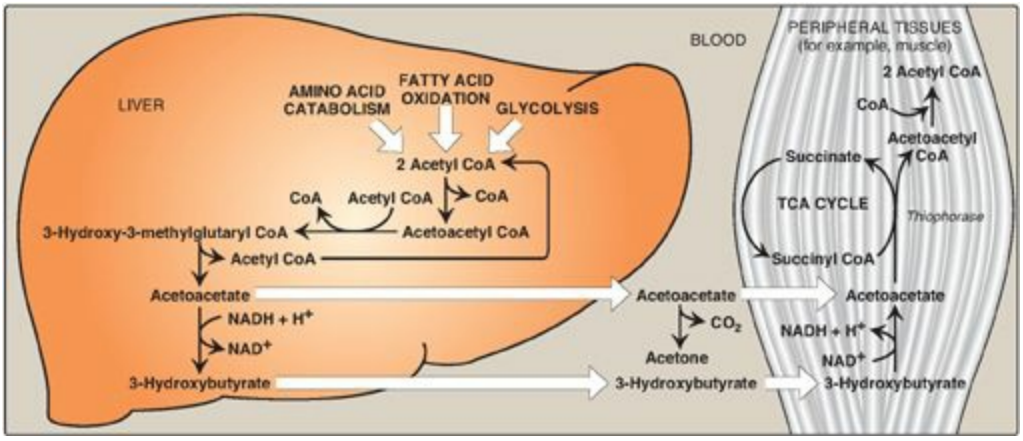


B. Use of ketone bodies by the peripheral tissues: ketolysis

Although the liver constantly synthesizes low levels of ketone bodies, their production becomes much more significant 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 (Figure 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 conversion to two acetyl CoAs. This pulls the reaction forward. Extrahepatic tissues, including the brain but excluding cells lacking mitochondria (for example, 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.

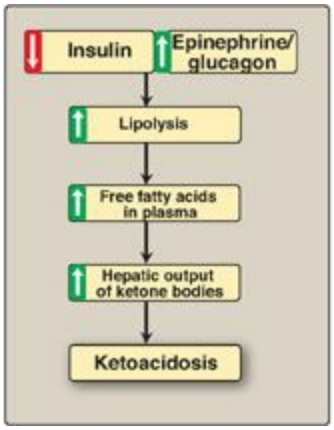
Figure 16.23 Ketone body synthesis in the liver and use in peripheral tissues. Liver and red blood cells cannot use ketone bodies. [Note: Thiophorase is also known as succinyl CoA:acetoacetate CoA transferase.] CoA = coenzyme A; NAD(H) = nicotinamide adenine dinucleotide; TCA = tricarboxylic acid.



C. Excessive production of ketone bodies 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. In diabetic individuals with severe ketosis, urinary excretion of the ketone bodies may be as high as 5,000 mg/24 hr, and the blood concentration may reach 90 mg/dl (versus less than 3 mg/dl in normal individuals). A frequent symptom of diabetic ketoacidosis (DKA) is a fruity odor on the breath, which results from increased production of acetone. An elevation of the ketone body concentration in the blood results in acidemia. [Note: The carboxyl group of a ketone body has a pK_a of about 4. Therefore, each ketone body loses a proton (H^+) as it circulates in the blood, which lowers the pH. Also, in DKA, 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 severe acidosis (ketoacidosis).] Ketoacidosis may also be seen in cases of prolonged fasting (see p. 330) and excessive ethanol consumption (see p. 318).

Figure 16.24 Mechanism of diabetic ketoacidosis seen in type 1 diabetes.


Insulin
Epinephrine/ glucagon
Lipolysis
Free fatty acids in plasma
Hepatic output of ketone bodies
Ketoacidosis

VI. CHAPTER SUMMARY

Generally a linear hydrocarbon chain with a terminal carboxyl group, a fatty acid can be **saturated** or **unsaturated**. Two fatty acids are dietary essentials: **linoleic** and **α-linolenic acids**. Fatty acids are synthesized in the cytosol of **liver** following a meal containing excess carbohydrate and protein. Carbons used to synthesize fatty acids are provided by **acetyl coenzyme A** (**CoA**), energy by **ATP**, and reducing equivalents by **nicotinamide adenine dinucleotide phosphate** ([**NADPH**]**;** Figure 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 catalyzed by **biotin**-requiring **acetyl CoA carboxylase** (**ACC**) . **Citrate** allosterically **activates** ACC and **long-chain fatty acyl CoAs inhibit it**. ACC can also be activated by **insulin** and inactivated by **adenosine monophosphate–activated protein kinase** (**AMPK**) in response to **epinephrine**, **glucagon**, or a **rise in AMP**. The remaining steps in fatty acid synthesis are catalyzed by the multifunctional enzyme, **fatty acid synthase**, 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 endoplasmic reticulum (ER). When fatty acids are required for energy, adipocyte **hormone-sensitive lipase** (**activated** by **epinephrine**, and **inhibited** by **insulin**), along with other lipases, degrades stored triacylglycerol (TAG). The fatty acid products are carried by **serum albumin** to the liver and peripheral tissues, where oxidation of the fatty acids provides energy. The **glycerol** backbone of the degraded TAG is carried by the blood to the **liver**, where it serves as an important **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. A translocase and the enzymes **carnitine palmitoyltransferases** (**CPT**) **I** and **II** are required. **CPT-I** is **inhibited** by **malonyl CoA**, thereby preventing simultaneous synthesis and degradation of fatty acids. In the mitochondria, fatty acids are oxidized, producing **acetyl CoA**, **nicotinamide adenine dinucleotide** (**NADH**), and **flavin adenine dinucleotide** (**FADH2**). The first step in the β-oxidation pathway is catalyzed by one of four acyl CoA dehydrogenases, each with chain-length specificity. **Medium-chain fatty acyl CoA dehydrogenase** (**MCAD**) **deficiency** causes a decrease in fatty acid oxidation (process stops once a medium chain fatty acid is produced), resulting in hypoketonemia and severe hypoglycemia. Oxidation of fatty acids with an odd number of carbons proceeds two carbons at a time (producing acetyl CoA) until three-carbon **propionyl CoA** remains. This compound is carboxylated to **methylmalonyl CoA** (by **biotin-requiring propionyl CoA carboxylase**), which is then converted to **succinyl CoA** (a gluconeogenic precursor) by **vitamin B2**-requiring **methylmalonyl CoA mutase**. A genetic error in the mutase or vitamin B_{12} deficiency causes **methylmalonic acidemia** and **aciduria**. β-Oxidation of **very-long-chain fatty acids** and α-oxidation of branched-chain fatty acids occur in

the peroxisome. ω-Oxidation, a minor pathway, occurs in the ER. Liver mitochondria can convert acetyl CoA derived from fatty acid oxidation into the ketone bodies **acetoacetate** and **3-hydroxybutyrate**. Peripheral tissues possessing mitochondria can oxidize 3-hydroxybutyrate to acetoacetate, which can be reconverted to 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 the ability 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**.

Study Questions

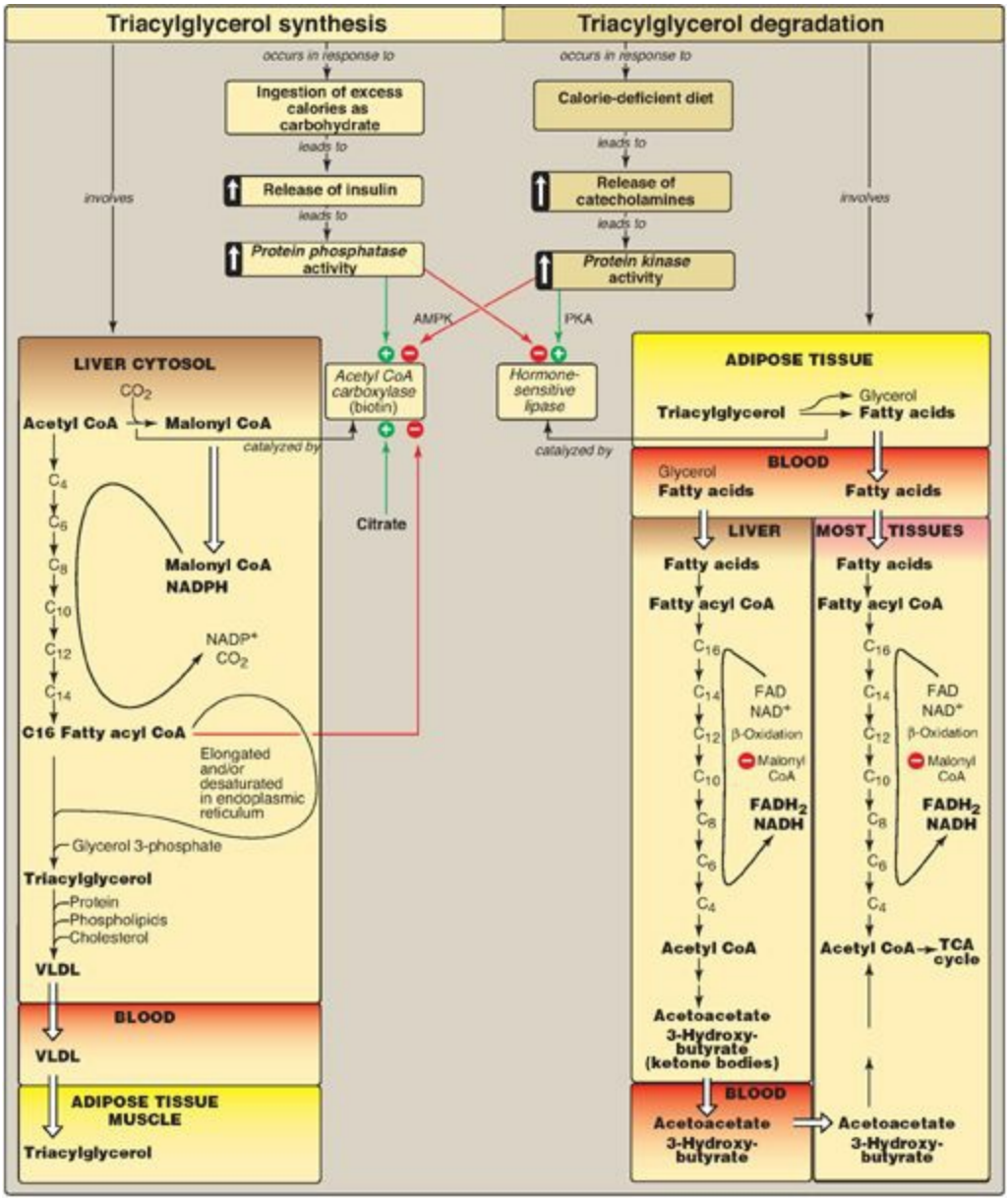
Choose the ONE correct answer.

16.1 When oleic acid, 18:1(9), is desaturated at carbon 6 and then elongated, what is the product?

A. 19:2(7,9)
B. 20:2 (n-6)
C. 20:2(6,9)
D. 20:2(8,11)

Correct answer = D. Fatty acids are elongated in the 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 further away from carbon 1. 20:2(8,11) is an n-9 (ω-9) fatty acid.

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; NAD(H) = nicotinamide adenine dinucleotide; TCA = tricarboxylic acid; VLDL = very-low-density lipoprotein.



16.2 A 4-month-old child is being evaluated for fasting hypoglycemia. Laboratory tests at admission reveal low levels of ketone bodies, free carnitine, and 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 were examined, he would be found 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 boy was hospitalized following a seizure. History revealed that for several days prior, his appetite was decreased due to a "stomach virus." At admission, his blood glucose was 24 mg/dl (age-referenced normal is 60–100). His urine was negative for ketone bodies and positive for a variety of dicarboxylic acids. Blood carnitine levels were normal. A tentative diagnosis of medium-chain fatty acyl coenzyme A dehydrogenase (MCAD) deficiency is made. In patients with MCAD deficiency, the fasting hypoglycemia is a consequence of:

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 less than 12 carbons in length results in decreased production of acetyl coenzyme (CoA), the allosteric activator of pyruvate carboxylase, a gluconeogenic enzyme, and, 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 Explain why with Zellweger syndrome both very-long-chain fatty acids (VLCFAs) and phytanic acid accumulate, whereas with X-linked adrenoleukodystrophy, only VLCFAs accumulate.

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 able to be formed. In X-linked adrenoleukodystrophy, the defect is an inability to transport very-long-chain fatty acids into the peroxisome, but other peroxisomal functions, such as α-oxidation, are normal.

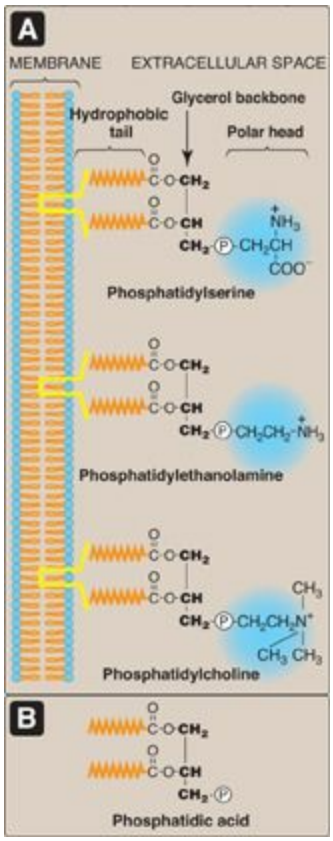
Phospholipid, Glycosphingolipid, and Eicosanoid Metabolism

17

I. OVERVIEW OF PHOSPHOLIPIDS

Phospholipids are polar, ionic compounds composed of an alcohol that is attached by a phosphodiester bond to either diacylglycerol (DAG) or sphingosine. Like fatty acids, phospholipids are amphipathic in nature. That is, each has a hydrophilic head, which is the phosphate group plus whatever alcohol is attached to it (for example, serine, ethanolamine, and choline, highlighted in blue in Figure 17.1A), and a long, hydrophobic tail containing fatty acids or fatty acid–derived hydrocarbons (shown in orange in Figure 17.1A). 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 Figure 17.1A). 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 in the solubilization of cholesterol.

Figure 17.1 A. Structures of some glycerophospholipids. B. Phosphatidic acid. Ⓟ = phosphate (an anion).



II. STRUCTURE OF PHOSPHOLIPIDS

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.

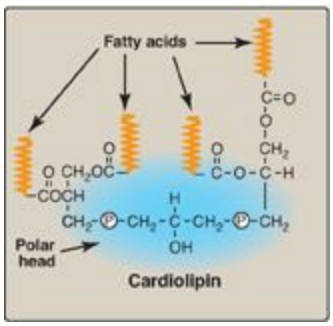
A. Glycerophospholipids

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 (Figure 17.1B). PA is the simplest phosphoglyceride and is the precursor of the other members of this group.

1. **Glycerophospholipids from phosphatidic acid and an alcohol:** The phosphate group on PA can be esterified to another compound containing an alcohol group (see Figure 17.1). For example:

Serine	+ PA →	phosphatidylserine (PS)
Ethanolamine	+ PA →	phosphatidylethanolamine (PE) (cephalin)
Choline	+ PA →	phosphatidylcholine (PC) (lecithin)
Inositol	+ PA →	phosphatidylinositol (PI)
Glycerol	+ PA →	phosphatidylglycerol (PG)

Figure 17.2 Structure of cardiolipin (diphosphatidylglycerol). Ⓟ = phosphate.

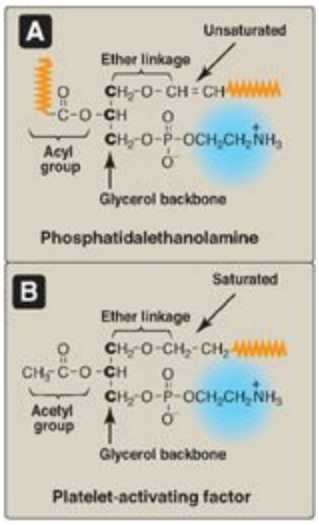


2. **Cardiolipin:** Two molecules of PA esterified through their phosphate groups to an additional molecule of glycerol is called cardiolipin, or diphosphatidylglycerol (Figure 17.2). Cardiolipin is found in membranes in bacteria and eukaryotes. In eukaryotes, cardiolipin is virtually exclusive to the inner mitochondrial membrane, where it maintains the structure and function of certain respiratory complexes of the electron transport chain. [Note: Cardiolipin is antigenic and is recognized by antibodies raised against Treponema pallidum, the bacterium that causes syphilis.]

3. **Plasmalogens:** When the fatty acid 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, phosphatidalethanolamine, which is abundant in nerve tissue (Figure 17.3A), is the plasmalogen that is similar in structure to phosphatidylethanolamine. Phosphatidalcholine (abundant in heart muscle) is the other quantitatively significant ether lipid in mammals. [Note: Plasmalogens use "al" rather than "yl" in their names.]

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 fatty acid) at carbon 2 of the glycerol backbone (Figure 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 p. 148). 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.]

Figure 17.3 The ether glycerophospholipids. A. The plasmalogen phosphatidalethanolamine. B. Platelet-activating factor. (≈≈≈ is a long, hydrophobic hydrocarbon chain.).



B. Sphingophospholipids: sphingomyelin

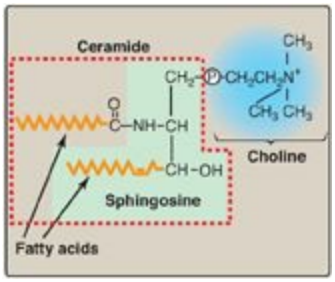
The backbone of sphingomyelin is the amino alcohol sphingosine, rather than glycerol (Figure 17.4). A long-chain fatty acid is attached to the amino group of sphingosine through an amide linkage, producing a ceramide, which can also serve as a precursor of glycolipids (see p. 209). The alcohol group 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. [Note: The myelin sheath is a layered, membranous structure that insulates and protects neuronal fibers of the central nervous system (CNS).]

III. PHOSPHOLIPID SYNTHESIS

Glycerophospholipid synthesis involves either the donation of PA from cytidine diphosphate (CDP)-diacylglycerol to an alcohol or the donation of the phosphomonoester of the alcohol from CDP-alcohol to 1,2-diacylglycerol (Figure 17.5). In both cases, the CDP-bound structure is considered an "activated intermediate," and cytidine monophosphate (CMP) is released as a side product of glycerophospholipid synthesis. A key concept in phosphoglyceride synthesis, therefore, is activation, either of 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 uridine diphosphate (UDP) (see p. 126).] The fatty acids esterified to the glycerol alcohol groups can vary widely, contributing to the heterogeneity of this group of compounds, with saturated fatty acids typically found at carbon 1 and unsaturated ones at carbon 2. Most phospholipids are synthesized in the smooth endoplasmic reticulum (ER). From there, they are transported to the Golgi apparatus 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 occurs in peroxisomes.]

Figure 17.4 Structure of sphingomyelin, showing sphingosine (in green box) and ceramide components (in dashed box). Ⓟ = phosphate.



A. Phosphatidic acid

PA is the precursor of many other phosphoglycerides. The steps in its synthesis from glycerol phosphate and two fatty acyl coenzyme A (CoA) molecules were illustrated in Figure 16.14, p. 189, in which PA is shown as a precursor of triacylglycerol.

> Essentially all cells except mature erythrocytes can synthesize phospholipids, whereas triacylglycerol synthesis occurs essentially only in liver, adipose tissue, lactating mammary glands, and intestinal mucosal cells.

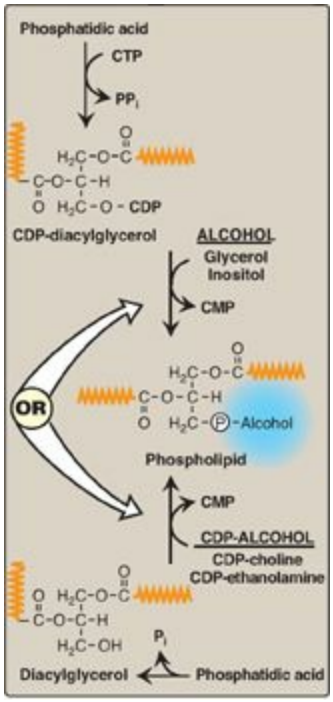
B. Phosphatidylcholine and phosphatidylethanolamine

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 below).]

1. **Synthesis from preexisting 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 Figure 17.5).

Figure 17.5 Phospholipid synthesis requires activation of either diacylglycerol or an alcohol by linkage to cytidine diphosphate (CDP). CMP = cytidine monophosphate; CTP = cytidine triphosphate; P_i = inorganic phosphate; PP_i = pyrophosphate. (~~~ is a fatty acid hydrocarbon chain.)



a. **Significance of choline reutilization:** The reutilization of choline is important because, whereas 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. 358) of 550 mg for men and 425 mg for women. [Note: Choline is also used for the synthesis of acetylcholine, a neurotransmitter.]

b. **Role of 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. 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.] 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 maturity of the fetus can be gauged by determining the ratio of DPPC to sphingomyelin, usually written as the L (for lecithin)-to-S ratio, in amniotic fluid. A value of two or above is evidence of maturity, because it reflects the major shift from sphingomyelin to DPPC synthesis that occurs in the pneumocytes at about 32 weeks of gestation.

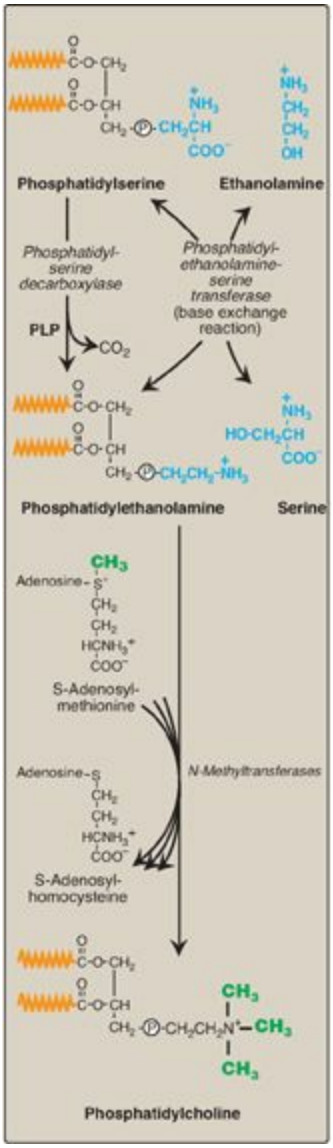
Lung maturation can be accelerated by giving the mother glucocorticoids shortly before delivery to induce expression of specific genes. Postnatal administration of natural or synthetic surfactant (by intratracheal instillation) is also used. [Note: Acute respiratory distress syndrome (ARDS), seen in 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 and carbon dioxide.]

2. **Synthesis of phosphatidylcholine from phosphatidylserine in the liver:** 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 serum lipoproteins. To provide the needed PC, PS is decarboxylated to PE by PS decarboxylase, an enzyme requiring pyridoxal phosphate (PLP) as a coenzyme. PE then undergoes three methylation steps to produce PC, as illustrated in Figure 17.6. S-adenosylmethionine is the methyl group donor (see p. 264).

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 Figure 17.6). This reaction, although reversible, is used primarily to produce the PS required for membrane synthesis.

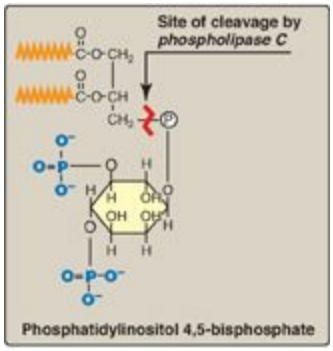
Figure 17.6 Synthesis of phosphatidylcholine from phosphatidylserine in the liver. (is a fatty acid hydrocarbon chain.) Ⓟ = phosphate; PLP = pyridoxal phosphate.



D. Phosphatidylinositol

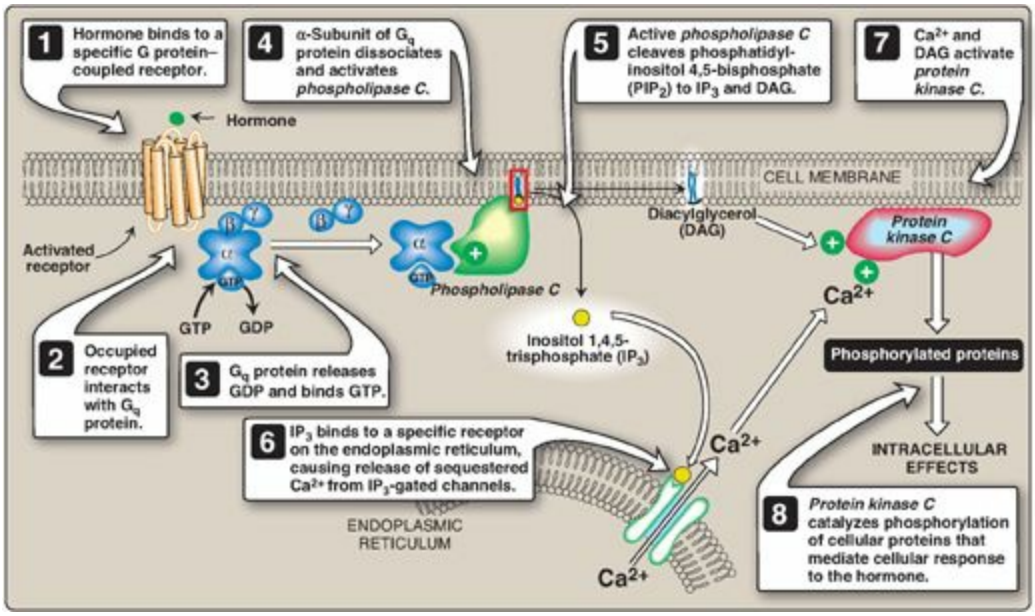
PI is synthesized from free inositol and CDP-diacylglycerol as shown in Figure 17.5. PI is an unusual phospholipid in that it most frequently contains stearic acid on carbon 1 and arachidonic acid on carbon 2 of the glycerol. PI, therefore, serves as a reservoir of arachidonic acid in membranes and, thus, provides the substrate for prostaglandin synthesis when required (see p. 213 for a discussion of these compounds). [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 enzymes known as "flippases" and "floppases."]

Figure 17.7 Structure of phosphatidylinositol 4,5-bisphosphate. Cleavage by phospholipase C produces inositol 1,4,5-trisphosphate and diacylglycerol. (〰 is a fatty acid hydrocarbon chain.) Ⓟ = phosphate.



1. **Role in signal transmission across membranes:** The phosphorylation of membrane-bound PI produces polyphosphoinositides such as phosphatidylinositol 4,5-bisphosphate ([PIP_2] Figure 17.7). The hydrolytic cleavage of PIP_2 by phospholipase C occurs in response to the binding of a variety of neurotransmitters, hormones, and growth factors to G protein–coupled receptors (such as the α-1 adrenergic receptor) on the cell membrane and activation of the G_q alpha subunit (Figure 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 transmission across the membrane is thus accomplished.

Figure 17.8 Role of inositol trisphosphate and diacylglycerol in intracellular signaling. GTP = guanosine triphosphate; GDP = guanosine diphosphate.



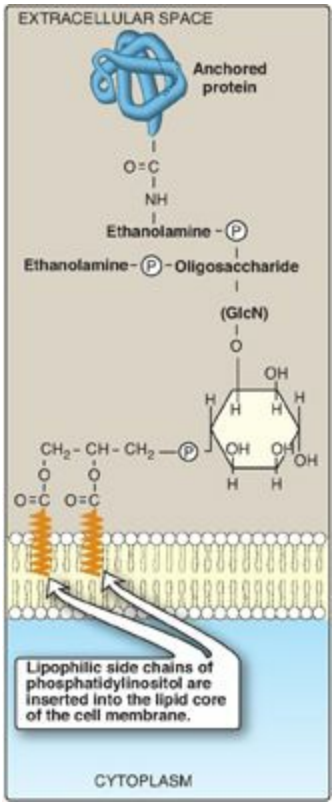
2. **Role in membrane protein anchoring:** Specific proteins can be covalently attached through a carbohydrate bridge to membrane-bound PI (Figure 17.9). Alkaline phosphatase, a digestive enzyme found on the surface of the small intestine that attacks organic phosphates, is an example of a protein attached to such 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 surface of the plasma membrane. The protein can be cleaved from its anchor by the action of phospholipase C (see Figure 17.8), releasing DAG. [Note: A deficiency in the synthesis of GPI in hematopoietic cells results in the hemolytic disease paroxysmal nocturnal hemoglobinuria.]

E. Phosphatidylglycerol and cardiolipin

Phosphatidylglycerol occurs in relatively large amounts in mitochondrial membranes and is a precursor of cardiolipin (diphosphatidyglycerol). It is synthesized by a two-step reaction from CDP-diacylglycerol and glycerol 3-phosphate. Cardiolipin (see Figure 17.2) is synthesized by the transfer of diacylglycerophosphate from CDP-diacylglycerol to a preexisting molecule of phosphatidylglycerol.

Figure 17.9 Example of a glycosyl phosphatidylinositol (GPI) membrane protein anchor. GlcN = glucosamine; Ⓟ = phosphate.



F. Sphingomyelin

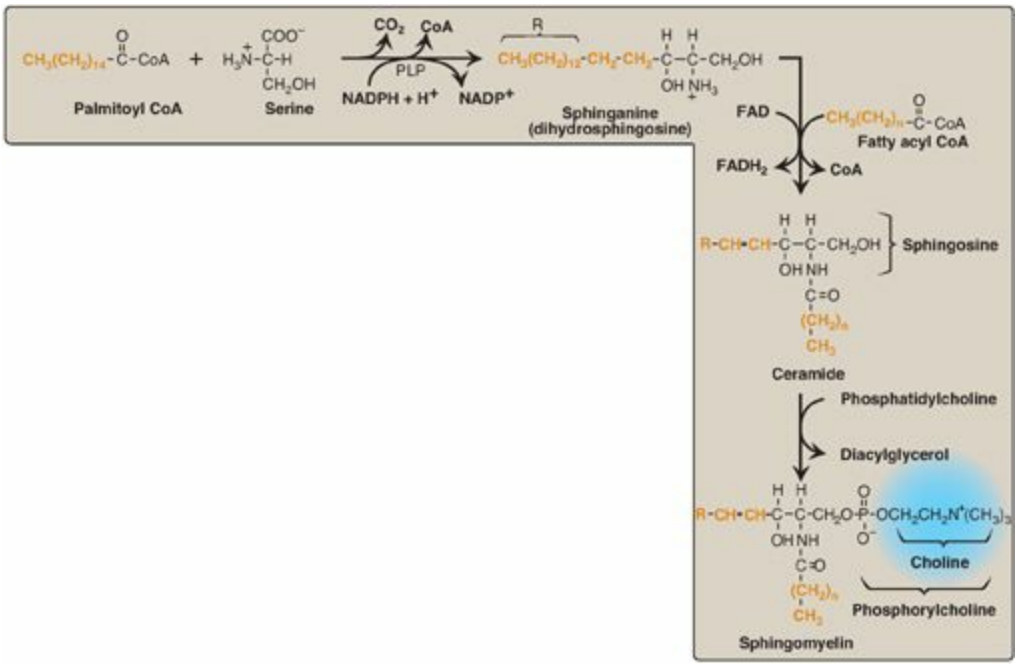
Sphingomyelin, a sphingosine-based phospholipid, is a major structural lipid in the membranes of nerve tissue. The synthesis of sphingomyelin is shown in Figure 17.10. Briefly, palmitoyl CoA condenses with serine, as CoA and the carboxyl group (as CO_2) of serine are lost. [Note: This reaction, like the decarboxylation reactions involved in the synthesis of PE from PS and of regulators from amino acids (for example, the catecholamines from tyrosine, see p. 286) requires pyridoxal phosphate (a derivative of vitamin B_6) as a coenzyme (see p. 378).] The product is reduced in a nicotinamide

adenine dinucleotide phosphate (NADPH)-requiring reaction to sphinganine (dihydrosphingosine), which is acylated at the amino group with one of a variety of long-chain fatty acids and then desaturated to produce a ceramide, the immediate precursor of sphingomyelin (and other sphingolipids, as described on p. 208).

> 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 fatty acids such as lignoceric acid and nervonic acid, whereas gray matter of the brain has sphingomyelin that contains primarily stearic acid.]

Figure 17.10 Synthesis of sphingomyelin. PLP = pyridoxal phosphate; NADP(H) = nicotinamide adenine dinucleotide phosphate; FAD(H_2) = flavin adenine dinucleotide; CoA = coenzyme A.



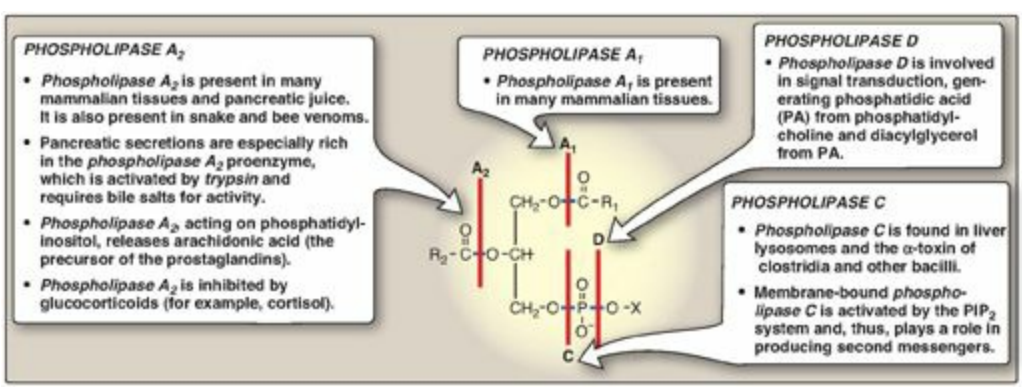
IV. DEGRADATION OF PHOSPHOLIPIDS

The degradation of phosphoglycerides is performed by phospholipases found in all tissues and pancreatic juice (for a discussion of phospholipid digestion, see p. 175). 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 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 fatty acid 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 (for example, DAG and IP_3) or that are the substrates for synthesis of messengers (for example, 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 fatty acids from membrane-bound phospholipids, which can be replaced with different fatty acids using fatty acyl CoA transferase. This mechanism is used as one way to create the unique lung surfactant DPCC (see p. 204) and to insure 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.]

Figure 17.11 Degradation of glycerophospholipids by phospholipases. PIP_2 = phosphatidylinositol 4,5-bisphosphate; R_1 and R_2 = fatty acids; X = an alcohol.

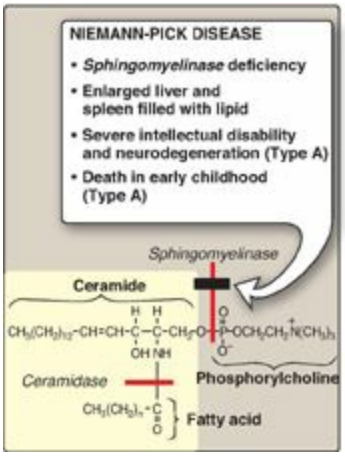


B. Sphingomyelin

Sphingomyelin is degraded by sphingomyelinase, a lysosomal enzyme that hydrolytically removes phosphorylcholine, leaving a ceramide. The ceramide is, in turn, cleaved by ceramidase into sphingosine and a free fatty acid (Figure 17.12). [Note:

The ceramide and sphingosine released 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 disease 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 less than 1% of normal enzymic activity), the liver and spleen are the primary sites of lipid deposits and are, therefore, greatly enlarged. The lipid consists primarily of the sphingomyelin that cannot be degraded (Figure 17.13). Infants with this lysosomal storage disease experience rapid and progressive neurodegeneration as a result of deposition of sphingomyelin in the CNS, and they die in early childhood. A less severe variant (Type B, which shows 5% or more 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.

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.]

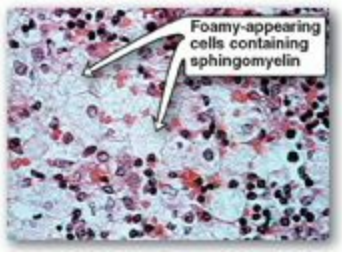


V. OVERVIEW OF GLYCOLIPIDS

Glycolipids are molecules that contain both carbohydrate and lipid components. Like the phospholipid sphingomyelin, glycolipids are derivatives of ceramides in which a long-chain fatty acid is attached to the amino alcohol sphingosine. They are, therefore, more precisely called glycosphingolipids. [Note: Ceramides, then, 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 (for example, adhesion and recognition), growth, and development.

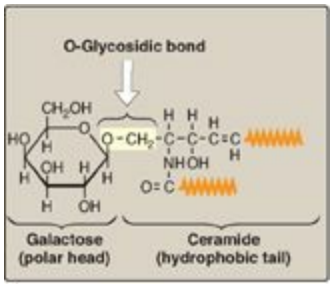
> Membrane glycosphingolipids associate with cholesterol and GPI-anchored proteins to form lipid rafts, laterally mobile microdomains of the plasma membrane that function to organize and regulate signaling and trafficking functions of membranes.

Figure 17.13 Accumulation of lipids in spleen cells from a patient with Niemann-Pick disease.



Glycosphingolipids are antigenic and are the source of blood group antigens, 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.] They also are used 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 17.14 Structure of a neutral glycosphingolipid, galactocerebroside. ( is a hydrophobic hydrocarbon chain.)


O-Glycosidic bond
Galactose
(polar head)
Ceramide
(hydrophobic tail)

VI. STRUCTURE OF GLYCOSPHINGOLIPIDS

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 (Figure 17.14). The number and type of carbohydrate moieties present determine the type of glycosphingolipid.

A. Neutral glycosphingolipids

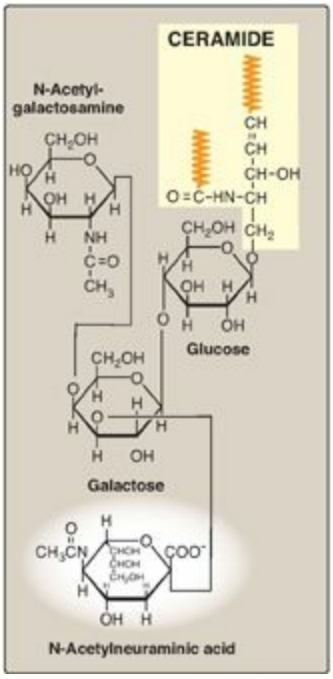
The simplest neutral (uncharged) glycosphingolipids are the cerebrosides. These are ceramide monosaccharides that contain either a molecule of galactose (forming ceramide-galactose or galactocerebroside, the most common cerebroside found in myelin, as shown in Figure 17.14) or glucose (forming ceramide-glucose or glucocerebroside, which serves primarily as 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 fatty acid attached to the sphingosine.] As their name implies, cerebrosides are found predominantly in the brain and peripheral nervous tissue, 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.

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 Figure 17.15) in gangliosides, or by sulfate groups in sulfatides.

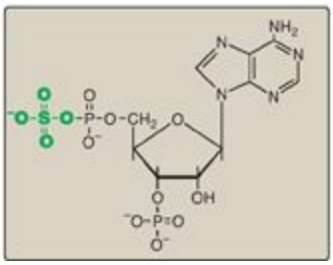
1. **Gangliosides:** These are the most complex glycosphingolipids and are found primarily in the ganglion cells of the CNS, particularly at the nerve endings. They are derivatives of ceramide oligosaccharides and contain one or more molecules of NANA. The notation for these compounds is G (for ganglioside) 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 Figure 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 Figure 17.20, p. 212).

Figure 17.15 Structure of the ganglioside G_{M2}. (is a hydrophobic hydrocarbon chain.)



2. **Sulfatides:** These sulfoglycosphingolipids are sulfated galactocerebrosides that are negatively charged at physiologic pH. Sulfatides are found predominantly in the brain and kidneys.

Figure 17.16 Structure of 3**I**-phosphoadenosine-5**I**-phosphosulfate.



VII. SYNTHESIS AND DEGRADATION OF GLYCOSPHINGOLIPIDS

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 p. 166).

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.]

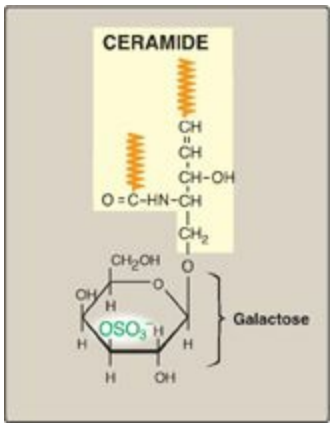
B. Addition of sulfate groups

A sulfate group from the sulfate carrier 3′-phosphoadenosine-5′-phosphosulfate ([PAPS], Figure 17.16) is added by a sulfotransferase to the 3′-hydroxyl group of the galactose in a galactocerebroside, forming the sulfatide galactocerebroside 3-sulfate (Figure 17.17). [Note: PAPS is also the sulfur donor in glycosaminoglycan synthesis (see p. 162) and steroid hormone catabolism (see p. 240).] An overview of the synthesis of sphingolipids is shown in Figure 17.18.

C. Degradation of glycosphingolipids

Glycosphingolipids are internalized by endocytosis as described for the glycosaminoglycans. All of the enzymes required for the degradative process are present in lysosomes, which fuse with the endocytotic vesicles. The lysosomal enzymes hydrolytically and irreversibly cleave specific bonds in the glycosphingolipid. As seen with the glycosaminoglycans (see p. 163) and glycoproteins (see p. 170), 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. [Note: Defects in the degradation of the polysaccharide chains in these three glycoconjugates, therefore, result in lysosomal storage diseases.]

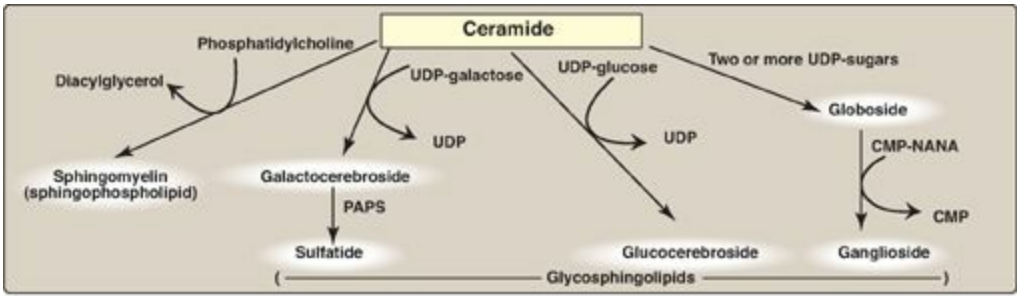
Figure 17.17 Structure of galactocerebroside 3-sulfate. (〰 is a hydrophobic hydrocarbon chain.)



D. Sphingolipidoses

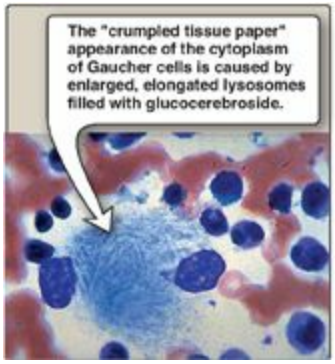
In a normal individual, synthesis and degradation of glycosphingolipids are balanced, so that the amount of these compounds present in membranes is constant. If a specific lysosomal acid hydrolase required for degradation is partially or totally missing, a sphingolipid accumulates. Lysosomal lipid storage 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 17.20 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 (for example, the saposins) that facilitate access of the hydrolases to short carbohydrate chains as degradation proceeds.]

Figure 17.18 Overview of sphingolipid synthesis. UDP = uridine diphosphate; CMP = cytidine monophosphate; NANA = N-acetylneuraminic acid; PAPS = 3′-phosphoadenosine-5′-phosphosulfate.



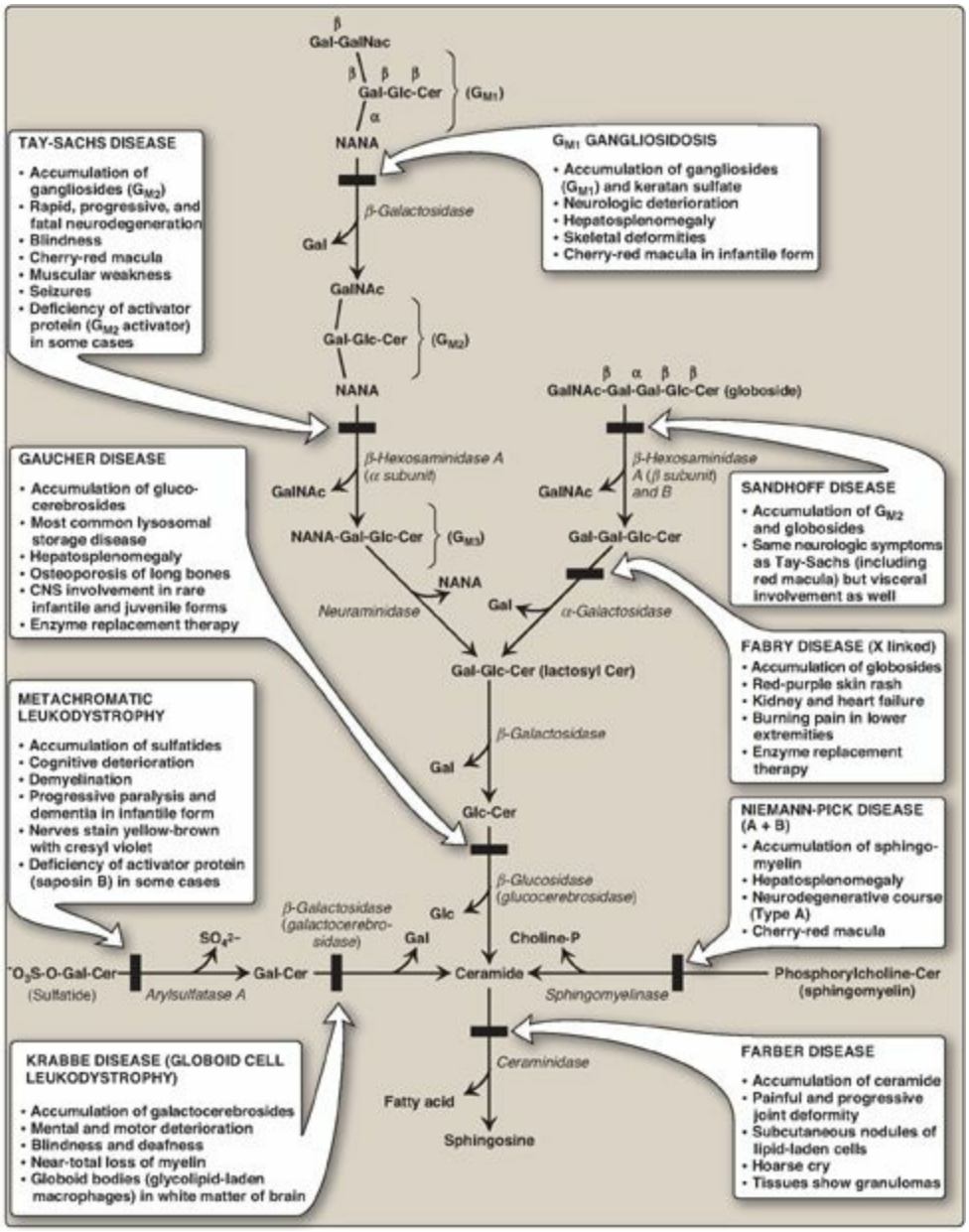
1. **Common properties:** A specific lysosomal hydrolytic enzyme is deficient in the classic form of each disorder. Therefore, usually only a single sphingolipid (the substrate for the deficient enzyme) accumulates in the involved organs in each disease. [Note: The rate of biosynthesis of the accumulating 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 diseases, 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 17.19 Aspirated bone marrow cells from a patient with Gaucher disease.



2. **Diagnosis and treatment:** A specific sphingolipidosis can be diagnosed by measuring enzyme activity in cultured fibroblasts or peripheral leukocytes or by analysis of DNA (see p. 473). Histologic examination of the affected tissue is also useful. [Note: Shell-like inclusion bodies are seen in Tay-Sachs, and a wrinkled tissue paper appearance of the cytosol is seen in Gaucher disease (Figure 17.19).] 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) and by substrate reduction therapy through pharmacologic reduction of glucosylcer-amide, the substrate for the deficient enzyme.

Figure 17.20 Degradation of sphingolipids showing the lysosomal enzymes affected in related genetic diseases, the sphingolipidoses. All of the diseases are autosomal recessive except Fabry disease, 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.


Gal-GalNac
Gal-Glc-Cer
(GM1)
NANA
β-Galactosidase
Gal
GalNAc
Gal-Glc-Cer
(GM2)
NANA
GalNAc-Gal-Gal-Glc-Cer (globoside)
β-Hexosaminidase A (α subunit)
β-Hexosaminidase A (β subunit) and B
GalNAc
GalNAc
NANA-Gal-Glc-Cer
(GM3)
Gal-Gal-Glc-Cer
NANA
Neuraminidase
Gal
α-Galactosidase
Gal-Glc-Cer (lactosyl Cer)
β-Galactosidase
Gal
Glc-Cer
β-Glucosidase (glucocerebrosidase)
β-Galactosidase (galactocerebrosidase)
Glc
Gal
Choline-P
SO4 2-
3O3S-O-Gal-Cer
(Sulfatide)
Arylsulfatase A
Gal-Cer
Ceramide
Sphingomyelinase
Phosphorylcholine-Cer (sphingomyelin)
Ceraminidase
Fatty acid
Sphingosine
TAY-SACHS DISEASE
• Accumulation of gangliosides (GM2)
• Rapid, progressive, and fatal neurodegeneration
• Blindness
• Cherry-red macula
• Muscular weakness
• Seizures
• Deficiency of activator protein (GM2 activator) in some cases
GM1 GANGLIOSIDOSIS
• Accumulation of gangliosides (GM1) and keratan sulfate
• Neurologic deterioration
• Hepatosplenomegaly
• Skeletal deformities
• Cherry-red macula in infantile form
GAUCHER DISEASE
• Accumulation of gluco-cerebrosides
• Most common lysosomal storage disease
• Hepatosplenomegaly
• Osteoporosis of long bones
• CNS involvement in rare infantile and juvenile forms
• Enzyme replacement therapy
SANDHOFF DISEASE
• Accumulation of GM2 and globosides
• Same neurologic symptoms as Tay-Sachs (including red macula) but visceral involvement as well
FABRY DISEASE (X linked)
• Accumulation of globosides
• Red-purple skin rash
• Kidney and heart failure
• Burning pain in lower extremities
• Enzyme replacement therapy
METACHROMATIC LEUKODYSTROPHY
• Accumulation of sulfatides
• Cognitive deterioration
• Demyelination
• Progressive paralysis and dementia in infantile form
• Nerves stain yellow-brown with cresyl violet
• Deficiency of activator protein (saposin B) in some cases
NIEMANN-PICK DISEASE (A + B)
• Accumulation of sphingo-myelin
• Hepatosplenomegaly
• Neurodegenerative course (Type A)
• Cherry-red macula
KRABBE DISEASE (GLOBOID CELL LEUKODYSTROPHY)
• Accumulation of galactocerebrosides
• Mental and motor deterioration
• Blindness and deafness
• Near-total loss of myelin
• Globoid bodies (glycolipid-laden macrophages) in white matter of brain
FARBER DISEASE
• Accumulation of ceramide
• Painful and progressive joint deformity
• Subcutaneous nodules of lipid-laden cells
• Hoarse cry
• Tissues show granulomas

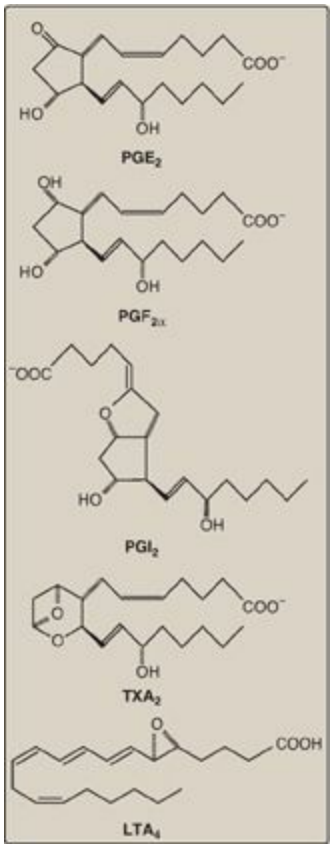
VIII. EICOSANOIDS: PROSTAGLANDINS AND RELATED COMPOUNDS

Prostaglandins, and the related compounds thromboxanes and leukotrienes, are collectively known as eicosanoids to reflect their origin from polyunsaturated fatty acids 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 (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. They also act locally rather than after transport in the blood to distant sites, as occurs with endocrine hormones such as insulin. 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 G protein–coupled receptors (see p. 94), which are different in different organ systems, and typically result in changes in cyclic adenosine monophosphate production. Examples of prostaglandins and related structures are shown in Figure 17.21.

A. Synthesis of prostaglandins and thromboxanes

Arachidonic acid, an ω-6 fatty acid containing 20 carbons and four double bonds (an eicosatetraenoic fatty acid), is the immediate precursor of the predominant type of prostaglandins in humans (series 2 or those with two double bonds, as shown in Figure 17.22). It is derived by the elongation and desaturation of the essential fatty acid linoleic acid, also an ω-6 fatty acid. Arachidonic acid is incorporated into membrane phospholipids (typically PI) at carbon 2, from which it is released by phospholipase A_2 in response to a variety of signals (Figure 17.23). [Note: Series 1 prostaglandins contain one double bond and are derived from an ω-6 eicosatrienoic fatty acid, dihomo-γ-linolenic acid, whereas series 3 contain three double bonds and are derived from eicosapentaenoic acid (EPA), an ω-3 fatty acid. See p. 363.]

Figure 17.21 Examples of prostaglandin structures. [Note: Prostaglandins are named as follows: PG plus a third letter (for example, A, D, E, or F), 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 known as prostacyclin. Thromboxanes are designated by TX and leukotrienes by LT.]



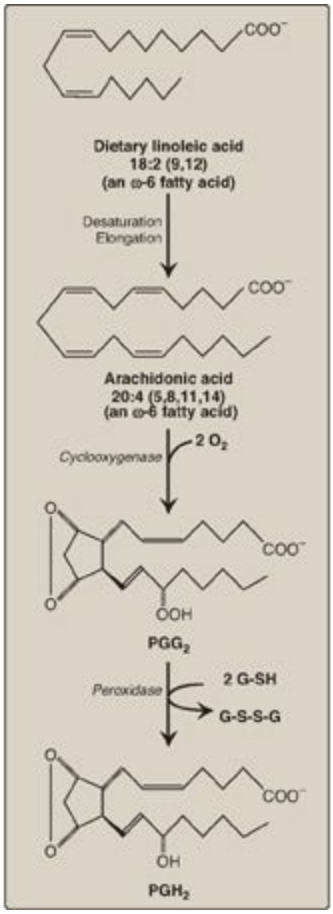
1. **Synthesis of PGH_2:** The first step in prostaglandin synthesis is the oxidative cyclization of free arachidonic acid to yield PGH_2 by prostaglandin endoperoxide synthase (PGH synthase). This enzyme is an ER membrane-bound protein that has two catalytic activities: fatty acid cyclooxygenase (COX), which requires two molecules of O_2, and peroxidase, which is dependent on reduced glutathione (see p. 148). PGH_2 is converted to a variety of prostaglandins and thromboxanes, as shown in Figure 17.23, by cell-specific synthases.

   a. **Isozymes of PGH synthase:** Two isozymes of PGH 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 prostaglandin synthesis subsequent to the induction of COX-2 mediates the pain, heat, redness, and swelling of inflammation and the fever of infection.]

2. **Inhibition of prostaglandin synthesis:** The synthesis of prostaglandins can be inhibited by a number of unrelated compounds. For example, cortisol (a steroidal anti-inflammatory agent) inhibits phospholipase A_2 activity (see Figure 17.23) and, therefore, the precursor of the prostaglandins, arachidonic acid, is not made available 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 prostaglandin, 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 and resolvins, lipid mediators with anti-inflammatory effects that are made from arachidonic acid and EPA, respectively. Inhibitors specific for COX-2 (the coxibs, for example, celecoxib) were designed to reduce pathologic inflammatory processes mediated by COX-2 while maintaining the physiologic functions of COX-1. However, their use has been associated with increased risk of heart attacks attacks, likely as a result of decreased PGI_2 synthesis (see below), and some have been withdrawn from the market.

Figure 17.22 Oxidation and cyclization of arachidonic acid by the two catalytic activities (cyclooxygenase and peroxidase) of prostaglandin endoperoxide synthase. G-SH = reduced glutathione; G-S-S-G = oxidized glutathione.



B. Synthesis of leukotrienes

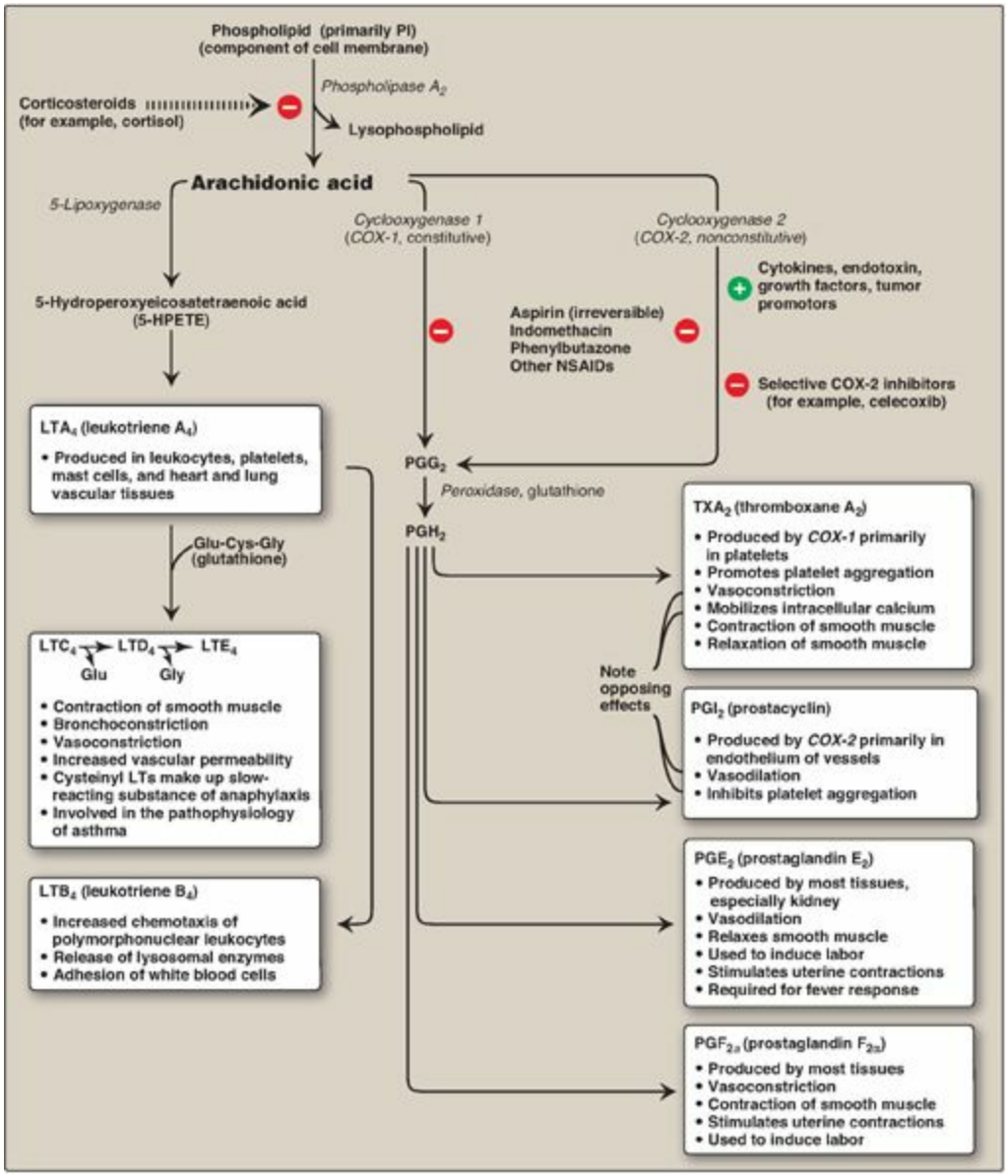
Arachidonic acid is converted to a variety of linear hydroperoxy (–OOH) acids by a separate pathway involving a family of lipoxygenases (LOXs). For example, 5-lipoxygenase converts arachidonic acid to 5-hydroperoxy-6,8,11,14 eicosatetraenoic acid ([5-HPETE], see Figure 17.23). 5-HPETE is converted to a series of leukotrienes containing four double bonds, the nature of the final products varying according to the tissue. Leukotrienes are mediators of allergic response and inflammation. Their synthesis is not inhibited by NSAIDs. [Note: Aspirin-induced asthma is a response to overproduction of leukotrienes with NSAID use.] Inhibitors of 5-lipoxygenase and

leukotriene receptor antagonists are used in the treatment of asthma.

C. Role of prostaglandins in platelet homeostasis

Thromboxane A_2 (TXA_2) is produced by COX-1 in activated platelets. It promotes adherence and aggregation of circulating platelets and contraction of vascular smooth muscle, thereby promoting formation of blood clots (thrombi). (See online Chapter 34.) 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 (Figure 17.24). The inhibition of COX-1 cannot be overcome in platelets, which lack nuclei. However, the inhibition of COX-2 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 17.23 Overview of the biosynthesis and function of some important prostaglandins (PGs), leukotrienes (LTs), and a thromboxane (TX) from arachidonic acid. [Note: The arachidonic acid in the membrane phospholipid was derived from the ω-6 essential fatty acid, linoleic, also an ω-6 fatty acid.] PI = phosphatidylinositol; NSAIDs = nonsteroidal anti-inflammatory drugs; Glu = glutamate; Cys = cysteine; Gly = glycine.


Phospholipid (primarily PI)
(component of cell membrane)
Corticosteroids
(for example, cortisol)
Phospholipase A_2
Lysophospholipid
Arachidonic acid
5-Lipoxygenase
Cyclooxygenase 1
(COX-1, constitutive)
Cyclooxygenase 2
(COX-2, nonconstitutive)
Cytokines, endotoxin, growth factors, tumor promotors
5-Hydroperoxyeicosatetraenoic acid
(5-HPETE)
Aspirin (irreversible)
Indomethacin
Phenylbutazone
Other NSAIDs
Selective COX-2 inhibitors
(for example, celecoxib)
LTA_4 (leukotriene A_4)
• Produced in leukocytes, platelets, mast cells, and heart and lung vascular tissues
PGG_2
Peroxidase, glutathione
PGH_2
Glu-Cys-Gly
(glutathione)
TXA_2 (thromboxane A_2)
• Produced by COX-1 primarily in platelets
• Promotes platelet aggregation
• Vasoconstriction
• Mobilizes intracellular calcium
• Contraction of smooth muscle
• Relaxation of smooth muscle
LTC_4 → LTD_4 → LTE_4
Glu
Gly
Note opposing effects
PGI_2 (prostacyclin)
• Produced by COX-2 primarily in endothelium of vessels
• Vasodilation
• Inhibits platelet aggregation
• Contraction of smooth muscle
• Bronchoconstriction
• Vasoconstriction
• Increased vascular permeability
• Cysteinyl LTs make up slow-reacting substance of anaphylaxis
• Involved in the pathophysiology of asthma
PGE_2 (prostaglandin E_2)
• Produced by most tissues, especially kidney
• Vasodilation
• Relaxes smooth muscle
• Used to induce labor
• Stimulates uterine contractions
• Required for fever response
LTB_4 (leukotriene B_4)
• Increased chemotaxis of polymorphonuclear leukocytes
• Release of lysosomal enzymes
• Adhesion of white blood cells
$PGF_{2\alpha}$ (prostaglandin $F_{2\alpha}$)
• Produced by most tissues
• Vasoconstriction
• Contraction of smooth muscle
• Stimulates uterine contractions
• Used to induce labor

IX. CHAPTER SUMMARY

Phospholipids are **polar**, **ionic** compounds composed of an **alcohol** (for example, **choline** or **ethanolamine**) attached by a phosphodiester bond to either **diacylglycerol** (DAG), producing **phosphatidylcholine** or **phosphatidylethanolamine**, or to the amino alcohol **sphingosine** (Figure 17.25). Addition of a long-chain fatty acid to sphingosine produces a **ceramide**. Addition of a **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 **respiratory distress syndrome**. **Phosphatidylinositol** (**PI**) serves as a reservoir for **arachidonic acid** in membranes. The phosphorylation of membrane-bound PI produces **phosphatidylinositol 4,5-bisphosphate** (**PIP2**). This compound is degraded by **phospholipase C** in response to the binding of a variety of neurotransmitters, hormones, and growth factors to membrane G protein–coupled receptors. The products of this degradation, **inositol 1,4,5-trisphosphate** (**IP3**) 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 phosphatidylinositol (**glycosyl phosphatidylinositol**, or **GPI**), forming a GPI anchor. A deficiency in the synthesis of GPI in hematopoietic cells results in a 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 + B**) **disease**. Glycosphingolipids are derivatives of **ceramides** to which carbohydrates have been attached. When one sugar molecule is added to the ceramide, a **cerebroside** is produced. If an oligosaccharide is added, a **globoside** is produced. If an acidic N-acetylneuraminic acid molecule is added, a **ganglioside** is produced. 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 produces a **sphingolipidosis**, in which a characteristic sphingolipid accumulates. **Prostaglandins** (**PGs**) , **thromboxanes** (**TXs**), and **leukotrienes** (**LTs**) are produced in very small amounts in almost all tissues, act locally, and have an extremely short half-life. **They serve as mediators of the inflammatory response. Arachidonic acid** is the immediate precursor of the predominant class of PGs in humans (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 A2** (inhibited by cortisol). Synthesis of the **PGs** and **TXs** begins with the oxidative cyclization of free arachidonic acid to yield PGH_2 by **prostaglandin endoperoxide synthase** (PGH synthase), an endoplasmic reticulum membrane protein that has two catalytic activities: **fatty acid cyclooxygenase** (**COX**) and **peroxidase**. There are two isozymes of PGH synthase: **COX-1** (constitutive) and **COX-2** (nonconstitutive). Aspirin irreversibly inhibits both. Opposing effects of PGI_2 and TXA_2 limit clot formation. **LTs** are linear molecules produced from arachidonic acid by the **5-lipoxygenase** pathway. They mediate allergic response and are not inhibited by aspirin or other NSAIDs.

Figure 17.24 Irreversible acetylation of cyclooxygenase (COX)-1 and COX-2 by aspirin.

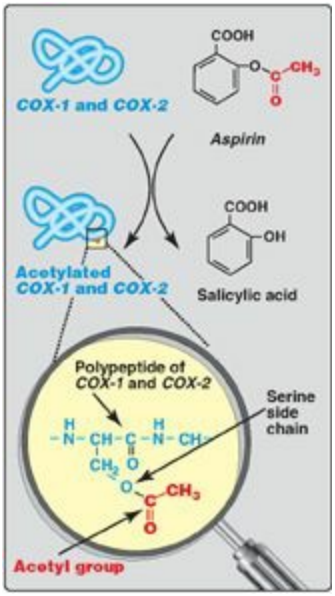
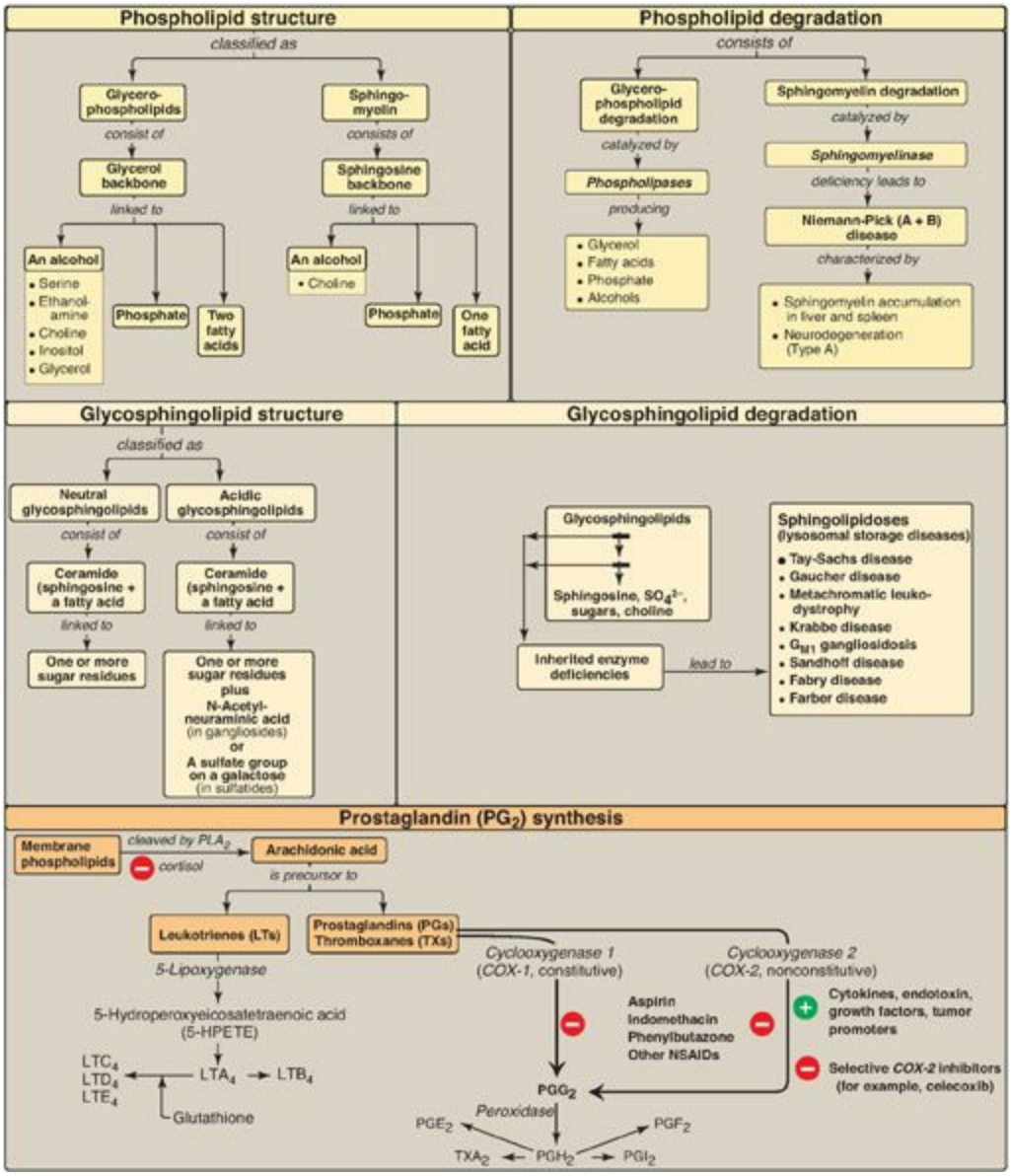


Figure 17.25 Key concept map for complex lipids. PLA_2 = phospholipase A_2; SO_4^{2-} = sulfate ion; NSAIDs = nonsteroidal anti-inflammatory drugs.


Phospholipid structure
classified as
Glycero-phospholipids
consist of
Glycerol backbone
linked to
An alcohol
• Serine
• Ethanol-amine
• Choline
• Inositol
• Glycerol
Phosphate
Two fatty acids
Sphingo-myelin
consists of
Sphingosine backbone
linked to
An alcohol
• Choline
Phosphate
One fatty acid
Phospholipid degradation
consists of
Glycero-phospholipid degradation
catalyzed by
Phospholipases
producing
• Glycerol
• Fatty acids
• Phosphate
• Alcohols
Sphingomyelin degradation
catalyzed by
Sphingomyelinase
deficiency leads to
Niemann-Pick (A + B) disease
characterized by
• Sphingomyelin accumulation in liver and spleen
• Neurodegeneration (Type A)
Glycosphingolipid structure
classified as
Neutral glycosphingolipids
consist of
Ceramide (sphingosine + a fatty acid
linked to
One or more sugar residues
Acidic glycosphingolipids
consist of
Ceramide (sphingosine + a fatty acid
linked to
One or more sugar residues plus
N-Acetyl-neuraminic acid (in gangliosides)
or
A sulfate group on a galactose (in sulfatides)
Glycosphingolipid degradation
Glycosphingolipids
Sphingosine, SO_4^{2-}, sugars, choline
Inherited enzyme deficiencies
lead to
Sphingolipidoses (lysosomal storage diseases)
• Tay-Sachs disease
• Gaucher disease
• Metachromatic leuko-dystrophy
• Krabbe disease
• G_{M1} gangliosidosis
• Sandhoff disease
• Fabry disease
• Farber disease
Prostaglandin (PG_2) synthesis
Membrane phospholipids
cleaved by PLA_2
cortisol
Arachidonic acid
is precursor to
Leukotrienes (LTs)
5-Lipoxygenase
5-Hydroperoxyeicosatetraenoic acid (5-HPETE)
LTC_4
LTD_4
LTE_4
LTA_4
LTB_4
Glutathione
Prostaglandins (PGs)
Thromboxanes (TXs)
Cyclooxygenase 1 (COX-1, constitutive)
Cyclooxygenase 2 (COX-2, nonconstitutive)
Aspirin
Indomethacin
Phenylbutazone
Other NSAIDs
Cytokines, endotoxin, growth factors, tumor promoters
Selective COX-2 inhibitors (for example, celecoxib)
PGG_2
Peroxidase
PGE_2
PGF_2
TXA_2
PGH_2
PGI_2

Study Questions

Choose the ONE best answer.

17.1 Aspirin-induced asthma (AIA) 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 best explains the symptoms seen in patients with AIA? NSAIDs:

A. inhibit the activity of the cystic fibrosis transmembrane conductance regulator protein, resulting in thickened secretions that block airways.
B. inhibit cyclooxygenase but not lipoxygenase, resulting in the flow of arachidonic acid to leukotriene synthesis.
C. activate the cyclooxygenase activity of PGH synthase, resulting in increased synthesis of prostaglandins that promote vasodilation.
D. activate phospholipases, resulting in decreased amounts of dipalmytoylphosphatidylcholine and alveolar collapse (atelectasis).

Correct answer = B. Nonsteroidal anti-inflammatory drugs (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 transmembrane conductance regulator protein protein, defects in which are the cause of cystic fibrosis. 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 of gestation, rapidly gave evidence of respiratory distress. Clinical laboratory and imaging (X-ray) results supported the diagnosis of infant respiratory distress syndrome. Which of the following statements about this syndrome is true?

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 greater than two.
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 ratio in amniotic is greater than two, a newborn's lungs are considered to be sufficiently mature (premature lungs would be expected to have a ratio lower than two). The RDS would not be due to too few type II pneumocytes, which would simply be secreting sphingomyelin rather than DPPC at 28 weeks of gestation. The mother is given a glucocorticoid, not surfactant, prior to giving birth. Surfactant would be administered to the baby postnatally to reduce surface tension.

17.3 A 10-year-old boy 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. Enzymic analysis revealed a deficiency of α-galactosidase, and enzyme replacement therapy was recommended. The most likely diagnosis is:

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, and kidney and cardiac complications. Protein in his urine indicates kidney damage. Enzyme replacement therapy is available.

17.4 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.

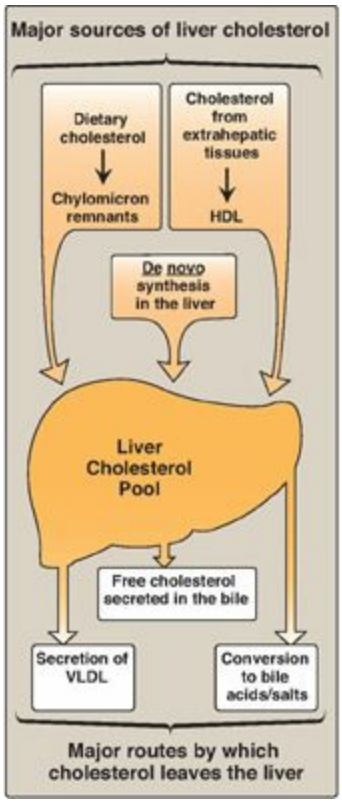
Cholesterol, Lipoprotein, and Steroid Metabolism

18

I. OVERVIEW

Cholesterol, the characteristic steroid alcohol of animal tissues, 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. It is, therefore, 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 liver's cholesterol pool from a number of sources including dietary cholesterol as well as that 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). HDL = highdensity lipoprotein; VLDL = very-lowdensity lipoprotein.



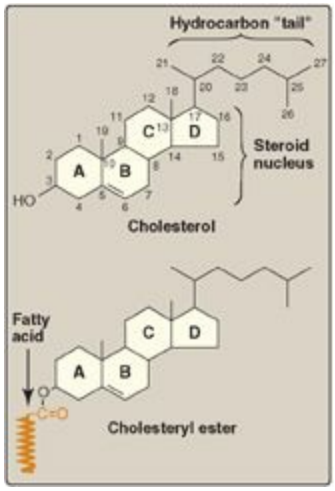
II. STRUCTURE OF CHOLESTEROL

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 (Figure 18.2).

A. Sterols

Steroids with eight to ten 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. [Note: Intestinal uptake of cholesterol is mediated, at least in part, by the protein Niemann-Pick C1-like 1 protein (NPC1-L1), the target of the drug ezetimibe that reduces absorption of dietary cholesterol (see p. 176). 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 transporter result in the rare condition of sitosterolemia. Because some cholesterol is transported back as well, plant sterols reduce the absorption of dietary cholesterol. Daily ingestion of plant sterol esters supplied, for example, in spreads or juices, is one of a number of dietary strategies to reduce plasma cholesterol levels (see p. 363).]

Figure 18.2 Structure of cholesterol and its ester.



B. Cholesteryl esters

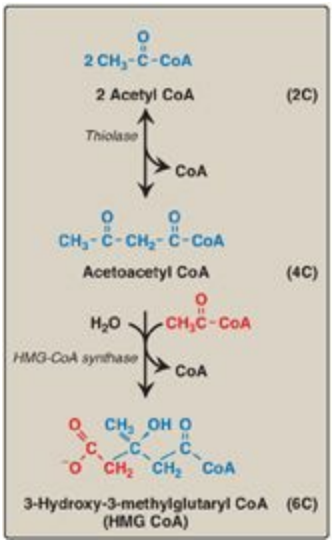
Most plasma cholesterol is in an esterified form (with a fatty acid attached at carbon 3, as shown in Figure 18.2), which makes the structure even more hydrophobic than free (unesterified) cholesterol. Cholesteryl esters are not found in membranes and are normally present only in low levels in most cells. Because of their hydrophobicity, cholesterol and its esters must be transported in association with protein as a component of a lipoprotein particle (see p. 227) or be solubilized by phospholipids and

bile salts in the bile (see p. 226).

III. SYNTHESIS OF CHOLESTEROL

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 body's cholesterol pool. As with fatty acids, 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 adenosine triphosphate (ATP). Synthesis requires enzymes in both the cytosol and the membrane of the smooth endoplasmic reticulum (ER). The pathway is responsive to changes in cholesterol concentration, and regulatory mechanisms exist to balance the rate of cholesterol synthesis within the body 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. Synthesis of 3-hydroxy-3-methylglutaryl coenzyme A

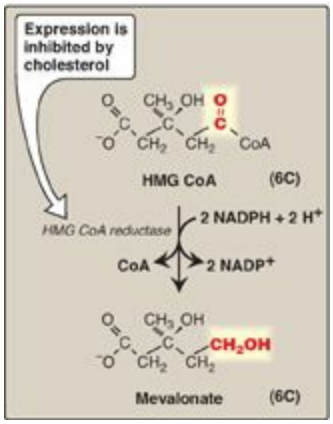
The first two reactions in the cholesterol synthetic pathway are similar to those in the pathway that produces ketone bodies (see Figure 16.22, p. 196). They result in the production of 3-hydroxy-3-methylglutaryl CoA ([HMG CoA] Figure 18.3). First, two acetyl CoA molecules condense to form acetoacetyl 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. Synthesis of mevalonate

The next step, the reduction of HMG CoA to mevalonate, is catalyzed by HMG CoA reductase and 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 (Figure 18.4). [Note: HMG CoA reductase is an integral membrane protein of the ER, with its catalytic domain projecting into the cytosol.] Regulation of reductase activity is discussed below.

Figure 18.4 Synthesis of mevalonate. HMG CoA = hydroxymethylglutaryl coenzyme A; NADP(H) = nicotinamide adenine dinucleotide phosphate.



C. Synthesis of cholesterol

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. Nonsterol isoprenoids include dolichol (see p. 167) and ubiquinone, or coenzyme Q (see p. 75).]

[3] IPP is isomerized to 3,3-dimethylallyl pyrophosphate (DPP).

[4] IPP and DPP condense to form ten-carbon geranyl pyrophosphate (GPP).

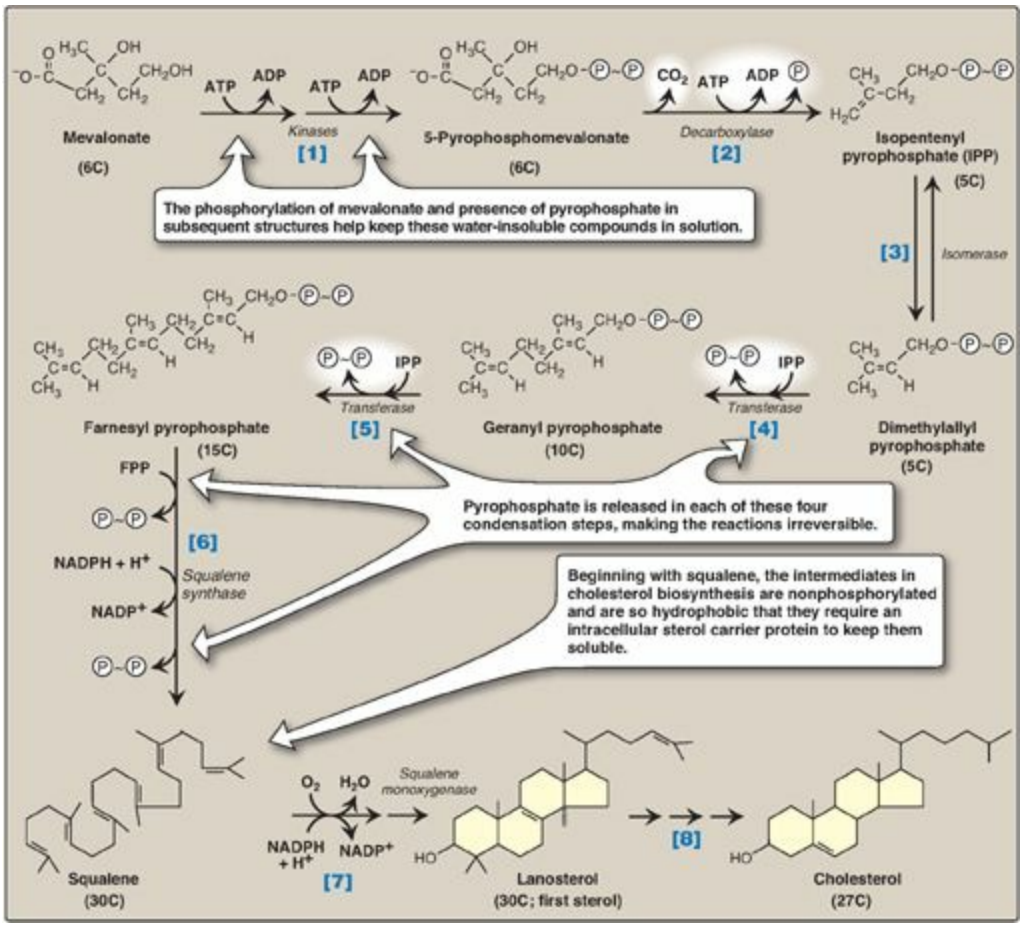
[5] A second molecule of IPP then condenses with GPP to form 15-carbon farnesyl pyrophosphate (FPP). [Note: Covalent attachment of farnesyl to proteins, a process known as "prenylation," is one mechanism for anchoring proteins (such as ras) to plasma membranes.]

[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 three 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 reactions catalyzed by ER-associated enzymes that use molecular oxygen 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, ER-associated 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 p. 386).]

Figure 18.5 Synthesis of cholesterol from mevalonate. ADP = adenosine diphosphate; Ⓟ = phosphate; Ⓟ~Ⓟ = pyrophosphate; NADP(H) = nicotinamide adenine dinucleotide phosphate.



D. Regulation of cholesterol synthesis

HMG CoA reductase is the major control point for cholesterol biosynthesis and is subject to different kinds of metabolic control.

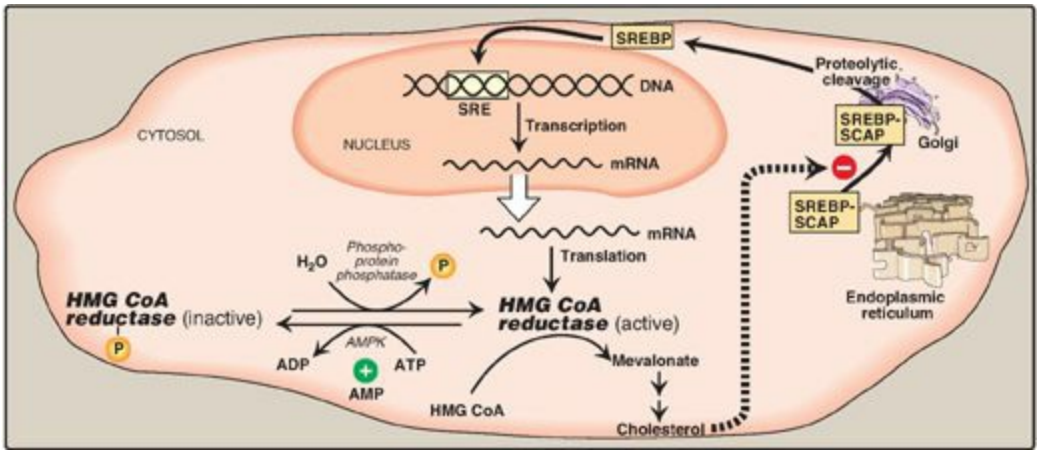
1. **Sterol-dependent regulation of gene expression:** Expression of the gene for HMG CoA reductase is controlled by the transcription factor, SREBP-2 (sterol regulatory element–binding protein-2) that binds DNA at the cis-acting sterol regulatory element (SRE) upstream of the reductase gene. SREBP-2 is an integral protein of the ER membrane, and associates with a second ER membrane protein, SCAP (SREBP cleavage–activating protein). When sterol levels in the cell are low, the SREBP–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 (Figure 18.6). If sterols are abundant, however, they bind SCAP at its sterol-sensing domain and induce the binding of SCAP to yet other ER membrane proteins, the insigs (insulin-induced gene [products]). This results in the retention of the SCAP–SREBP complex in the ER, thereby preventing the activation of SREBP-2, and leading to downregulation of cholesterol synthesis. [Note: SREBP-1 upregulates expression of enzymes involved in fatty acid synthesis in response to insulin (see p. 184).]

2. **Sterol-accelerated enzyme degradation:** The reductase itself is a sterol-sensing integral protein of the ER membrane. When sterol levels in the cell are high, the enzyme binds to insig proteins. Binding leads to ubiquitination and proteasomal degradation of the reductase (see p. 247).

3. **Sterol-independent phosphorylation/dephosphorylation:** HMG CoA reductase activity is controlled covalently through the actions of adenosine monophosphate (AMP)-activated protein kinase ([AMPK] see p. 183) and a phosphoprotein phosphatase (see Figure 18.6). The phosphorylated form of the enzyme is inactive, whereas the dephosphorylated form is active. [Note: Because AMPK is activated by AMP, cholesterol synthesis, like fatty acid synthesis, is decreased when ATP availability is decreased.]

4. **Hormonal regulation:** The amount of HMG CoA reductase is controlled hormonally. An increase in insulin and thyroxine favors upregulation of the expression of the gene for the reductase. Glucagon and the glucocorticoids have the opposite effect.

Figure 18.6 Regulation of hydroxymethylglutaryl coenzyme A (HMG CoA) reductase. SRE = sterol regulatory element; SREBP = sterol regulatory element-binding protein; SCAP = SREBP cleavage-activating protein; AMPK = adenosine monophosphate-activated protein kinase; ADP = adenosine diphosphate; P = phosphate; mRNA = messenger RNA.

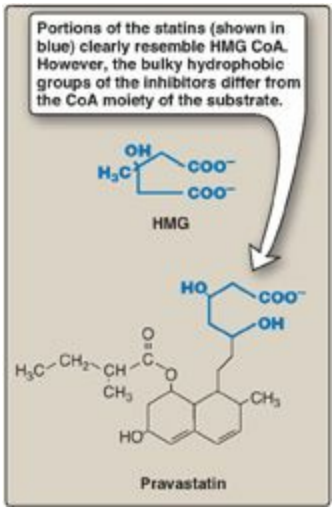


5. Inhibition by drugs: The statin drugs (atorvastatin, fluvastatin, lovastatin, pravastatin, rosuvastatin, and simvastatin) are structural analogs of HMG CoA, and are (or are metabolized to) reversible, competitive inhibitors of HMG CoA reductase (Figure 18.7). They are used to decrease plasma cholesterol levels in patients with hypercholesterolemia.

IV. DEGRADATION OF CHOLESTEROL

The ring structure of cholesterol cannot be metabolized to CO_2 and H_2O in humans. Rather, the intact sterol 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. 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.

Figure 18.7 Structural similarity of hydroxymethylglutaric acid (HMG) and pravastatin, a clinically useful cholesterol-lowering drug of the "statin" family. CoA = coenzyme A.



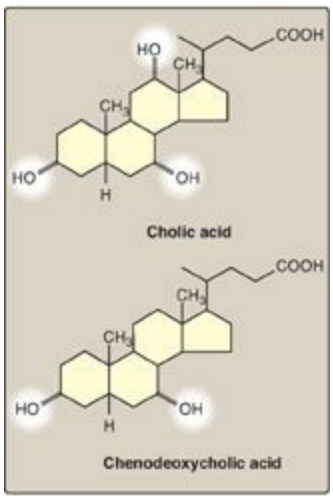
V. BILE ACIDS AND BILE SALTS

Bile consists of a watery mixture of organic and inorganic compounds. Phosphatidylcholine, or lecithin (see p. 202), 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 of the bile acids

The bile acids contain 24 carbons, with two or three hydroxyl groups and a side chain that terminates in a carboxyl group. The carboxyl group has a pK_a of about 6. In the duodenum (pH approximately 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 the methyl groups that are β (they lie "above" the plane of the rings). Therefore, the molecules have both a polar and a nonpolar face and can act as emulsifying agents in the intestine, helping prepare dietary triacylglycerol and other complex lipids for degradation by pancreatic digestive enzymes.

Figure 18.8 Bile acids. [Note: The ionized forms are bile salts.]



B. Synthesis of bile acids

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 18.8, are called "primary" bile acids. [Note: 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 ER-associated cytochrome P450 monooxygenase found only in

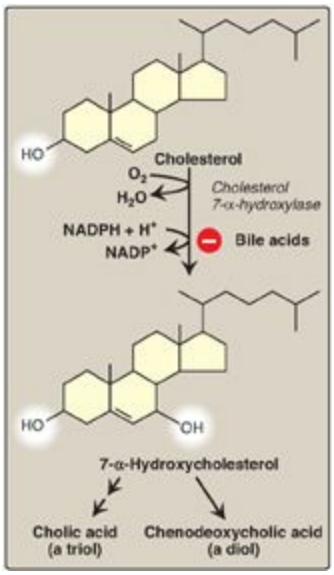
liver. Expression of the enzyme is downregulated by bile acids (Figure 18.9)]

C. Synthesis of conjugated bile acids

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 (Figure 18.10). The ratio of glycine to taurine forms in the bile is approximately 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. 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 18.9 Synthesis of the bile acids, cholic acid and chenodeoxycholic acid, from cholesterol.

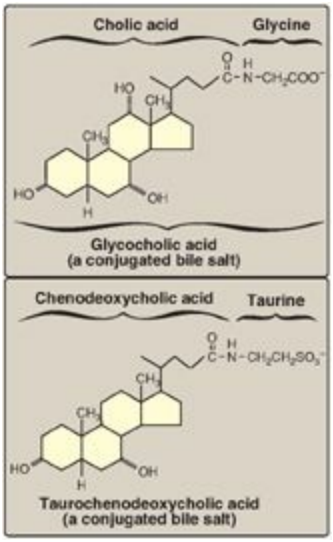


D. Action of intestinal flora on bile salts

Bacteria in the intestine can deconjugate (remove glycine and taurine) bile salts. They can also remove the hydroxyl group at carbon 7, producing "secondary" bile salts such as deoxycholic acid from cholic acid and lithocholic acid from chenodeoxycholic acid

(Figure 18.11).

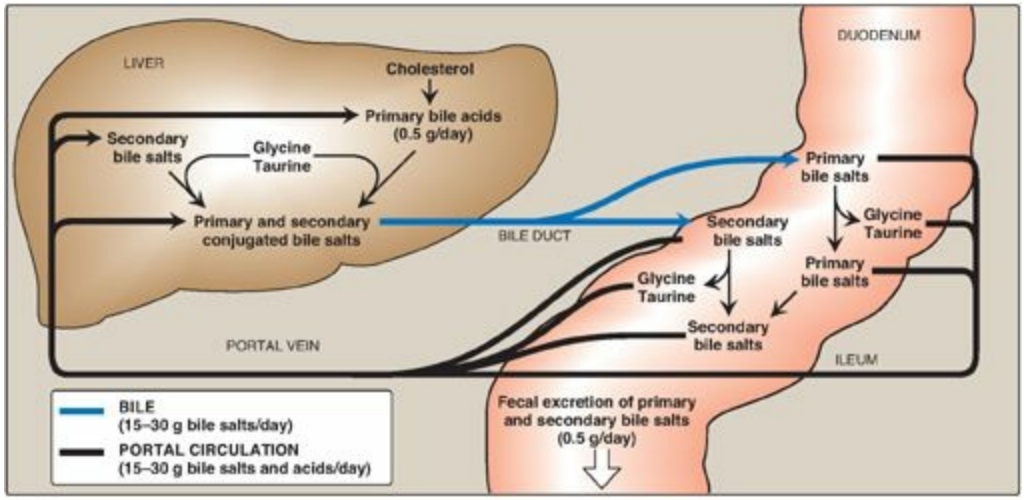
Figure 18.10 Conjugated bile salts. Note "cholic" in the names.



E. Enterohepatic circulation

Bile salts secreted into the intestine are efficiently reabsorbed (greater than 95%) and reused. The liver actively secretes bile salts into the bile. In the intestine, they are reabsorbed in the terminal ileum via a Na^+-bile salt cotransporter and returned to the blood via a separate transport system. [Note: Lithocolic acid is only poorly absorbed.] They are efficiently taken up from blood by the hepatocytes via an isoform of the cotransporter and reused. [Note: Albumin binds bile salts noncovalently and transports them through the blood as was seen with fatty acids (see p. 181).] The continuous process of secretion of bile salts into the bile, their passage through the duodenum where some are deconjugated then dehydroxylated to secondary bile salts, their uptake in the ileum, and their subsequent return to the liver as a mixture of primary and secondary forms is termed the enterohepatic circulation (see Figure 18.11). Between 15 and 30 g of bile salts are secreted from the liver into the duodenum each day, yet only about 0.5 g (less than 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; prevent their reabsorption; and, so, promote 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 p. 365).]

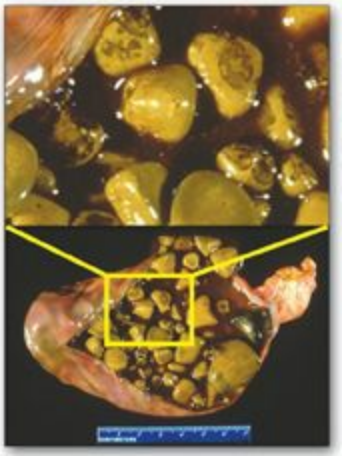
Figure 18.11 Enterohepatic circulation of bile salts. [Note: Primary forms are converted to secondary forms by dehydroxylation.]



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 phosphatidylcholine present, the cholesterol may precipitate in the gallbladder, leading to cholesterol gallstone disease, or cholelithiasis (Figure 18.12). 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 (for example, gemfibrozil) to reduce cholesterol (and triacylglycerol) 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 over 85% of cases of cholelithiasis, with bilirubin and mixed stones accounting for the rest].

Figure 18.12 Gallbladder with gallstones.



VI. PLASMA LIPOPROTEINS

The plasma lipoproteins are spherical macromolecular complexes of lipids and specific proteins (apolipoproteins). The lipoprotein particles include chylomicrons, very-low-density lipoproteins (VLDLs), low-density lipoproteins (LDLs), and high-density lipoproteins (HDLs). They differ in lipid and protein composition, size, density (Figure 18.13), and site of origin. [Note: Because lipoprotein particles constantly interchange lipids and apolipoproteins with each other, 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, the transport system is less perfect than in other animals and, as a result, humans experience a gradual deposition of lipid (especially cholesterol) in tissues. This is a potentially life-threatening occurrence when the lipid deposition contributes to plaque formation, causing the narrowing of blood vessels (atherosclerosis).

A. Composition of plasma lipoproteins

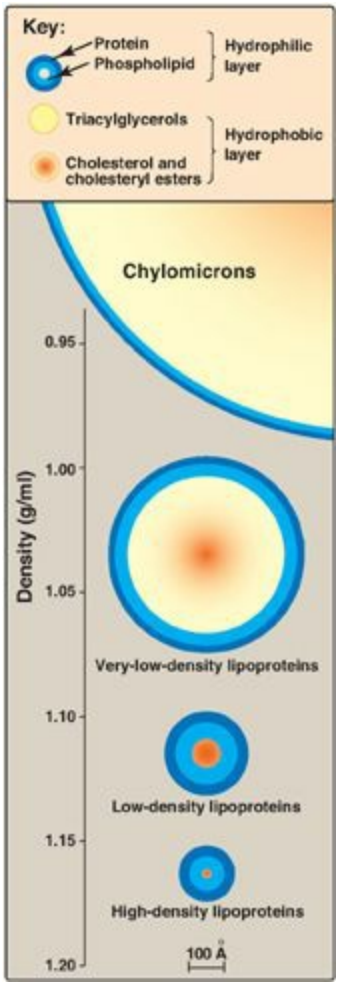
Lipoproteins are composed of a neutral lipid core (containing triacylglycerol [TAG] and cholesteryl esters) surrounded by a shell of amphipathic apolipoproteins, phospholipid, and unesterified (free) cholesterol (Figure 18.14). These amphipathic compounds are oriented so that their polar portions are 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. Total C = LDL – C + HDL – C + VLDL – C, where VLDL – C is calculated by dividing TAG by 5 because TAG represent 20% of the volume of VLDL. The goal value for total cholesterol is less than 200 mg/dl.]

1. **Size and density of lipoprotein particles:** 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 18.15, 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 (for example, apolipoprotein [apo] C-I, apo C-II, and apo C-III). [Note: Functions of all of the apolipoproteins are not yet known.]

Figure 18.13 Approximate size and density of serum lipoproteins. Each family of lipoproteins exhibits a range of sizes and densities, and this figure shows typical values. The width of the rings approximates 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 a surface component, whereas cholesteryl esters are located in the interior of the lipoproteins.]



B. Metabolism of chylomicrons

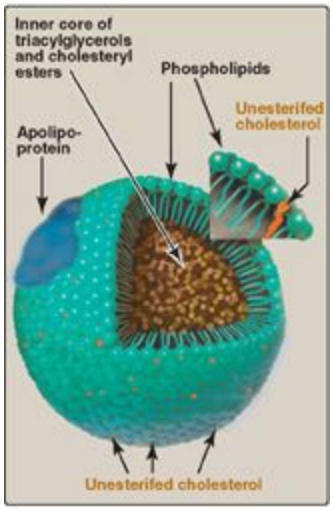
Chylomicrons are assembled in intestinal mucosal cells and carry dietary (exogenous) TAG, cholesterol, fat-soluble vitamins, and cholesteryl esters to the peripheral tissues (Figure 18.16). [Note: TAGs account for close to 90% of the lipids in a chylomicron.]

1. **Synthesis of apolipoproteins:** Apo B-48 is unique to chylomicrons. Its synthesis begins on the rough ER, and it is glycosylated as it moves through the ER 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 p. 457) of a cytosine to a uracil in intestinal apo B-

100 messenger RNA (mRNA) creates a nonsense (stop) codon (see p. 433), allowing translation of only 48% of the mRNA.]

2. **Assembly of chylomicrons:** The enzymes involved in TAG, cholesterol, and phospholipid synthesis are located in the smooth ER. Assembly of the apolipoproteins and lipid into chylomicrons requires microsomal triglyceride transfer protein ([MTP], see p. 178), 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 18.14 Structure of a typical lipoprotein particle.

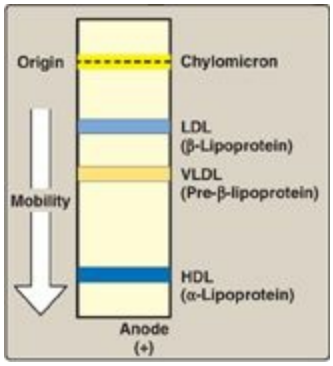


3. **Modification of nascent chylomicron particles:** The particle released by the intestinal mucosal cell is called a "nascent" chylomicron because it is functionally incomplete. When it reaches the plasma, the particle is rapidly modified, receiving apolipoproteins E (which is recognized by hepatic receptors) and 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 (see below). The source of these apolipoproteins is circulating HDL (see Figure 18.16).

4. **Degradation of triacylglycerol by lipoprotein lipase:** LPL is an extracellular enzyme that is anchored by heparan sulfate to the capillary walls of most tissues, but predominantly those of adipose tissue and cardiac and skeletal muscle. Adult liver does not have this enzyme. [Note: A hepatic lipase is found on the surface of endothelial cells of the liver. It plays some role in TAG degradation in chylomirons and VLDL and is particularly important in HDL metabolism (see p. 236).] LPL, activated by apo C-II on circulating lipoprotein particles, hydrolyzes the TAG contained in these particles to yield fatty acids and glycerol. The fatty acids are stored (by the adipose) or used for energy (by the muscle). If they are not immediately taken up by a cell, the long-chain fatty acids are transported by serum

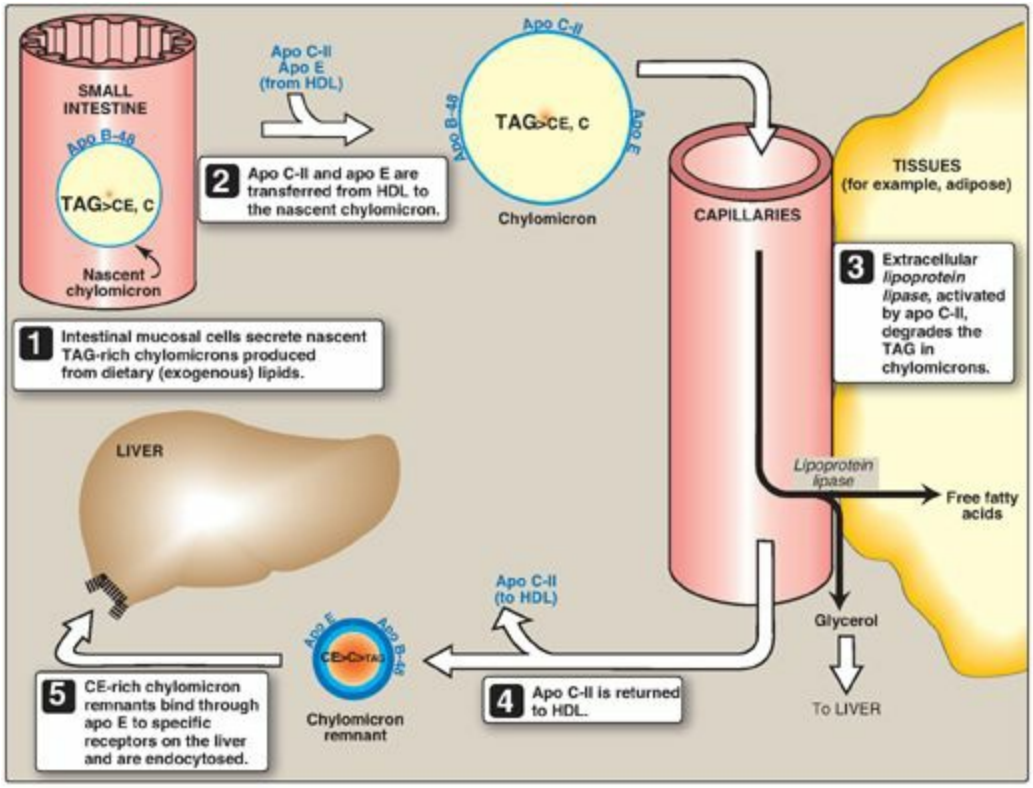
albumin until their uptake does occur. The glycerol is used by the liver, for example, in lipid synthesis or gluconeogenesis. [Note: Patients with a deficiency of LPL or apo C-II (type 1 hyperlipoproteinemia, or familial LPL-deficiency) show a dramatic accumulation (1,000 mg/dl or greater) of chylomicron-TAG in the plasma (hypertriacylglycerolemia) even in the fasted state. These individuals are at increased risk for acute pancreatitis.]

Figure 18.15 Electrophoretic mobility of plasma lipoproteins. The order of low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) is reversed if ultracentrifugation is used as the separation technique. HDL = highdensity lipoprotein.



5. **Regulation of lipoprotein lipase activity:** 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 fatty acids to provide much of the energy needed for cardiac function.]

Figure 18.16 Metabolism of chylomicrons. Apo B-48, apo C-II, and apo E are apolipoproteins found as specific components of plasma lipoproteins. The lipoprotein particles are not drawn to scale (see Figure 18.13 for details of their size and density). TAG = triacylglycerol; C = cholesterol; CE = cholesteryl ester; HDL = high-density lipoprotein particle.



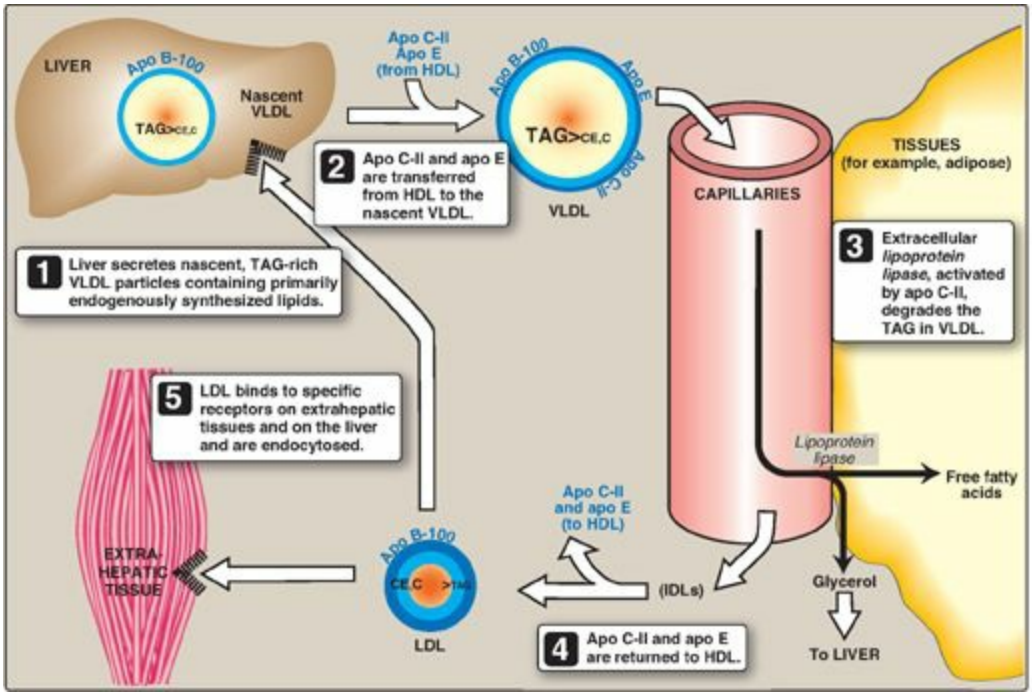
6. **Formation of chylomicron remnants:** As the chylomicron circulates, and more than 90% of the TAG in its core is degraded by LPL, the particle decreases in size and increases in density. In addition, the C apolipoproteins (but not apo E) are returned to HDL. The remaining particle, called a "remnant," is rapidly removed from the circulation by the liver, whose cell membranes contain lipoprotein receptors that recognize apo E (see Figure 18.16). 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 fatty acids. The receptor is recycled. (A more detailed discussion of the mechanism of receptor-mediated endocytosis is illustrated for LDL in Figure 18.20.)

C. Metabolism of very-low-density lipoproteins

VLDLs are produced in the liver (Figure 18.17). They are composed predominantly of endogenous TAG (approximately 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 (see p. 228). [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.]

Figure 18.17 Metabolism of very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL). Apo B-100, apo C-II, and apo E are apolipoproteins found as specific components of plasma lipoprotein particles. The lipoproteins are not drawn to scale (see

Figure 18.13 for details of their size and density). TAG = triacylglycerol; HDL = high-density lipoprotein particle; IDLs = intermediate-density lipoprotein particles; C = cholesterol; CE = cholesteryl ester.



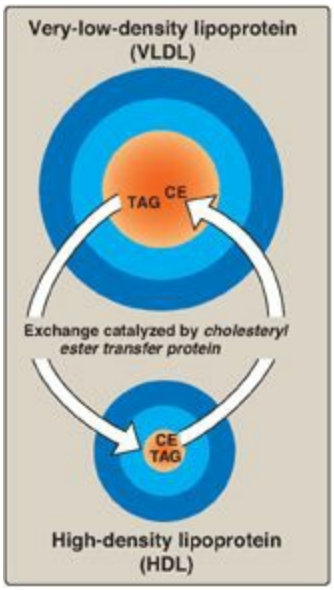
1. **Release from liver:** VLDLs are secreted directly into the blood by the liver as nascent particles containing apo B-100. They must obtain apo C-II and apo E from circulating HDL (see Figure 18.17). As with chylomicrons, apo C-II is required for activation of LPL. [Note: Abetalipoproteinemia is a rare hypolipoproteinemia caused by a defect in MTP, leading to an inability to load apo B with lipid. As a consequence, few VLDLs or chylomicrons are formed, and TAGs accumulate in the liver and intestine.]

2. **Modification in the circulation:** As VLDLs pass through the circulation, TAG is degraded by LPL, causing the VLDLs 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 18.18.

3. **Conversion to low-density lipoproteins:** With these modifications, the VLDL is converted in the plasma to LDL. Intermediate-sized particles, the intermediate-density lipoproteins (IDLs), or VLDL remnants, are observed during this transition. IDLs can also be taken up by liver cells through receptor-mediated endocytosis that uses apo E as the ligand. [Note: 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 chylomicron remnants and IDLs. These individuals have familial type III hyperlipoproteinemia (familial dysbetalipoproteinemia, or broad beta disease), with

hypercholesterolemia and premature atherosclerosis. Not as yet well understood is the fact that the E-4 isoform confers increased susceptibility to and decreased 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 18.18 Transfer of cholesteryl ester (CE) from HDL to VLDL in exchange for triacylglycerol (TAG).



D. Metabolism of low-density lipoproteins

LDL particles contain much less TAG than their VLDL predecessors and have a high concentration of cholesterol and cholesteryl esters (Figure 18.19).

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 cell surface 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 18.20. [Note: The numbers in brackets below refer to corresponding numbers on that figure.] A similar mechanism of receptor-mediated endocytosis is used for the cellular 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 taken in by endocytosis. [Note: A deficiency of functional LDL receptors causes a significant elevation in plasma LDL-C. Patients with such deficiencies have type II hyperlipidemia (familial hypercholesterolemia, or FH) and premature atherosclerosis. Autosomal-dominant hypercholesterolemia can also be caused by increased activity of a protease,

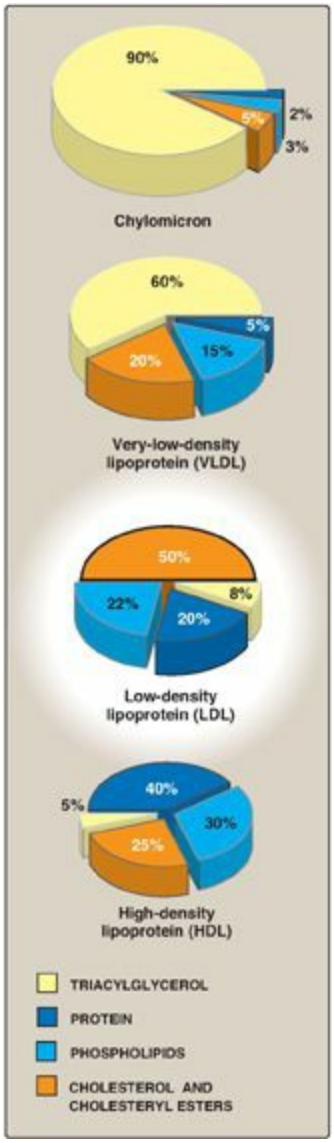
proprotein convertase subtilisin/kexin type 9 (PCSK9), which promotes degradation of the receptor, and by defects in apo B-100 that reduce its binding to the receptor.]

[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 LDLs stay free within the lumen of the vesicle. [Note: This structure is called CURL, the compartment for uncoupling of receptor and ligand.]

[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, fatty acids, and phospholipids. These compounds can be reutilized by the cell. [Note: Storage diseases caused by rare autosomal-recessive deficiencies in the ability to hydrolyze lysosomal cholesteryl esters (late-onset Wolman disease), or to transport free cholesterol out of the lysosome (Niemann-Pick disease, Type C) have been identified.]

Figure 18.19 Composition of the plasma lipoproteins. Note the high concentration of cholesterol and cholesteryl esters in LDL.

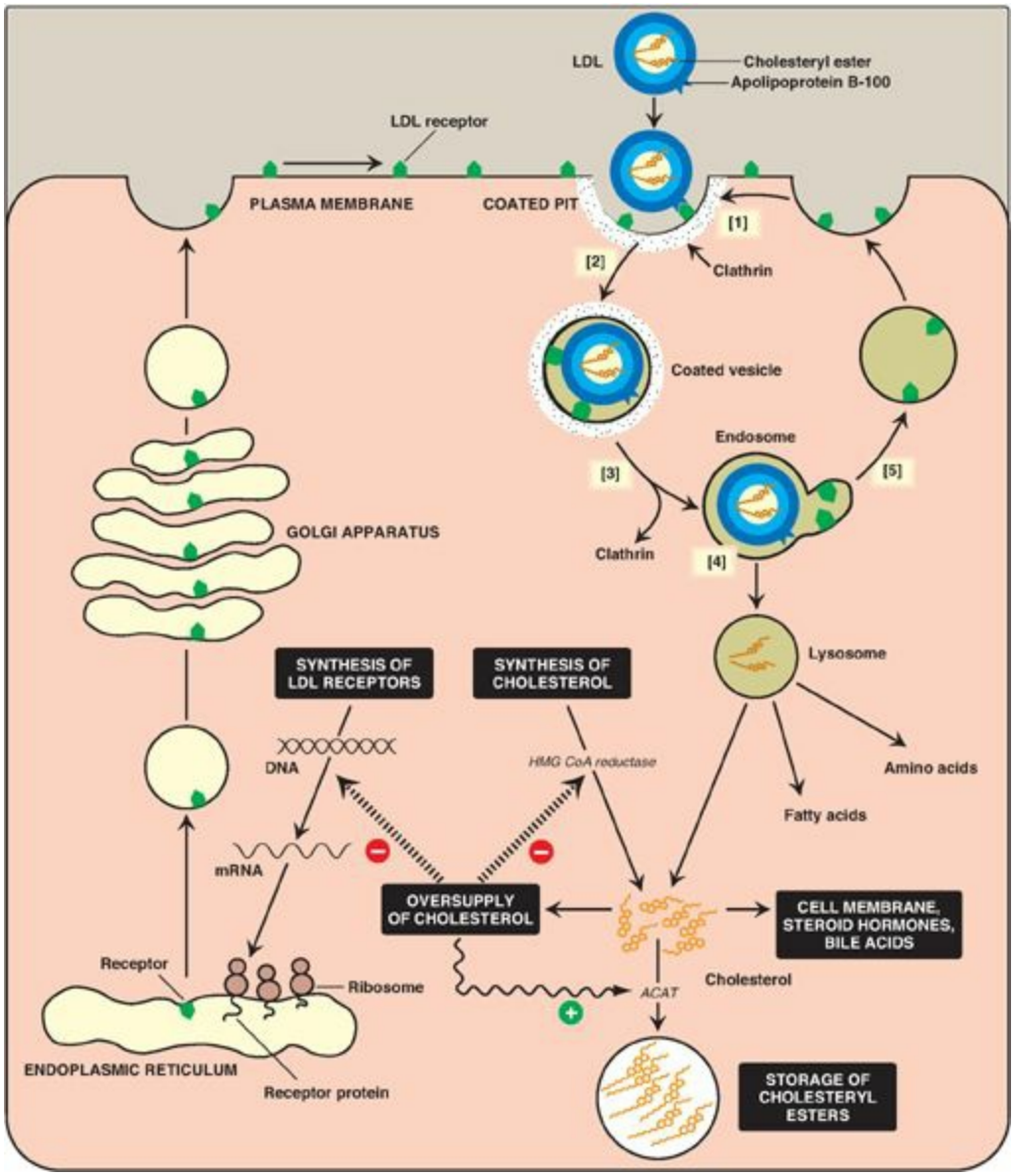


2. **Effect of endocytosed cholesterol on cellular cholesterol homeostasis:** The chylomicron remnant-, IDL-, and LDL-derived cholesterol affects cellular cholesterol content in several ways (see Figure 18.20). First, expression of the gene for HMG CoA reductase is inhibited by high cholesterol, as a result of which, de novo cholesterol synthesis decreases. 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 cholesterol into cells. [Note: Regulation of the LDL receptor gene involves a SRE and SREBP-2, as was seen in the regulation of the gene for HMG CoA reductase (see p. 222). 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 fatty acid from a fatty acyl CoA to cholesterol, producing a cholesteryl ester that can be stored in the cell (Figure 18.21). The activity of ACAT is enhanced in the presence of increased intracellular cholesterol.

3. **Uptake of chemically modified LDL 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

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 components or apo B have been oxidized. Unlike the LDL receptor, the scavenger receptor 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 (Figure 18.22).

Figure 18.20 Cellular uptake and degradation of low-density lipoprotein (LDL). [Note: Oversupply of cholesterol accelerates the degradation of HMG CoA reductase. It also decreases synthesis of the reductase by preventing expression of its gene as seen with the LDL receptor.] ACAT = acyl CoA:cholesterol acyltransferase; HMG CoA = hydroxymethylglutaryl coenzyme A; mRNA = messenger RNA.



E. Metabolism of high-density lipoproteins

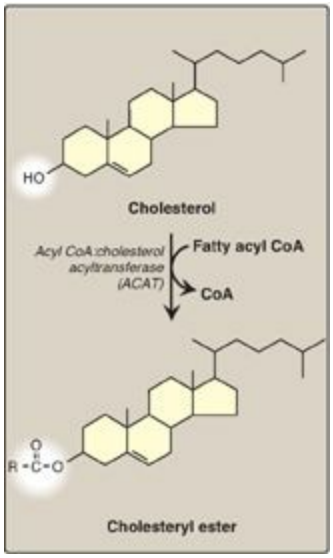
HDLs comprise a heterogeneous family of lipoproteins with a complex metabolism that is not yet completely understood. HDL particles are formed in blood by the addition of

lipid to apo A-1, an apolipoprotein made by the liver and intestine and secreted into blood. [Note: HDLs are also formed within the liver and intestine.] Apo A-1 accounts for about 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 o f LPL) and apo E (the apolipoprotein required for the receptor-mediated endocytosis of IDLs and chylomicron remnants).

2. **Uptake of unesterified cholesterol:** Nascent HDLs are disc-shaped particles containing primarily phospholipid (largely phosphatidylcholine) and apolipoproteins A, C, and E. They take up cholesterol from nonhepatic (peripheral) tissues and return it to the liver as cholesteryl esters (Figure 18.23). [Note: HDL particles are excellent acceptors of unesterified cholesterol as a result of their high concentration of phospholipids, which are important solubilizers of cholesterol.]

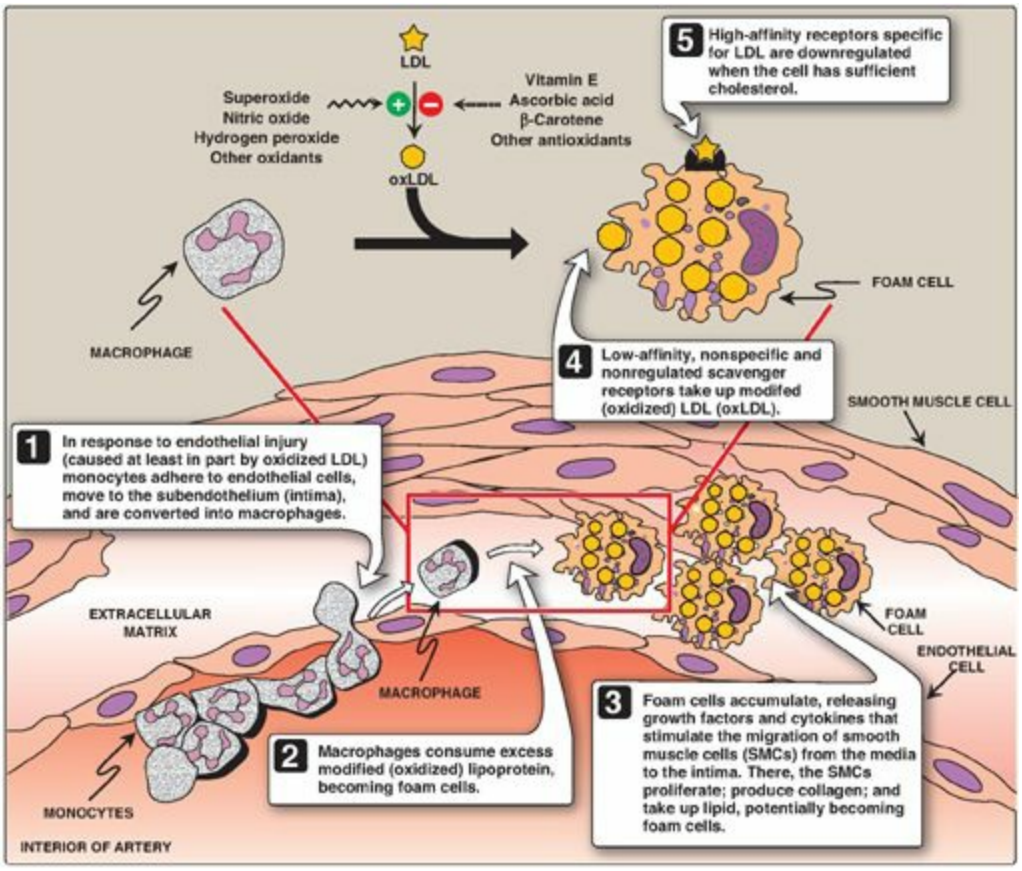
Figure 18.21 Synthesis of intracellular cholesteryl ester by ACAT. [Note: Lecithin:cholesterol acyl transferase (LCAT) is the extracellular enzyme that esterifies cholesterol using phosphatidylcholine (lecithin) as the source of the fatty acid.] CoA = coenzyme A.



3. **Esterification of cholesterol:** When cholesterol is taken up by HDL, it is immediately esterified by the plasma enzyme lecithin:cholesterol acyltransferase (LCAT, also known as PCAT, in which "P" stands for phosphatidylcholine, the source of the fatty acid). This enzyme is synthesized and secreted by the liver. LCAT binds to nascent HDL, and is activated by apo A-I. LCAT transfers the fatty acid from carbon 2 of phosphatidylcholine 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. CETP (see p. 231) moves some of the cholesteryl esters from HDL to VLDL in exchange for TAG, relieving product inhibition of LCAT. Because VLDLs are catabolized to LDL, the cholesteryl esters transferred by CETP are ultimately taken up by the liver (see p. 236).

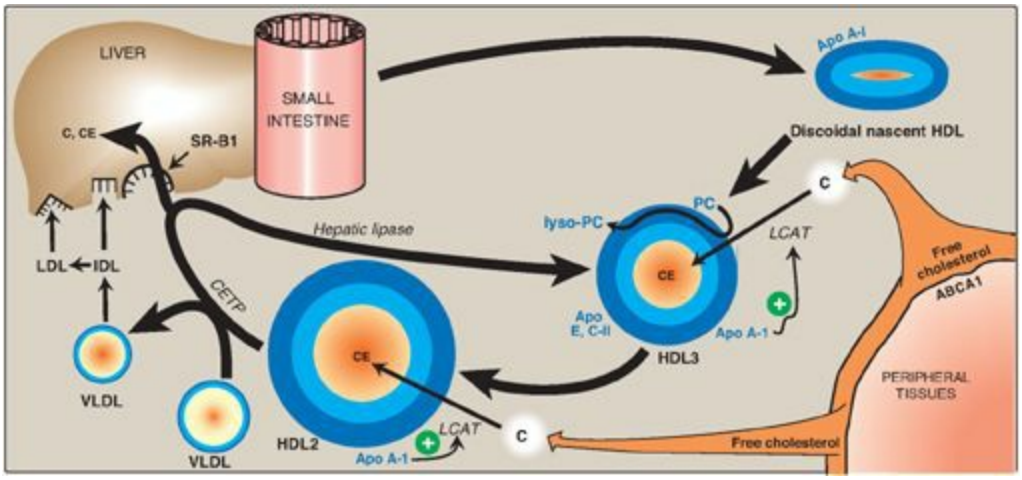
Figure 18.22 Role of oxidized lipoproteins in plaque formation in an arterial wall. LDL = low-density lipoprotein.



4. **Reverse cholesterol transport:** The selective transfer of cholesterol from peripheral cells to HDL, from HDL to the liver for bile acid synthesis or disposal via the bile, and to steroidogenic cells for hormone synthesis, is a key component of cholesterol homeostasis. This process of reverse cholesterol transport is, in part, the basis for the inverse relationship seen between plasma HDL concentration and atherosclerosis and for HDL's designation as the "good" cholesterol carrier. [Note: Exercise and estrogen raise HDL levels.] Reverse cholesterol transport 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 steroidogenic cells), the selective transfer of the cholesteryl esters into these cells, and the release of lipid-depleted HDL (HDL3). The efflux of cholesterol from peripheral cells is mediated, at least in part, 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.] The uptake of cholesteryl esters

by the liver is mediated by a cell-surface receptor, SR-B1 (scavenger receptor class B type 1) that binds HDL (see p. 234 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: Hepatic lipase, with its ability to degrade both TAG and phospholipids, also participates in the conversion of HDL2 to HDL3.]

Figure 18.23 Metabolism of high-density lipoprotein (HDL) particles. Apo = apolipoprotein; ABCA1 = transport protein; C = cholesterol; CE = cholesteryl ester; LCAT = lecithin:cholesterol acyltransferase; VLDL = very-low-density lipoprotein; IDL = intermediate-density lipoprotein; LDL = low-density lipoprotein; 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, X-linked adrenoleukodystrophy, respiratory distress syndrome due to decreased surfactant secretion, and cystic fibrosis.

F. Role of lipoprotein (a) in heart disease

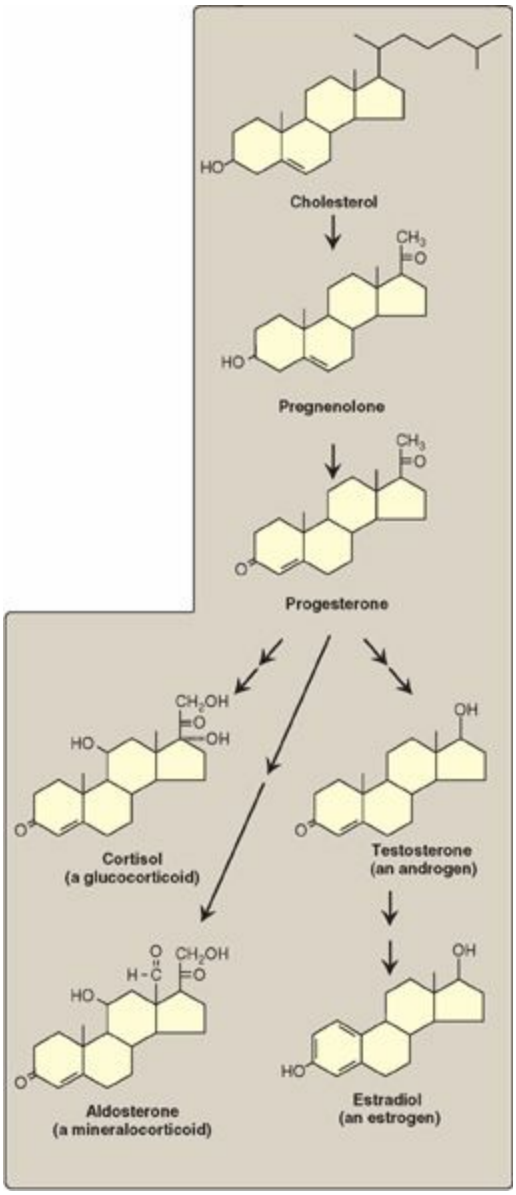
Lipoprotein (a), or Lp(a), is a particle that, when present in large quantities in the plasma, is associated with an increased risk of coronary heart disease. Lp(a) is nearly identical in structure to an LDL particle. Its distinguishing feature is the presence of an additional apolipoprotein molecule, apo(a), that is covalently linked at a single site to apo B-100. Circulating levels of Lp(a) are determined primarily by genetics. However, factors such as diet may play some role, as trans fatty acids have been shown to increase Lp(a), whereas ω-3 fatty acids decrease it. [Note: Apo(a) is structurally homologous to plasminogen, the precursor of a blood protease whose target is fibrin, the main protein component of blood clots (See Chapter 34 online). It is hypothesized that elevated Lp(a) slows the breakdown of blood clots that trigger heart attacks

because it competes with plasminogen for binding to fibrin. The physiologic function of Lp(a) in unknown. Niacin reduces Lp(a), as well as LDL-cholesterol and TAGs, and raises HDL.]

VII. STEROID HORMONES

Cholesterol is the precursor of all classes of steroid hormones: glucocorticoids (for example, cortisol), mineralocorticoids (for example, aldosterone), and the sex hormones (that is, androgens, estrogens, and progestins) as shown in Figure 18.24). [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. Plasma 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 (for example, 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 18.25.

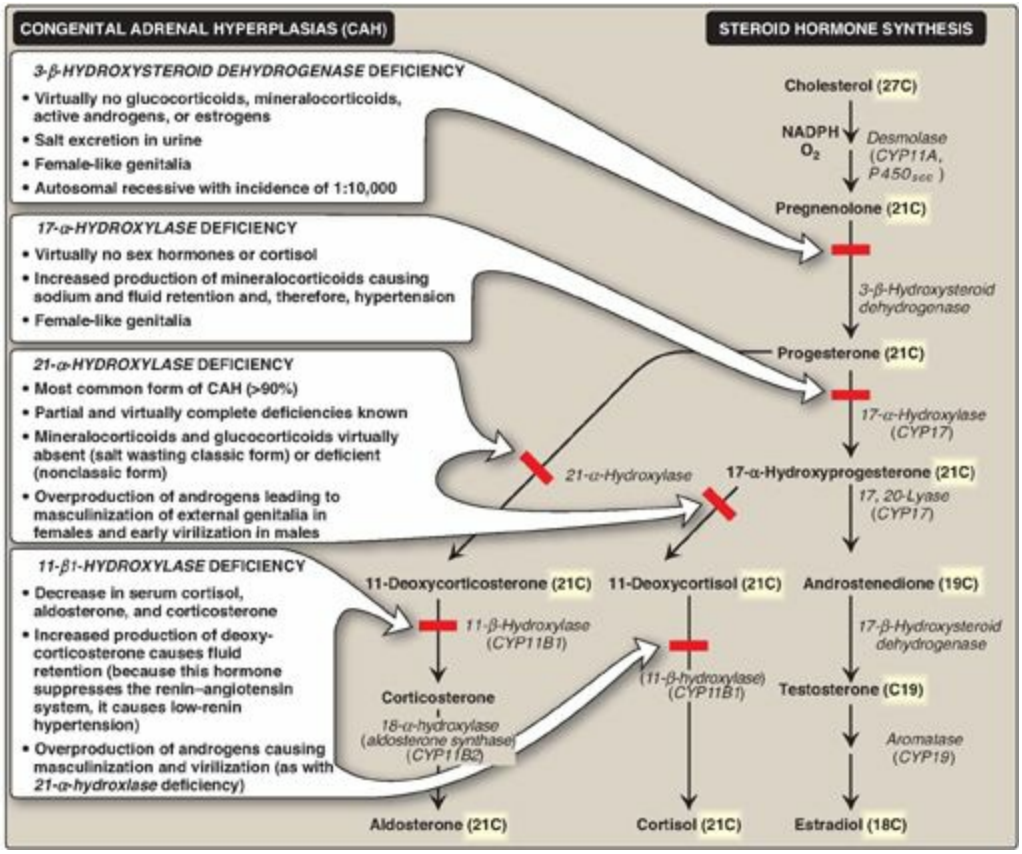
Figure 18.24 Key steroid hormones.



A. Synthesis of steroid hormones

Synthesis involves shortening the hydrocarbon chain of cholesterol and hydroxylation of 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 (desmolase, $P450_{scc}$), a cytochrome P450 (CYP) mixed-function oxidase of the inner mitochondrial membrane (see p. 149). 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 StAR (steroidogenic acute regulatory) protein. Pregnenolone is the parent compound for all steroid hormones (see Figure 18.25). It is oxidized and then isomerized to progesterone, which is further modified to the other steroid hormones by hydroxylation reactions that occur in the ER and mitochondria. Like desmolase, the enzymes primarily are CYP proteins. 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 Figure 18.25). Collectively these disorders are known as the congenital adrenal hyperplasias. [Note: Addison disease, due to autoimmune destruction of the adrenal cortex, is characterized by adrenocortical insufficiency.]

Figure 18.25 Steroid hormone synthesis and associated diseases. [Note: 3-β-Hydroxysteroid dehydrogenase, CYP17, and CYP11B2 are bifunctional enzymes. Synthesis of testosterone and the estrogens from cholesterol occurs primarily outside of the adrenal gland.] NADPH = nicotinamide adenine dinucleotide phosphate; CYP = cytochrome P450.



B. Secretion of adrenal cortical steroid hormones

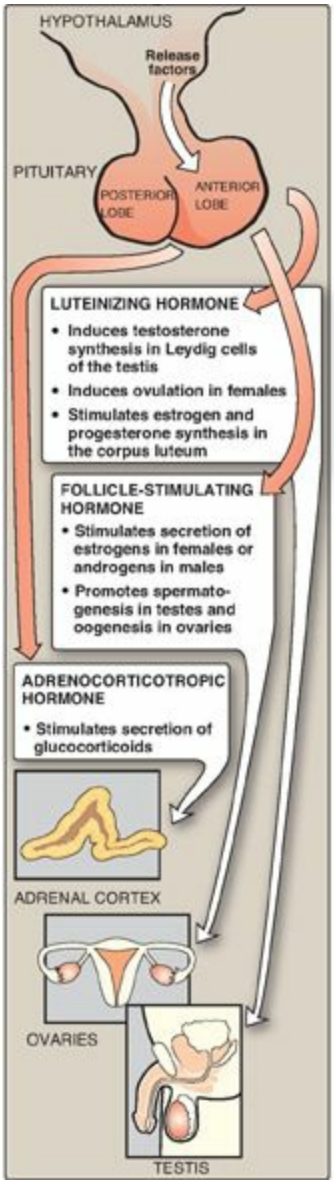
Steroid hormones are secreted on demand from their tissues of origin in response to hormonal signals. The corticosteroids and androgens are made in different regions of the adrenal cortex and are secreted into blood in response to different signals.

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 (Figure 18.26). In response to severe stress (for example, 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). The polypeptide ACTH, the "stress hormone," stimulates the adrenal cortex to synthesize and secrete the glucocorticoid cortisol. Cortisol allows the body to respond to stress through its effects on intermediary metabolism (for example, increased gluconeogenesis) and the inflammatory and immune responses. As cortisol levels rise, the release of CRH and ACTH is inhibited. [Note: ACTH binds to a membrane G protein–coupled receptor, resulting in cyclic AMP (cAMP) production and activation of protein kinase A (PKA) (see p. 94). PKA phosphorylates and activates both the esterase that converts cholesteryl ester to free cholesterol and StAR protein.]

2. **Aldosterone:** Its production in the outer layer (zona glomerulosa) of the adrenal cortex is induced by a decrease in the plasma Na^+/K^+ ratio, and by the hormone angiotensin II. Angiotensin II (an octapeptide) is produced from angiotensin I (a decapeptide) by angiotensin-converting enzyme (ACE), an enzyme found predominantly in the lungs but also distributed widely in the body. [Note:

Angiotensin I is produced in the blood by cleavage of an inactive precursor, angiotensinogen, secreted by the liver. Cleavage is accomplished by the enzyme renin, made and secreted by the kidney.] Angiotensin II binds to cell-surface receptors. However, in contrast to ACTH, its effects are mediated through the phosphatidylinositol 4,5-bisphosphate pathway (see p. 205) and not by cAMP. Aldosterone's primary effect is on the kidney tubules, where it stimulates sodium and water uptake and potassium excretion (Figure 18.27). [Note: An effect of aldosterone is an increase in blood pressure. Competitive inhibitors of ACE are used to treat renin-dependent hypertension.]

Figure 18.26 Pituitary hormone stimulation of steroid hormone synthesis and secretion.



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 in peripheral tissues to testosterone, a stronger androgen, and to estrogens.

Estrogens are derived from androstenedione and testosterone by aromatase (CYP19) . Aromatase inhibitors are used in the treatment of estrogen-responsive breast cancer in postmenopausal women.

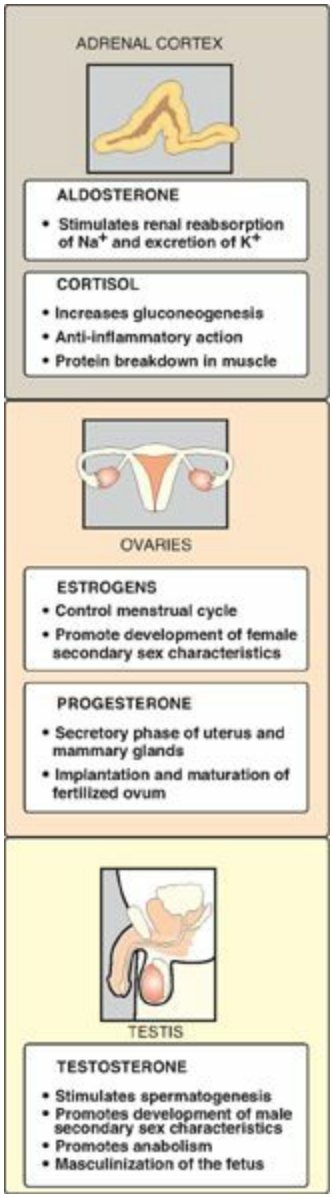
C. Secretion of steroid hormones from gonads

The testes and ovaries 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 Figure 18.27). FSH regulates the growth of ovarian follicles and stimulates testicular spermatogenesis.

D. Mechanism of steroid hormone action

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 promoter activation and increased transcription of targeted genes (Figure 18.28). An HRE is found in the promoter or an enhancer element (see p. 424) for genes that respond to a specific steroid hormone, thus insuring 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 sequence on the DNA. Receptors for the steroid hormones, plus those for thyroid hormone, retinoic acid (see p. 382), and 1,25-dihydroxycholecalciferol (vitamin D, see p. 386), are members of a "superfamily" of structurally related gene regulators that function in a similar way.]

Figure 18.27 Actions of steroid hormones.



E. Further metabolism of steroid hormones

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 3I-phosphoadenosyl-5I-phosphosulfate, see p. 162). Approximately 20%–30% of these metabolites are secreted into the bile and then excreted in the feces, whereas the remainder are released into the blood and filtered from the plasma in the kidney, passing into the urine. These conjugated metabolites are fairly water soluble and do not need protein carriers.

VIII. CHAPTER SUMMARY

Cholesterol is a **hydrophobic** compound, with a single hydroxyl group located at carbon 3 of the A ring, to which a fatty acid can be attached, producing an even more hydrophobic **cholesteryl ester**. Cholesterol is synthesized by virtually all human tissues, although primarily by **liver**, **intestine**, **adrenal cortex**, and **reproductive tissues** (Figure 18.29). All the carbon atoms in cholesterol are provided by **acetyl coenzyme A** (**CoA**), and **nicotinamide adenine dinucleotide phosphate** provides the reducing equivalents. The pathway is driven by hydrolysis of the high-energy thioester bond of acetyl CoA and the terminal phosphate bond of adenosine triphosphate. Cholesterol is synthesized in the **cytoplasm**. The **rate-limiting and regulated step** in cholesterol synthesis is catalyzed by the smooth endoplasmic reticulum–membrane protein, **hydroxymethylglutaryl coenzyme A** (**HMG CoA**) **reductase**, which produces **mevalonate** from HMG CoA. The enzyme is regulated by a number of mechanisms: 1) **Expression of the gene for the reductase** is activated when cholesterol levels are low, via the transcription factor, **sterol regulatory element–binding protein-2** (**SREBP-2**), bound to a sterol regulatory element (**SRE**), resulting in increased enzyme and, therefore, more cholesterol, synthesis; 2) **degradation** of the reductase is accelerated when cholesterol levels are high; 3) reductase activity is controlled by **adenosine monophosphate** (**AMP**)**–activated protein kinase** ([**AMPK**], which phosphorylates and **inactivates** the reductase) and an **insulin-activated protein phosphatase** (which dephosphorylates and **activates** it); and 4) expression of the gene for the reductase is upregulated by insulin and downregulated by 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.

Cholesterol can be eliminated from the body either by **conversion to bile salts** or by **secretion into the bile**. **Bile salts** and **phosphatidylcholine** are quantitatively the most important organic components of bile. The rate-limiting step in bile acid synthesis is catalyzed by **cholesterol-7-gα-hydroxylase**, which is **inhibited** by **bile acids**. Before the bile acids leave the liver, they are conjugated to a molecule of either **glycine** or **taurine**, producing the **conjugated bile salts glycocholic** or **taurocholic acid** and **glycochenodeoxycholic** or **taurochenodeoxycholic acid**. Bile salts (deprotonated) are more amphipathic than bile acids (protonated) and, therefore, are more effective emulsifiers of dietary fat. In the intestine, bacteria can remove the glycine and taurine and can remove a hydroxyl group from the steroid nucleus, producing the **secondary bile salts**, **deoxycholic** and **lithocholic acids**. More than 95% of the bile salts are efficiently reabsorbed in the intestinal ileum by a sodium–bile salt cotransporter, returned to the blood, and carried by albumin back to the liver where they are taken up by the

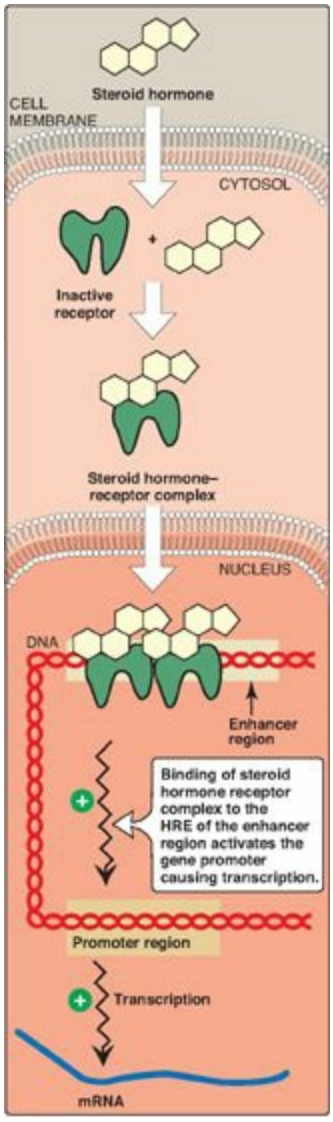
hepatic form of the cotransporter and reused (**enterohepatic circulation, which bile acid sequestrants reduce**). If more cholesterol enters the bile than can be solubilized by the available bile salts and phosphatidylcholine, **cholesterol gallstone disease** (**cholelithiasis**) can occur.

The plasma lipoproteins include **chylomicrons**, **very-low-density lipoproteins** (**VLDLs**) , **low-density lipoproteins** (**LDLs**), and **high-density lipoproteins** (**HDLs**). They function to keep lipids (primarily **triacyl-glycerol** [**TAG**] and **cholesteryl esters**) soluble as they transport them between tissues. Lipoproteins are composed of a **neutral lipid** (**TAG**, **cholesteryl esters**, or both) core surrounded by a shell of amphipathic **apolipoproteins**, **phospholipid**, and **unesterified cholesterol**. **Chylomicrons** are assembled in **intestinal mucosal cells** from **dietary lipids** (primarily **TAG**). Each nascent chylomicron particle has one molecule of **apolipoprotein** (**apo**) **B-48**. They are released from the cells into the lymphatic system and travel to the blood, where they receive **apo C-II** and **apo E** from **HDLs**. Apo C-II activates endothelial **lipoprotein lipase (LPL)**, which degrades the TAG in chylomicrons to fatty acids and glycerol. The **fatty acids** that are released are **stored** (in the **adipose**) or used for **energy** (by the **muscle**). The **glycerol** is metabolized by the **liver**. Patients with a **deficiency** of **LPL** or **apo C-II** show a dramatic accumulation of chylomicrons in the plasma (**type I hyperlipoproteinemia**, or **familial LPL deficiency**) even if fasted. After most of the TAG is removed, apo C-II is returned to the HDL, and the **chylomicron remnant**, carrying most of the **dietary cholesterol**, binds to a **receptor** on the **liver** that recognizes **apo E**. The particle is **endocytosed**, and its contents degraded by **lysosomal enzymes**. Defective uptake of these remnants causes type III hyperlipoproteinemia. **Nascent VLDLs** are produced in the **liver** and are composed predominantly of **TAG**. They contain a single molecule of **apo B-100**. Like nascent chylomicrons, VLDLs receive **apo C-II** and **apo E** from **HDL** in the plasma. The **function** of VLDL is to **carry hepatic TAG** to the **peripheral tissues** where **LPL** degrades the lipid. Additionally, the VLDL particle receives **cholesteryl esters** from **HDL in exchange for TAG**. This process is accomplished by **cholesteryl ester transfer protein** (**CETP**). Eventually, VLDL in the **plasma** is **converted to LDL**, a much smaller, denser particle. Apo C-II and apo E are returned to HDL, but the LDL retains **apo B-100**, which is recognized by **receptors** o n **peripheral tissues** and the **liver**. LDLs undergo **receptor-mediated endocytosis**, and their contents are degraded in the **lysosomes**. A **deficiency of functional LDL receptors** causes **type II hyperlipoproteinemia** (**familial hypercholesterolemia**). The endocytosed cholesterol decreases synthesis of **HMG CoA reductase** (and of **LDL receptors**) through prevention of SREBP-2 binding to the SRE. Some of it can also be esterified by **acyl CoA:cholesterol acyltransferase** (**ACAT**) and stored. **HDL** 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 **unesterified cholesterol** from from peripheral tissues via ABCA1and **esterifying it** using **lecithin:cholesterol acyl transferase(LCAT)**, a liver-synthesized plasma enzyme that is activated by **apo A-1**; and 3) delivering these cholesteryl esters to the liver (**reverse cholesterol transport**) for uptake via scavenger receptor-B1(SR-B1).

Cholesterol is the precursor of all classes of steroid hormones, which include **glucocorticoids**, **mineralocorticoids**, and the **sex hormones** (**androgens**, **estrogens**, and **progestins**) . **Synthesis**, using primarily **cytochrome P450 mixed-function oxidases**, occurs in the **adrenal cortex** (**cortisol**, **aldosterone**, and **androgens**), **ovaries** and **placenta** (**estrogens** and **progestins**), and **testes** (**testosterone**). The initial and rate-limiting step is the conversion of cholesterol to **pregnenolone** by the side-chain cleavage enzyme **P450scc.** Deficiencies in synthesis Iead to **congenital adrenal hyperplasia.** 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**) in association with coactivator proteins, thereby causing **promoter activation** and **increased transcription** of targeted genes. In association with corepressors, **transcription is decreased**.

Figure 18.28 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 proteins that relax the DNA. mRNA = messenger RNA.


Steroid hormone
CELL MEMBRANE
CYTOSOL
Inactive receptor
Steroid hormone–receptor complex
NUCLEUS
DNA
Enhancer region
Binding of steroid hormone receptor complex to the HRE of the enhancer region activates the gene promoter causing transcription.
Promoter region
Transcription
mRNA

Study Questions

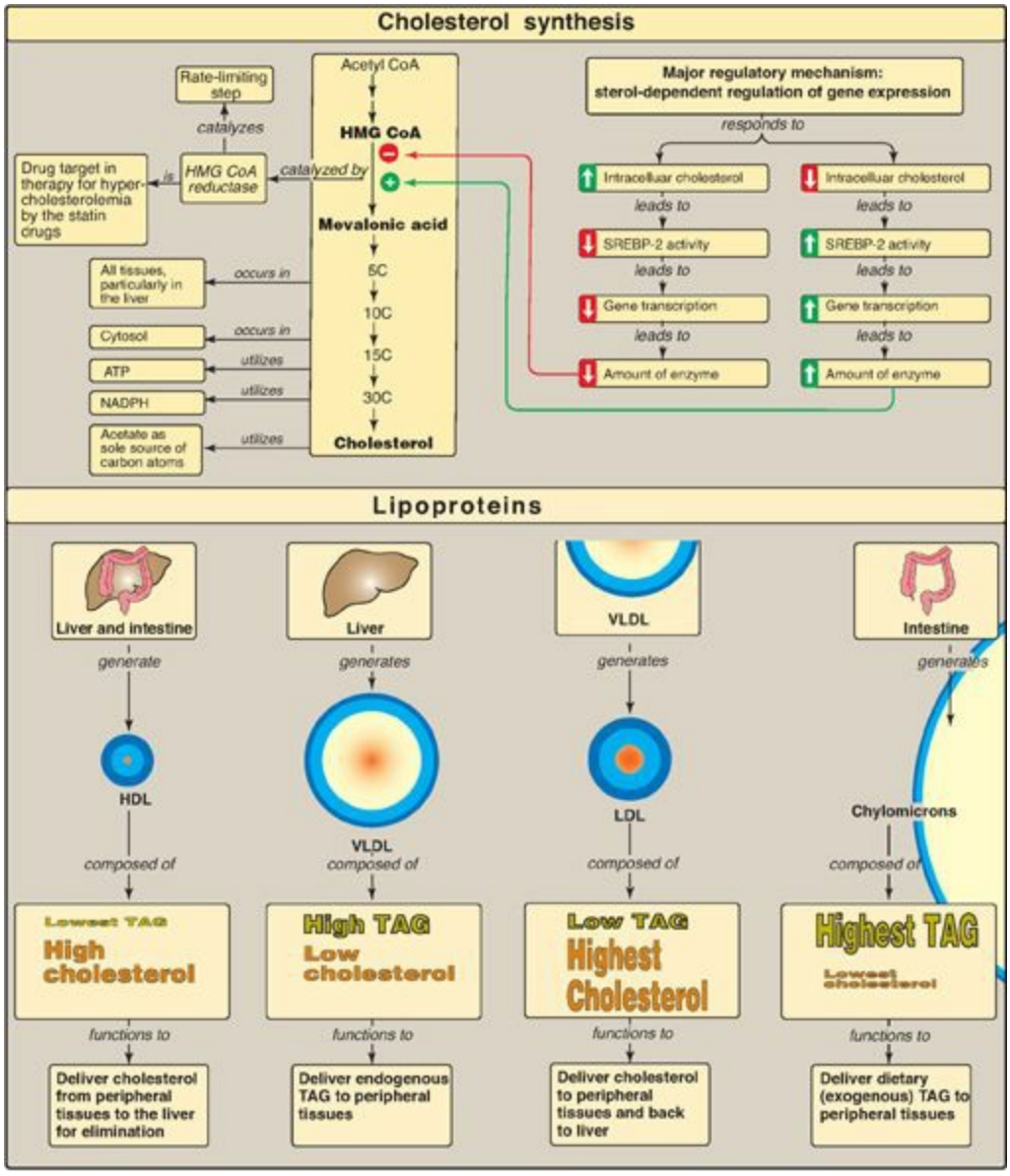
Choose the ONE best answer.

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 adenosine triphosphate 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 adenosine triphosphate results in a rise in adenosine monophosphate (AMP), which activates AMP kinase (AMPK), thereby phosphorylating and inactivating the enzyme. 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.

Figure 18.29 Concept map for cholesterol and the lipoproteins. HMG CoA = hydroxymethylglutaryl coenzyme A; SREBP = sterol regulatory element-binding protein; HDL = high-density lipoprotein; VLDL = very-low-density lipoprotein; LDL = lowdensity lipoprotein; TAG = triacylglycerol; NADP(H) = nicotinamide adenine dinucleotide phosphate.



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 1/5 of the volume of VLDL in fasted blood.

For Questions 18.3 and 18.4:

A young girl with a history of severe abdominal pain was taken to her local hospital at 5 a.m. in severe distress. Blood was drawn, and the plasma appeared milky, with the triacylglycerol level in excess of 2,000 mg/dl (normal = 4–150 mg/dl). The patient was placed on a diet extremely limited in fat but supplemented with medium-chain triglycerides.

Correct answer = A. The milky appearance of her blood was a result of triacylglycerol-rich chylomicrons. Because 5 a.m. 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" in plasma.

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 = C. The triacylglycerol (TAG) in chylomicrons is degraded by endothelial lipoprotein lipase, which requires apo C-II as a coenzyme. Deficiency of the enzyme or coenzyme results in decreased ability to degrade chylomicrons to their remnants, which get cleared by the liver. Apo A-I is the coenzyme for lecithin:cholesterol acyltransferase; apo B-48 is the ligand for the hepatic receptor that binds chylomicron remnants; 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).

18.4 Which one of the following proteins is most likely to be deficient in this patient?

A. Apo A-I

B. Apo B-48
C. Apo C-II
D. Cholesteryl ester transfer protein
E. Microsomal triglyceride transfer protein

18.5 Complete the table below for an individual with classic 21-α-hydroxylase deficiency relative to a normal individual.

Variable	Increased	Decreased
Aldosterone		
Cortisol		
Androstenedione		
Adrenocorticotropic hormone		
Blood glucose		
Blood pressure		

How might the results be changed if this individual were deficient in 17-α-hydroxylase, rather than 21-α-hydroxylase?

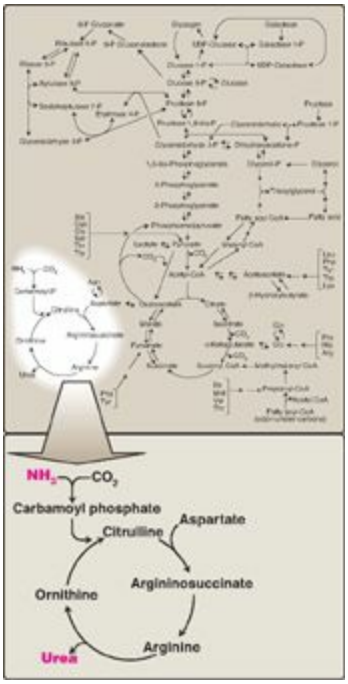
21-α-Hydroxylase deficiency causes mineralocorticoids (aldosterone) and glucocorticoids (cortisol) to be virtually absent. Because aldosterone increases blood pressure, and cortisol increases blood glucose, their deficiencies result in a decrease in blood pressure and blood glucose, respectively. Cortisol 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, therefore, causes androstenedione levels to rise.
With 17-α-hydroxylase deficiency, sex hormone synthesis would be inhibited. Mineralocorticoid production would be increased, leading to hypertension.

Amino Acids: Disposal of Nitrogen 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 normal protein degradation. 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 (Figure 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 CO_2 and water, glucose, fatty acids, or ketone bodies by the central pathways of metabolism described in Chapters 8–13 and 16.

Figure 19.1 Urea cycle shown as part of the essential pathways of energy metabolism. (See Figure 8.2, p. 92, for a more detailed view of intermediary metabolism.)



II. OVERALL NITROGEN METABOLISM

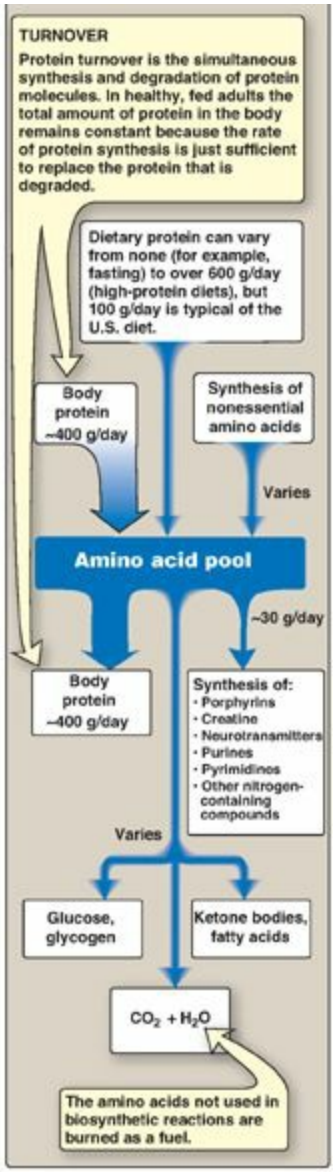
Amino acid catabolism is part of the larger process of the metabolism of nitrogen-containing molecules. Nitrogen enters the body in a variety of compounds present in food, the most important being amino acids contained in dietary protein. Nitrogen leaves the body as urea, ammonia, and other products derived from amino acid metabolism. The role of body proteins in these transformations involves two important concepts: the amino acid pool and protein turnover.

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 (Figure 19.2). Conversely, the amino 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 (see Figure 19.2). Although the amino acid pool is small (comprising about 90–100 g of amino acids) in comparison with the amount of protein in the body (about 12 kg in a 70-kg man), it is conceptually at the center of whole-body nitrogen metabolism.

> 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.

Figure 19.2 Sources and fates of amino acids.



B. Protein turnover

Most proteins in the body are constantly being synthesized and then degraded, 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 (that is, essentially constant), and cellular levels of the protein are controlled by selective degradation.

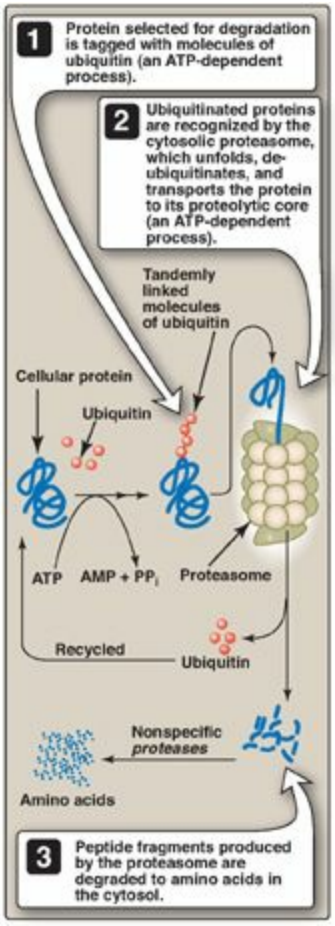
1. **Rate of turnover:** In healthy adults, the total amount of protein in the body remains constant because the rate of protein synthesis is just sufficient to replace the protein that is degraded. This process, called protein turnover, leads to the hydrolysis and resynthesis of 300–400 g of body protein each day. The rate of protein turnover varies widely for individual proteins. Short-lived proteins (for example, 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 adenosine triphosphate (ATP)-dependent ubiquitin-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. 162) 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 proteolytic pathway:** Proteins selected for degradation by the cytosolic ubiquitin-proteasome system are first modified by the covalent attachment of ubiquitin (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 on the protein substrate by a three-step, enzyme-catalyzed, ATP-dependent process. [Note: Enzyme 1 (E1, or activating enzyme) activates Ub, which is then transferred to E2 (conjugating enzyme). E3 (a ligase) identifies the protein to be degraded and interacts with E2-Ub.] The consecutive addition of four or more Ub molecules to the target protein generates a polyubiquitin chain. Proteins tagged with Ub are recognized by a large, barrel-shaped, macromolecular, proteolytic complex called a proteasome (Figure 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. Ub is recycled. It is noteworthy that the selective degradation of proteins by the ubiquitin-proteosome complex (unlike simple hydrolysis by proteolytic enzymes) requires energy in the form of ATP.

   b. **Chemical signals for protein degradation:** 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. For example, some proteins that have been chemically altered by oxidation or tagged with ubiquitin are preferentially degraded. The half-life of a protein is also influenced by the amino (N)-terminal residue. For example, proteins that have serine as the N-terminal amino acid are long-lived, with a half-life of more than 20 hours, whereas those with aspartate at their N-terminus have a half-life of only 3 minutes. 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 degraded and, therefore, have short half-lives.

Figure 19.3 The ubiquitin-proteasome degradation pathway of proteins. AMP = adenosine monophosphate; PP_i = pyrophosphate.


1
Protein selected for degradation is tagged with molecules of ubiquitin (an ATP-dependent process).
2
Ubiquitinated proteins are recognized by the cytosolic proteasome, which unfolds, de-ubiquitinates, and transports the protein to its proteolytic core (an ATP-dependent process).
Tandemly linked molecules of ubiquitin
Cellular protein
Ubiquitin
ATP
AMP + PPi
Proteasome
Recycled
Ubiquitin
Nonspecific proteases
Amino acids
3
Peptide fragments produced by the proteasome are degraded to amino acids in the cytosol.

III. DIGESTION OF DIETARY PROTEINS

Most of the nitrogen in the diet is consumed in the form of protein, typically amounting to 70–100 g/day in the American diet (see Figure 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.] They must, therefore, 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 (Figure 19.4).

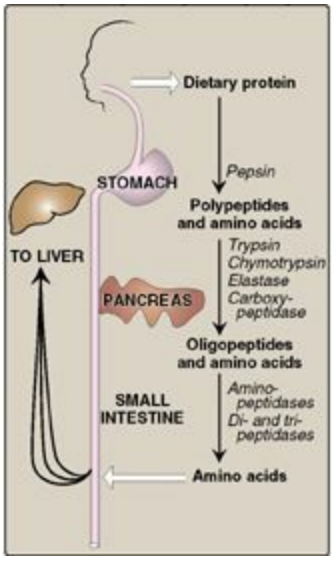
A. Digestion by gastric secretion

The digestion of proteins begins in the stomach, which secretes gastric juice, a unique solution containing hydrochloric acid and the proenzyme pepsinogen.

1. **Hydrochloric acid:** Stomach acid is too dilute (pH 2–3) 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.] Pepsinogen is activated to pepsin, either by hydrochloric acid or autocatalytically by pepsin molecules that have already been activated. Pepsin releases peptides and a few free amino acids from dietary proteins.

Figure 19.4 Digestion of dietary proteins by the proteolytic enzymes of the gastrointestinal tract.



B. Digestion by pancreatic enzymes

On entering the small intestine, large 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 (cleave within) and exopeptidases (cut at an end). [Note: Bicarbonate (HCO_3^-), also secreted by the pancreas, raises the pH.]

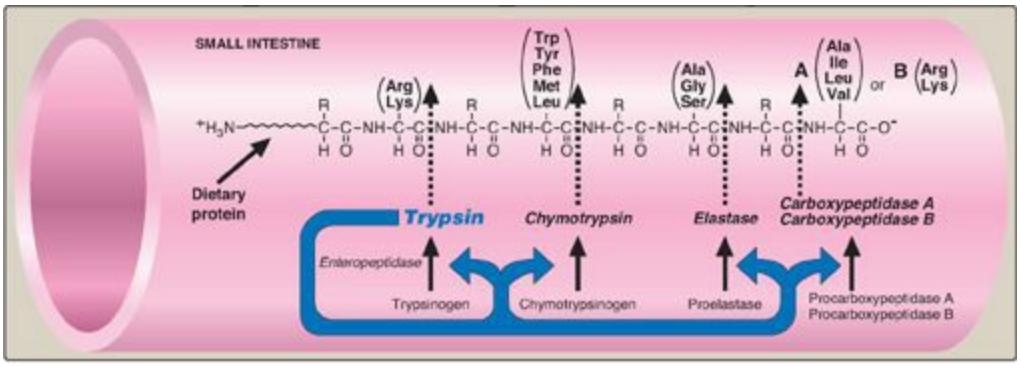
1. **Specificity:** Each of these enzymes has a different specificity for the amino acid R-groups adjacent to the susceptible peptide bond (Figure 19.5). For example, trypsin cleaves only when the carbonyl group of the peptide bond is contributed by arginine or lysine. These enzymes, like pepsin described above, are synthesized and secreted as inactive zymogens.

2. **Release of zymogens:** The release and activation of the pancreatic zymogens is mediated by the secretion of cholecystokinin and secretin, two polypeptide hormones of the digestive tract (see p. 176).

3. **Activation of zymogens:** Enteropeptidase (formerly called enterokinase), an enzyme synthesized by and present on the luminal surface of intestinal mucosal cells of the brush border membrane, 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. Enteropeptidase, thus, unleashes a cascade of proteolytic activity because trypsin is the common activator of all the pancreatic zymogens (see Figure 19.5).

4. **Abnormalities in protein digestion:** In individuals with a deficiency in pancreatic secretion (for example, due to 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. 177) as well as undigested protein.

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.]



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.

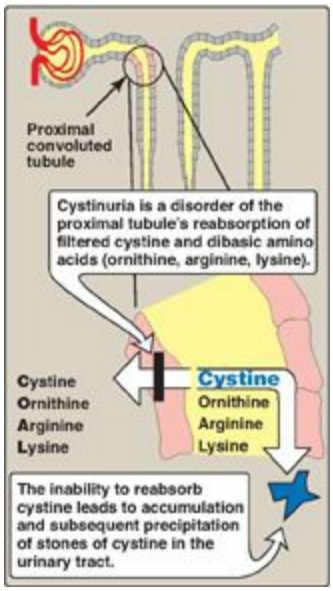
C. Digestion of oligopeptides by enzymes of the small intestine

The luminal surface of the intestine contains aminopeptidase, an exopeptidase that repeatedly cleaves the N-terminal residue from oligopeptides to produce even smaller peptides and free amino acids.

D. Absorption of amino acids and small peptides

Free amino acids are taken into the enterocytes by a sodium-linked secondary transport system of the apical membrane. Di- and tripeptides, however, are taken up by a proton-linked transport system. The peptides are hydrolyzed in the cytosol to amino acids that are released into the portal system by facilitated diffusion. 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 are important examples of amino acids that are not metabolized by the liver but, instead, are sent from the liver primarily to muscle via the blood.]

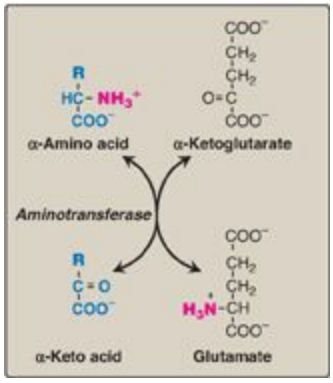
Figure 19.6 Genetic defect seen in cystinuria. [Note: Cystinuria is distinct from cystinosis, a rare defect in the transport of cystine out of lysosomes that results in the formation of cystine crystals within the lysosome and tissue damage.]



IV. TRANSPORT OF AMINO ACIDS INTO CELLS

The concentration of free amino acids in the extracellular fluids is significantly lower than that within the cells of the body. This concentration gradient is maintained because active transport systems, driven by the hydrolysis of ATP, are required for movement of amino acids from the extracellular space into cells. At least seven different transport systems are known that have overlapping specificities for different amino acids. Because the small intestine and the proximal tubule of the kidney have common transport systems for amino acid uptake, a defect in any one of these systems results in an inability to absorb particular amino acids into the gut 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 (Figure 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 transport of tryptophan can result in Hartnup disorder and pellagra-like (see p. 380) dermatologic and neurologic symptoms.]

Figure 19.7 Aminotransferase reaction using α-ketoglutarate as the amino group acceptor.



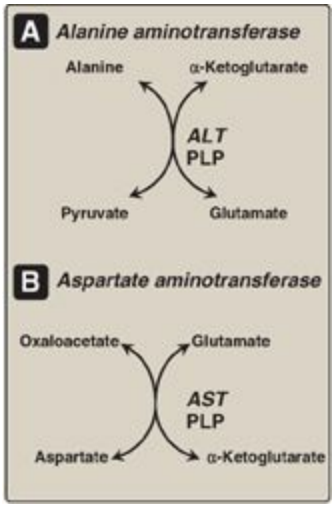
V. REMOVAL OF NITROGEN FROM AMINO ACIDS

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. 253).

A. Transamination: the funneling of amino groups to glutamate

The first step in the catabolism of most amino acids is the transfer of their α-amino group to α-ketoglutarate (Figure 19.7), producing an α-keto acid (derived from the original amino acid) and glutamate. α-Ketoglutarate plays a pivotal role in amino acid metabolism by accepting the amino groups from most amino acids, thereby becoming glutamate. Glutamate produced by transamination can be oxidatively deaminated (see 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 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. 265–266).]

Figure 19.8 Reactions catalyzed during amino acid catabolism. A. Alanine aminotransferase (ALT). B. Aspartate aminotransferase (AST). PLP = pyridoxal phosphate (see p. 251).



1. **Substrate specificity of aminotransferases:** 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

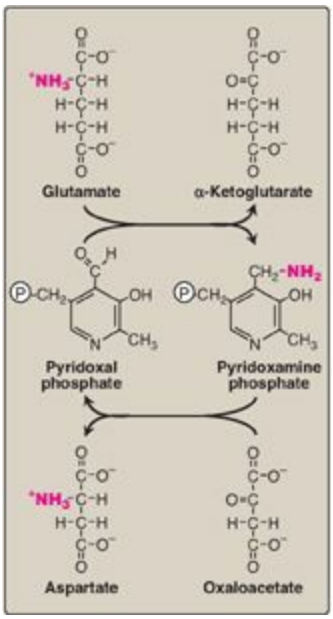
alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as shown in Figure 19.8).

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: Glutamate, in effect, 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 transfers amino groups from glutamate to oxaloacetate, forming aspartate, which is used as a source of nitrogen in the urea cycle (see p. 253). Like other transaminations, the AST reaction is reversible.

2. Mechanism of action of aminotransferases: All aminotransferases require the coenzyme pyridoxal phosphate (a derivative of vitamin B_6; see p. 378), which is covalently linked to the ε-amino group of a specific lysine residue at the active site of the enzyme. Aminotransferases act by transferring the amino group of an amino acid to the pyridoxal part of the coenzyme to generate pyridoxamine phosphate. The pyridoxamine form of the coenzyme then reacts with an α-keto acid to form an amino acid, at the same time regenerating the original aldehyde form of the coenzyme. Figure 19.9 shows these two component reactions for the reaction catalyzed by AST.

Figure 19.9 Cyclic interconversion of pyridoxal phosphate and pyridoxamine phosphate during the aspartate aminotransferase reaction. Ⓟ = phosphate group.



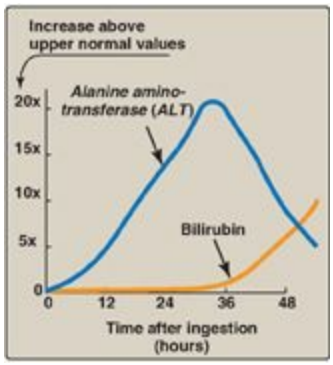
3. Equilibrium of transamination reactions: For most transamination reactions,

the equilibrium constant is near 1. This allows the reaction to function in both amino acid degradation through removal of α-amino groups (for example, 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 (for example, when the supply of amino acids from the diet is not adequate to meet the synthetic needs of cells).

4. **Diagnostic value of plasma aminotransferases:** Aminotrans-ferases 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. **Liver disease:** Plasma AST and ALT are elevated in nearly all liver 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 (so-called "liver function tests") are often useful in determining the course of liver damage. Figure 19.10 shows the early release of ALT into the serum, following ingestion of a liver toxin. [Note: Elevated serum bilirubin results from hepatocellular damage that decreases the hepatic conjugation and excretion of bilirubin (see p. 284).]

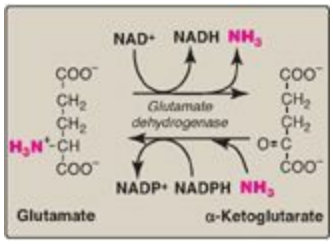
Figure 19.10 Pattern of serum ALT and bilirubin in the plasma, following poisoning with the toxic mushroom Amanita phalloides.



   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.

Figure 19.11 Oxidative deamination by glutamate dehydrogenase. [Note: The enzyme

is unusual in that it uses both NAD^+ (nicotinamide adenine dinucleotide) and NADPH (nicotinamide adenine dinucleotide phosphate).]



B. Oxidative deamination of amino acids

In contrast to transamination reactions that transfer amino groups, oxidative deamination reactions result in the liberation of the amino group as free ammonia (Figure 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 un-ionized form (NH_3) that crosses membranes.]

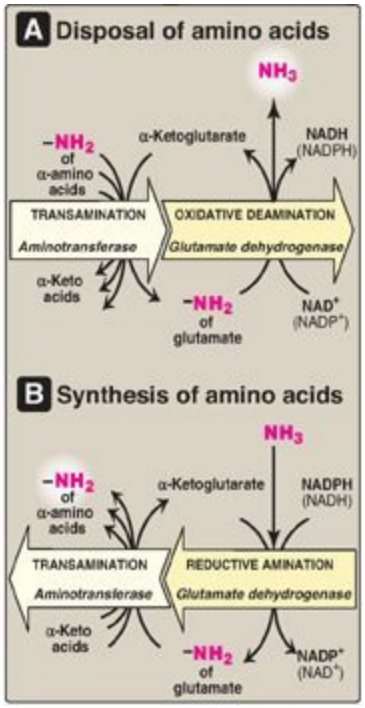
1. **Glutamate dehydrogenase:** As described above, the amino groups of most amino acids are ultimately funneled to glutamate by means of transamination with α-ketoglutarate. Glutamate is unique in that it is the only amino acid that undergoes rapid oxidative deamination, a reaction catalyzed by glutamate dehydrogenase (see Figure 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:** Glutamate dehydrogenase, a mitochondrial enzyme, is unusual in that it can use either nicotinamide adenine dinucleotide (NAD^+) or its phosphorylated reduced form (NADPH) as a coenzyme (see Figure 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 Figure 19.12A), and NADPH is used in reductive amination (the simultaneous gain of ammonia coupled with the reduction of the carbon skeleton, as shown in Figure 19.12B).

    b. **Direction of reactions:** 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 (see Figure 19.12A). High ammonia levels are required to drive the reaction to glutamate synthesis.

c. Allosteric regulators: Guanosine triphosphate is an allosteric inhibitor of glutamate dehydrogenase, whereas adenosine diphosphate (ADP) is an activator. Therefore, when energy levels are low in the cell, amino acid degradation by glutamate dehydrogenase is high, facilitating energy production from the carbon skeletons derived from amino acids.

Figure 19.12 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.



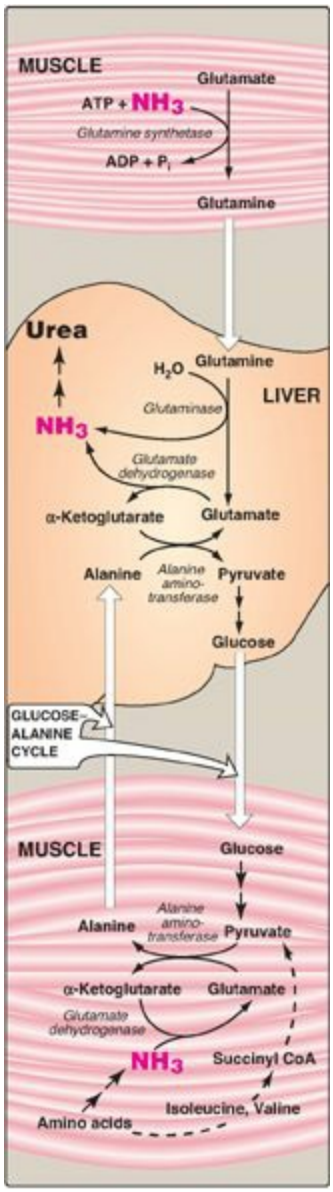
2. D-Amino acid oxidase: D-Amino acids (see p. 5) are found in plants and in the cell walls of microorganisms but are not used in the synthesis of mammalian proteins. D-Amino acids are, however, present in the diet and are efficiently metabolized by the kidney and liver. D-Amino acid oxidase (DAO) is a flavin adenine dinucleotide–dependent peroxisomal enzyme that catalyzes the oxidative deamination of these amino acid isomers, thereby producing α-keto acids, ammonia, and hydrogen peroxide. 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.] L-amino acid oxidases are known, but their physiologic significance is unclear.

C. Transport of ammonia to the liver

Two mechanisms are available in humans for the transport of ammonia from the peripheral tissues to the liver for its ultimate 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 (Figure 19.13). The glutamine is transported in the blood to the liver where it is cleaved by glutaminase to produce glutamate and free ammonia (see p. 256). 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 branched-chain amino acids isoleucine and valine. Alanine is transported by the blood to the liver, where it is converted to pyruvate, again by transamination. The pyruvate is used to synthesize glucose, which can enter the blood and be used by muscle, a pathway called the glucose–alanine cycle.

Figure 19.13 Transport of ammonia (NH_3) from muscle to the liver. ADP = adenosine diphosphate; Pi = inorganic phosphate; CoA = coenzyme A.



VI. UREA CYCLE

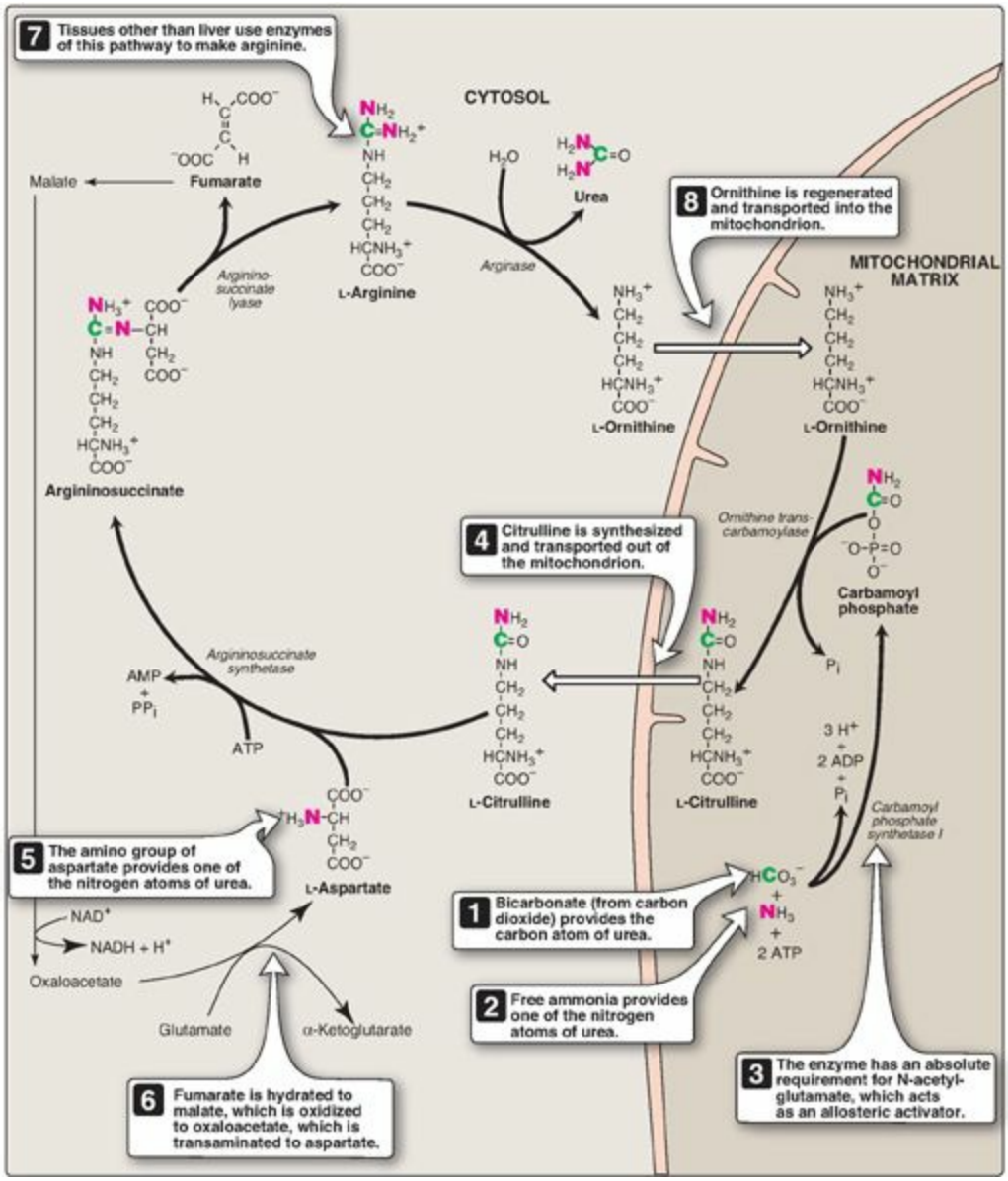
Urea (H_2NCONH_2) is the major disposal form of amino groups derived from amino acids and accounts for about 90% of the nitrogen-containing components of urine. One nitrogen of the urea molecule is supplied by free ammonia and the other nitrogen by aspartate. [Note: Glutamate is the immediate precursor of both ammonia (through oxidative deamination by glutamate dehydrogenase) 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 to the kidneys for excretion in the urine.

A. Reactions of the cycle

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 (Figure 19.14). [Note: Gluconeogenesis (see p. 117) and heme synthesis (see p. 278) also involve both the mitochondrial matrix and the cytosol.]

1. **Formation of carbamoyl phosphate:** 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 glutamate dehydrogenase (see Figure 19.11). Ultimately, the nitrogen atom derived from this ammonia becomes one of the nitrogens of urea. CPS I requires N-acetylglutamate as a positive allosteric activator (see Figure 19.14). [Note: Carbamoyl phosphate synthetase II participates in the biosynthesis of pyrimidines (see p. 302). It does not require N-acetylglutamate, uses glutamine as the nitrogen source, and occurs in 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

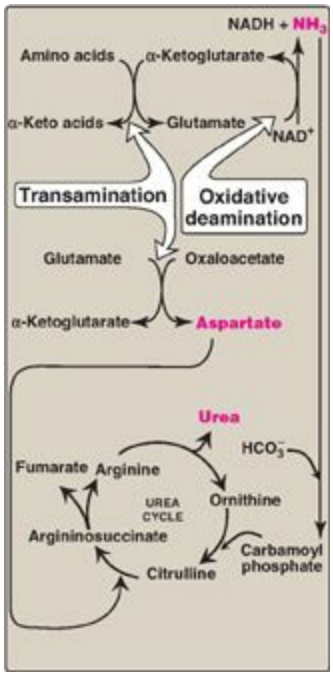


2. **Formation of citrulline:** The carbamoyl portion of carbamoyl phosphate is transferred to ornithine by ornithine transcarbam-oylase (OTC) as the high-energy phosphate is released as inorganic phosphate. The reaction product, citrulline, is transported to the cytosol. [Note: Ornithine and citrulline are basic amino acids that participate in the urea cycle, moving across the inner mitochondrial membrane via a cotransporter. They are not incorporated into cellular proteins because there are no codons for these amino acids (see p. 432).] Ornithine is regenerated with each turn of the urea cycle, much in the same way that oxaloacetate is regenerated by the reactions of the citric acid cycle (see p. 109).

3. **Synthesis of argininosuccinate:** 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.

Figure 19.15 Flow of nitrogen from amino acids to urea. Amino groups for urea

synthesis are collected in the form of ammonia and aspartate. NAD(H) = nicotinamide adenine dinucleotide.

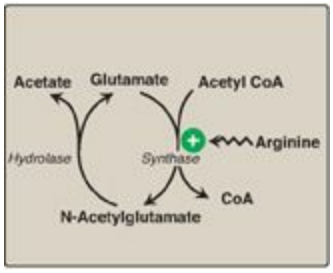


4. **Cleavage of argininosuccinate:** Argininosuccinate is cleaved by argininosuccinate lyase to yield arginine and fumarate. The arginine formed by this reaction serves as the immediate precursor of urea. Fumarate produced in the urea cycle is hydrated to malate, providing a link with several metabolic pathways. For example, the malate can be transported into the mitochondria via the malate–aspartate shuttle, reenter the tricarboxylic acid cycle, and get oxidized to oxaloacetate, which can be used for gluconeogenesis (see p. 120). [Note: Malate oxidation generates NADH and, subsequently, ATP.] Alternatively, the oxaloacetate can be converted to aspartate via transamination (see Figure 19.8) and can enter the urea cycle (see Figure 19.14).

5. **Cleavage of arginine to ornithine and urea:** Arginase 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 by these reactions.

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 (see Figure 19.19). A portion of the urea diffuses from the blood into the intestine and is cleaved to CO_2 and NH_3 by bacterial urease. This 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 NH_3 production.

Figure 19.16 Formation and degradation of Nacetylglutamate, an allosteric activator of carbamoyl phosphate synthetase I. CoA = coenzyme A.



B. Overall stoichiometry of the urea cycle

$$\text{Aspartate} + NH_3 + HCO_3^- + 3\text{ATP} + H_2O \rightarrow$$

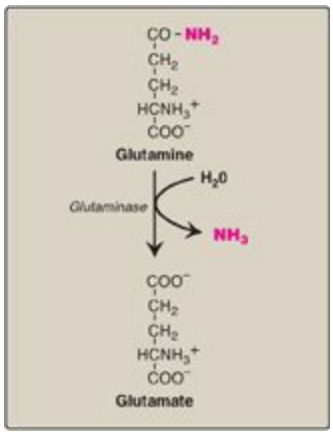
$$\text{urea} + \text{fumarate} + 2\text{ADP} + AMP + 2\ P_i + PP_i$$

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. 70). One nitrogen of the urea molecule is supplied by free NH_3, and the other nitrogen by aspartate. Glutamate is the immediate precursor of both ammonia (through oxidative deamination by glutamate dehydrogenase) 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 (Figure 19.15).

C. Regulation of the urea cycle

N-Acetylglutamate (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 (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 19.17 Hydrolysis of glutamine to form ammonia (NH_3).



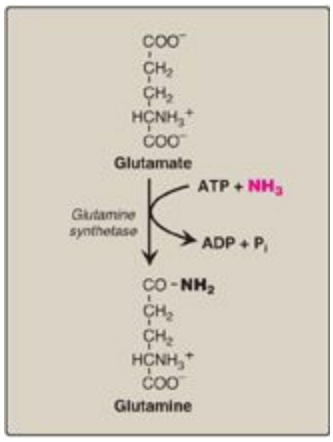
VII. METABOLISM OF AMMONIA

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 level of ammonia in the blood must be kept very low, because even slightly elevated concentrations (hyperammonemia) are toxic to the central nervous system (CNS). Therefore, there must be a metabolic mechanism by which nitrogen is moved from peripheral tissues to the liver for ultimate disposal as urea, at the same time maintaining low levels of circulating ammonia.

A. Sources of ammonia

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 (that is, the linking of aminotransferase and glutamate dehydrogenase reactions), producing ammonia. [Note: Liver catabolizes straight-chain amino acids, primarily.] However, substantial amounts of ammonia can be obtained from other sources.

Figure 19.18 Synthesis of glutamine. ADP = adenosine diphosphate; P_i = inorganic phosphate.



1. **From glutamine:** An important source of plasma glutamine is from the catabolism of branched-chain amino acids in skeletal muscle. This glutamine is taken up by cells of the intestine, the liver, and the kidney. The liver and kidneys generate ammonia from glutamine by the actions of glutaminase (Figure 19.17) and glutamate dehydrogenase. 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 glutamate dehydrogenase, is a glucogenic precursor in liver and kidney.] Ammonia is also generated by intestinal glutaminase. The intestinal mucosal cells 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. **From bacterial action in the intestine:** 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. **From 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. 286).

4. **From purines and pyrimidines:** In the catabolism of purines and pyrimidines, amino groups attached to the ring atoms are released as ammonia (see Figure 22.15 and p. 304).

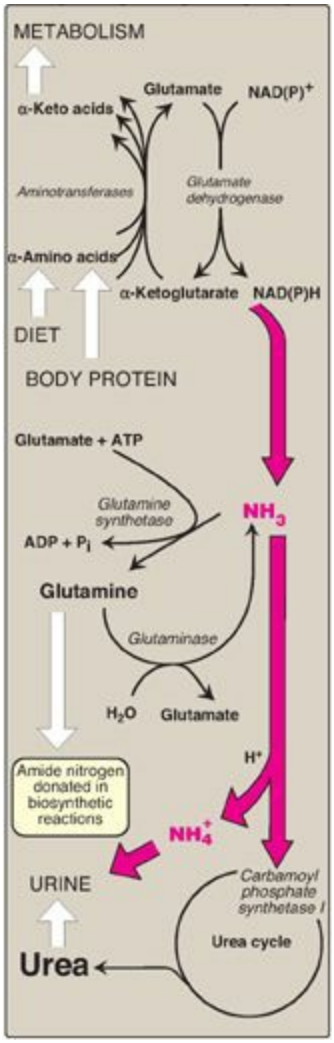
B. Transport of ammonia in the circulation

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 or alanine, rather than as free ammonia (see Figure 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 (Figure 19.18). The ATP-requiring formation of glutamine from glutamate and ammonia by glutamine synthetase occurs primarily in skeletal muscle and 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 and the urea cycle in periportal (close to inflow of blood) hepatocytes and via glutamine synthetase as an ammonia "scavenger" in the perivenous hepatocytes.] The metabolism of ammonia is summarized in Figure 19.19.

Figure 19.19 Metabolism of ammonia (NH_3). [Note: Glutamate dehydrogenase is one of several sources of NH_3.] Urea content in the urine is reported as urinary urea nitrogen, or UUN. Urea in blood is reported as BUN (blood urea nitrogen). The enzymes glutamate dehydrogenase, glutamine synthetase, and carbamoyl phosphate synthetase I fix NH_3 into organic molecules.



C. Hyperammonemia

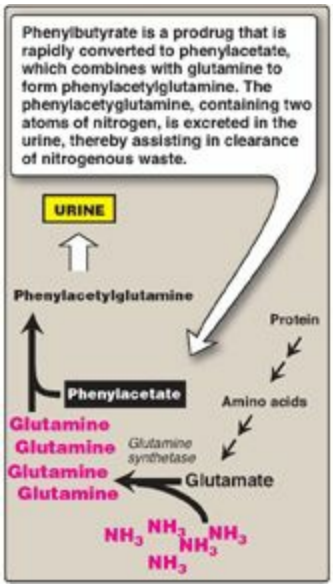
The capacity of the hepatic urea cycle exceeds the normal rates of ammonia generation, and the levels of serum ammonia are normally low (5–35 μmol/l). However, when liver function is compromised, due either to genetic defects of the urea cycle or liver disease, blood levels can rise above 1,000 μmol/l. 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 hyperammonemia:** Liver disease is a common cause of 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.

2. **Congenital hyperammonemia:** Genetic deficiencies of each of the five enzymes

of the urea cycle have been described, with an overall incidence estimated to be 1:25,000 live births. X-linked ornithine transcarbamoylase deficiency is the most common of these disorders, 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. [Note: The hyperammonemia seen with arginase deficiency is less severe because arginine contains two waste nitrogens and can be excreted in the urine.] Historically, urea cycle defects had high morbidity (neurologic manifestations) and mortality. Treatment included restriction of dietary protein in the presence of sufficient calories to prevent catabolism. Administration of compounds that bind covalently to amino acids, producing nitrogen-containing molecules that are excreted in the urine, has improved survival. For example, phenylbutyrate given orally is converted to phenylacetate. This condenses with glutamine to form phenylacetylglutamine, which is excreted (Figure 19.20).

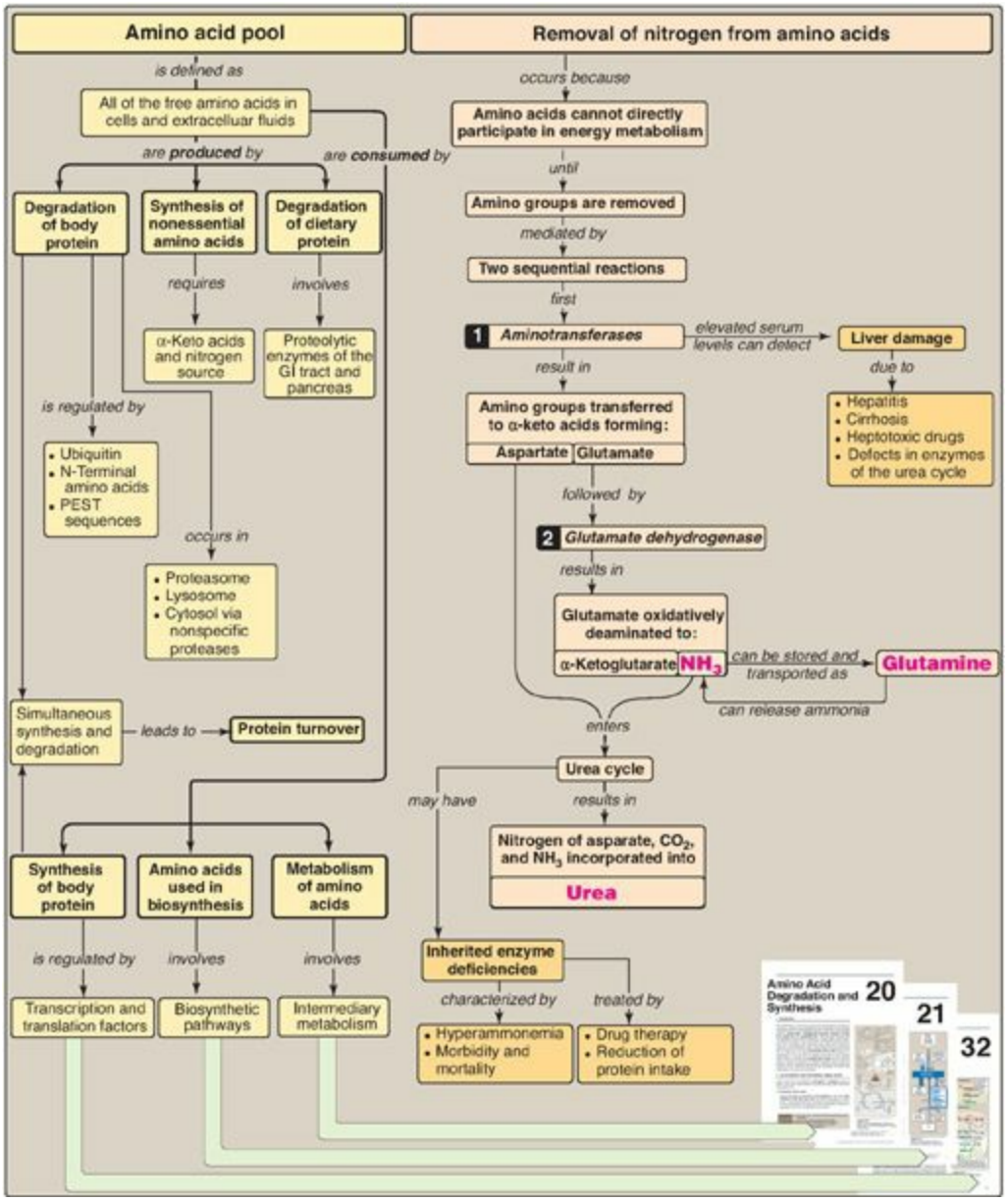
Figure 19.20 Treatment of patients with urea cycle defects by administration of phenylbutyrate to aid in excretion of ammonia (NH_3).



VIII. 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 (Figure 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 cells via sodium-linked secondary active transport. Note that 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 adenosine triphosphate (ATP)-dependent, cytosolic, selective **ubiquitin–proteasome** and ATP-independent, 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 **glutamate dehydrogenase**, forming **ammonia** and the corresponding **α-keto acids**. A portion of the **free ammonia** is excreted in the **urine**, some of which 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. The two major causes of **hyperammonemia** (with its neurologic effects) are liver disease and inherited deficiencies of urea cycle enzymes such as X-linked **ornithine transcarbamolyase**.

Figure 19.21 Key concept map for nitrogen metabolism. GI = gastrointestinal; PEST = proline, glutamate, serine, threonine; NH_3 = ammonia.

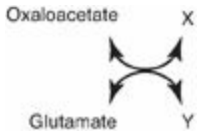

Amino acid pool
Removal of nitrogen from amino acids
is defined as
All of the free amino acids in cells and extracellular fluids
are produced by
are consumed by
Degradation of body protein
Synthesis of nonessential amino acids
Degradation of dietary protein
requires
involves
α-Keto acids and nitrogen source
Proteolytic enzymes of the GI tract and pancreas
is regulated by
Ubiquitin
N-Terminal amino acids
PEST sequences
occurs in
Proteasome
Lysosome
Cytosol via nonspecific proteases
Simultaneous synthesis and degradation
leads to
Protein turnover
Synthesis of body protein
Amino acids used in biosynthesis
Metabolism of amino acids
is regulated by
involves
involves
Transcription and translation factors
Biosynthetic pathways
Intermediary metabolism
occurs because
Amino acids cannot directly participate in energy metabolism
until
Amino groups are removed
mediated by
Two sequential reactions
first
1 Aminotransferases
elevated serum levels can detect
Liver damage
due to
Hepatitis
Cirrhosis
Heptotoxic drugs
Defects in enzymes of the urea cycle
result in
Amino groups transferred to α-keto acids forming:
Aspartate
Glutamate
followed by
2 Glutamate dehydrogenase
results in
Glutamate oxidatively deaminated to:
α-Ketoglutarate
NH3
can be stored and transported as
Glutamine
can release ammonia
enters
Urea cycle
may have
results in
Nitrogen of asparate, CO2, and NH3 incorporated into
Urea
Inherited enzyme deficiencies
characterized by
treated by
Hyperammonemia
Morbidity and mortality
Drug therapy
Reduction of protein intake
Amino Acid Degradation and Synthesis
20
21
32

Study Questions:

Choose the ONE best answer.

19.1 In the transamination reaction shown to the right, which of the following are the products X and Y?

A. Alanine, α-ketoglutarate
B. Asparate, α-ketoglutarate
C. Glutamate, alanine
D. Pyruvate, aspartate



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 proton-linked transport system.
B. In healthy, fed individuals, the input to the amino acid pool exceeds the output.
C. Liver uses ammonia to buffer protons.
D. Muscle-derived glutamine is metabolized in liver and kidney tissue to ammonia plus 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 deamidated to ammonia plus glutamate. The glutamate is deaminated to ammonia plus α-

ketoglutarate, which can be used for gluconeogenesis. Free amino acids are taken into enterocytes by a sodium-linked transport system. Healthy, fed individuals are in nitrogen balance, in which nitrogen input equals output. Liver converts ammonia to urea, and kidney uses 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– 19.5:

A female neonate did well until approximately 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 5–35 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 transcarbamoylase

Correct answer = C. Genetic deficiencies of each of the five enzymes of the urea cycle, as well as deficiencies in N-acetyglutamate 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 (argininosucinate 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

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 always accompanies hyperammonemia. Asparagine and lysine do not serve this sequestering role. Urea would be decreased due to 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 transcarbamoylase to form citrulline. Citrulline, containing one waste nitrogen, will be excreted.

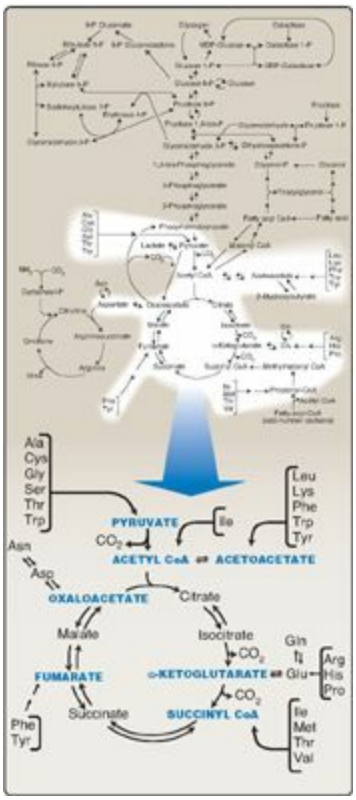
Amino Acid Degradation and Synthesis

20

I. OVERVIEW

The catabolism of the amino acids involves the removal of α-amino groups, followed by the degradation of the resulting carbon skeletons. These pathways converge to form seven intermediate products: oxaloacetate, pyruvate, α-ketoglutarate, fumarate, succinyl coenzyme A (CoA), acetyl CoA, and acetoacetate. These products directly enter the pathways of intermediary metabolism, resulting either in the synthesis of glucose or lipid or in the production of energy through their oxidation to 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 20.14 (see p. 269). Nonessential amino acids (Figure 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, the essential amino acids cannot be synthesized (or produced in sufficient amounts) by the body and, therefore, 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 Figure 8.2, p. 92, for a more detailed view of these processes.) CoA = coenzyme A.

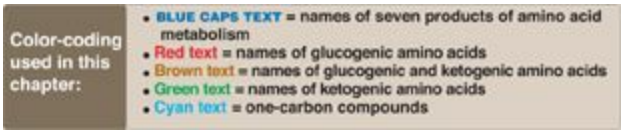


II. GLUCOGENIC AND KETOGENIC AMINO ACIDS

Amino acids can be classified as glucogenic, ketogenic, or both, based on which of the seven intermediates are produced during their catabolism (see Figure 20.2).

A. Glucogenic amino acids

Amino acids whose catabolism yields pyruvate or one of the intermediates of the TCA cycle are termed glucogenic. These intermediates are substrates for gluconeogenesis (see p. 117) and, therefore, can give rise to the net synthesis of glucose in the liver and kidney.



B. Ketogenic amino acids

Amino acids whose catabolism yields either acetoacetate or one of its precursors (acetyl CoA or acetoacetyl CoA) are termed ketogenic (see Figure 20.2). Acetoacetate is one of the ketone bodies, which also include 3-hydroxybutyrate and acetone (see p. 195). 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.

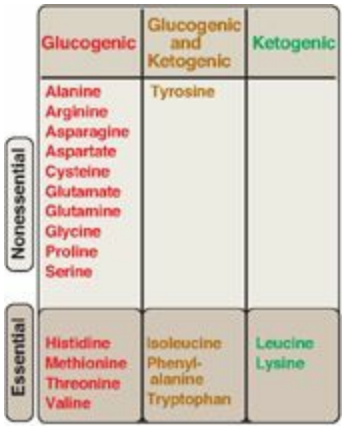
III. CATABOLISM OF THE CARBON SKELETONS OF AMINO ACIDS

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.

A. Amino acids that form oxaloacetate

Asparagine is hydrolyzed by asparaginase, liberating ammonium (NH_4^+) and aspartate (Figure 20.3). Aspartate loses its amino group by transamination to form oxaloacetate (see Figure 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 leukemic patients. Asparaginase lowers the level of asparagine in the plasma, thereby depriving cancer cells of a required nutrient.]

Figure 20.2 Classification of amino acids. [Note: Some amino acids can become conditionally essential. For example, supplementation with glutamine and arginine has been shown to improve outcomes in patients with trauma, postoperative infections, and immunosuppression.]



B. Amino acids that form α-ketoglutarate via glutamate

1. **Glutamine:** This amino acid is hydrolyzed to glutamate and ammonium by the enzyme glutaminase (see p. 256). Glutamate is converted to α-ketoglutarate by transamination or through oxidative deamination by glutamate dehydrogenase (see p. 252).

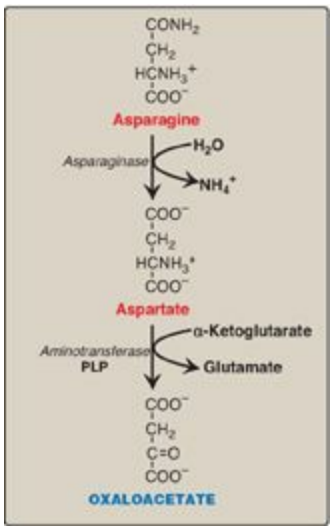
2. **Proline:** This amino acid is oxidized to glutamate. Glutamate is transaminated or oxidatively deaminated to form α-ketoglutarate.

3. **Arginine:** This amino acid is hydrolyzed by arginase to produce ornithine (and urea). [Note: This reaction occurs primarily in the liver as part of the urea cycle (see p. 255).] Ornithine is subsequently converted to α-ketoglutarate, with glutamate

semialdehyde as an intermediate.

4. **Histidine:** This amino acid is oxidatively deaminated by histidase to urocanic acid, which subsequently forms N-formiminoglutamate ([FIGlu] Figure 20.4). FIGlu donates its formimino group to tetrahydrofolate (THF), leaving glutamate, which is degraded as described above. [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. 267 for a discussion of folic acid, THF, and one-carbon metabolism.)

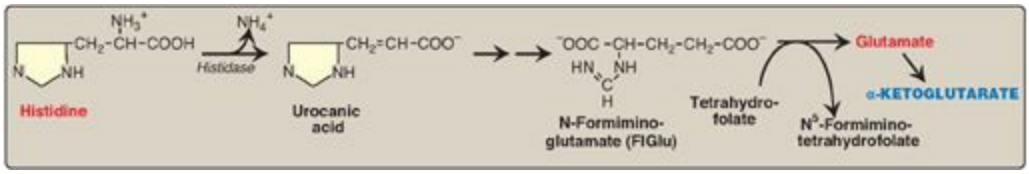
Figure 20.3 Metabolism of asparagine and aspartate. [Note: Recall that carbons from aspartate give rise to fumarate in the urea cycle (see p. 254).] PLP = pyridoxal phosphate.



C. Amino acids that form pyruvate

1. **Alanine:** This amino acid loses its amino group by transamination to form pyruvate (Figure 20.5). [Note: Alanine is the major gluconeogenic amino acid.]

Figure 20.4 Degradation of histidine.



2. **Serine:** This amino acid can be converted to glycine and N^5,N^{10}-methylenetetrahydrofolate (Figure 20.6A). Serine can also be converted to pyruvate by serine dehydratase (Figure 20.6B).

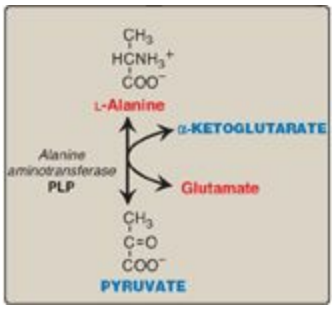
3. **Glycine:** This amino acid can be converted to serine by the reversible addition of a methylene group from N^5,N^{10}-methylenetetrahydrofolic acid (see Figure 20.6A) or oxidized to CO_2 and NH_4^+. [Note: Glycine can be deaminated to glyoxylate, which

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).]

4. **Cysteine:** This amino acid undergoes desulfuration to yield pyruvate. [Note: The sulfate released can be used to synthesize 3-phosphoadenosine-5**I**-phosphosulfate (PAPS), an activated sulfur donor to a variety of acceptors.] Cysteine can 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 20.5 Transamination of alanine to form pyruvate. PLP = pyridoxal phosphate.

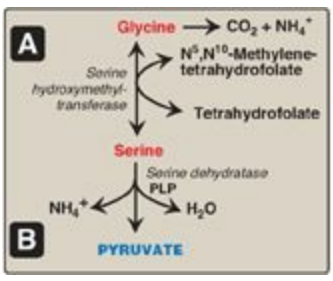


D. Amino acids that form fumarate

1. **Phenylalanine and tyrosine:** Hydroxylation of phenylalanine produces tyrosine (Figure 20.7). This reaction, catalyzed by tetrahydrobiopterin-requiring phenylalanine hydroxylase, initiates the catabolism of phenylalanine. Thus, the metabolism of phenylalanine and tyrosine merge, leading ultimately to the formation of fumarate and acetoacetate. Phenylalanine and tyrosine are, therefore, both glucogenic and ketogenic.

2. **Inherited deficiencies:** Inherited deficiencies in the enzymes of phenylalanine and tyrosine metabolism lead to the diseases phenylketonuria (see p. 270), tyrosimenia (see p. 269), and alkaptonuria (see p. 274) as well as the condition of albinism (see p. 273).

Figure 20.6 A. Interconversion of serine and glycine and oxidation of glycine. B. Dehydration of serine to form pyruvate. PLP = pyridoxal phosphate.

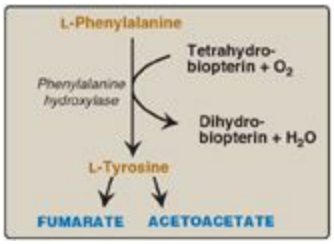


E. Amino acids that form succinyl coenzyme A: methionine

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 (Figure 20.8). Methionine is also the source of homocysteine, a metabolite associated with atherosclerotic vascular disease and thrombosis (see p. 265).

1. **Synthesis of S-adenosylmethionine:** Methionine condenses with adenosine triphosphate (ATP), forming SAM, a high-energy compound that is unusual in that it contains no phosphate. The formation of SAM is driven, in effect, by hydrolysis of all three phosphate bonds in ATP (see Figure 20.8).

Figure 20.7 Degradation of phenylalanine.



2. **Activated methyl group:** The methyl group attached to the tertiary sulfur in SAM is "activated" and can be transferred by methyltransferases to a variety of acceptor molecules such as norepinephrine in the synthesis of epinephrine (see p. 286). The methyl group is usually transferred to nitrogen (as with epinephrine) or oxygen atoms (as with the catechols; see p. 286) 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. **Hydrolysis of S-adenosylhomocysteine:** After donation of the methyl group, SAH is hydrolyzed to homocysteine (Hcy) and adenosine. Hcy has two fates. If there is a deficiency of methionine, Hcy may be remethylated to methionine (see Figure 20.8). If methionine stores are adequate, Hcy may enter the transsulfuration pathway, where it is converted to cysteine.

   a. **Resynthesis of methionine:** 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. 375). [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. **Synthesis of cysteine:** Hcy condenses with serine, forming cystathionine, which is hydrolyzed to α-ketobutyrate and cysteine (see Figure 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 p. 194). 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 20.8 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.

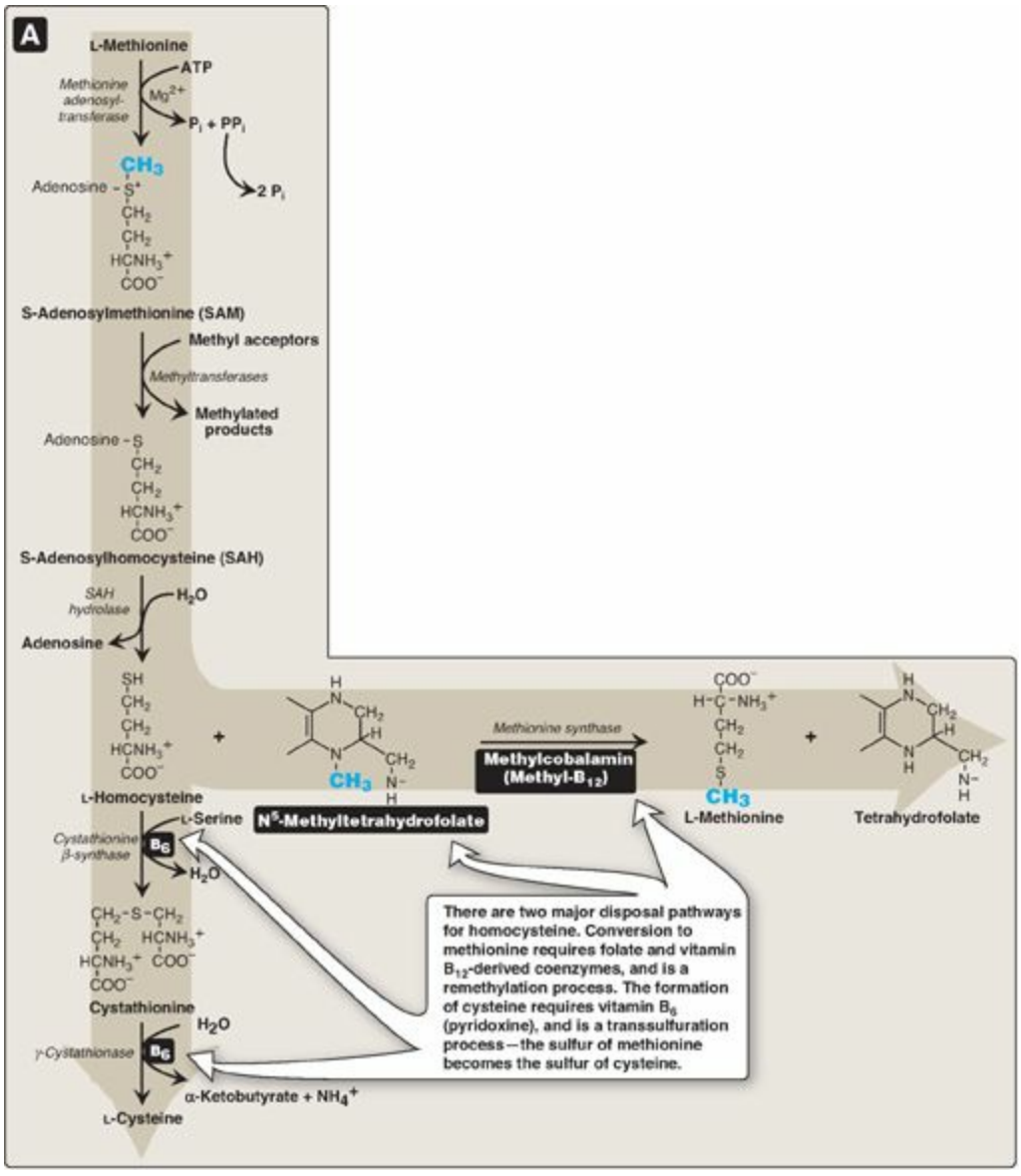
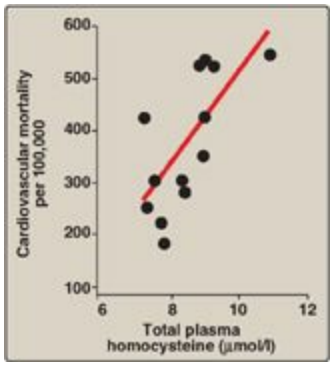


Figure 20.9 Association between cardiovascular disease mortality and total plasma homocysteine.



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 disease (Figure 20.9). Mild elevations are seen in about 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 or cysteine. Supplementation with these vitamins has been shown to reduce circulating levels of Hcy. However, in patients with established cardiovascular disease, 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. These individuals experience premature vascular disease, with about 25% dying from thrombotic complications before age 30 years.] Deficiencies in the remethylation reaction also result in a rise in Hcy.

> Elevated homocysteine and decreased folic acid levels in pregnant women are associated with increased incidence of neural tube defects (improper closure, as in spina bifida) in the fetus. Periconceptual supplementation with folate reduces the risk of such defects.

F. Other amino acids that form succinyl coenzyme A

Degradation of valine, isoleucine, and threonine also results in the production of succinyl CoA, a TCA cycle intermediate and glucogenic compound.

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 (Figure 20.10).

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. 193).]

G. Amino acids that form acetyl coenzyme A or acetoacetyl coenzyme A

Leucine, isoleucine, lysine, and tryptophan form acetyl CoA or acetoacetyl CoA directly, without pyruvate serving as an intermediate (through the pyruvate dehydrogenase reaction; see p. 109). As noted earlier, phenylalanine and tyrosine also give rise to acetoacetate during their catabolism (see Figure 20.7). Therefore, there are a total of six partly or wholly ketogenic amino acids.

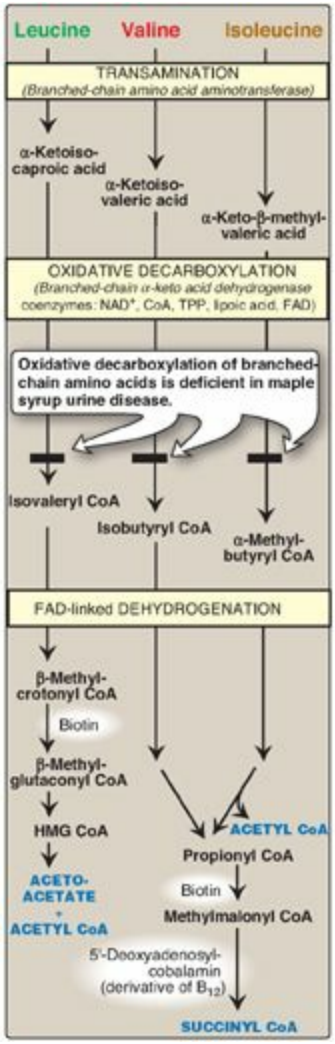
1. **Leucine:** This amino acid is exclusively ketogenic in its catabolism, forming acetyl CoA and acetoacetate (see Figure 20.10). The initial steps in the catabolism of leucine are similar to those of the other BCAAs, isoleucine and valine (see below).

2. **Isoleucine:** This amino acid is both ketogenic and glucogenic, because its metabolism yields acetyl CoA and propionyl CoA. The first three steps in the metabolism of isoleucine are similar to the initial steps in the degradation of the other BCAAs, valine and leucine (see Figure 20.10).

3. **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.

4. **Tryptophan:** This amino acid is both glucogenic and ketogenic because its catabolism yields alanine and acetoacetyl CoA. [Note: Quinolinate from tryptophan catabolism is used in the synthesis of nicotinamide adenine dinucleotide (NAD, see p. 379).]

Figure 20.10 Degradation of leucine, valine, and isoleucine. [Note: β-Methylcrotonyl CoA carboxylase is one of four biotinrequiring carboxylases we have encountered. 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. Catabolism of the branched-chain amino acids

The BCAAs isoleucine, leucine, and valine are essential amino acids. In contrast to other amino acids, they are metabolized primarily by the peripheral tissues (particularly muscle), rather than by the liver. Because these three amino acids have a similar route of catabolism, it is convenient to describe them as a group (see Figure 20.10).

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.

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. This complex uses thiamine pyrophosphate, lipoic acid, 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 pyruvate dehydrogenase (PDH) complex (see p. 110) and the oxidation of α-ketoglutarate to succinyl CoA by α-ketoglutarate dehydrogenase complex (see p. 112). The Enzyme 3 (E3) component is identical in BCKD, PDH, and α-ketoglutarate dehydrogenase.]

3. **Dehydrogenation:** Oxidation of the products formed in the BCKD reaction yields α-β-unsaturated acyl CoA derivatives and $FADH_2$. This reaction is analogous to the FAD-linked dehydrogenation in the β-oxidation of fatty acids (see p. 192). [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 sent out into the blood from muscle (see p. 253).]

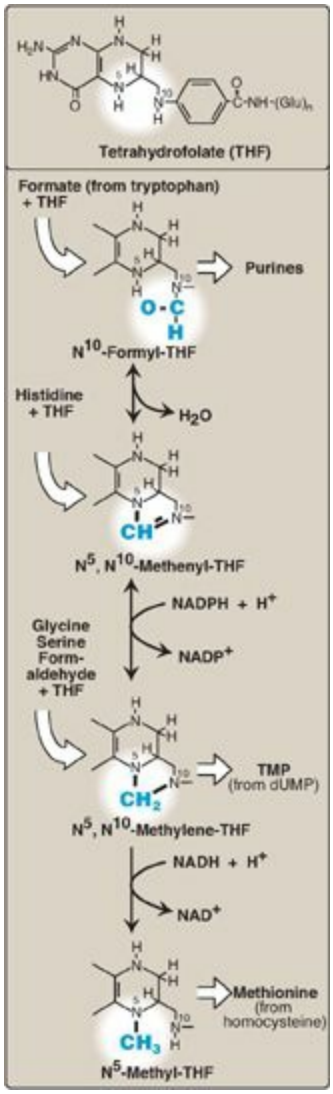
IV. FOLIC ACID AND AMINO ACID METABOLISM

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, the dehydrated form of carbonic acid, is carried by the vitamin biotin, 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 phosphates (NADPH). The one-carbon unit carried by THF is bound to nitrogen N^5 or N^{10} or to both N^5 and N^{10}. Figure 20.11 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 due to decreased availability of the purines and of the thymidine monophosphate needed for DNA synthesis (see p. 303).]

Figure 20.11 Summary of the interconversions and uses of the carrier tetrahydrofolate. [Note: N^5, N^{10}-Methenyl-THF also arises from N^5-formimino-THF (see Figure 20.4).] NADP(H) = nicotinamide adenine dinucleotide phosphate; NAD(H) = nicotinamide adenine dinucleotide; TMP = thymidine monophosphate; dUMP = deoxyuridine monophosphate.


Tetrahydrofolate (THF)
Formate (from tryptophan) + THF
Purines
N10-Formyl-THF
Histidine + THF
H2O
N5, N10-Methenyl-THF
Glycine Serine Form-aldehyde + THF
NADPH + H+
NADP+
TMP (from dUMP)
N5, N10-Methylene-THF
NADH + H+
NAD+
Methionine (from homocysteine)
N5-Methyl-THF

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 later in Figure 20.14. [Note: Some amino acids found in proteins, such as hydroxyproline and hydroxylysine (see p. 45), are modified after their incorporation into the protein (posttranslational modification; see p. 443).]

A. Synthesis from α-keto acids

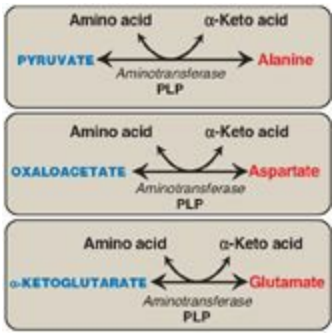
Alanine, aspartate, and glutamate are synthesized by transfer of an amino group to the α-keto acids pyruvate, oxaloacetate, and α-ketoglutarate, respectively. These transamination reactions (Figure 20.12; also see p. 250) are the most direct of the biosynthetic pathways. Glutamate is unusual in that it can also be synthesized by the reverse of oxidative deamination, catalyzed by glutamate dehydrogenase, when ammonia levels are high (see p. 252).

B. Synthesis by amidation

1. **Glutamine:** This amino acid, which contains an amide linkage with ammonia at the γ-carboxyl, is formed from glutamate by glutamine synthetase (see Figure 19.18, p. 256). 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. 256 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.

Figure 20.12 Formation of alanine, aspartate, and glutamate from the corresponding α-keto acids. PLP = pyridoxal phosphate.



C. Proline

Glutamate via glutamate semialdehyde is converted to proline by cyclization and reduction reactions. [Note: The semialdehyde can also be transaminated to ornithine.]

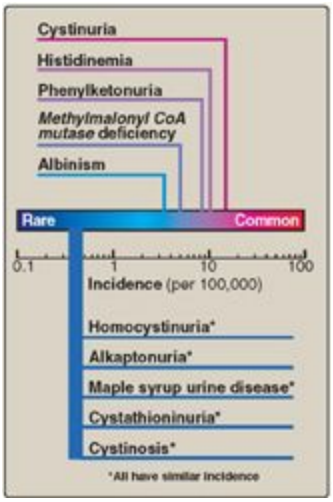
D. Serine, glycine, and cysteine

1. **Serine:** This amino acid arises from 3-phosphoglycerate, an intermediate in glycolysis (see Figure 8.18, p. 101), 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}-methylene-THF as the one-carbon donor (see Figure 20.6A). [Note: Selenocysteine (Sec), the 21st genetically encoded amino acid, is synthesized from serine and selenium while serine is attached to transfer RNA. Sec is found in several proteins such as glutathione peroxidase (see p. 148).]

2. **Glycine:** This amino acid is synthesized from serine by removal of a hydroxymethyl group, also by serine hydroxymethyltransferase (see Figure 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 Figure 20.8). (Hcy is derived from methionine as described on p. 264.) Because methionine is an essential amino acid, cysteine synthesis can be sustained only if the dietary intake of methionine is adequate.

Figure 20.13 Incidence of inherited diseases of amino acid metabolism. [Note: Cystinuria is the most common genetic error of amino acid transport.]

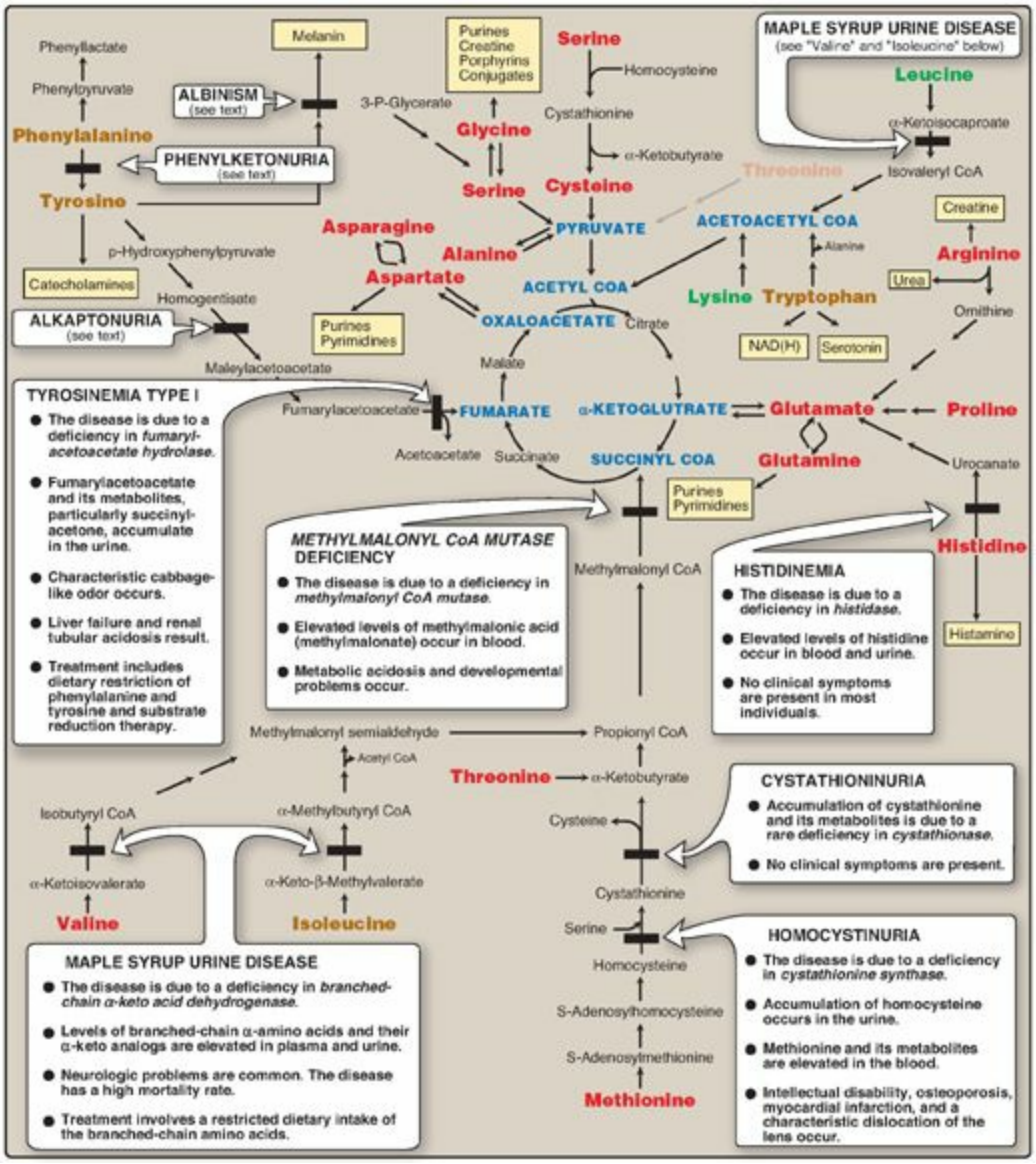


E. Tyrosine

Tyrosine is formed from phenylalanine by phenylalanine hydroxylase. The reaction requires molecular oxygen and the coenzyme tetrahydrobiopterin (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.

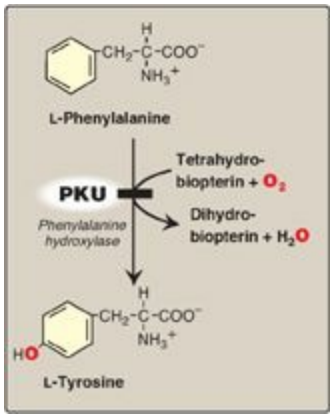
Figure 20.14 Summary of the metabolism of amino acids in humans. Genetically determined enzyme deficiencies are summarized in white boxes. Nitrogen-containing compounds derived from amino acids are shown in small, yellow boxes. Classification of amino acids is color coded: Red = glucogenic; brown = glucogenic 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.



VI. METABOLIC DEFECTS IN AMINO ACID METABOLISM

Inborn errors of metabolism are commonly caused by mutant genes that generally result in abnormal proteins, most often enzymes. 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 inherited defects of amino acid metabolism almost invariably result in intellectual disability or other developmental abnormalities as a consequence of harmful accumulation of metabolites. Although more than 50 of these disorders have been described, many are rare, occurring in less than 1 per 250,000 in most populations (Figure 20.13). Collectively, however, they constitute a very significant portion of pediatric genetic diseases (Figure 20.14). Phenylketonuria (PKU) is an important disease of amino acid metabolism because it is relatively common and responds to dietary treatment.

Figure 20.15 A deficiency in phenylalanine hydroxylase results in the disease phenylketonuria (PKU).



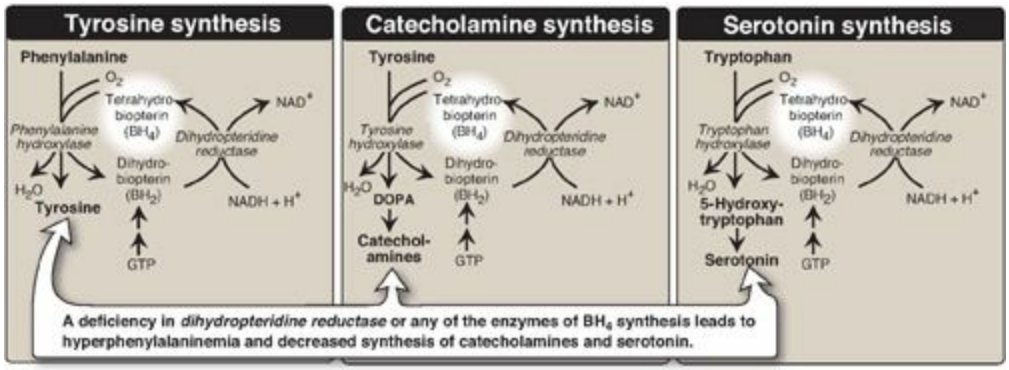
> Screening of newborns for a number of treatable disorders, including those of amino acid metabolism, is done by tandem mass spectrometry of blood obtained from a heel prick. By law, all states must screen for over 20 disorders, with some screening for over 30. All states screen for PKU.

A. Hyperphenylalanemia and phenylketonuria

PKU, caused by a deficiency of phenylalanine hydroxylase ([PAH] Figure 20.15), is the most common clinically encountered inborn error of amino acid metabolism (incidence 1:15,000). Biochemically, it is characterized by accumulation of phenylalanine, resulting in hyperphenylalanemia, and a deficiency of tyrosine. It is treated by dietary restriction of phenylalanine. Hyperphenylalanemia may also be caused by deficiencies in any of the several enzymes required to synthesize BH_4 or in dihydropteridine reductase, which regenerates BH_4 from BH_2 (Figure 20.16). 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 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 20.16 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 = 3,4-dihydroxyphenylalanine.



1. Characteristics of classic phenylketonuria:

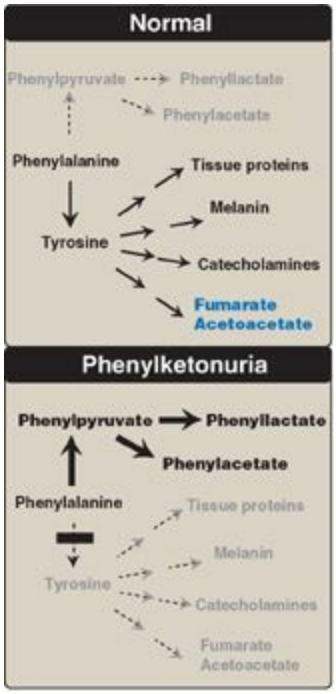
a. Elevated phenylalanine: Phenylalanine is present in high concentrations (ten times normal) in tissues, plasma, and urine. Phenyllactate, phenylacetate, and phenylpyruvate, which are not normally produced in significant amounts in the presence of functional PAH, are also elevated in PKU (Figure 20.17). These metabolites give urine a characteristic musty ("mousey") odor. [Note: The disease acquired its name from the presence of a phenylpyruvate, a phenylketone in the urine.]

b. Central nervous system symptoms: Severe intellectual disability, developmental delay, microcephaly, and seizures are characteristic findings in untreated PKU. The patient with untreated PKU typically shows symptoms of intellectual disability by age 1 year and rarely achieves an intelligence quotient (IQ) greater than 50 (Figure 20.18). [Note: These clinical manifestations are now rarely seen as a result of neonatal screening programs.]

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

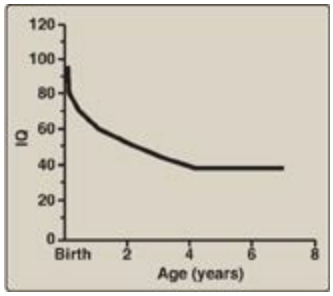
the pigment melanin**,** is inhibited in PKU.

Figure 20.17 Pathways of phenylalanine metabolism in normal individuals and in patients with phenylketonuria.



2. Neonatal screening and diagnosis: Early diagnosis of PKU is important because the disease is treatable by dietary means. Due to 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 24–48 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.

Figure 20.18 Typical intellectual ability in untreated patients of different ages with phenylketonuria. IQ = intelligence quotient.

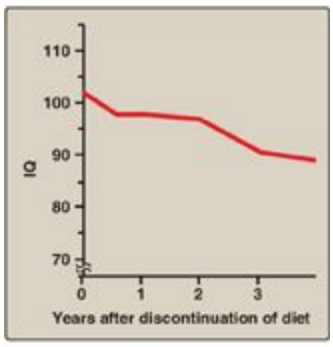


3. Prenatal diagnosis: Classic PKU is caused by any of 100 or more different mutations in the gene that codes for PAH. The frequency of any given mutation

varies among populations, and the disease is often doubly heterozygous (that is, the PAH gene has a different mutation in each allele). Despite this complexity, prenatal diagnosis is possible (see p. 477).

4. **Treatment:** Most natural protein contains phenylalanine, an essential amino acid, and 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 7–10 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 phenyalanine-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 (Figure 20.19). Lifelong restriction of dietary phenylalanine is, therefore, recommended. [Note: Individuals with PKU are advised to avoid aspartame, an artificial sweetener that contains phenylalanine.]

Figure 20.19 Changes in IQ scores after discontinuation of low-phenylalanine diet in patients with phenylketonuria. IQ = intelligence quotient.



5. **Maternal phenylketonuria:** If women with PKU who are not on a low-phenylalanine diet become pregnant, the offspring are affected with "maternal PKU syndrome." High blood phenylalanine levels in the mother cause microcephaly and congenital heart abnormalities in the fetus (phenylalanine is a teratogen). 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 must be maintained throughout the pregnancy.

B. Maple syrup urine disease

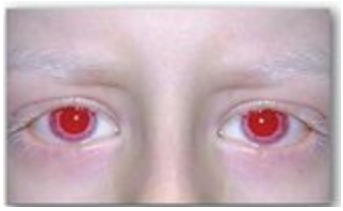
Maple syrup urine disease (MSUD) is a rare (1:185,000), autosomal recessive disorder in which there is a partial or complete deficiency in BCKD, a mitochondrial enzyme complex that oxidatively decarboxylates leucine, isoleucine, and valine (see Figure 20.10). These BCAAs and their corresponding α-keto 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 due to the rise in leucine), and a characteristic maple syrup-like odor of the urine due to the rise in isoleucine. If untreated, the disease is fatal. If treatment is delayed, intellectual disability results.

1. **Classification:** The term "maple syrup urine disease" includes a classic type and several variant forms of the disorder. 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 neonatal screening are available, and most affected individuals are compound heterozygotes.

3. **Treatment:** MSUD is treated with a synthetic formula that is free of BCAAs, 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 is 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.]

Figure 20.20 Patient with oculocutaneous albinism, showing white eyebrows and lashes and eyes that appear red in color.



C. Albinism

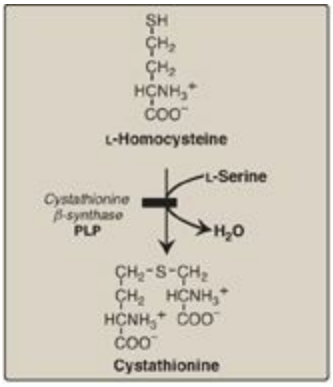
Albinism refers to a group of conditions in which a defect in tyrosine metabolism

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 (Figure 20.20) , tyrosinase-negative oculocutaneous albinism (type 1 albinism), results from an absent or defective copper-requiring tyrosinase. 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 are at increased risk for skin cancer.

D. Homocystinuria

The homocystinurias are a group of disorders involving defects in the metabolism of Hcy. These autosomal-recessive diseases are characterized by high plasma and urinary levels of Hcy and methionine and low levels of cysteine. The most common cause of homocystinuria is a defect in the enzyme cystathionine β-synthase, which converts Hcy to cystathionine (Figure 20.21). Individuals who are 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. Patients can be responsive or nonresponsive to oral administration of pyridoxine (vitamin B_6), which is converted to pyridoxal phosphate, the coenzyme of cystathionine β-synthase. Vitamin B_6–responsive patients usually have a milder and later onset of clinical symptoms compared with B_6-nonresponsive patients. Treatment includes restriction of methionine intake and supplementation with vitamins B_6, B_{12}, and folate.

Figure 20.21 Enzyme deficiency in homocystinuria. PLP = pyridoxal phosphate.



E. Alkaptonuria (alcaptonuria)

Alkaptonuria is a rare metabolic condition involving a deficiency in homogentisic acid oxidase, resulting in the accumulation of homogentisic acid (HA), an intermediate in the degradative pathway of tyrosine (see p. 269.) The condition has three characteristic symptoms: homogentisic aciduria (the urine contains elevated levels of

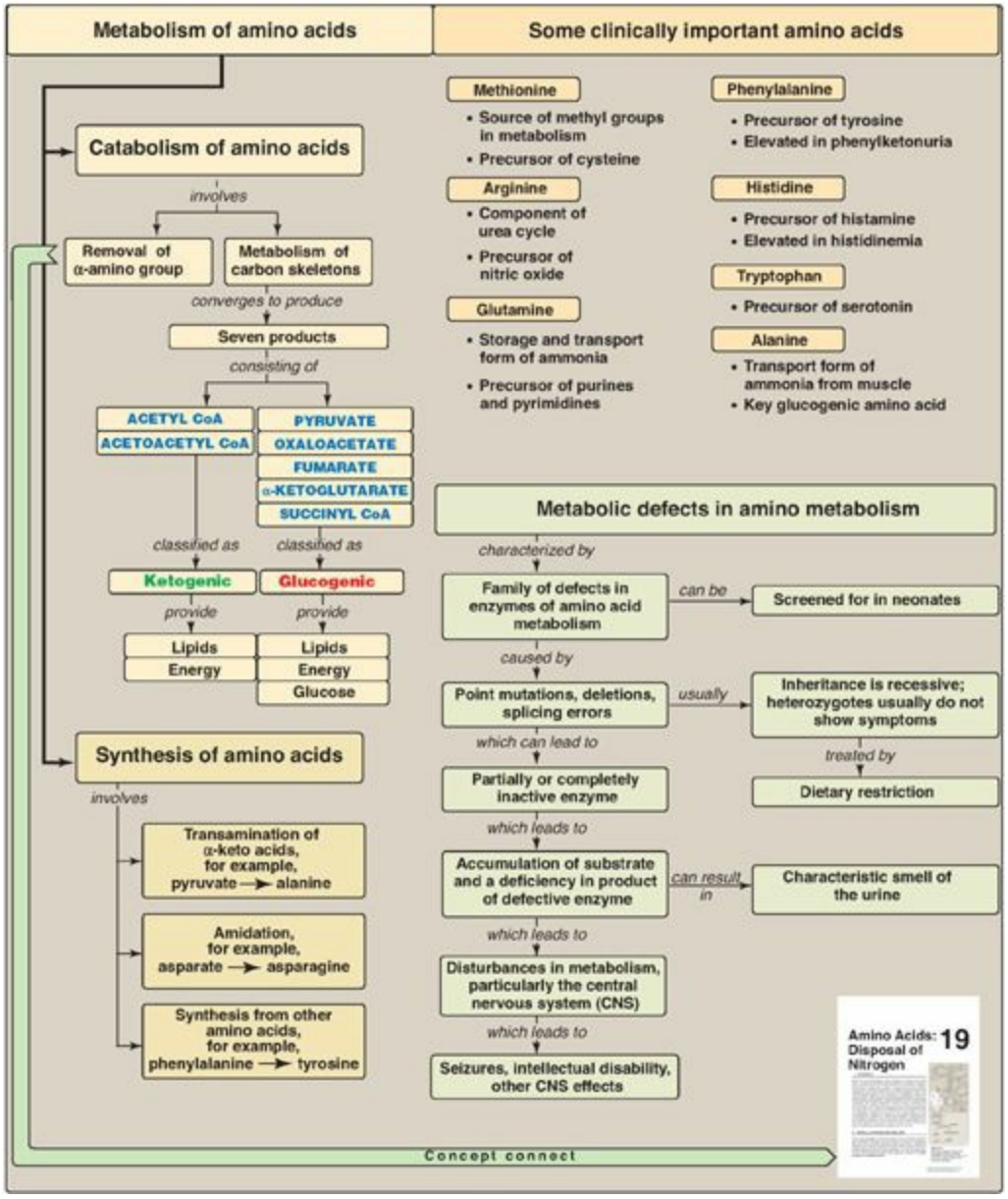
HA, which is oxidized to a dark pigment on standing, as shown in Figure 20.22A), large joint arthritis, and deposition of black pigment (ochronosis) in cartilage and collagenous tissue (Figure 20.22B). Patients with alkaptonuria are usually asymptomatic until about age 40 years. Dark staining of diapers can indicate the disease in infants, but usually no symptoms are present until later in life. Diets low in phenylalanine and tyrosine reduce the levels of HA and decrease the amount of pigment deposited in body tissues. Although alkaptonuria is not life-threatening, the associated arthritis may be severely crippling.

Figure 20.22 A patient with alkaptonuria. A. Urine. B. Vertebrae.

VII. CHAPTER SUMMARY

Amino acids whose catabolism yields **pyruvate** or one of the **intermediates of the tricarboxylic acid cycle** are termed **glucogenic** (Figure 20.23). They can give rise to the net formation of **glucose** in the **liver** and the **kidney**. 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 **acetoacetate** or one of its precursors, **acetyl coenzyme A** (**CoA**) or **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. **Phenylketonuria** (**PKU**) is caused by a **deficiency** of **phenylalanine hydroxylase** (**PAH**), the enzyme that converts phenylalanine to tyrosine. **Hyperphenylalaninemia** may also be caused by deficiencies in the enzymes that synthesize or regenerate the coenzyme for PAH, **tetrahydrobiopterin**. Untreated patients with PKU suffer from severe intellectual disability, developmental delay, microcephaly, seizures and a characteristic mousey smell of the urine. Treatment involves controlling dietary phenylalanine. Tyrosine becomes an essential dietary component for people with PKU. **Maple syrup urine disease** is caused by a partial or complete **deficiency** in **branched-chain α-keto acid dehydrogenase**, the enzyme that decarboxylates **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 dietary leucine, isoleucine, and valine. Other important genetic diseases associated with amino acid metabolism include **albinism**, **homocystinuria**, **methylmalonyl CoA mutase deficiency**, **alkaptonuria**, **histidinemia**, **tyrosinemia**, and **cystathioninuria**.

Figure 20.23 Key concept map for amino acid metabolism. CoA = coenzyme A.


Metabolism of amino acids
Some clinically important amino acids
Catabolism of amino acids
involves
Removal of α-amino group
Metabolism of carbon skeletons
converges to produce
Seven products
consisting of
ACETYL CoA
ACETOACETYL CoA
PYRUVATE
OXALOACETATE
FUMARATE
α-KETOGLUTARATE
SUCCINYL CoA
classified as
classified as
Ketogenic
Glucogenic
provide
provide
Lipids
Energy
Lipids
Energy
Glucose
Synthesis of amino acids
involves
Transamination of α-keto acids, for example, pyruvate → alanine
Amidation, for example, asparate → asparagine
Synthesis from other amino acids, for example, phenylalanine → tyrosine
Methionine
• Source of methyl groups in metabolism
• Precursor of cysteine
Arginine
• Component of urea cycle
• Precursor of nitric oxide
Glutamine
• Storage and transport form of ammonia
• Precursor of purines and pyrimidines
Phenylalanine
• Precursor of tyrosine
• Elevated in phenylketonuria
Histidine
• Precursor of histamine
• Elevated in histidinemia
Tryptophan
• Precursor of serotonin
Alanine
• Transport form of ammonia from muscle
• Key glucogenic amino acid
Metabolic defects in amino metabolism
characterized by
Family of defects in enzymes of amino acid metabolism
can be
Screened for in neonates
caused by
Point mutations, deletions, splicing errors
usually
Inheritance is recessive; heterozygotes usually do not show symptoms
which can lead to
treated by
Partially or completely inactive enzyme
Dietary restriction
which leads to
Accumulation of substrate and a deficiency in product of defective enzyme
can result in
Characteristic smell of the urine
which leads to
Disturbances in metabolism, particularly the central nervous system (CNS)
which leads to
Seizures, intellectual disability, other CNS effects
Amino Acids: Disposal of Nitrogen 19
Concept connect

Study Questions:

Choose the ONE best answer.

For Questions 20.1–20.3, match the deficient enzyme with the associated clinical sign or laboratory finding in urine.

A. Black pigmentation of cartilage
B. Cabbage-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. 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. A deficiency in tyrosinase results in decreased formation of melanin from tyrosine in skin, hair, and eyes. A cabbage-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 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 recommended to be discontinued in adulthood.
C. Supplementation with vitamin B_6 is required.
D. Tyrosine is an essential amino acid.

Correct answer = D. In patients with phenylketonuria, tyrosine cannot be synthesized from phenylalanine and, hence, becomes essential and must be supplied in the diet. Phenylanine 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 7–10 days of life to prevent intellectual disability, and life-long restriction of phenylalanine is recommended to prevent cognitive decline. Additionally, elevated levels of phenylalanine are teratogenic to a developing fetus.

20.5 Which one of the following statements concerning amino acids is correct?

A. Alanine is ketogenic.
B. Amino acids that are catabolized to acetyl coenzyme A are glucogenic.
C. Branched-chain amino acids are catabolized primarily in liver.
D. Cysteine is essential for individuals consuming a diet severely limited in methionine.

Correct answer = D. Methionine is the precursor of cysteine, which becomes essential if methionine is severely restricted. Alanine is the primary glucogenic amino acid. Acetyl coenzyme A (CoA) cannot be used for the net synthesis of glucose. Amino acids catabolized to acetyl CoA are ketogenic. Branched-chain amino acids are catabolized primarily in skeletal muscle.

20.6 In an individual with the Enzyme 3–deficient form of maple syrup urine disease, why would lactic acidosis be an expected finding?

The three α-keto acid dehydrogenase complexes (pyruvate dehydrogenase [PDH], α-ketoglutarate dehydrogenase, and branched-chain α-keto acid dehydrogenase [BCKD]) have a common Enzyme 3 (E3) (dihydrolipoyl dehydrogenase). 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

Conversion of Amino Acids to Specialized Products

21

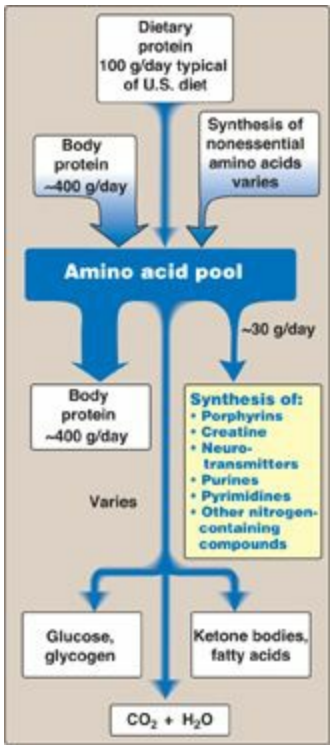
I. OVERVIEW

In addition to serving as building blocks for proteins, amino acids are precursors of many nitrogen-containing compounds that have important physiologic functions (Figure 21.1). These molecules include porphyrins, neurotransmitters, hormones, purines, and pyrimidines.

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. 280). Heme is the prosthetic group for hemoglobin, myoglobin, the cytochromes, the cytochrome P450 (CYP) monooxygenase system, catalase, nitric oxide synthase, and peroxidase. These hemeproteins are rapidly synthesized and degraded. For example, 6–7 g of hemoglobin are synthesized each day to replace heme lost through the normal turnover of erythrocytes. The simultaneous synthesis and degradation of the associated porphyrins and recycling of the bound iron ions is coordinated with the turnover of hemeproteins.

Figure 21.1 Amino acids as precursors of nitrogen-containing compounds.

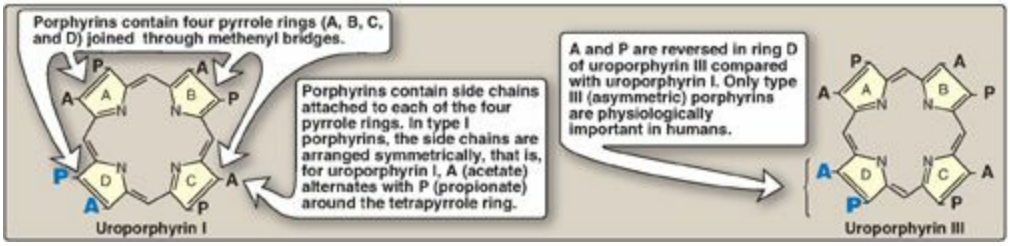


A. Structure of porphyrins

Porphyrins are cyclic molecules formed by the linkage of four pyrrole rings through methenyl bridges (Figure 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 that are 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) contains vinyl ($–CH=CH_2$), methyl, and propionate groups.

Figure 21.2 Structures of uroporphyrin I and uroporphyrin III.



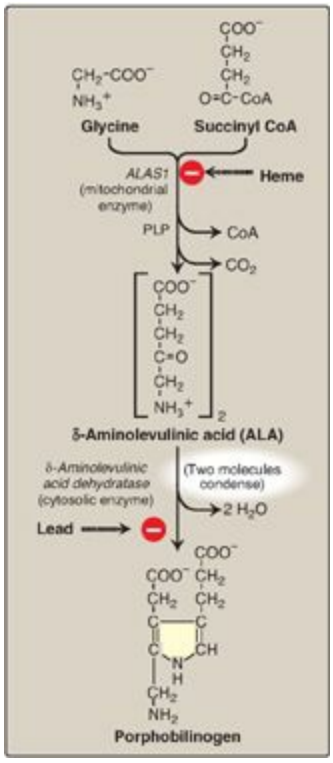
2. **Distribution of side chains:** The side chains of porphyrins can be ordered around the tetrapyrrole nucleus in four different ways, designated by Roman numerals I to IV. Only type III porphyrins, which contain an asymmetric substitution on ring D (see Figure 21.2), are physiologically important in humans. [Note: Protoporphyrin IX is a member of the type III series.]

3. **Porphyrinogens:** These porphyrin precursors (for example, uroporphyrinogen) exist in a chemically reduced, colorless form and serve as intermediates between porphobilinogen and the oxidized, colored protoporphyrins in heme biosynthesis.

B. Biosynthesis of heme

The major sites of heme biosynthesis are the liver, which synthesizes a number of heme proteins (particularly the CYP proteins), and the erythrocyte-producing cells of the bone marrow, which are active in hemoglobin synthesis. [Note: Over 85% of all heme synthesis occurs in erythroid tissue.] 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. In contrast, heme synthesis in erythroid cells is relatively constant and is matched to the rate of globin synthesis. 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 (see Figure 21.8). [Note: Mature red blood cells (RBCs) lack mitochondria and are unable to synthesize heme.]

Figure 21.3 Pathway of porphyrin synthesis: Formation of porphobilinogen. ALAS = δ-aminolevulinic acid synthase; CoA = coenzyme A; PLP = pyridoxal phosphate. (Continued in Figures 21.4 and 21.5.)



1. **Formation of δ-aminolevulinic acid:** All the carbon and nitrogen atoms of the porphyrin molecule are provided by glycine (a nonessential amino acid) and succinyl coenzyme A (a citric acid cycle intermediate) that condense to form δ-aminolevulinic acid (ALA) in a reaction catalyzed by ALA synthase ([ALAS] Figure 21.3) This reaction requires pyridoxal phosphate (PLP) as a coenzyme and is the committed and rate-limiting step in porphyrin biosynthesis. [Note: There are two isoforms of ALAS, 1 and 2, 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.]

   a. **Effect of heme (hemin):** 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 thereby 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.]

   b. **Effect of drugs:** Administration of any of a large number of drugs results in a significant increase in hepatic ALAS1 activity. These drugs are metabolized by the microsomal CYP monooxygenase system, a hemeprotein oxidase system found in the liver (see p. 149). 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 heme in liver cells. The lower intracellular heme concentration leads to an increase in the synthesis of ALAS1 and prompts a corresponding increase in the synthesis of

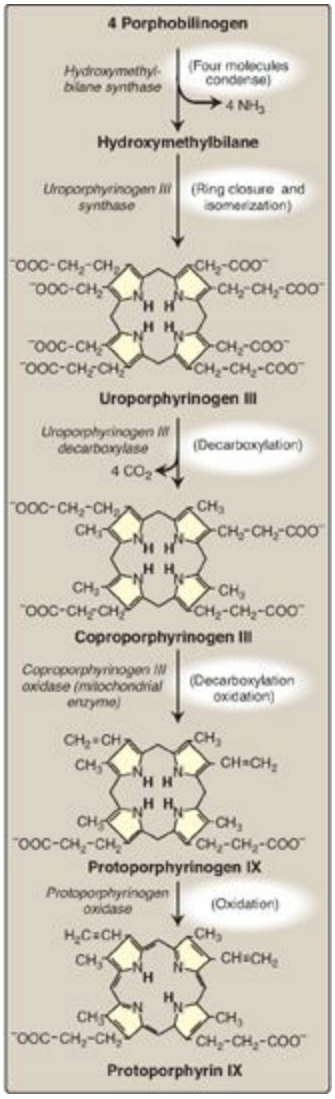
ALA.

2. **Formation of porphobilinogen:** The condensation of two molecules of ALA to form porphobilinogen by zinc-containing ALA dehydratase (porphobilinogen synthase) is extremely sensitive to inhibition by heavy metal ions (for example, lead) that replace the zinc (see Figure 21.3). This inhibition is, in part, responsible for the elevation in ALA and the anemia seen in lead poisoning.

3. **Formation of uroporphyrinogen:** The condensation of four porphobilinogens produces the linear tetrapyrrole hydroxymethylbilane, which is cyclized and isomerized by uroporphyrinogen III synthase to produce the asymmetric uroporphyrinogen III. This cyclic tetrapyrrole undergoes decarboxylation of its acetate groups, generating coproporphyrinogen III (Figure 21.4). These reactions occur in the cytosol.

4. **Formation of heme:** Coproporphyrinogen III enters the mitochondrion, and two propionate side chains are decarboxylated by coproporphyrinogen III oxidase to vinyl groups generating protoporphyrinogen IX, which is oxidized to protoporphyrin IX. The introduction of iron (as Fe^{2+}) into protoporphyrin IX can occur spontaneously, but the rate is enhanced by ferrochelatase, an enzyme that, like ALA dehydratase, is inhibited by lead (Figure 21.5).

Figure 21.4 Pathway of porphyrin synthesis: formation of protoporphyrin IX. (Continued from Figure 21.3.) [Note: Deficiency in uroporphyrinogen III synthase prevents isomerization, resulting in production of type I porphyrins.]



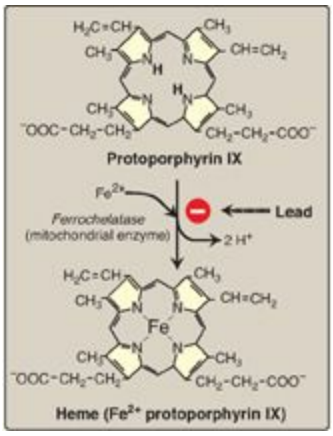
C. Porphyrias

Porphyrias are rare, inherited (or occasionally acquired) defects in heme synthesis, resulting in the accumulation and increased excretion of porphyrins or porphyrin precursors (see Figure 21.8). [Note: With few exceptions, porphyrias are inherited as autosomal-dominant 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" 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 (that is, their skin itches and burns [pruritus] when exposed to visible light). [Note: Photosenstivity 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 reactive

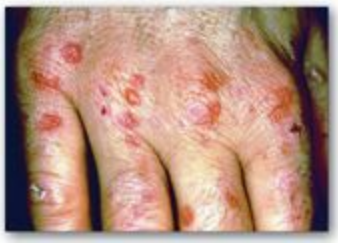
oxygen species can oxidatively damage membranes and cause the release of destructive enzymes from lysosomes.]

Figure 21.5 Pathway of porphyrin synthesis: formation of heme. (Continued from Figures 21.3 and 21.4.) Fe^{2+} = ferrous iron.



a. Chronic hepatic porphyria: Porphyria cutanea tarda, the most common porphyria, is a chronic disease of the liver. The disease is associated with a deficiency in uroporphyrinogen decarboxylase, but clinical expression of the enzyme 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. Clinical onset is typically during the fourth or fifth decade of life. Porphyrin accumulation leads to cutaneous symptoms (Figure 21.6) as well as urine that is red to brown in natural light (Figure 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, acute intermittent porphyria, hereditary coproporphyria, and variegate porphyria) are characterized by acute attacks of gastrointestinal (GI), neuropsychiatric, and motor symptoms that may be accompanied by photosensitivity. Porphyrias leading to accumulation of ALA and porphobilinogen, such as acute intermittent porphyria, 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.

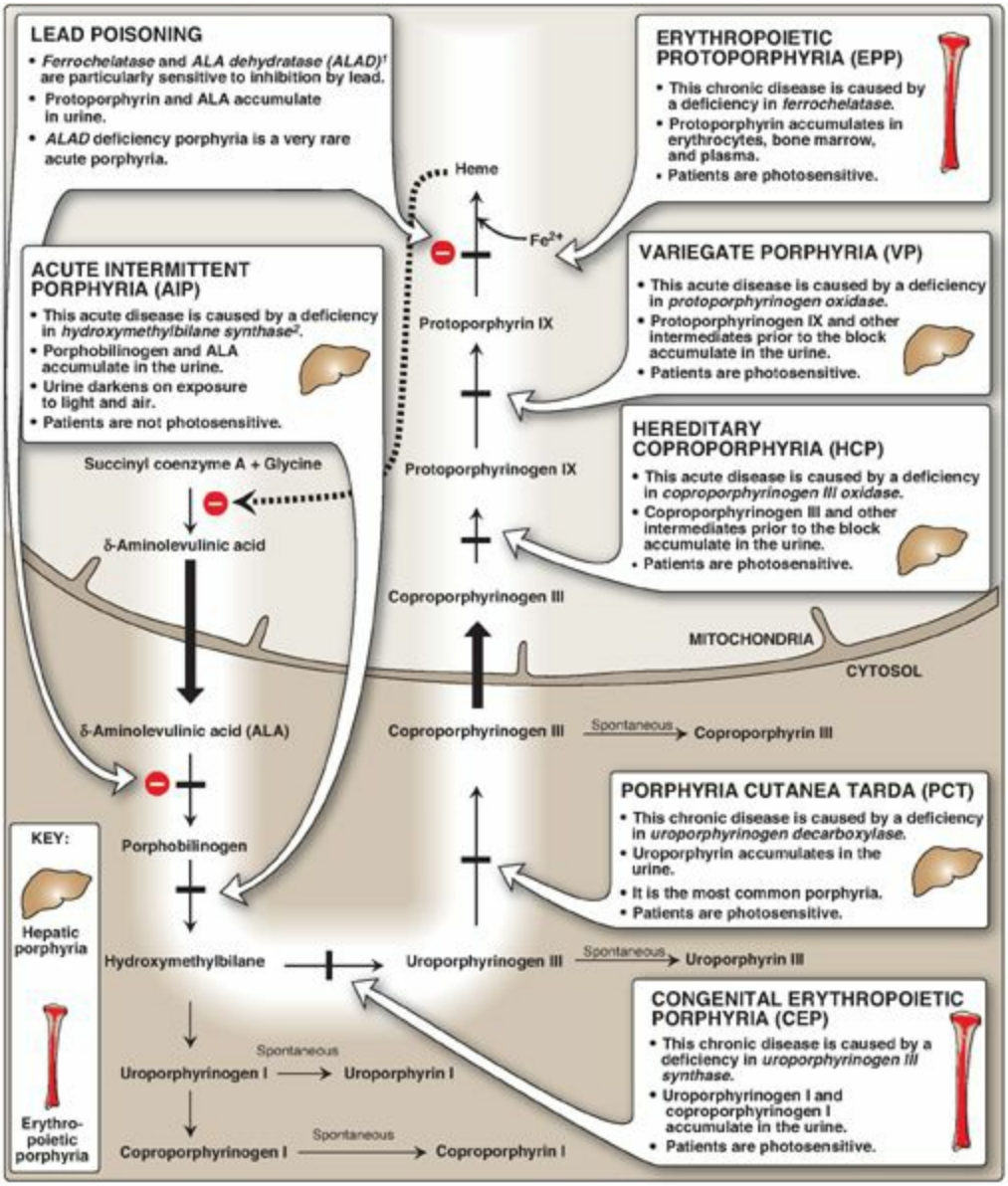
c. Erythropoietic porphyrias: The chronic erythropoietic porphyrias (congenital erythropoietic porphyria and erythropoietic protoporphyria) are characterized by skin rashes and blisters that appear in early childhood. [Note: Patients with erythropoietic protoporphyria are also at risk for hepatobiliary disease.]

2. Increased δ-aminolevulinic acid synthase activity: One common feature of the porphyrias is a decreased synthesis of heme. In the liver, heme normally functions as a repressor of the gene for ALAS1. Therefore, the absence of this end product results in an increase in the synthesis of ALAS1 (derepression). 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.

Figure 21.7 Urine from a patient with porphyria cutanea tarda (right) and from a patient with normal porphyrin excretion (left).

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, which decreases the synthesis of ALAS1. Protection from sunlight, ingestion of β-carotene (a free-radical scavenger), and phlebotomy 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: Deficiencies in ALA synthase-1 (ALAS1) are unknown. Deficiencies in ALAS2 result in an anemia, not a porphyria.]



D. Degradation of heme

After approximately 120 days in the circulation, red blood cells are taken up and degraded by the reticuloendothelial system, particularly in the liver and spleen (Figure 21.9). Approximately 85% of heme destined for degradation comes from senescent RBCs. The remainder is from the degradation of heme proteins other than hemoglobin.

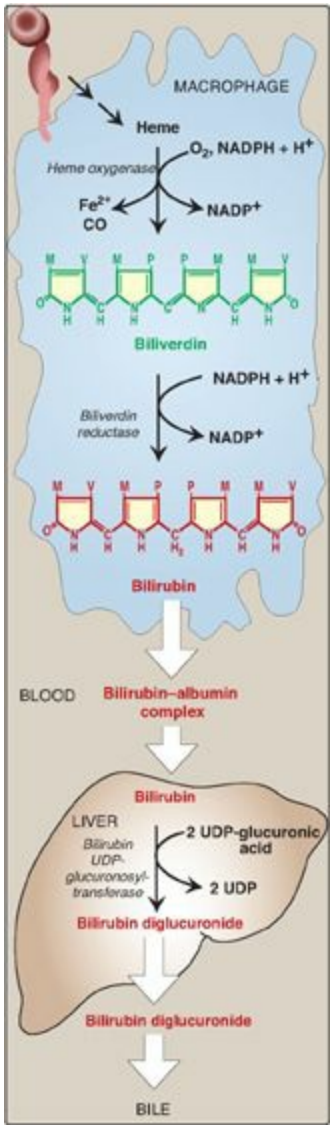
1. **Formation of bilirubin:** The first step in the degradation of heme is catalyzed by the microsomal heme oxygenase system of the reticuloendothelial cells. In the presence of nicotinamide adenine dinucleotide phosphate and O_2, the enzyme catalyzes three successive oxygenations that result in opening of the porphyrin ring (converting cyclic heme to linear biliverdin), production of carbon monoxide (CO), and release of Fe^{2+} (see Figure 21.9). [Note: The CO has biologic function, acting as a signaling molecule and anti-inflammatory.] 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. **Uptake of bilirubin by the liver:** Bilirubin is only slightly soluble in plasma and, therefore, is transported 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. 285).] Bilirubin dissociates from the carrier albumin molecule; enters a hepatocyte via facilitated diffusion; and binds to intracellular proteins, particularly the protein ligandin.

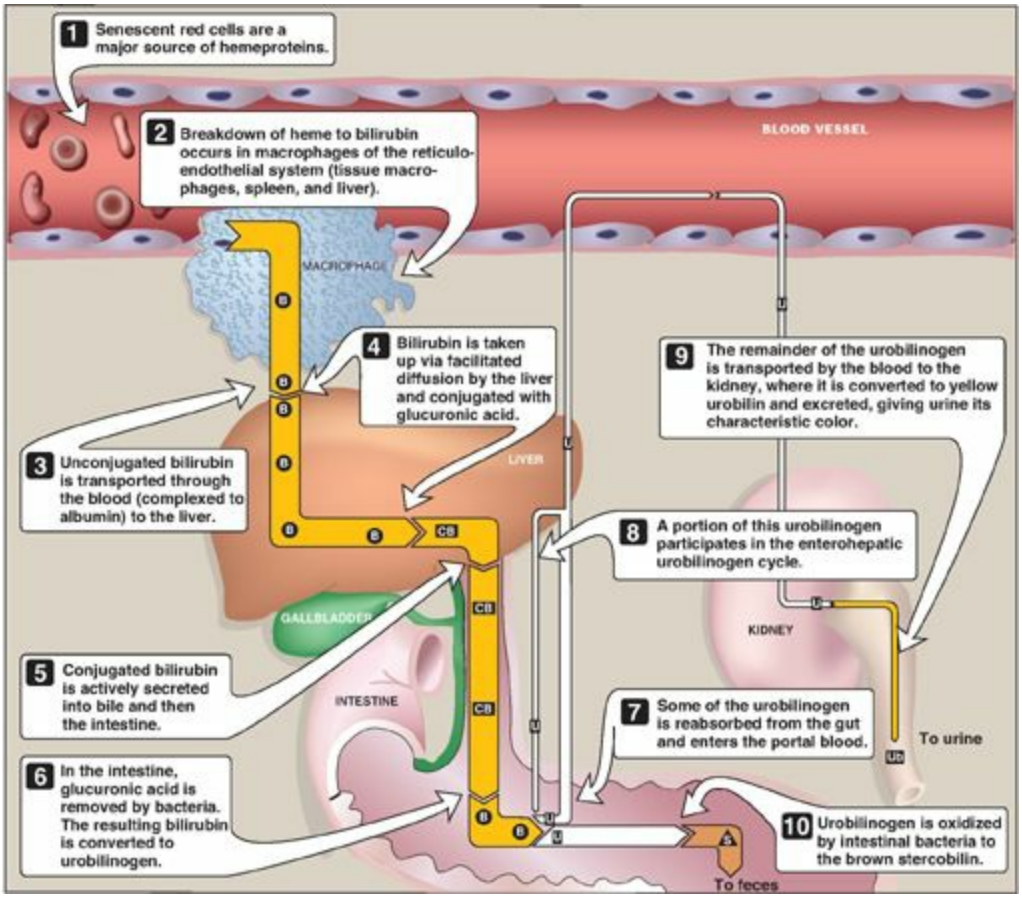
3. **Formation of bilirubin diglucuronide:** In the hepatocyte, the solubility of bilirubin is increased by the addition of two molecules of glucuronic acid, producing bilirubin diglucuronide. [Note: This process is referred to as conjugation.] The reaction is catalyzed by microsomal bilirubin UDP-glucuronosyltransferase (bilirubin UGT) using uridine diphosphate (UDP)-glucuronic acid as the glucuronate donor. [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 deficiency.]

Figure 21.9 Formation of bilirubin from heme and its conversion to bilirubin diglucuronide. UDP = uridine diphosphate; CO = carbon monoxide; NADP(H) = nicotinamide adenine dinucleotide phosphate.



4. **Secretion of bilirubin into bile:** Bilirubin diglucuronide (conjugated bilirubin [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 Ⓑ = bilirubin; CB = conjugated bilirubin; U = urobilinogen; Ub = urobilin; S = stercobilin.

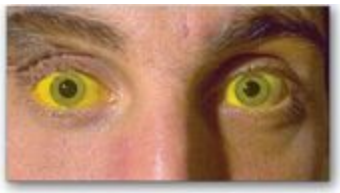


5. **Formation of urobilins in the intestine:** Bilirubin diglucuronide is hydrolyzed and reduced by bacteria in the gut to yield urobilinogen, a colorless compound. Most of the urobilinogen is oxidized by intestinal bacteria to stercobilin, which gives feces the characteristic brown color. However, some of the urobilinogen 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 (also called icterus) refers to the yellow color of skin, nail beds, and sclerae (whites of the eyes) caused by deposition of bilirubin, secondary to increased bilirubin levels in the blood (hyper-bilirubinemia) 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 about 1 mg/dl. Jaundice is seen at 2–3 mg/dl.]

Figure 21.11 Jaundiced patient, with the sclerae of his eyes appearing yellow.

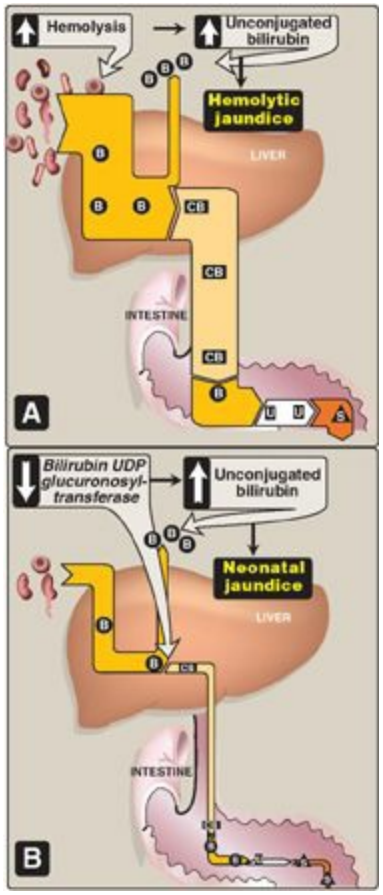


1. **Types of jaundice:** Jaundice can be classified into three major types described below. However, in clinical practice, jaundice is often more complex than indicated in this simple classification. For example, the accumulation of bilirubin may be a result of defects at more than one step in its metabolism.

   a. **Hemolytic jaundice:** The liver has the capacity to conjugate and excrete over 3,000 mg of bilirubin per 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 bilirubin diglucuronide. However, extensive hemolysis (for example, in patients with sickle cell anemia or pyruvate kinase or glucose 6-phosphate dehydrogenase deficiency) may produce bilirubin faster than it can be conjugated. UCB levels in the blood become elevated, causing jaundice (Figure 21.12A). [Note: With hemolysis, more CB is made and excreted into the bile, the amount of urobilinogen entering the enterohepatic circulation is increased, and urinary urobilinogen is increased.]

   b. **Hepatocellular jaundice:** Damage to liver cells (for example, in patients with cirrhosis or hepatitis) can cause UCB levels in the blood to increase 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 (AST and ALT, respectively; see p. 251) are elevated. [Note: If CB is made but is not efficiently secreted from the liver into bile (intrahepatic cholestasis), it can diffuse ("leak") into the blood, causing a conjugated hyperbilirubinemia.]

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



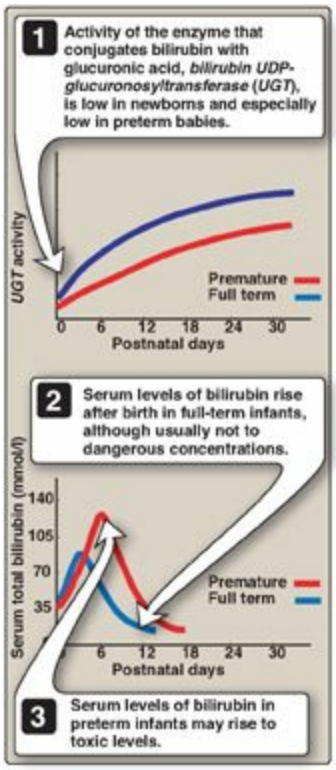
c. Obstructive jaundice: In this instance, jaundice is not caused by overproduction of bilirubin or decreased conjugation but, instead, results from obstruction of the common bile duct (extrahepatic cholestasis). For example, the presence of a tumor or bile stones may block the duct, preventing passage of CB into the intestine. Patients with obstructive jaundice experience GI pain and nausea and produce stools that are a pale, clay color. The liver "regurgitates" CB into the blood (hyperbilirubinemia). The CB is eventually excreted in the urine (which darkens upon standing), and is referred to as "urinary bilirubin." Urinary urobilinogen is absent.

2. Jaundice in newborns: The majority of 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 21.13. Elevated UCB, in excess of the binding capacity of albumin (20–25 mg/dl), can diffuse into the basal ganglia, cause 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. Determination of bilirubin concentration: 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 one 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 about 4% of the total bilirubin is conjugated or direct-reacting, because most is secreted into bile.]

Figure 21.13 Neonatal jaundice. UDP = uridine diphosphate.



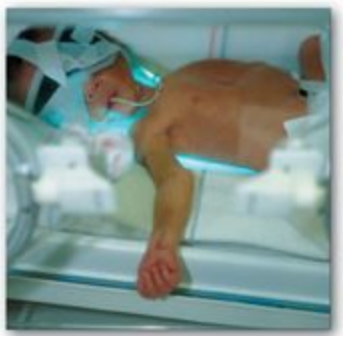
III. OTHER NITROGEN-CONTAINING COMPOUNDS

A. Catecholamines

Dopamine, norepinephrine, and epinephrine are biologically active (biogenic) amines that are collectively termed catecholamines. Dopamine and norepinephrine are synthesized in the brain and function as neurotransmitters. Norepinephrine is also synthesized in the adrenal medulla, as is epinephrine.

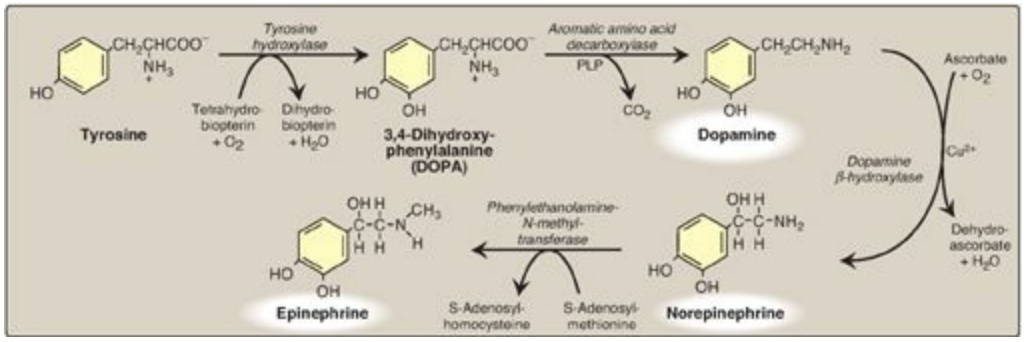
1. **Function:** Outside the CNS, norepinephrine and its methylated derivative, epinephrine, are hormone regulators of carbohydrate and lipid metabolism. Norepinephrine and epinephrine are released from storage vesicles in the adrenal medulla in response to fright, exercise, cold, and low levels of blood 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 21.14 Phototherapy in neonatal jaudice.



2. **Synthesis:** The catecholamines are synthesized from tyrosine, as shown in Figure 21.15. Tyrosine is first hydroxylated by tyrosine hydroxylase to form 3,4-dihydroxyphenylalanine (DOPA) in a reaction analogous to that described for the hydroxylation of phenylalanine (see p. 270). The tetrahydrobiopterin (BH_4)-requiring enzyme is abundant in the CNS, the sympathetic ganglia, and the adrenal medulla and is the rate-limiting step of the pathway. DOPA is decarboxylated in a reaction requiring PLP (see p. 378) to form dopamine, which is hydroxylated by dopamine β-hydroxylase to yield norepinephrine in a reaction that requires ascorbate (vitamin C) and copper. Epinephrine is formed from norepinephrine by an N-methylation reaction using S-adenosylmethionine (SAM) as the methyl donor (see p. 264).

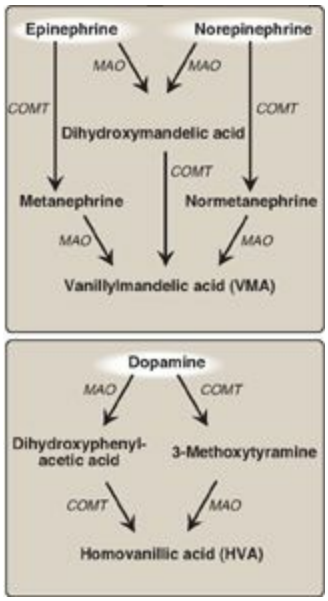
Figure 21.15 Synthesis of catecholamines. PLP = pyridoxal phosphate; Cu^{2+} = copper.



> 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 L-DOPA (levodopa) is the most common treatment. Dopamine cannot cross the blood brain barrier.

3. **Degradation:** The catecholamines are inactivated by oxidative deamination catalyzed by monoamine oxidase (MAO) and by O-methylation carried out by catechol-O-methyltransferase (COMT) using SAM as the methyl donor (Figure 21.16). The two reactions can occur in either order. The aldehyde products of the MAO reaction are oxidized to the corresponding acids. The metabolic products of these reactions are excreted in the urine as vanillylmandelic acid (VMA) from epinephrine and norepinephrine and homovanillic acid from dopamine. [Note: VMA is increased with pheochromocytomas, rare tumors of the adrenal gland characterized by excessive production of catecholamines.]

Figure 21.16 Metabolism of the catecholamines by catechol-O-methyltranferase (COMT) and monoamine oxidase (MAO).



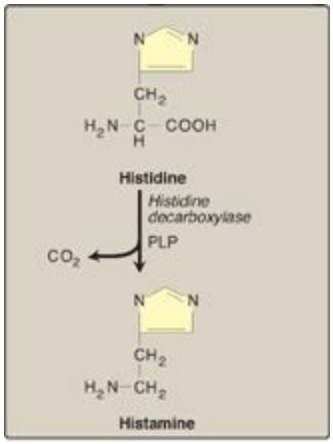
4. **Monoamine oxidase inhibitors:** MAO is found in neural and other tissues, such as the intestine and liver. In the neuron, this enzyme oxidatively deaminates and

inactivates any excess neurotransmitter molecules (norepinephrine, dopamine, or serotonin) that may leak out of synaptic vesicles when the neuron is at rest. MAO inhibitors may irreversibly or reversibly inactivate the enzyme, permitting neurotransmitter molecules to escape degradation and, therefore, to both accumulate within the presynaptic neuron and to leak into the synaptic space. This causes activation of norepinephrine and serotonin receptors and may be responsible for the antidepressant action of these drugs.

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 requiring PLP (Figure 21.17). It is secreted by mast cells as a result of allergic reactions or trauma. Histamine has no clinical applications, but agents that interfere with the action of histamine have important therapeutic applications.

Figure 21.17 Biosynthesis of histamine. PLP = pyridoxal phosphate.



C. Serotonin

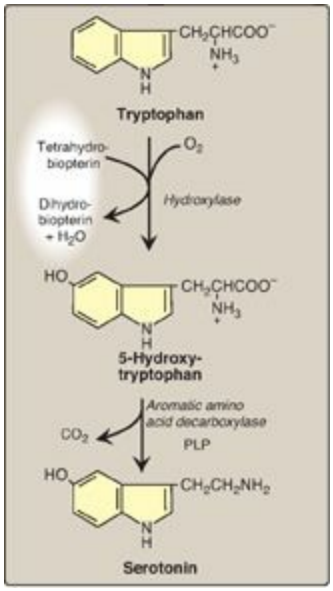
Serotonin, also called 5-hydroxytryptamine (5-HT), is synthesized and stored at several sites in the body (Figure 21.18). The largest amount by far is found in the intestinal mucosa. Smaller amounts occur in the CNS, where it functions as a neurotransmitter, and in platelets. Serotonin is synthesized 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 serotonin, which is degraded by MAO. Serotonin has multiple physiologic roles including pain perception, 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.]

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 (Figure 21.19) to maintain the intracellular level of adenosine triphosphate (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 liver and kidney tissue from glycine and the guanidino group of arginine, plus a methyl group from SAM (see Figure 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. 65).]

Figure 21.18 Synthesis of serotonin. [Note: Serotonin is converted to melatonin in the pineal gland.] PLP = pyridoxal phosphate.



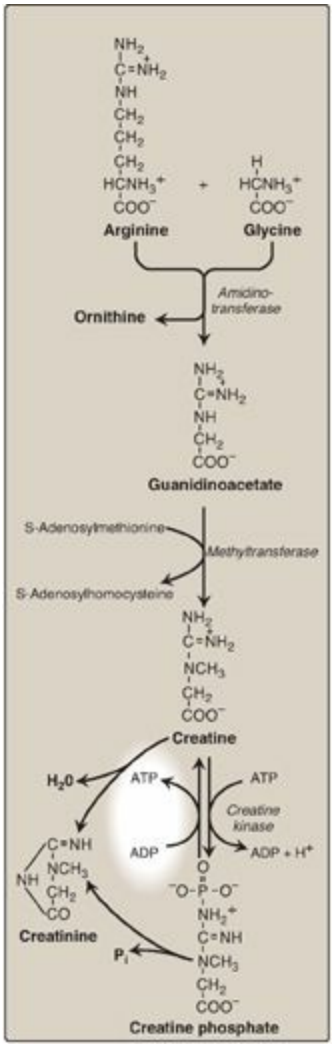
2. **Degradation:** Creatine and creatine phosphate spontaneously cyclize at a slow but constant rate to form creatinine, which is excreted in the urine. The amount excreted is proportional to the total creatine phosphate content of the body and, therefore, can be used to estimate muscle mass. When muscle mass decreases for any reason (for example, from paralysis or muscular dystrophy), the creatinine content of the urine falls. In addition, any rise in blood creatinine is a sensitive indicator of kidney malfunction, because creatinine normally is rapidly removed from the blood and excreted. A typical adult male excretes about 1–2 g of creatinine per day.

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. [Note: A defect in melanin production results in oculocutaneous albinism, the most common type being due to defects in copper-containing tyrosinase (see p. 273).]

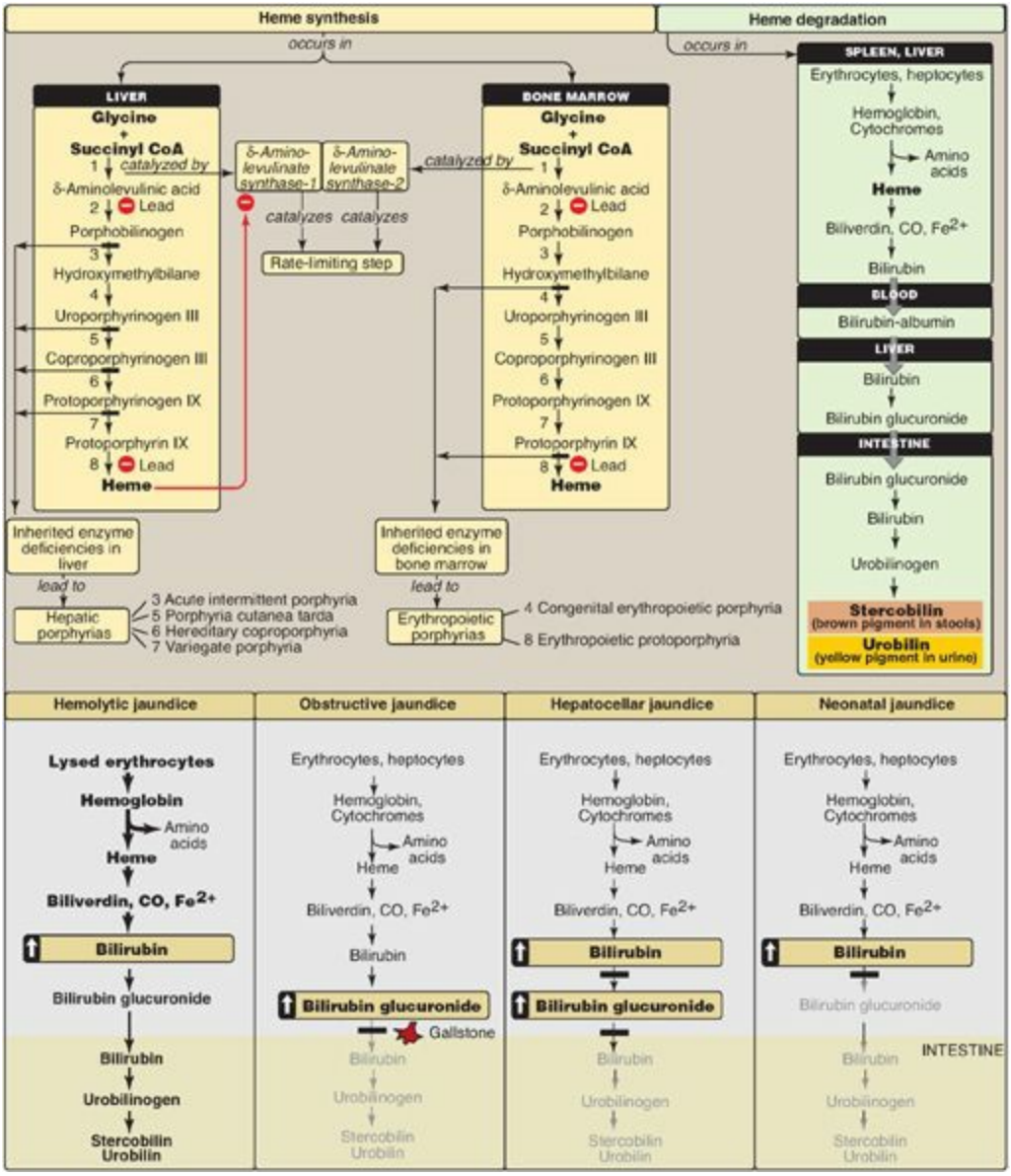
Figure 21.19 Synthesis of creatine. ADP = adenosine diphosphate; P_i = inorganic phosphate.



IV. CHAPTER SUMMARY

Amino acids are precursors of many nitrogen-containing compounds including **porphyrins**, which, in combination with ferrous (Fe^{2+}) iron, form **heme** (Figure 21.20). The major sites of **heme biosynthesis** are the **liver**, which synthesizes a number of heme proteins (particularly cytochrome P450 enzymes), and the **erythrocyte-producing cells** of the bone marrow, which are active in hemoglobin synthesis. In the liver, the rate of heme synthesis is highly variable, responding to alterations in the cellular heme pool caused by fluctuating demands for hemeproteins. In contrast, heme synthesis in erythroid cells is relatively constant and is matched to the rate of globin synthesis. **Porphyrin synthesis** start with **glycine** and **succinyl coenzyme A**. The **committed step** in heme synthesis is the formation of δ-aminolevulinic acid (ALA). This reaction is catalyzed by **ALA synthase-1** (**ALAS1**) in liver (inhibited by **hemin**, the oxidized form of heme that accumulates in the cell when heme is being underutilized) and **ALAS2** in erythroid tissues (regulated by **iron**) . **Porphyrias** are caused by inherited (primarily **autosomal-dominant**) or acquired defects in heme synthesis, resulting in the accumulation and increased excretion of porphyrins or porphyrin precursors. Enzymic defects early in the pathway cause **abdominal pain** and **neuropsychiatric symptoms**, whereas later defects cause **photosensitivity**. **Degradation** of hemeproteins occurs in the **reticuloendothelial system**, particularly in the **liver** and **spleen**. The first step in the degradation of heme is the production by **heme oxygenase** of the green pigment **biliverdin**, which is subsequently reduced to **bilirubin**. Bilirubin is transported to the liver, where its solubility is increased by the addition of two molecules of **glucuronic acid**. Bilirubin diglucuronide is transported into the **bile canaliculi**, where it is first hydrolyzed and reduced by bacteria in the gut to yield **urobilinogen**, then oxidized by intestinal bacteria to **stercobilin**. **Jaundice** 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 type of jaundice are **hemolytic jaundice**, **obstructive jaundice**, and **hepatocellular jaundice**. Other important N-containing compounds derived from amino acids include the **catecholamines** (dopamine, norepinephrine, and epinephrine), **creatine**, **histamine**, **serotonin**, and **melanin**.

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 into bile.] CoA = coenzyme A; CO = carbon monoxide.


Heme synthesis
Heme degradation
occurs in
occurs in
LIVER
Glycine
Succinyl CoA
1 catalyzed by
δ-Aminolevulinic acid
2 Lead
Porphobilinogen
3
Hydroxymethylbilane
4
Uroporphyrinogen III
5
Coproporphyrinogen III
6
Protoporphyrinogen IX
7
Protoporphyrin IX
8 Lead
Heme
δ-Amino-levulinate synthase-1
δ-Amino-levulinate synthase-2
catalyzed by
catalyzes
catalyzes
Rate-limiting step
BONE MARROW
Glycine
Succinyl CoA
1
δ-Aminolevulinic acid
2 Lead
Porphobilinogen
3
Hydroxymethylbilane
4
Uroporphyrinogen III
5
Coproporphyrinogen III
6
Protoporphyrinogen IX
7
Protoporphyrin IX
8 Lead
Heme
Inherited enzyme deficiencies in liver
lead to
Hepatic porphyrias
3 Acute intermittent porphyria
5 Porphyria cutanea tarda
6 Hereditary coproporphyria
7 Variegate porphyria
Inherited enzyme deficiencies in bone marrow
lead to
Erythropoietic porphyrias
4 Congenital erythropoietic porphyria
8 Erythropoietic protoporphyria
SPLEEN, LIVER
Erythrocytes, heplocytes
Hemoglobin, Cytochromes
Amino acids
Heme
Biliverdin, CO, Fe2+
Bilirubin
BLOOD
Bilirubin-albumin
LIVER
Bilirubin
Bilirubin glucuronide
INTESTINE
Bilirubin glucuronide
Bilirubin
Urobilinogen
Stercobilin
(brown pigment in stools)
Urobilin
(yellow pigment in urine)
Hemolytic jaundice
Lysed erythrocytes
Hemoglobin
Amino acids
Heme
Biliverdin, CO, Fe2+
Bilirubin
Bilirubin glucuronide
Bilirubin
Urobilinogen
Stercobilin
Urobilin
Obstructive jaundice
Erythrocytes, heptocytes
Hemoglobin, Cytochromes
Amino acids
Heme
Biliverdin, CO, Fe2+
Bilirubin
Bilirubin glucuronide
Gallstone
Bilirubin
Urobilinogen
Stercobilin
Urobilin
Hepatocellar jaundice
Erythrocytes, heptocytes
Hemoglobin, Cytochromes
Amino acids
Heme
Biliverdin, CO, Fe2+
Bilirubin
Bilirubin glucuronide
Bilirubin
Urobilinogen
Stercobilin
Urobilin
Neonatal jaundice
Erythrocytes, heptocytes
Hemoglobin, Cytochromes
Amino acids
Heme
Biliverdin, CO, Fe2+
Bilirubin
Bilirubin glucuronide
Bilirubin
Urobilinogen
Stercobilin
Urobilin
INTESTINE

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 liver in individuals treated with certain drugs such as the barbiturate phenobarbital.
D. occurs in the cytosol.
E. requires biotin as a coenzyme.

Correct answer = A. δ-Aminolevulinic acid synthase is cytosolic 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 man 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 about 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 about 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, <27mg). 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 decarboxylase, but clinical expression of the enzyme deficiency is influenced by hepatic injury caused by environmental (for example, ethanol) and infectious (for example, 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 enzyme. The laboratory and clinical findings are inconsistent with other porphyrias.

21.3 A patient presents with jaundice, abdominal pain, and nausea. Clinical laboratory studies give the following results:

Serum bilirubin	Urine urobilinogen	Urinary bilirubin
Increase in conjugated bilirubin	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 conjugated bilirubin (CB) into the intestine (stool will be pale in color). The liver "regurgitates" the CB into the blood (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. Bilirubiun UDP-glucuronosyltransferase
C. Ferrochelatase
D. Heme oxygenase
E. Porphobilinogen synthase

Correct answer = A. This child has the acquired porphyria of lead

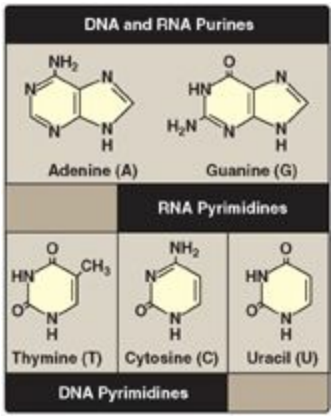
poisoning. Lead inhibits δ-aminolevulinic acid dehydratase and, consequently, heme synthesis. The decrease in heme derepresses δ-aminolevulinic acid synthase-1 (the hepatic isozyme), resulting in an increase in its activity. The decrease in heme also results in decreased hemoglobin synthesis, and anemia is seen. Ferrochelatase is directly inhibited by lead. The other choices are enzymes of heme degradation.

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 or cells proliferate. Nucleotides also serve as carriers of activated intermediates in the synthesis of some carbohydrates, lipids, and conjugated proteins (for example, 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 currency" 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: Little of the purines and pyrimidines supplied by diet are utilized and are degraded instead.]

Figure 22.1 Purines and pyrimidines commonly found in DNA and RNA.



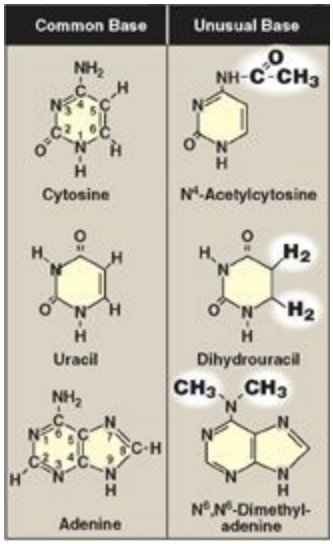
II. NUCLEOTIDE STRUCTURE

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.

A. Purine and pyrimidine structures

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 (Figure 22.1). Unusual (modified) bases are occasionally found in some species of DNA and RNA (for example, in some viral DNA) and 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

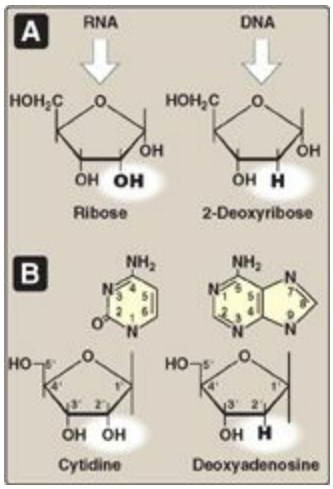
The addition of a pentose sugar to a base through a glycosidic bond produces a nucleoside. If the sugar is ribose, a ribonucleoside is produced, and if the sugar is 2-deoxyribose, a deoxyribonucleoside is produced (Figure 22.3A). The ribonucleosides of A, G, C, and U are named adenosine, guanosine, cytidine, and uridine, respectively. The deoxyribonucleosides of A, G, C, and T have the added prefix, "deoxy-" (for example, 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 (Figure 22.3B). Note that the 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 5I-OH of the pentose, forming a nucleoside 5I-phosphate or a 5I-nucleotide. The type of pentose is denoted by the prefix in the names "5I-ribonucleotide" and "5I-deoxyribonucleotide." If one phosphate group is attached to the 5I-carbon of the pentose, the structure is a nucleoside monophosphate, like adenosine monophosphate [AMP] also called adenylate). If a second or third phosphate is added to the nucleoside, a nucleoside diphosphate (for example, adenosine diphosphate [ADP] or triphosphate (for example, adenosine triphosphate [ATP]) results (Figure 22.4). The second and third phosphates are each connected to the nucleotide by a "high-energy" bond. [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 pyrimidinecontaining nucleosides.



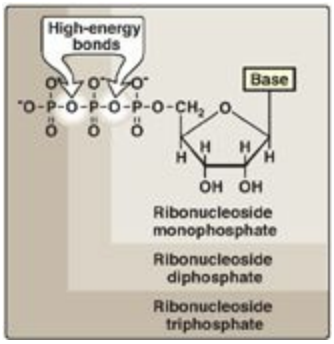
III. SYNTHESIS OF PURINE NUCLEOTIDES

The atoms of the purine ring are contributed by a number of compounds, including amino acids (aspartate, glycine, and glutamine), CO_2, and N^{10}-formyltetrahydrofolate (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. (See p. 147 for the synthesis of ribose 5-phosphate by the pentose phosphate pathway.)

A. Synthesis of 5-phosphoribosyl-1-pyrophosphate

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 (Figure 22.6). This X-linked enzyme is activated by inorganic phosphate and inhibited by purine nucleotides (end-product inhibition). [Note: The sugar moiety of PRPP is ribose, and, therefore, 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. 297).]

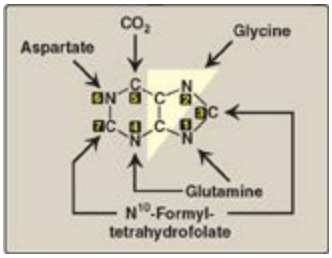
Figure 22.4 Ribonucleoside monophosphate, diphosphate, and triphosphate.



B. Synthesis of 5-phosphoribosylamine

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, glu-tamine:phosphoribosylpyrophosphate amidotransferase, is inhibited by the purine 5**I**-nucleotides AMP and guanosine monophosphate ([GMP] also called 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 amidotransferase. Therefore, any small change in the PRPP concentration causes a proportional change in rate of the reaction (see p. 59).]

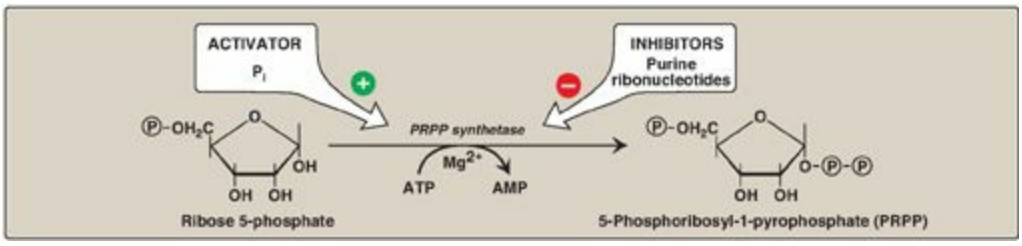
Figure 22.5 Sources of the individual atoms in the purine ring. The order in which the atoms are added is shown by the numbers in the black boxes (see Figure 22.7).



C. Synthesis of inosine monophosphate, the "parent" purine nucleotide

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. Four steps in this pathway require ATP as an energy source, and two steps in the pathway require N^{10}-formyltetrahydrofolate as a one-carbon donor (see p. 267). [Note: Hypoxanthine is found in tRNA (see p. 437).]

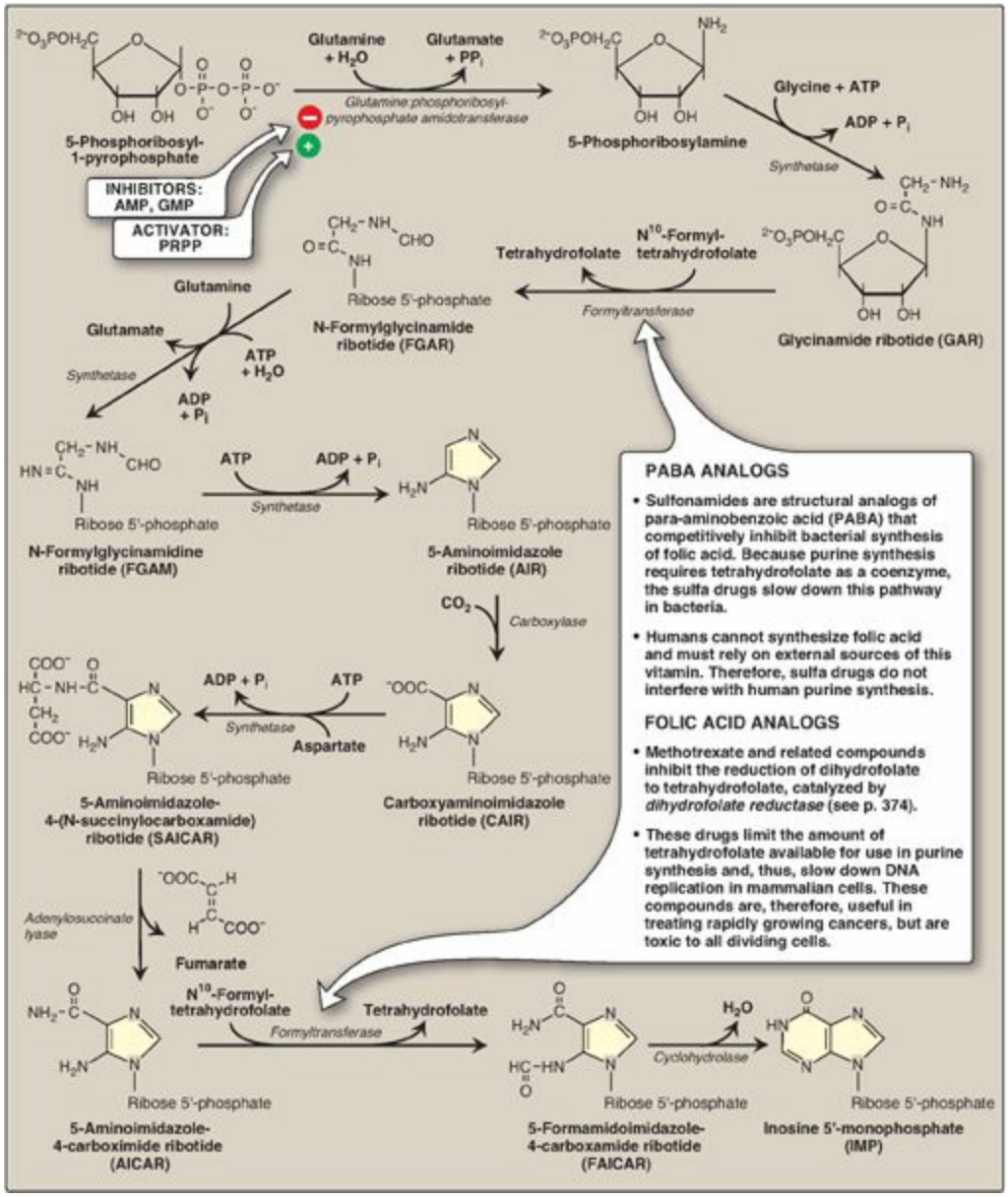
Figure 22.6 Synthesis of PRPP, showing the activator and inhibitors of the reaction. [Note: This is not the committed step of purine synthesis because PRPP is used in other pathways.] Ⓟ = phosphate; P_i = inorganic phosphate; AMP = adenosine monophosphate.



D. Synthetic inhibitors of purine synthesis

Some synthetic inhibitors of purine synthesis (for example, the sulfonamides), are designed to inhibit the growth of rapidly dividing microorganisms without interfering with human cell functions (see Figure 22.7). Other purine synthesis inhibitors, such as structural analogs of folic acid (for example, methotrexate), are used pharmacologically to control the spread of cancer by interfering with the synthesis of nucleotides and, therefore, of DNA and RNA (see Figure 22.7).

Figure 22.7 De novo synthesis of purine nucleotides, showing the inhibitory effect of some structural analogs. AMP = adenosine monophosphate; ADP = adenosine diphosphate; GMP = guanosine monophosphate; PRPP = 5-phosphoribosyl- 1-pyrophosphate; P_i = inorganic phosphate; PP_i = pyrophosphate.



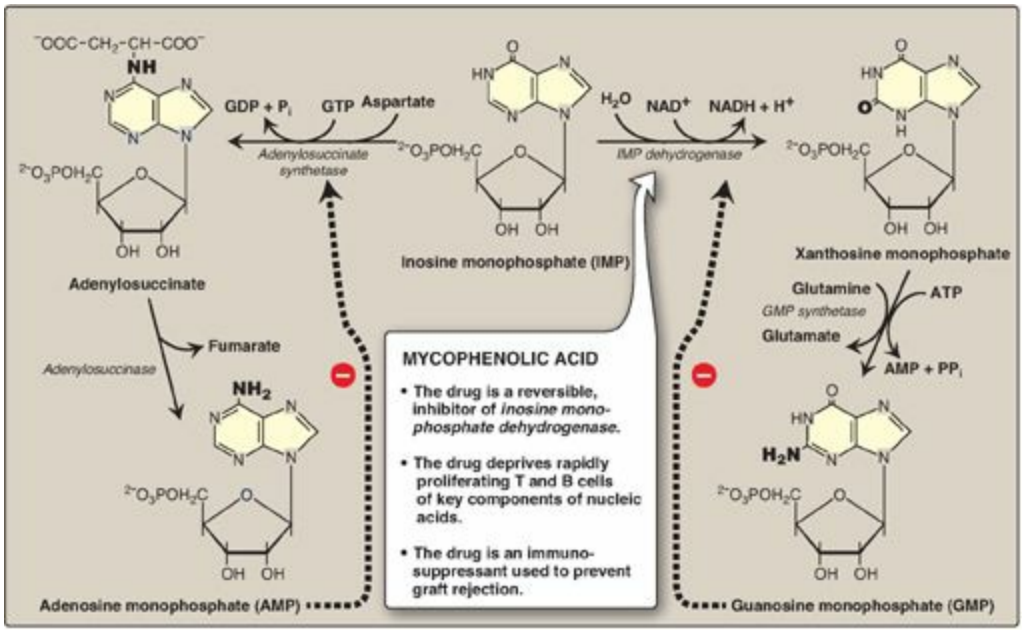
> Inhibitors of human purine synthesis are extremely toxic to tissues, especially to developing structures such as in a fetus, or to cell types that normally replicate rapidly, including those of bone marrow, skin, gastrointestinal (GI) tract, immune system, or hair follicles. As a result, individuals taking such anticancer drugs can experience adverse effects, including anemia, scaly skin, GI tract disturbance, immunodeficiencies, and hair loss.

E. Synthesis of adenosine and guanosine monophosphate

The conversion of IMP to either AMP or GMP uses a two-step, energy-requiring pathway (Figure 22.8). Note that the synthesis of AMP requires guanosine triphosphate (GTP) as an energy source, whereas the synthesis of GMP requires ATP. 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 synthesis is turned off at the amidotransferase step.

Figure 22.8 Conversion of IMP to AMP and GMP showing feedback inhibition. [Note: AMP is also called adenylate. GMP is also called guanylate.] NAD(H) = nicotinamide adenine dinucleotide; GDP = guanosine diphosphate; GTP = guanosine triphosphate; AMP = adenosine monophosphate; P_i = inorganic phosphate; PP_i = pyrophosphate.

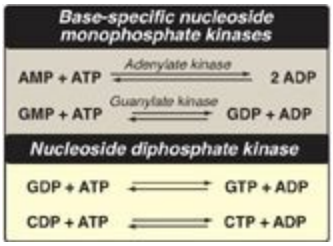


F. Conversion of nucleoside monophosphates to nucleoside diphosphates and triphosphates

Nucleoside diphosphates are synthesized from the corresponding nucleoside monophosphates by base-specific nucleoside monophosphate kinases (Figure 22.9). [Note: These kinases do not discriminate between ribose or 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 nucleoside diphosphates and triphosphates.
AMP = adenosine monophosphate;
ADP = adenosine diphosphate;
GMP = guanosine monophosphate;
GDP = guanosine diphosphate;
GTP = guanosine triphosphate;
CDP = cytidine diphosphate;

CTP = cytidine triphosphate.

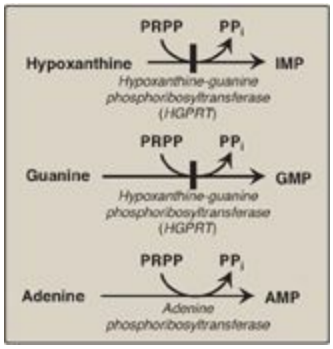


G. Salvage pathway for purines

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. **Salvage of purine bases to nucleotides:** Two enzymes are involved: adenine phosphoribosyltransferase (APRT) and hypoxanthine-guanine phosphoribosyltransferase (HGPRT). Both enzymes use PRPP as the source of the ribose 5-phosphate group (Figure 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 = inosine monophosphate;
GMP = guanosine monophosphate;
AMP = adenosine monophosphate;
PRPP = 5-phosphoribosyl-1- pyrophosphate;
PP_i = pyrophosphate.



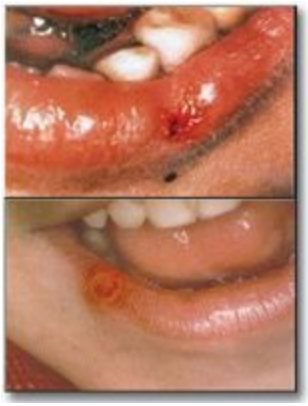
2. **Lesch-Nyhan syndrome:** Lesch-Nyhan is a rare, X-linked recessive disorder associated with a virtually complete deficiency of HGPRT. The deficiency results in an inability to salvage hypoxanthine or guanine, from which excessive amounts of

uric acid, the end product of purine degradation, are then produced (see p. 298). In addition, the lack of this salvage pathway causes increased PRPP levels and decreased IMP and GMP levels. As a result, glutamine:phosphoribosylpyrophosphate amidotransferase (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 Lesch-Nyhan a heritable 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 (for example, biting of lips and fingers) as shown in Figure 22.11).

IV. SYNTHESIS OF DEOXYRIBONUCLEOTIDES

The nucleotides described thus far all contain ribose (ribonucleotides). The nucleotides required for DNA synthesis, however, are 2**I**-deoxyribonucleotides, which are produced from ribonucleoside diphosphates by the enzyme ribonucleotide reductase during the S-phase of the cell cycle (see p. 407). [Note: The same enzyme acts on pyrimidine ribonucleotides.]

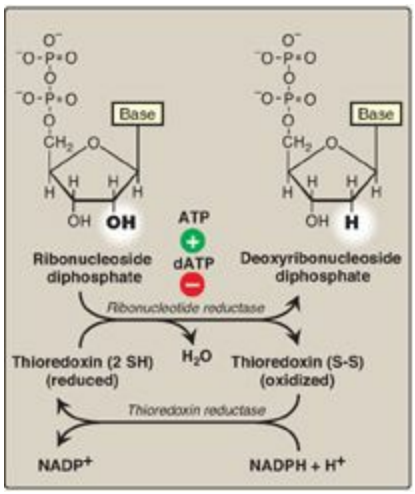
Figure 22.11 Lesions on the lips of Lesch-Nyhan patients caused by self-mutilation.



A. Ribonucleotide reductase

Ribonucleotide reductase (ribonucleoside diphosphate reductase) is composed of two nonidentical dimeric subunits, R1 and R2, 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**I**-hydroxyl group are two sulfhydryl groups on the enzyme itself, which, during the reaction, form a disulfide bond (Figure 22.12).

Figure 22.12 Conversion of ribonucleotides to deoxyribonucleotides. NADP(H) = nicotinamide adenine dinucleotide phosphate; dATP = deoxyadenosine triphosphate.



1. **Regeneration of reduced enzyme:** In order for ribonucleotide reductase to

continue to produce deoxyribonucleotides, the disulfide bond created during the production of the 2**I**-deoxy carbon must be reduced. The source of the reducing equivalents for this purpose is thioredoxin, a peptide coenzyme of ribonucleotide reductase. Thioredoxin contains two cysteine residues separated by two amino acids in the peptide chain. The two sulfhydryl groups of thioredoxin donate their hydrogen atoms to ribonucleotide reductase, forming a disulfide bond in the process (see p. 19).

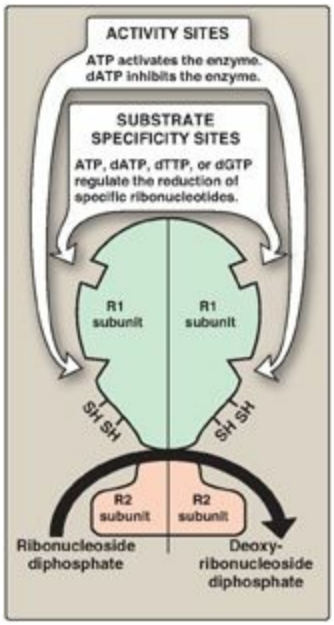
2. **Regeneration of reduced thioredoxin:** Thioredoxin must be converted back to its reduced form in order to continue to perform its function. The necessary reducing equivalents are provided by NADPH + H^+, and the reaction is catalyzed by thioredoxin reductase (see Figure 22.12).

B. Regulation of deoxyribonucleotide synthesis

Ribonucleotide reductase is responsible for maintaining a balanced supply of the deoxyribonucleotides required for DNA synthesis. To achieve this, the regulation of the enzyme is complex. In addition to the catalytic (active) site, there are allosteric sites on the enzyme involved in regulating its activity (Figure 22.13).

1. **Activity sites:** The binding of dATP to allosteric sites (known as the activity sites) on the enzyme 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 deficiency (see p. 301). In contrast, ATP bound to these sites activates the enzyme.

Figure 22.13 Regulation of ribonucleotide reductase. dATP = deoxyadenosine triphosphate; dTTP = deoxythymidine triphosphate; dGTP = deoxyguanosine triphosphate.



2. **Substrate specificity sites:** The binding of nucleoside triphosphates to additional allosteric sites (known as the substrate specificity sites) on the enzyme 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 sites causes a conformational change that allows reduction of GDP to dGDP at the catalytic site.

The drug hydroxyurea (hydroxycarbamide) inhibits ribonucleotide reductase, thereby inhibiting the generation of substrates for DNA synthesis. Hydroxyurea 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 disease (see p. 36). However, the increase in fetal hemoglobin seen with hydroxyurea is not due to its effect on ribonucleotide reductase.

V. DEGRADATION OF PURINE NUCLEOTIDES

Degradation of dietary nucleic acids occurs in the small intestine, where a family of pancreatic enzymes hydrolyzes the nucleic acids to nucleotides. Inside the intestinal mucosal cells, purine nucleotides are sequentially degraded by specific enzymes to nucleosides and free bases, with uric acid as the end product of this pathway. [Note: Purine nucleotides from de novo synthesis are degraded in the liver primarily. The free bases are sent out from liver and salvaged by peripheral tissues.]

A. Degradation of dietary nucleic acids in the small intestine

Ribonucleases and deoxyribonucleases, secreted by the pancreas, hydrolyze dietary RNA and DNA to oligonucleotides. Oligonucleotides are further hydrolyzed by pancreatic phosphodiesterases, producing a mixture of 3′- and 5′-mononucleotides. In the intestinal mucosal cells, a family of nucleotidases removes the phosphate groups hydrolytically, releasing nucleosides that are further 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 generally converted to uric acid in intestinal mucosal cells. 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 Formation of uric acid

A summary of the steps in the production of uric acid and 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 deaminase or from adenosine to produce inosine (hypoxanthine-ribose) by adenosine deaminase.

[2] IMP and GMP are converted into their 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 guanine. [Note: A mutase interconverts ribose 1- and ribose 5-phosphate.]

[4] Guanine is deaminated to form xanthine.

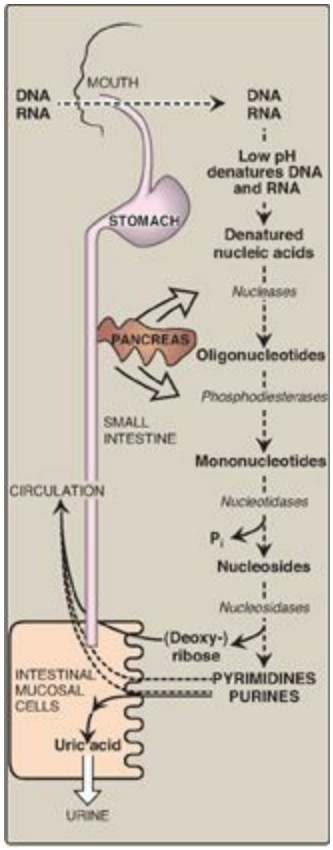
[5] Hypoxanthine is oxidized by xanthine oxidase to xanthine, which is further oxidized by xanthine oxidase to uric acid, the final product of human purine degradation. Uric acid is excreted primarily in the urine.

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 (Figure 22.16). Formation of uric acid stones in the kidney (urolithiasis) may also be seen. [Note: Hyperuricemia, while necessary, 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 (Figure 22.17) from an affected joint (or material from a tophus) using polarized light microscopy to confirm the presence of needle-shaped MSU crystals (Figure 22.18).

    a. **Underexcretion of uric acid:** In over 90% of individuals, hyperuricemia is caused by underexcretion of uric acid. Underexcretion can be primary, due to as-yet-unidentified inherent excretory defects, or secondary to known disease processes that affect how the kidney handles urate (for example, in lactic acidosis, lactate increases renal urate reabsorption, thereby decreasing its excretion) and to environmental factors such as the use of drugs (for example, thiazide diuretics) or exposure to lead (saturnine gout).

Figure 22.14 Digestion of dietary nucleic acids. [Note: Much of the metabolism of the mononucleotides occurs within the intestinal mucosal cells.] P_i = inorganic phosphate.



b. Overproduction of uric acid: 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. 58) for the production of PRPP, a lower K_m (see p. 59) for ribose 5-phosphate, or a decreased sensitivity to purine nucleotides, its allosteric inhibitors (see p. 62). In each case, increased availability of PRPP increases purine production, resulting in elevated levels of plasma uric acid. Lesch-Nyhan syndrome (see p. 296) also causes hyperuricemia as a result of the decreased salvage of hypoxanthine and guanine and the subsequent increased availability of PRPP. Secondary hyperuricemia is typically the consequence of increased availability of purines (for example, 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 Figure 11.8 on p. 130) or hereditary fructose intolerance (see p. 138).

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.

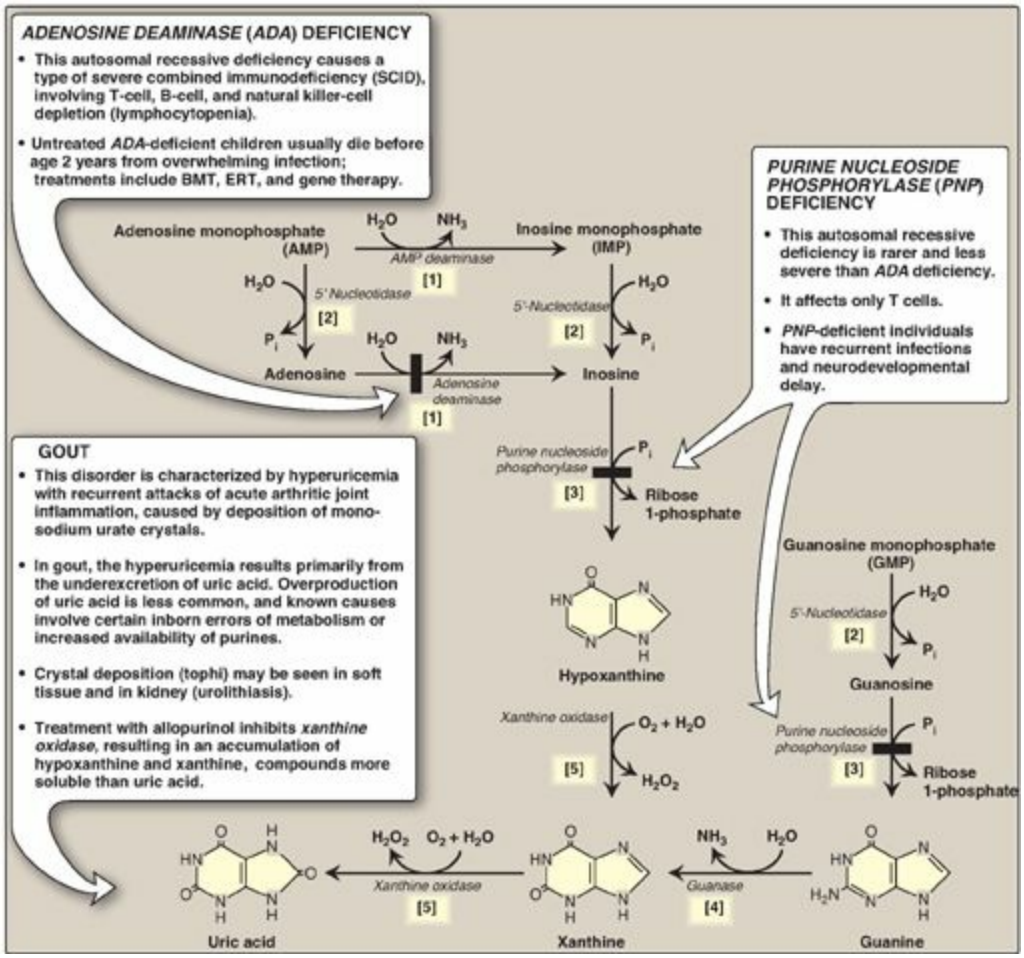
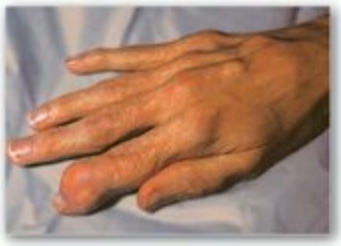


Figure 22.16 Tophaceous gout.

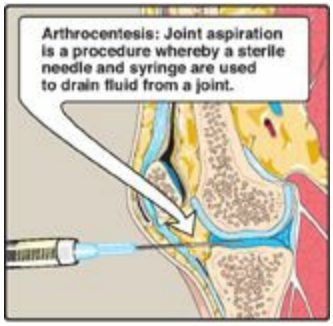


A diet rich in meat, seafood (particularly shellfish), and ethanol is associated with increased risk of gout, whereas a diet rich in low-fat dairy products is associated with a decreased risk.

c. Treatment of gout: 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 urate crystals. Uricosuric agents, such as probenecid or sulfinpyrazone, that increase

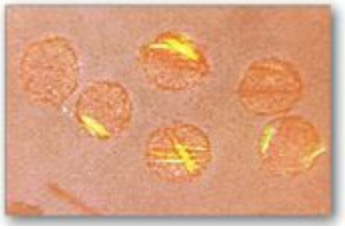
renal excretion of uric acid, are used in patients who are "underexcretors" of uric acid. Allopurinol, a structural analog of hypoxanthine, inhibits uric acid synthesis and is used in patients who are "overproducers" of uric acid. Allopurinol is converted in the body to oxypurinol, which inhibits xanthine oxidase (XO), resulting in an accumulation of hypoxanthine and xanthine (see Figure 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 non-purine 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 or arthritis, such as infection, gout, and rheumatoid disease.



2. **Adenosine deaminase deficiency:** Adenosine deaminase (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. 297). 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. It is estimated that, in the United States, ADA deficiency accounts for approximately 14% of all cases of SCID. Treatments include bone marrow transplantation, enzyme replacement therapy, and gene therapy. 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.]

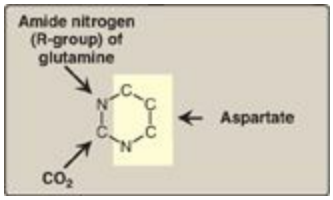
Figure 22.18 Gout can be diagnosed by the presence of negatively birefringent monosodium urate crystals in aspirated synovial fluid examined by polarized-light microscopy. Here, crystals are within polymorphonuclear leukocytes.



VI. PYRIMIDINE SYNTHESIS AND DEGRADATION

Unlike the synthesis of the purine ring, which is constructed on a preexisting 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 (Figure 22.19).

Figure 22.19 Sources of the individual atoms in the pyrimidine ring.



A. Synthesis of carbamoyl phosphate

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 (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. 253). Defects in ornithine transcarbamylase of the urea cycle promote pyrimidine synthesis due to increased availability of carbamoyl phosphate. A comparison of the two enzymes is presented in Figure 22.20.]

Figure 22.20 Summary of the differences between carbamoyl phosphate synthetase (CPS) I and II. PRPP = 5-phosphoribosyl-1-pyrophosphate; UTP = uridine triphosphate.

	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

B. Synthesis of orotic acid

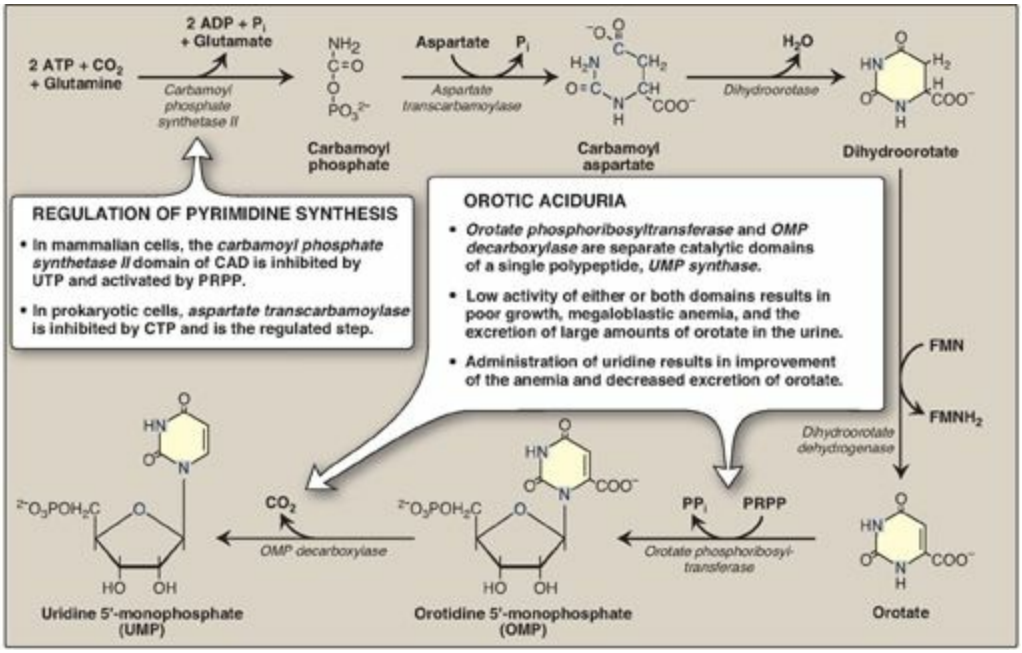
The second step in pyrimidine synthesis is the formation of carbamoylaspartate, catalyzed by aspartate transcarbamoylase. The pyrimidine ring is then closed by dihydroorotase. The resulting dihydroorotate is oxidized to produce orotic acid (orotate) as shown in Figure 22.21. The enzyme that produces orotate, dihydroorotate dehydrogenase, is a flavoprotein associated with the inner mitochondrial membrane. All other enzymes in pyrimidine biosynthesis are cytosolic. [Note: The first three

enzymic activities in this pathway (CPS II, aspartate transcarbamoylase, and dihydroorotase) are actually three different catalytic domains of a single polypeptide known as CAD from the first letter in the name of each domain. (See p. 19 for a discussion of domains.) This is an example of a multfunctional or multicatalytic polypeptide that facilitates the ordered synthesis of an important compound. Synthesis of the purine nucleotide IMP also involves multifunctional proteins.]

C. Formation of a pyrimidine nucleotide

The completed pyrimidine ring is converted to the nucleotide orotidine monophosphate (OMP) in the second stage of pyrimidine nucleotide synthesis (see Figure 22.21). PRPP is again 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, the parent pyrimidine mononucleotide, is converted to uridine monophosphate (UMP) by orotidylate decarboxylase, which removes the carboxyl group. Orotate phosphoribosyltransferase and orotidylate decarboxylase are also catalytic domains of a single polypeptide chain called UMP synthase. 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 Figure 22.21). 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). dUTPase, then, plays an important role in reducing availability of dUTP as a substrate for DNA synthesis, thereby preventing erroneous incorporation of uracil into DNA.]

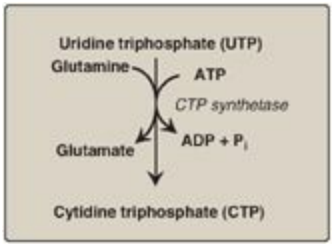
Figure 22.21 De novo pyrimidine synthesis. ADP = adenosine diphosphate; P_i = inorganic phosphate; FMN(H_2) = flavin mononucleotide; CTP = cytidine triphosphate; PRPP = 5-phosphoribosyl-1-pyrophosphate; PP_i = pyrophosphate.



D. Synthesis of cytidine triphosphate

Cytidine triphosphate (CTP) is produced by amination of UTP by CTP synthetase (Figure 22.22), with glutamine providing the nitrogen. [Note: Some CTP is dephosphorylated to cytidine diphosphate (CDP), which is a substrate for ribonucleotide reductase. The dCDP product can be phosphorylated to dCTP for DNA synthesis or dephosphoprylated 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.

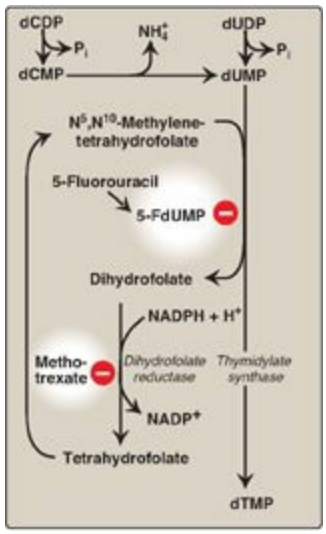


E. Synthesis of deoxythymidine monophosphate

dUMP is converted to deoxythymidine monophosphate (dTMP) by thymidylate synthase, which uses N^5,N^{10}-methylene tetrahydrofolate as the source of the methyl group (see p. 267 for a discussion of this coenzyme). This is an unusual reaction in that tetrahydrofolate (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] Figure 22.23). Inhibitors of thymidylate synthase include thymine 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. 60). DHF can be reduced to THF by dihydrofolate reductase (see Figure 28.3, p. 374), 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 Figure 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 decrease the growth rate of cancer cells. [Note: Acyclovir (a purine analog) and AZT (3**I**-azido-3**I**-deoxythymidine, a pyrimidine analog) are used to treat infections of herpes simplex virus and human immunodeficiency virus, respectively. Each inhibits the viral DNA polymerase.]

Figure 22.23 Synthesis of dTMP from dUMP, illustrating sites of action of antineoplastic drugs.



F. Degradation and salvage of pyrimidines

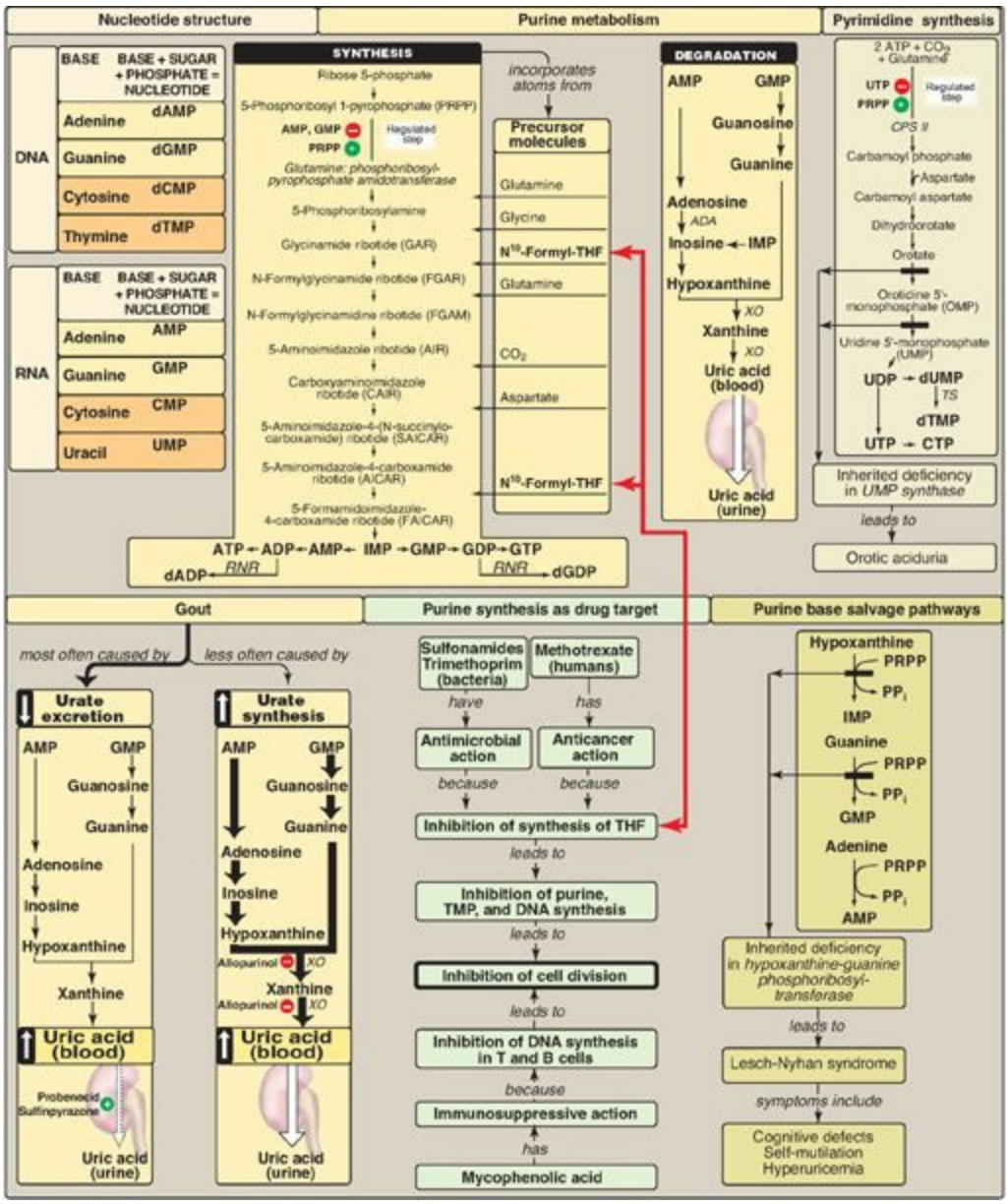
Unlike the purine ring, which is not cleaved in humans, the pyrimidine ring is opened and degraded to highly soluble products, β-alanine (from the degradation of CMP and UMP) and β-aminoisobutyrate (from TMP degradation), with the production of NH_3 and CO_2. Pyrimidine bases can be salvaged to nucleosides, 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.]

VII. CHAPTER SUMMARY

Nucleotides are composed of a **nitrogenous base** (**adenine** = **A**, **guanine** = **G**, **cytosine** = **C**, **uracil** = **U**, and **thymine** = **T**); a **pentose**; and one, two, or three **phosphate groups** (Figure 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** (for example, adenosine monophosphate [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** (for example, deoxyAMP), and will be found almost exclusively as a component of **DNA**. The **committed step** in **purine synthesis** uses **5-phosphoribosyl-1-pyrophosphate** ([**PRPP**], an "activated pentose" that provides the ribose-phosphate group for de novo purine and pyrimidine synthesis and salvage) and nitrogen from **glutamine** to produce phosphoribosyl amine. The enzyme is **glutamine:PRPP amidotransferase** and is **inhibited** by **AMP** and **guanosine monophosphate** ([**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 **adenine phosphoribosyltransferase (APRT)** and **hypoxanthine-guanine phosphoribosyltransferase** (**HGPRT**). A near-total deficiency of HGPRT causes **Lesch-Nyhan syndrome**, a severe, heritable form of hyperuricemia accompanied by compulsive self-mutilation. All deoxyribonucleotides are synthesized from ribonucleotides by the enzyme **ribonucleotide reductase**. This enzyme is highly regulated (for example, it is **strongly inhibited by deoxyadenosine triphosphate [dATP]**, a compound that is overproduced in bone marrow cells in individuals with **adenosine deaminase deficiency)**. This syndrome causes **severe combined immunodeficiency disease**. The end product of purine degradation is **uric acid**, a compound whose overproduction or undersecretion causes **hyperuricemia** that, if accompanied by the deposition of monosodium urate 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 **carbamoyl phosphate synthetase II**, is the **regulated** step in this pathway (it is **inhibited by uridine triphosphate [UTP]** and **activated by PRPP**). The UTP produced by this pathway can be converted to cytidine triphosphate. Deoxyuridine monophosphate can be converted to **deoxythymidine monophosphate** using **thymidylate synthase**, an enzyme targeted by anticancer drugs such as **5-fluorouracil**. The regeneration of tetrahydrofolate from dihydrofolate produced in the thymidylate synthase reaction requires **dihydrofolate reductase**, an enzyme targeted by the drug, **methotrexate**. Pyrimidine degradation results in soluble products.

Figure 22.24 Key concept map for nucleotide metabolism. THF = tetrahydrofolate; ADA= adenosine deaminase; XO = xanthine oxidase; TS = thymidylate synthase; RNR =

ribonucleotide reductase; CPS II = carbamyl phosphate synthetase II; AMP = adenosine monophosphate; GMP = guanosine monophosphate; CMP = cytidine monophosphate; TMP = thymidine monophosphate; IMP = inosine monophosphate; d = deoxy; PP_i = pyrophosphate.

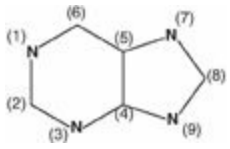


Study Questions

Choose the ONE correct 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 nitrogen (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 patient 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 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 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?

Blood ammonia level would be expected to be elevated in ornithine transcarbamylase deficiency but not in uridine monophosphate synthase deficiency.

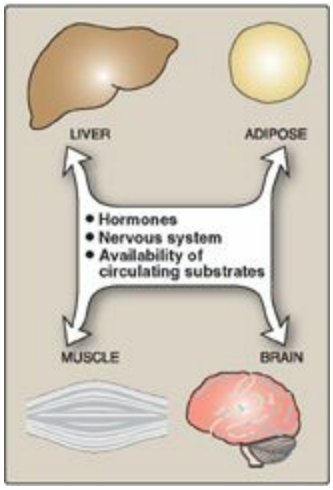
Metabolic Effects of Insulin and Glucagon

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 organs. Communication between tissues is mediated by the nervous system, by the availability of circulating substrates, and by variation in the levels of plasma hormones (Figure 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 "survival crises" (for example, 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.



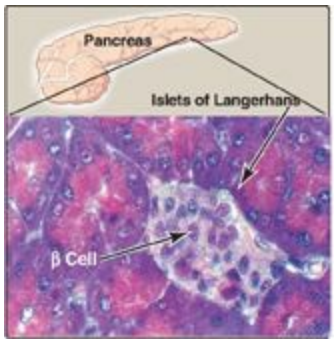
II. INSULIN

Insulin is a peptide hormone produced by the β cells of the islets of Langerhans, which are clusters of cells that are embedded in the endocrine portion of the pancreas (Figure 23.2). [Note: "Insulin" is from the Latin for island.] The islets make up only about 1%–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, triacylglycerols (TAGs), and protein.

A. Structure of insulin

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 bridges (Figure 23.3A). The insulin molecule also contains an intramolecular disulfide bridge between amino acid 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. 470).]

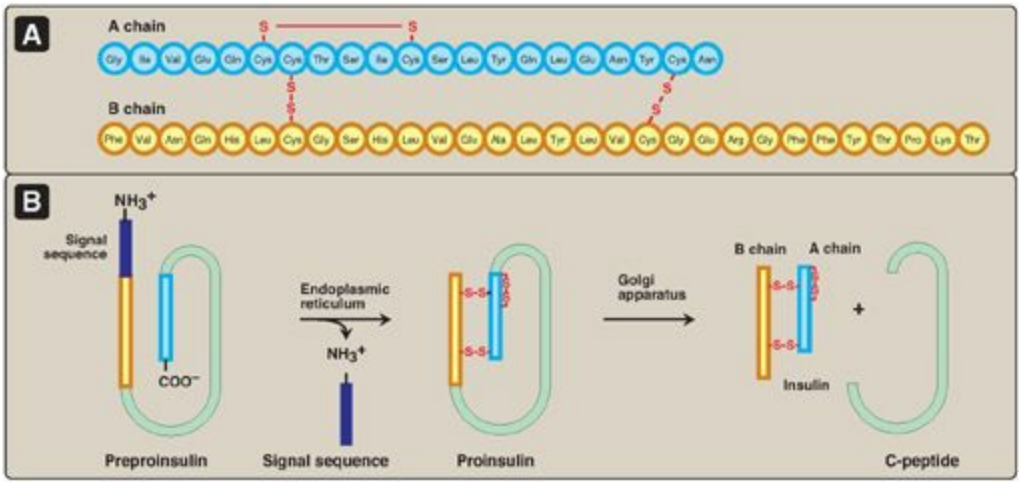
Figure 23.2 Islets of Langerhans.



B. Synthesis of insulin

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, that are sequentially cleaved to form the active hormone plus the connecting or C-peptide (see Figure 23.4). [Note: The C-peptide is essential for proper insulin folding. Also, because its half-life in the plasma is longer than that of insulin, the C-peptide is a good indicator of insulin production and secretion.] Insulin is stored in the cytosol in granules that, given the proper stimulus (see below), are released by exocytosis. (See p. 166 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 approximately 6 minutes. This short duration of action permits rapid changes in circulating levels of the hormone.

Figure 23.3 A. Structure of insulin. B. Formation of human insulin from preproinsulin.

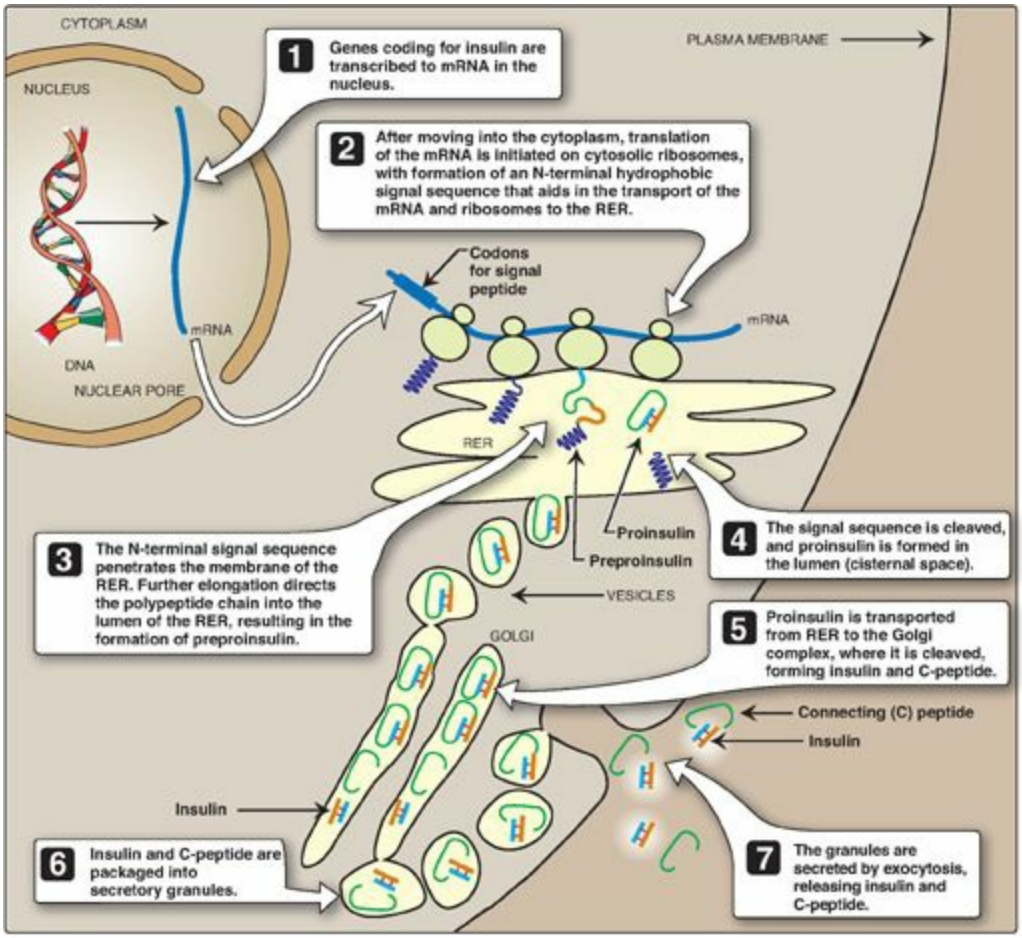


C. Regulation of insulin secretion

Secretion of insulin is regulated by bloodborne fuels and hormones.

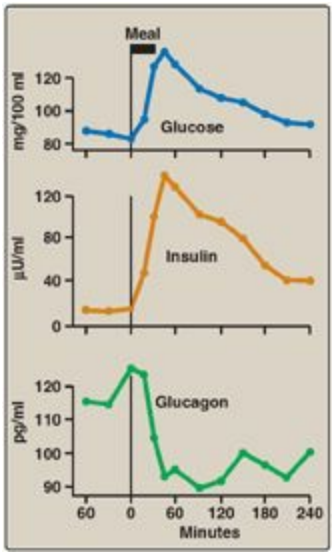
1. **Stimulation of insulin secretion:** Insulin secretion by the pancreatic β cells is closely coordinated with the release of glucagon by pancreatic α cells (Figure 23.5). The relative amounts of insulin and glucagon released by the pancreas are regulated so that the rate of hepatic glucose production is kept equal to the use of glucose by peripheral tissues. 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 ntracellular 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 (see Figure 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. 98). At blood glucose levels above 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 (see p. 98) maximizes the enzyme's responsiveness to changes in blood glucose level. Metabolism of glucose 6-phosphate generates adenosine triphosphate (ATP), leading to insulin secretion (see blue box below).

Figure 23.5 Changes in blood levels of glucose, insulin, and glucagon after ingestion of a carbohydrate-rich meal.

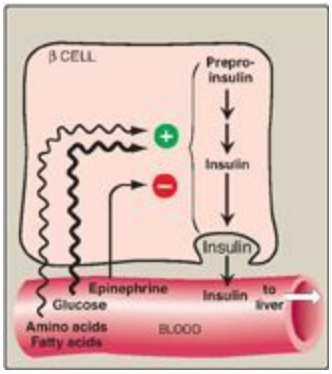


b. Amino acids: Ingestion of protein causes a transient rise in plasma amino acid levels (for example, arginine) that enhances the glucose-stimulated secretion of insulin. [Note: Fatty acids have a similar effect.]

c. Gastrointestinal peptide hormones: The intestinal peptides glucagon-like protein-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).

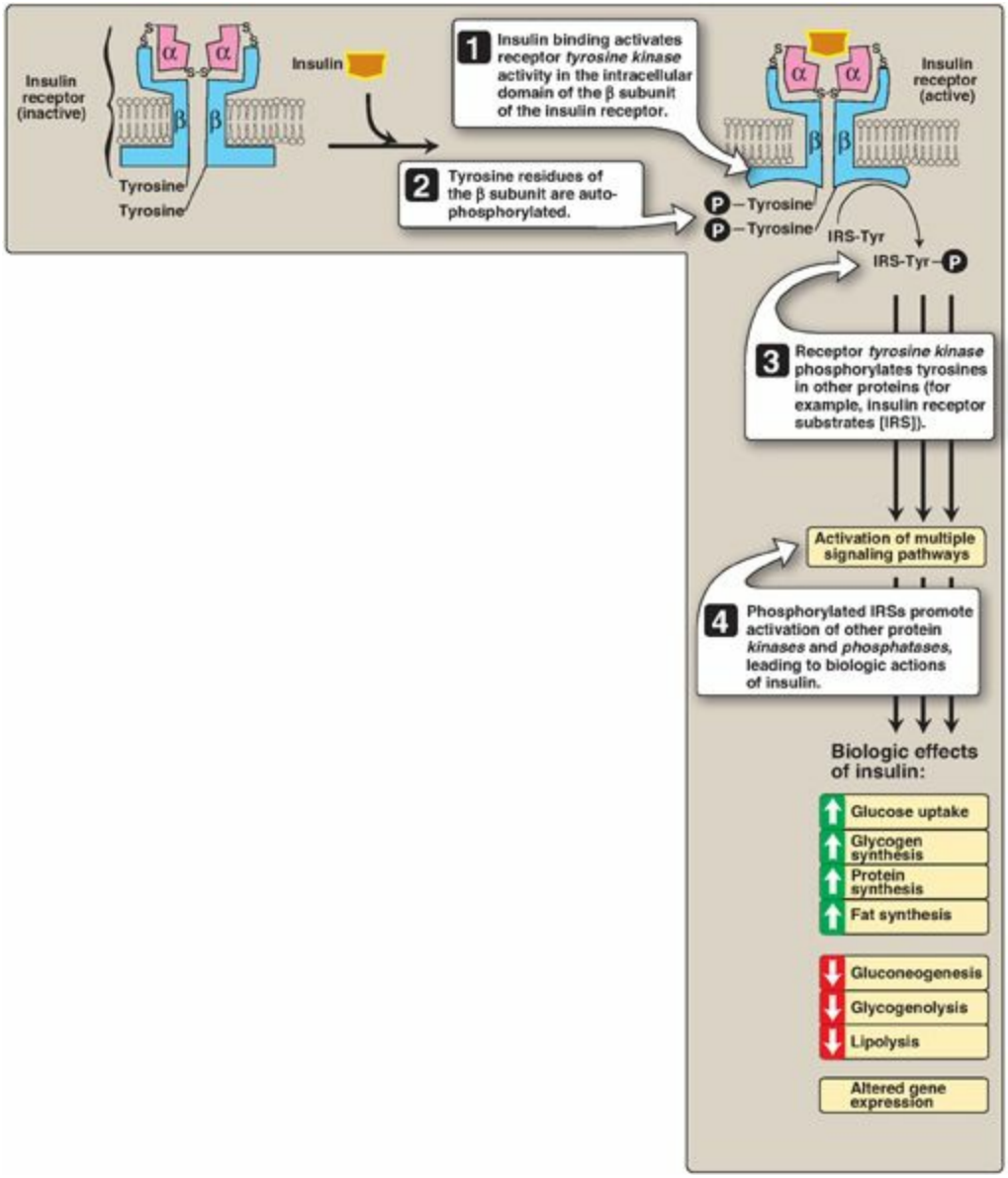
Glucose-dependent release of insulin into blood is mediated through a rise in calcium (Ca^{2+}) concentration in the β cell. Glucose taken into β cells is phosphorylated and metabolized, with subsequent production of ATP. ATP-sensitive potassium (K^+) channels close, causing depolarization of the plasma membrane, opening of voltage-gated Ca^{2+} channels, and influx of Ca^{2+} into the cell. Ca^{2+} causes vesicles containing insulin to be exocytosed from the β cell. Sulfonylureas, oral agents used to treat type 2 diabetes, increase insulin secretion by closing ATP-sensitive K^+ channels.

Figure 23.6 Regulation of insulin release from pancreatic β cells. [Note: Gastrointestinal peptide hormones also stimulate insulin release.]



2. **Inhibition of insulin secretion:** The synthesis and release of insulin are decreased when there is a scarcity of dietary fuels and also during periods of physiologic stress (for example, infection, hypoxia, and vigorous exercise). These effects are mediated primarily by the catecholamines norepinephrine and epinephrine, which are made from tyrosine in the sympathetic nervous system and the adrenal medulla and secreted. Secretion is largely controlled by the nervous system. 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. 121) and fatty acids from adipose tissue (produced by lipolysis; see p. 189). In addition, these biogenic amines can override the normal glucose-stimulated release of insulin. Thus, in emergency situations, the sympathetic nervous system 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 23.7 Insulin receptor. P = phosphate; Tyr = tyrosine.



D. Metabolic effects of insulin

Insulin promotes the storage of nutrients as 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 the liver and muscle, insulin increases glycogen synthesis. In the muscle and adipose, insulin increases glucose uptake by increasing the number of glucose transporters (GLUT-4; see p. 97) in the cell membrane. The IV administration of insulin, thus, 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 affects gene transcription.]

2. **Effects on lipid metabolism:** Adipose tissue responds rapidly to a rise in insulin, which causes a significant reduction in the release of fatty acids by inhibiting the activity of hormone-sensitive lipase, which degrades lipids in adipose tissue. Insulin

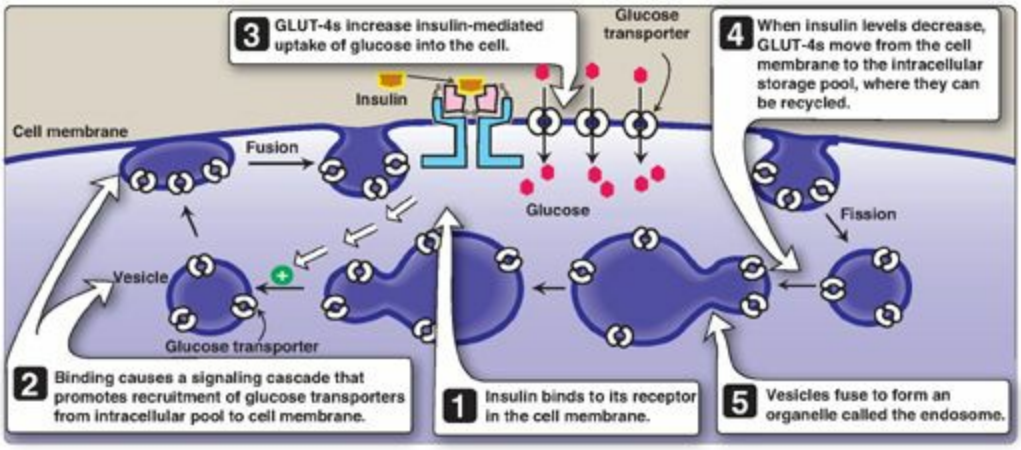
acts by promoting the dephosphorylation and, hence, inactivation of the enzyme (see p. 190). Insulin also increases the transport and metabolism of glucose into adipocytes, providing the glycerol 3-phosphate substrate for TAG synthesis. Expression of the gene for lipoprotein lipase (see p. 229) is increased by insulin in adipose tissue, thereby providing fatty acids for esterification to the glycerol. [Note: Insulin also promotes the conversion of glucose to TAG in the liver. The TAGs are secreted in very-low-density lipoproteins (VLDLs).]

3. **Effects on protein synthesis:** In most tissues, insulin stimulates the entry of amino acids into cells and protein synthesis. [Note: Insulin stimulates protein synthesis through activation of factors required for translation initiation.]

E. Mechanism of insulin action

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 (Figure 23.7).

Figure 23.8 Insulin-mediated recruitment of glucose transporters (GLUT-4s) from intracellular stores in skeletal and cardiac muscle and adipose tissue.

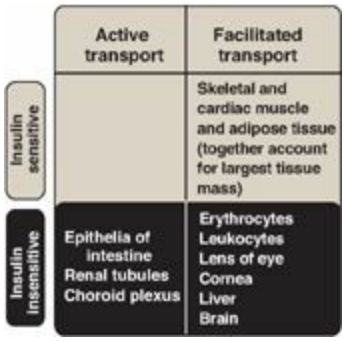


1. **Insulin receptor:** The insulin receptor is synthesized as a single polypeptide that is glycosylated and cleaved into α and β subunits, which are then assembled into a tetramer linked by disulfide bonds (see Figure 23.7). The extracellular α subunit contains the insulin-binding site. A hydrophobic 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 tyrosine-kinase receptor.

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 (see Figure 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, activating a number of pathways that affect gene expression, cell metabolism, and growth. The actions of insulin are terminated by dephosphorylation of the receptor.

Figure 23.9 Characteristics of glucose transport in various tissues.



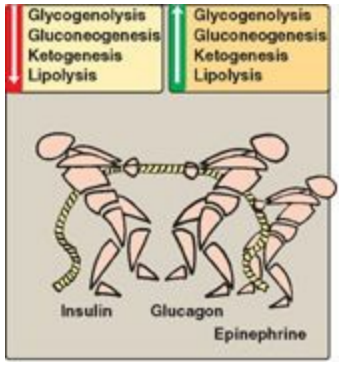
3. **Membrane effects of insulin:** Glucose transport in some tissues, such as muscle and adipose, increases in the presence of insulin (Figure 23.8). Insulin promotes movement of insulin-sensitive glucose transporters (GLUT-4s) from a pool located in intracellular vesicles to the cell membrane. [Note: 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 to the 3,4,5-trisphosphate form that binds to and activates phosphoinositide kinase 1. This kinase, in turn, activates Akt (protein kinase B), resulting in GLUT-4 movement.] In contrast, other tissues have insulin-insensitive systems for glucose transport (Figure 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, therby decreasing the number of surface receptors. This is one type of "downregulation."]

5. **Time course of insulin actions:** 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-1; see p. 184) and translation.

Figure 23.10 Opposing actions of insulin and glucagon plus epinephrine.



III. GLUCAGON

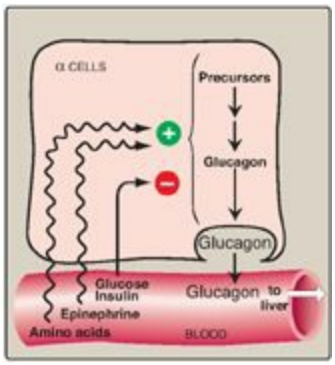
Glucagon is a peptide hormone secreted by the α cells of the pancreatic islets of Langerhans. Glucagon, along with epinephrine, norepinephrine, cortisol, and growth hormone (the "counterregulatory" hormones), opposes many of the actions of insulin (Figure 23.10). Most importantly, glucagon acts to maintain 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 Figure 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.

A. Stimulation of glucagon secretion

The α cell is responsive to a variety of stimuli that signal actual or potential hypoglycemia (Figure 23.11). Specifically, glucagon secretion is increased by low blood glucose, amino acids, and catecholamines.

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 below for a discussion of hypoglycemia).

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 only.]



2. **Amino acids:** Amino acids (for example, 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 produced by the adrenal medulla, norepinephrine produced by 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. Inhibition of glucagon 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 (see Figure 23.5). The regulation of glucagon secretion is summarized in Figure 23.11.

C. Metabolic effects of glucagon

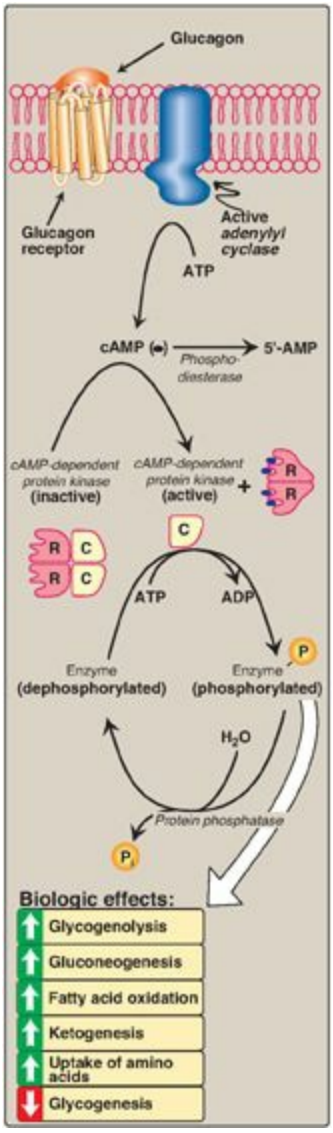
Glucagon is a catabolic hormone that promotes the maintenance of blood glucose levels. Its primary target is the liver.

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 breakdown of liver glycogen and an increase in hepatic gluconeogenesis.

2. **Effects on lipid metabolism:** The primary effect of glucagon on lipid metabolism is inhibition of fatty acid synthesis through phosphorylation of ACC (see p. 184). The decrease in malonyl CoA production as a result of ACC inhibition removes the break on long-chain fatty acid β-oxidation. Glucagon also plays a role in lipolysis in adipose, but the major activators of hormone sensitive lipase (via phosphorylation by protein kinase A) are the catecholamines. The free fatty acids released are taken up by liver and oxidized to acetyl CoA, which is used in ketone body synthesis.

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.

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; Ⓟ = phosphate.



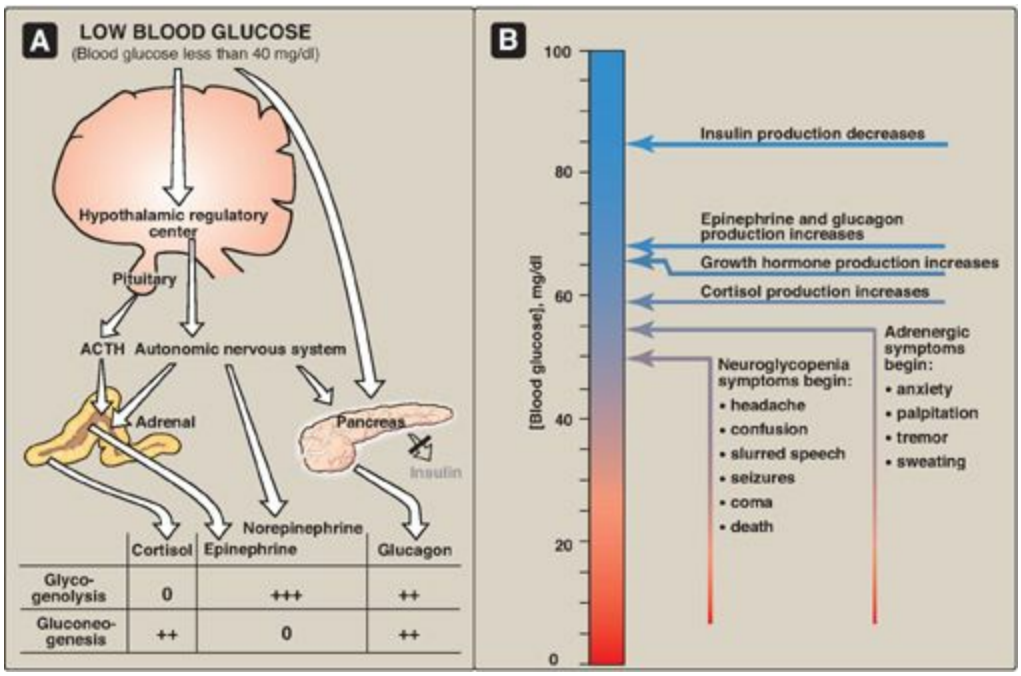
D. Mechanism of action of glucagon

Glucagon binds to high-affinity G protein–coupled receptors on the cell membrane of hepatocytes. The receptors for glucagon are distinct from those that bind insulin or epinephrine. [Note: Glucagon receptors are not found on skeletal muscle.] Glucagon binding results in activation of adenylyl cyclase in the plasma membrane (Figure 23.12; also see p. 94). This causes a rise in cyclic adenosine monophosphate (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. 130. [Note: Glucagon, like insulin, affects gene transcription. For example, glucagon induces expression of phosphoenolpyruvate carboxykinase (see p. 122).]

IV. HYPOGLYCEMIA

Hypoglycemia is characterized by 1) central nervous system (CNS) symptoms, including confusion, aberrant behavior, or coma; 2) a simultaneous blood glucose level equal to or less than 40 mg/dl; and 3) symptoms being resolved within minutes following the administration of glucose (Figure 23.13). Hypoglycemia is a medical emergency because the CNS has an absolute requirement for a continuous supply of bloodborne glucose to serve as fuel for energy metabolism. Transient hypoglycemia can cause cerebral dysfunction, whereas severe, prolonged hypoglycemia causes brain death. 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 elevated glucagon and the catecholamines, combined with the diminished release of insulin.

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 70-99 mg/100 ml.] + = weak stimulation; ++ = moderate stimulation; +++ = strong stimulation; 0 = no effect; ACTH = adrenocorticotropic hormone.



A. Symptoms of hypoglycemia

The symptoms of hypoglycemia can be divided into two categories. Adrenergic 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. Neuroglycopenia (that is, the impaired delivery of glucose to the brain) 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 below 40 mg/dl. The slow decline in glucose deprives the CNS of fuel, but fails to trigger an adequate adrenergic response.

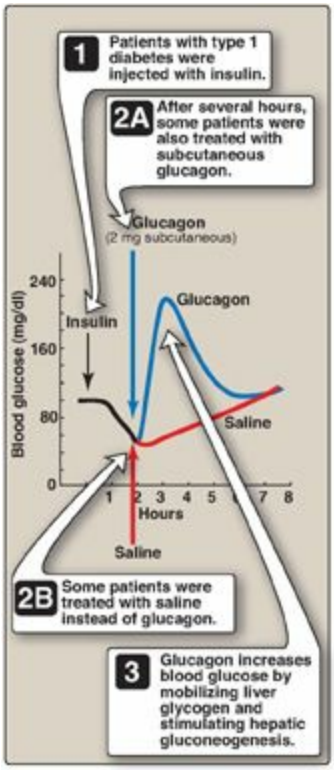
B. Glucoregulatory systems

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 autonomic nervous system) and release of adrenocorticotropic hormone (ACTH) and growth hormone by the anterior pituitary (see Figure 23.13). [Note: ACTH increases cortisol synthesis and secretion in the adrenal cortex (see p. 239.] Glucagon, the catecholamines, cortisol, and growth hormones are sometimes called the "counterregulatory" hormones because each opposes the action of insulin on glucose use.

1. **Glucagon and epinephrine:** Secretion of these 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, inhibits insulin secretion, and inhibits the insulin-mediated uptake of glucose by peripheral 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. 340). The prevention or correction of hypoglycemia fails when the secretion of both glucagon and epinephrine is deficient.

2. **Cortisol and growth hormone:** These 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 of hypoglycemia

Hypoglycemia may be divided into four types: 1) insulin-induced, 2) postprandial (sometimes called reactive hypoglycemia), 3) fasting hypoglycemia, and 4) alcohol-related.

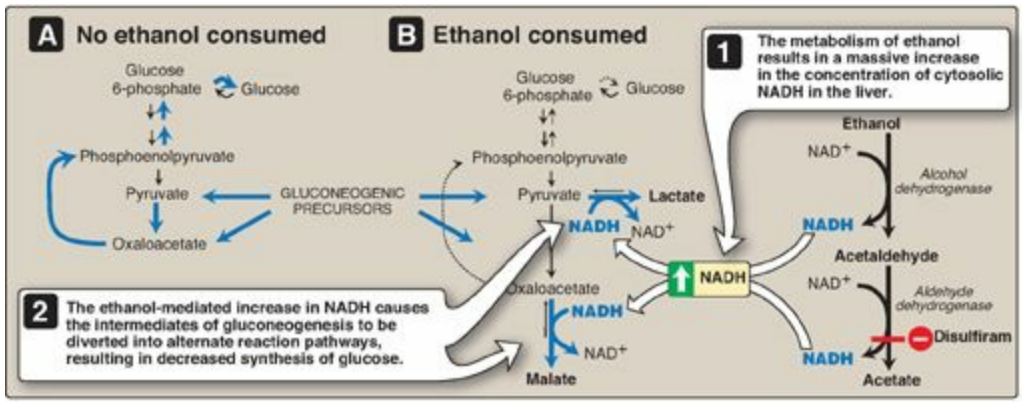
1. **Insulin-induced hypoglycemia:** Hypoglycemia occurs frequently in patients with diabetes who are receiving insulin treatment, particularly those striving to achieve tight control of blood glucose levels. Mild hypoglycemia in fully conscious patients is treated by oral administration of carbohydrate. Unconscious patients are typically given glucagon subcutaneously or intramuscularly (Figure 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 below). Alternately, fasting hypoglycemia may be the result of an increased rate of glucose use by the peripheral tissues due to 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, for example, defects in fatty acid oxidation, result in fasting hypoglycemia.]

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.



4. **Alcohol-related hypoglycemia:** Alcohol is metabolized in the liver by two oxidation reactions (Figure 23.15). Ethanol is first converted to acetaldehyde by 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 concentration of cytosolic NADH. The abundance of NADH favors the reduction of pyruvate to lactate and of oxaloacetate (OAA) to malate. Recall from p. 118 that pyruvate and OAA are intermediates in the synthesis of glucose. Thus, the ethanol-mediated increase in NADH causes these intermediates of gluconeogenesis 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. 195) and can result in alcoholic 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. Becuase alcohol consumption can also increase the risk for hypoglycemia in patients using insulin, those in an intensive insulin treatment protocol (see p. 340) are counseled about the increased risk of hypoglycemia that generally occurs many hours after alcohol ingestion. [Note: Chronic alcohol consumption can also result in alcoholic fatty liver due to increased hepatic synthesis of TAGs coupled with impaired formation or release of VLDLs. This occurs as a result

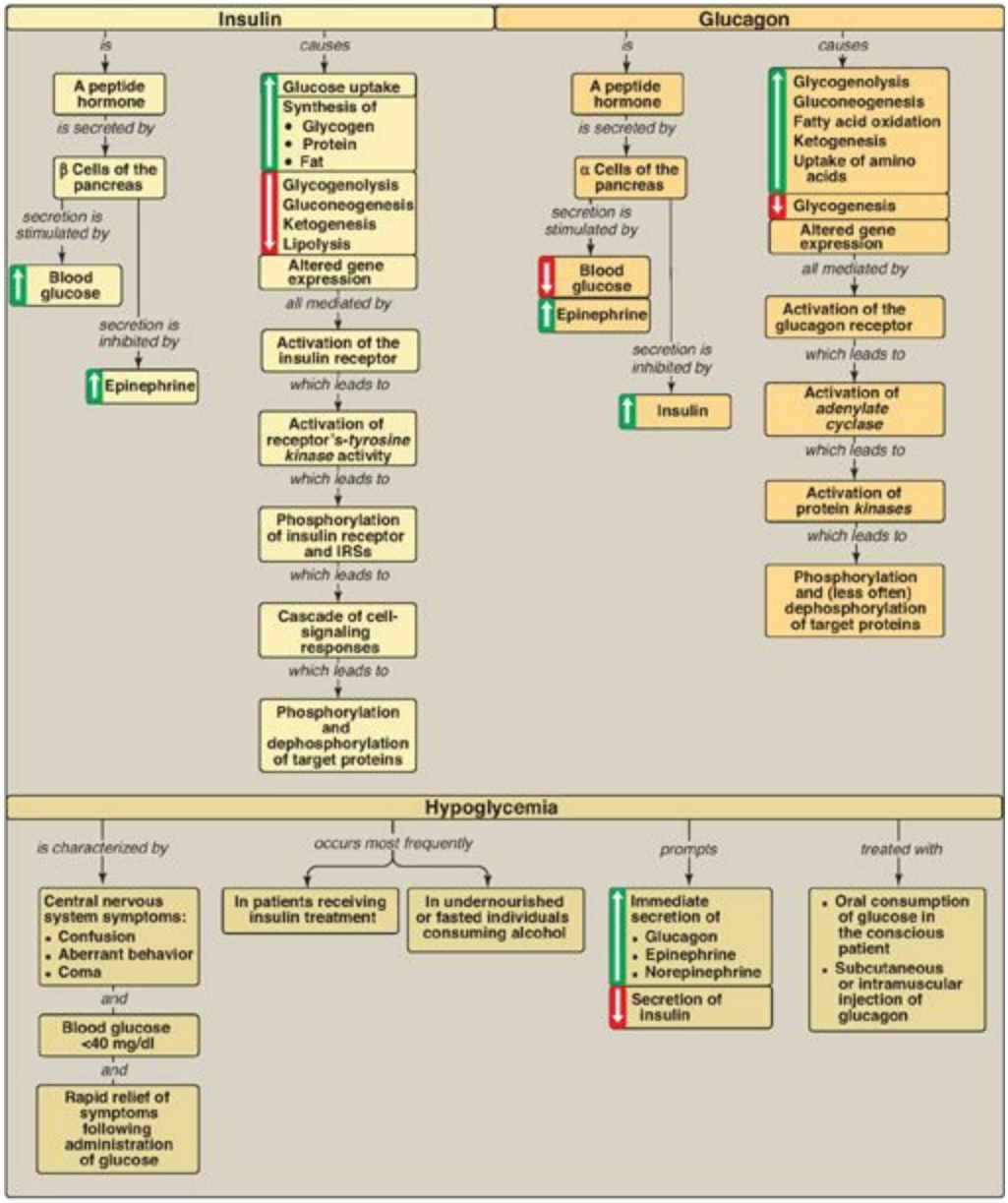
of decreased fatty acid oxidation due to a fall in the NAD^+/NADH ratio and increased lipogenesis due to the increased availability of fatty acids (decreased catabolism) and of glyceraldehyde 3-phosphate (the dehydrogenase is inhibited by the low NAD^+/NADH ratio). With continued alcohol consumption, alcoholic fatty liver can progress first to alcoholic hepatitis and then to alcoholic cirrhosis (Figure 23.16).]

Figure 23.16 Effects of chronic alcohol consumption on liver morphology.

V. CHAPTER SUMMARY

The integration of energy metabolism is controlled primarily by **insulin** and the opposing actions of **glucagon** and the catecholamines, particularly **epinephrine** (Figure 23.17). 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 (for example, 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 muscle and adipose) and the synthesis of glycogen, protein, and triacylglycerol: it is an **anabolic** hormone. These actions are mediated by binding to its **tyrosine kinase receptor**. Binding initiates a cascade of cell-signaling responses, including phosphorylation of a family of proteins called **insulin receptor substrate proteins**. **Glucagon** is a monomeric peptide hormone produced by the **α cells** of the pancreatic islets (both insulin and glucagon synthesis involves 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 uptake of amino acids: 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 receptors of hepatocytes. Binding results in the activation of **adenylyl cyclase**, which produces the second messenger **cyclic adenosine monophosphate** (**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 **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.

Figure 23.17 Key concept map for the metabolic effects of insulin and glucagon, and hypoglycemia. IRSs = insulin receptor substrates.


Insulin
is
A peptide hormone
is secreted by
β Cells of the pancreas
secretion is stimulated by
Blood glucose
secretion is inhibited by
Epinephrine
causes
Glucose uptake
Synthesis of
• Glycogen
• Protein
• Fat
Glycogenolysis
Gluconeogenesis
Ketogenesis
Lipolysis
Altered gene expression
all mediated by
Activation of the insulin receptor
which leads to
Activation of receptor's-tyrosine kinase activity
which leads to
Phosphorylation of insulin receptor and IRSs
which leads to
Cascade of cell-signaling responses
which leads to
Phosphorylation and dephosphorylation of target proteins
Glucagon
is
A peptide hormone
is secreted by
α Cells of the pancreas
secretion is stimulated by
Blood glucose
Epinephrine
secretion is inhibited by
Insulin
causes
Glycogenolysis
Gluconeogenesis
Fatty acid oxidation
Ketogenesis
Uptake of amino acids
Glycogenesis
Altered gene expression
all mediated by
Activation of the glucagon receptor
which leads to
Activation of adenylate cyclase
which leads to
Activation of protein kinases
which leads to
Phosphorylation and (less often) dephosphorylation of target proteins
Hypoglycemia
is characterized by
Central nervous system symptoms:
• Confusion
• Aberrant behavior
• Coma
and
Blood glucose <40 mg/dl
and
Rapid relief of symptoms following administration of glucose
occurs most frequently
In patients receiving insulin treatment
In undernourished or fasted individuals consuming alcohol
prompts
Immediate secretion of
• Glucagon
• Epinephrine
• Norepinephrine
Secretion of insulin
treated with
• Oral consumption of glucose in the conscious patient
• Subcutaneous or intramuscular injection of glucagon

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

Correct answer = A. The glucose transporter (GLUT-4) in adipose (and muscle) tissue is dependent on insulin. Insulin results in transport of GLUT-4 from intracellular vesicles to the cell membrane. The other tissues in the list contain GLUTs that are independent of insulin because they are always located on the cell membrane.

23.3 A 39-year-old woman 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 p.m. 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 was 45 mg/dl (normal = 70–99). 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-to-oxidized forms of nicotinamide adenine dinucleotide.
C. oxaloacetate and pyruvate.
D. use of acetyl coenzyme A in fatty acid 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 NADH 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 oxidation. The decrease in OAA shunts any acetyl coenzyme A produced to ketogenesis. Note that the inhibition of fatty acid degradation results in their reesterification into triacylglycerol that can result in fatty liver.

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

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 glucose transporters, resulting in hypoglycemia. The hypoglycemia is insufficient to suppress insulin production and secretion, however. 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?

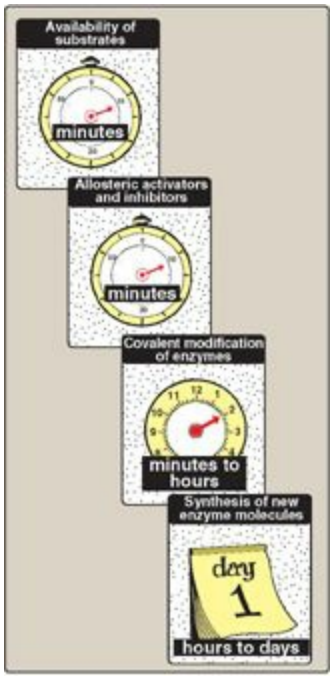
A glucagon-secreting tumor of the pancreas (glucagonoma) would result in hyperglycemia, not hypoglycemia. The constant production of glucagon would result in constant gluconeogenesis, using amino acids from proteolysis as substrates. This results in loss of body weight.

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 (TAG) occur, the latter primarily as components of chylomicrons synthesized by the intestinal mucosal cells (see p. 228). Islet tissue of the pancreas responds to the elevated levels of glucose with an increased secretion of insulin and a decreased release of glucagon. The elevated insulin-to-glucagon ratio and the ready availability of circulating substrates make the absorptive state an anabolic period characterized by increased synthesis of TAG and glycogen to replenish fuel stores and enhanced 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. ENZYMIC CHANGES IN THE ABSORPTIVE STATE

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 (Figure 24.1) and allows the body to adapt to a wide variety of physiologic situations. In the well-fed state, these regulatory mechanisms ensure that available nutrients are captured as glycogen, TAG, and protein.

A. 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. 99). In contrast, gluconeogenesis is inhibited by fructose 2,6-bisphosphate, an allosteric inhibitor of fructose 1,6-bisphosphatase (see p. 121).

B. Covalent modification

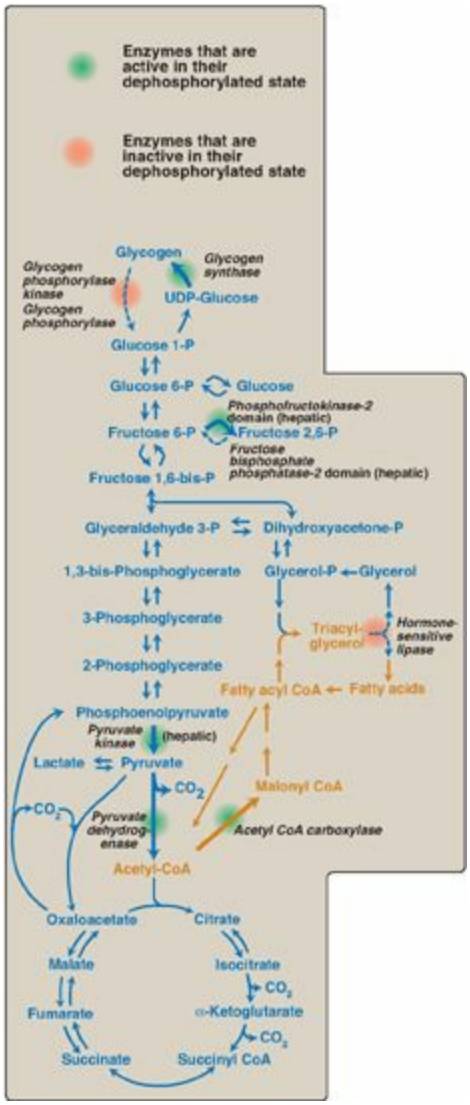
The activity of many enzymes is regulated by the addition (via kinases, such as cyclic adenosine monophosphate [cAMP]-activated protein kinase A [PKA] and adenosine monophosphate-activated protein kinase [AMPK]) or removal (via phosphatases) of phosphate groups from specific serine, threonine, or tyrosine residues of the protein. In the absorptive state, most of the covalently regulated enzymes are in the dephosphorylated form and are active (Figure 24.2). Three exceptions are glycogen phosphorylase kinase (see p. 132), glycogen phosphorylase (see p. 132), and hormone-sensitive lipase (HSL) of adipose tissue (see p. 190), which are inactive in their dephosphorylated form. [Note: In liver, the phosphatase domain of bifunctional phosphofructokinase-2 (PFK-2) is inactive when the protein is dephosphorylated (see p. 100).]

C. 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 influencing the activity of existing enzyme molecules. Enzymes subject to regulation of synthesis are often those that are needed under specific physiologic conditions. For example, in the fed state, elevated insulin levels result in an increase in the synthesis of key enzymes, such as acetyl coenzyme A (CoA) carboxylase ([ACC] see p. 184) and fatty acid synthase (see p.184), involved in anabolic metabolism. In the fasted state, glucagon induces expression of phosphoenolpyruvate carboxykinase (PEPCK) of gluconeogenesis (see p.120). Both hormones affect transcription factors.

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.



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

Liver is normally a glucose-producing rather than a glucose-using tissue. However, after a meal containing carbohydrate, the liver becomes a net consumer, retaining roughly 60 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 low affinity (high K_m) for glucose and, therefore, takes up glucose only when blood glucose is high (see p. 97). Additional mechanisms by which hepatic glucose metabolism is increased include the following. [Note: The numbers in colored circles in the text refer to Figure 24.3.]

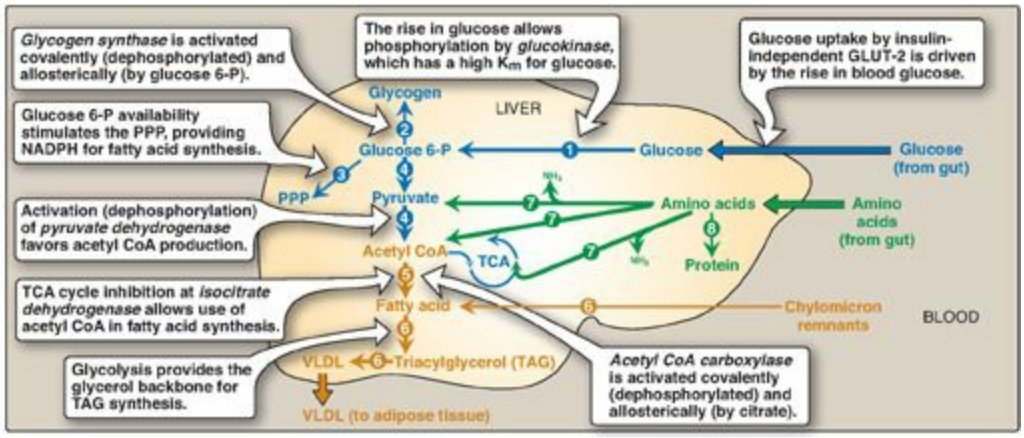
1. **Increased phosphorylation of glucose:** The elevated levels of glucose within the hepatocyte (as a result of elevated extracellular levels) allow glucokinase to phosphorylate glucose to glucose 6-phosphate (Figure 24.3, ❶). (Recall that glucokinase has a high K_m for glucose, is not subject to direct product inhibition, and has a sigmoidal reaction curve; see p.98.).

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 allosteric effector (see Figure 24.3, ❷).

3. **Increased activity of the pentose phosphate pathway:** 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 ([PPP] see p. 145). This pathway typically accounts for 5%–10% of the glucose metabolized by the liver (see Figure 24.3, ❸).

Figure 24.3 Major metabolic pathways in liver in the absorptive state. [Note: The acetyl 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; PPP = pentose phosphate pathway; TCA = tricarboxylic acid (cycle); CoA =

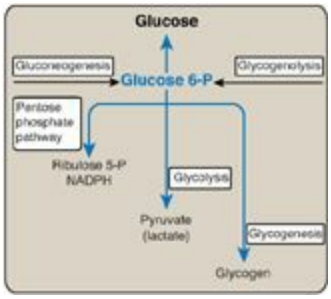
coenzyme A; VLDL = very-low-density lipoprotein; GLUT = glucose transporter; NADPH = nicotinamide adenine dinucleotide phosphate.



4. **Increased glycolysis:** In 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-to-glucagon ratio that results in increased amounts of the regulated enzymes of glycolysis: glucokinase, PFK-1, and pyruvate kinase ([PK] see p. 102). 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 (see Figure 24.3, ④). The acetyl CoA either is used as a substrate for fatty acid (FA) synthesis or is oxidized for energy in the tricarboxylic acid (TCA) cycle. (See Figure 24.4 for the central role of glucose 6-phosphate.)

5. **Decreased production of glucose:** Although glycolysis and glycogenesis (pathways that promote glucose storage) are stimulated in liver in the absorptive state, gluconeogenesis and glycogenolysis (pathways that generate glucose) are decreased. Pyruvate carboxylase (PC), which catalyzes the first step in gluconeogenesis, is largely inactive due to low levels of acetyl CoA, its allosteric activator (see p. 119). [Note: The acetyl CoA is being used for fatty acid synthesis.] The high insulin-to-glucagon ratio also favors inactivation of other gluconeogenic enzymes such as fructose 1,6-bisphosphatase (see Figure 8.17, p. 100). Glycogenolysis is inhibited by dephosphorylation of glycogen phosphorylase and phosphorylase kinase.

Figure 24.4 Central role of glucose 6-phosphate in metabolism. [Note: The presence of glucose 6-phosphatase in liver allows the production of free glucose from glycogenolysis and gluconeogenesis.] NADPH = nicotinamide adenine dinucleotide phosphate; P = phosphate.



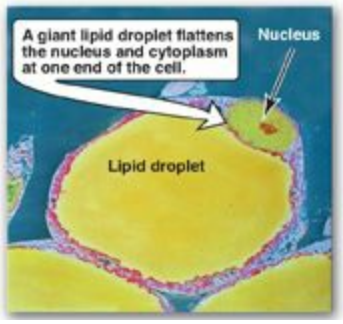
B. Fat metabolism

1. **Increased fatty acid synthesis:** Liver is the primary tissue for de novo synthesis of FAs (see Figure 24.3, 5). 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) 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. 183). [Note: Malonyl CoA inhibits carnitine palmitoyltransferase-I (CPT-I) of FA oxidation (see p.191). Citrate, thereby, directly activates FA synthesis and indirectly inhibits FA degradation.]

    a. **Source of cytosolic acetyl coenzyme A:** Pyruvate from aerobic glycolysis enters mitochondria and is decarboxylated by PDH. The acetyl CoA product is combined with oxaloacetate (OAA) to form citrate via citrate synthase. Citrate leaves the mitochondria (as a result of the inhibition of isocitrate dehydrogenase by adenosine triphosphate [ATP]) and enters the cytosol. Citrate is cleaved by ATP-citrate lyase (induced by insulin), producing the acetyl CoA substrate of ACC and OAA. The OAA is reduced to malate, which is oxidatively decarboxylated to pyruvate by malic enzyme as NADPH is formed (see p. 187).

2. **Increased triacylglycerol synthesis:** TAG synthesis is favored because fatty acyl CoAs are available both from de novo synthesis from acetyl CoA and from hydrolysis of the TAG component of chylomicron remnants removed from the blood by hepatocytes (see p. 178). Glycerol 3-phosphate, the backbone for TAG synthesis, is provided by glycolysis (see p. 189). The liver packages TAG into very-low-density lipoprotein (VLDL) particles that are secreted into the blood for use by extrahepatic tissues, particularly adipose and muscle tissues (see Figure 24.3, 6).

Figure 24.5 Colorized transmission electron micrograph of adipocytes.



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 (see Figure 24.3, 7). The liver has limited capacity to degrade the branched-chain amino acids (BCAAs) leucine, isoleucine, and valine. They pass through the liver essentially unchanged and are preferentially metabolized in muscle (see p. 266).

2. **Increased protein synthesis:** The body does not store protein in the same way that it maintains glycogen or TAG reserves (see p. 327). 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 postabsorptive period (see Figure 24.3, 8).

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 approximately 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 TAG (Figure 24.5).

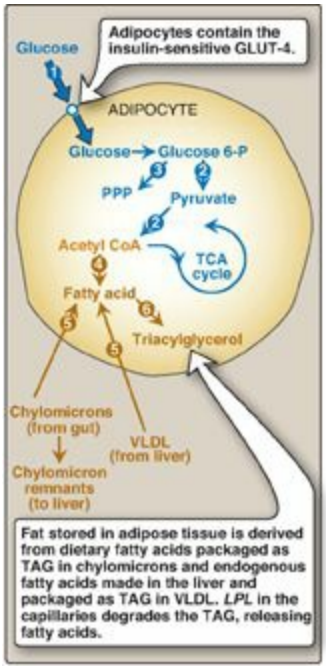
A. Carbohydrate metabolism

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 (Figure 24.6, ❶). The glucose is phosphorylated by hexokinase.

2. **Increased glycolysis:** The increased intracellular availability of glucose results in an enhanced rate of glycolysis (see Figure 24.6, ❷). In adipose tissue, glycolysis serves a synthetic function by supplying glycerol 3-phosphate for TAG synthesis (see p. 189). Recall that adipose tissue lacks glycerol kinase.

3. **Increased activity of the pentose phosphate pathway:** Adipose tissue can metabolize glucose by means of the PPP, thereby producing NADPH, which is essential for fat synthesis (see p. 186 and Figure 24.6, ❸). However, in humans, de novo synthesis is not a major source of FA in adipose tissue, except when refeeding a previously fasted individual (see Figure 24.6, ❹).

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; PPP = pentose phosphate pathway; CoA = coenzyme A; TCA = tricarboxylic acid; TAG = triacylglycerol; VLDL = very-low-density lipoprotein; LPL = lipoprotein lipase.



B. Fat metabolism

Most of the FAs 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 (see Figure 24.6, ⑤). The FAs are released from the lipoproteins by the action of lipoprotein lipase (LPL), an extracellular enzyme attached to the capillary walls in many tissues, particularly adipose and muscle. In adipose tissue, LPL is upregulated by insulin. Thus, in the fed state, elevated levels of glucose and insulin favor storage of TAG (see Figure 24.6, ⑥), all the carbons of which are supplied by glucose. [Note: Elevated insulin favors the dephosphorylated (inactive) form of HSL (see p. 190), thereby inhibiting lipolysis in the fed state.]

V. RESTING SKELETAL MUSCLE

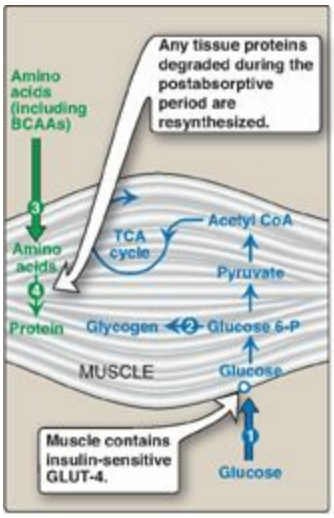
In the fed state, muscle takes up glucose via GLUT-4 (for energy and glycogen synthesis) and amino acids (for energy and protein synthesis). [Note: The energy metabolism of skeletal muscle is unique in being able to respond to substantial changes in the demand for ATP that accompanies muscle contraction. At rest, muscle accounts for approximately 30% of the O_2 consumption of the body, whereas during vigorous exercise, it is responsible for up to 90% of the total O_2 consumption. This graphically illustrates the fact that skeletal muscle, despite its potential for transient periods of anaerobic glycolysis, is an oxidative tissue. In contrast to liver, there is no covalent regulation of PFK-2 in skeletal muscle. In the cardiac isozyme, however, the kinase domain is activated by epinephrine-mediated phosphorylation.]

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 by GLUT-4 (see p. 97 and Figure 24.7, ❶), thereby reducing blood glucose. Glucose is phosphorylated to glucose 6-phosphate by hexokinase and metabolized to provide the energy needs of the cells.

2. **Increased glycogen synthesis:** The increased insulin-to-glucagon ratio and the availability of glucose 6-phosphate favor glycogen synthesis, particularly if glycogen stores have been depleted as a result of exercise (see p. 126, and Figure 24.7, ❷).

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.



B. Fat metabolism

FAs are released from chylomicrons and VLDL by the action of LPL (see pp. 228 and 231). However, fatty acids are of secondary importance as a fuel for resting muscle during the fed state, in which glucose is the primary source of energy.

C. Amino acid metabolism

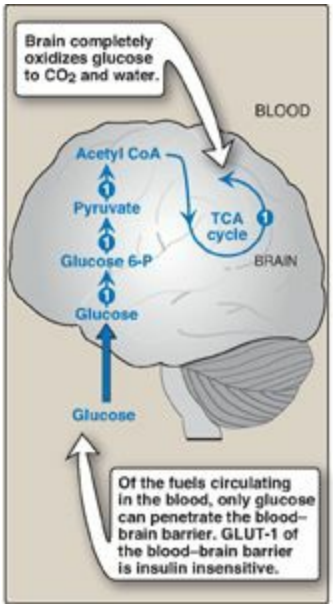
1. **Increased protein synthesis:** An increase in amino acid uptake and protein synthesis occurs in the absorptive period after ingestion of a meal containing protein (see Figure 24.7, ③ and ④). This synthesis replaces protein degraded since the previous meal.

2. **Increased uptake of branched-chain amino acids:** Muscle is the principal site for degradation of the BCAAs (leucine, isoleucine, and valine) because it contains the required transaminase (see p. 266). The BCAAs escape metabolism by the liver and are taken up by muscle, where they are used for protein synthesis (see Figure 24.7, ③) and as sources of energy.

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), completely oxidizing approximately 140 g/day to CO_2 and H_2O. The brain contains no significant stores of glycogen and is, therefore, completely dependent on the availability of blood glucose (Figure 24.8, ❶). [Note: If blood glucose levels fall below 40 mg/100 ml (normal fasted blood glucose is 70–99 mg/100 ml), cerebral function is impaired (see p. 315).] The brain also lacks significant stores of TAG, and the FAs circulating in the blood make little contribution to energy production because FAs bound to albumin do not efficiently cross the BBB. The intertissue exchanges characteristic of the absorptive period are summarized in Figure 24.9.

Figure 24.8 Major metabolic pathways in 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.



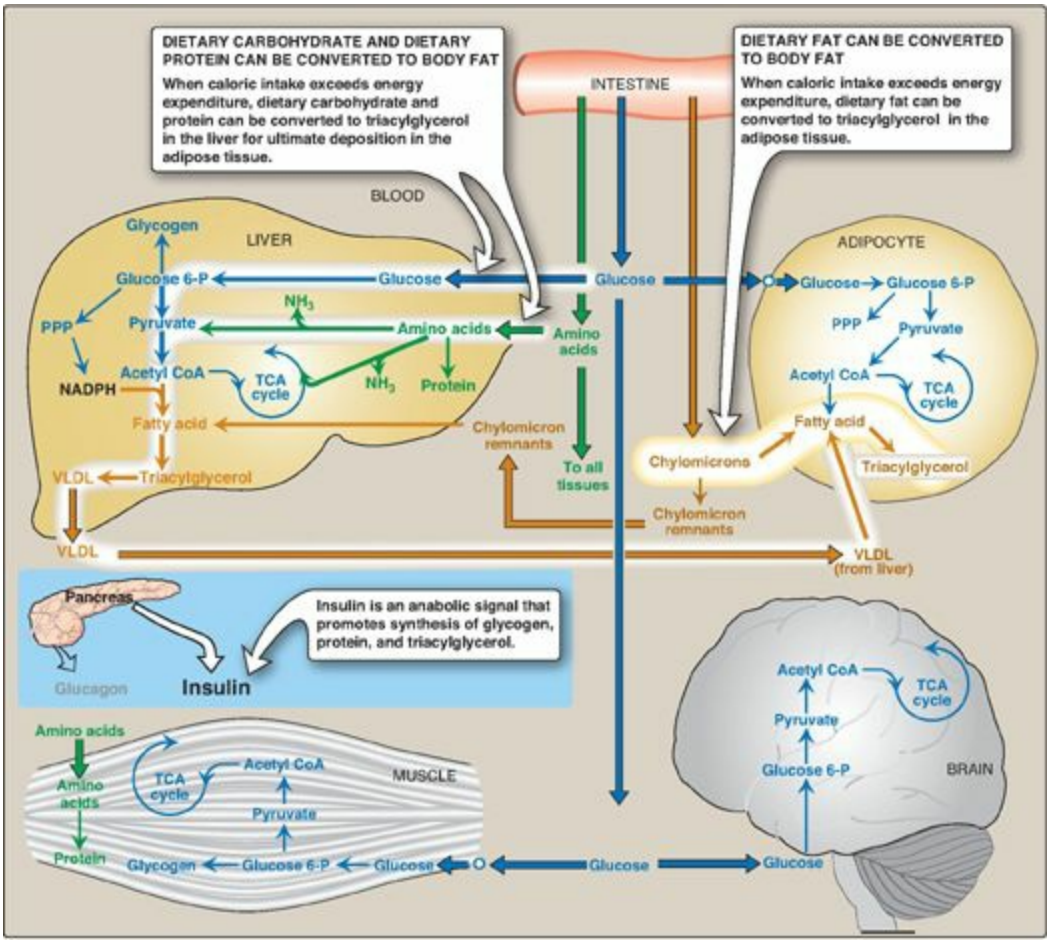
VII. OVERVIEW OF FASTING

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 (for example, 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 and epinephrine release. The decreased insulin/counterregulatory hormone ratio and the decreased availability of circulating substrates make the period of nutrient deprivation a catabolic period characterized by degradation of TAG, glycogen, and protein. This sets into motion an exchange of substrates among 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 fatty acids from adipose tissue and the synthesis and release of ketone bodies from the liver to supply energy to other tissues. [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. Note 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 (for example, as a structural component of the body, an enzyme, and so forth). Therefore, only about one third of the body's protein can be used for energy production without fatally compromising vital functions.]

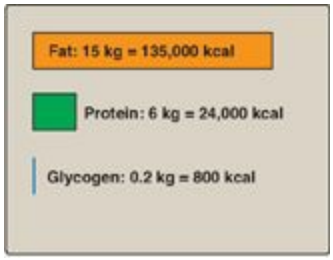
Figure 24.9 Intertissue relationships in the absorptive state and the hormonal signals that promote them. [Note: Small circles on the perimeter of muscle and the adipocyte indicate insulin-dependent glucose transporters.] P = phosphate; PPP = pentose phosphate pathway; CoA = coenzyme A; NADPH = nicotinamide adenine dinucleotide phosphate; TCA = tricarboxylic acid; VLDL = very-low-density lipoprotein.



B. Enzymic changes in fasting

In fasting (as in the 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 to those described for the absorptive state (see Figure 24.9). For example, although most of the enzymes regulated by covalent modification are dephosphorylated and active in the fed state, they are phosphorylated and inactive in the fasted state. Three exceptions are glycogen phosphorylase (see p. 132), glycogen phosphorylase kinase (see p. 132), and HSL of adipose tissue (see p. 190), 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 liver, lipolysis with release of FAs 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 24.10 Metabolic fuels present in a 70-kg man at the beginning of a fast. The fat stores are sufficient to meet energy needs for about 80 days.


Fat: 15 kg = 135,000 kcal
Protein: 6 kg = 24,000 kcal
Glycogen: 0.2 kg = 800 kcal

VIII. LIVER IN FASTING

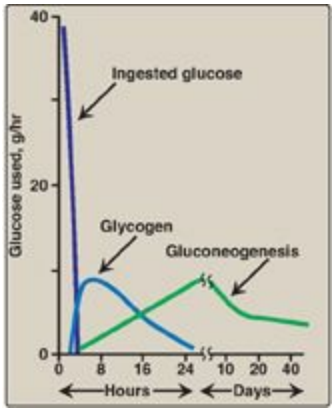
The primary role of liver in energy metabolism during fasting is maintenance of blood glucose through the production of glucose from glycogenolysis and gluconeogenesis for glucose-dependent tissues and the synthesis and distribution of ketone bodies for use by other tissues. Therefore, "hepatic" metabolism and "extrahepatic" or "peripheral" metabolism are distinguished.

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 (postabsorptive) 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 (see Figure 24.4).]

1. **Increased glycogen degradation:** Figure 24.11 shows the sources of blood glucose after ingestion of 100 g of glucose. During the brief absorptive period, ingested glucose is the major source of blood glucose. Several hours later, blood glucose levels have declined sufficiently to cause increased secretion of glucagon and decreased release of insulin. The increased glucagon-to-insulin ratio causes a rapid mobilization of liver glycogen stores (which contain about 80 g of glycogen in the fed state) due to PKA-mediated phosphorylation (and activation) of glycogen phosphorylase kinase that phosphorylates (and activates) glycogen phosphorylase (see p. 130). Figure 24.11 shows that liver glycogen is nearly exhausted after 10–18 hours of fasting, and therefore, hepatic glycogenolysis is a transient response to early fasting. Figure 24.12, 1, shows glycogen degradation as part of the overall metabolic response of the liver during fasting. [Note: Phosphorylation of glycogen synthase simultaneously inhibits glycogenesis.]

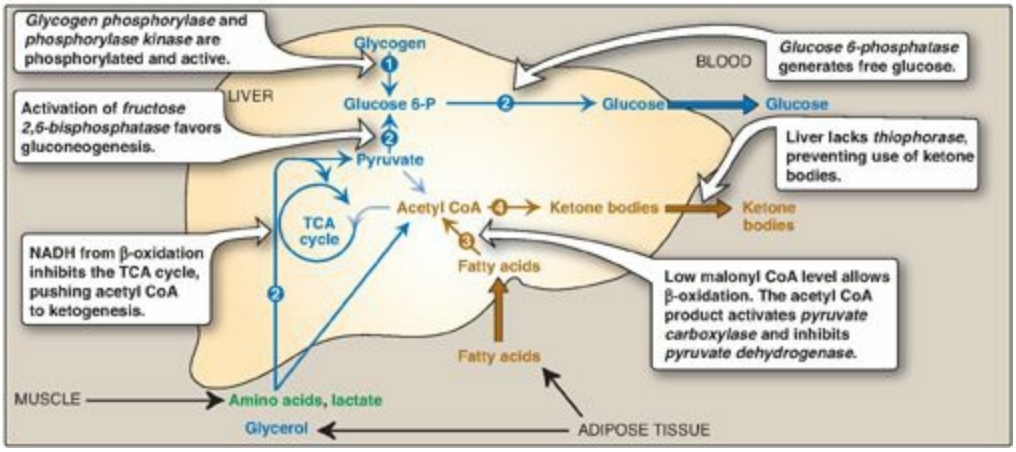
Figure 24.11 Sources of blood glucose after ingestion of 100 g of glucose. [Note: See Section B.2. for an explanation as to why gluconeogenesis declines.]



2. **Increased glucose synthesis:** The synthesis of glucose and its release into the circulation are vital hepatic functions during short- and long-term fasting (see Figure

24.12, ②). 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 (due to decreased availability of its inhibitor fructose 2,6-bisphosphate; see p. 121) and by induction of PEPCK by glucagon (see p. 122), begins 4–6 hours after the last meal and becomes fully active as stores of liver glycogen are depleted (see Figure 24.11). [Note: The decrease in fructose 2,6-bisphosphate simultaneously inhibits glycolysis at PFK-1 (see p. 99).]

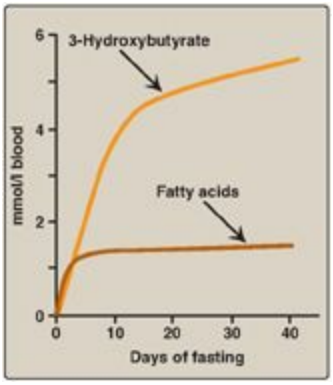
Figure 24.12 Major metabolic pathways in liver during fasting. [Note: The numbers in circles, which appear both in the figure and in the corresponding citation in the text, indicate important metabolic pathways for carbohydrate or fat.] P = phosphate; CoA = coenzyme A; TCA = tricarboxylic acid; NADH = nicotinamide adenine dinucleotide.



B. Fat metabolism

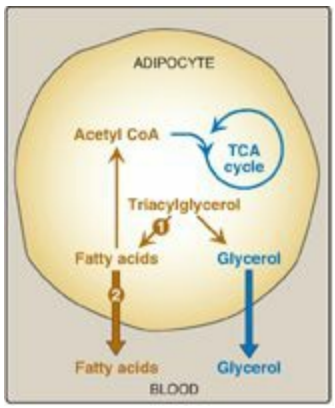
1. **Increased fatty acid oxidation:** The oxidation of FAs obtained from TAG hydrolysis in adipose tissue is the major source of energy in hepatic tissue in the postabsorptive state (see Figure 24.12, ③). The fall in malonyl CoA due to phosphorylation (inactivation) of ACC by AMPK removes the brake on CPT-1, allowing β-oxidation to occur (see p. 191). FA oxidation generates NADH, $FADH_2$, and acetyl CoA. The NADH inhibits the TCA cycle. The acetyl CoA is an allosteric activator of PC and an allosteric inhibitor of PDH, thereby favoring use of pyruvate in gluconeogenesis (see Figure 8.24). [Note: The 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.

Figure 24.13 Concentrations of fatty acids and 3-hydroxybutyrate in the blood during fasting. [Note: 3-Hydroxybutyrate is made from the reduction of acetoacetate.]



2. **Increased ketone body synthesis:** 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 (see p. 195) but not by the liver itself because liver lacks thiophorase. Ketogenesis, which starts during the first days of fasting (Figure 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 brain, once their level in the blood is sufficiently high. This reduces the need for gluconeogenesis from amino acid carbon skeletons, thus preserving essential protein (see Figure 24.11). Ketogenesis as part of the overall hepatic response to fasting is shown in Figure 24.12, ④. [Note: Ketone bodies are organic acids and, when present at high concentrations, can cause ketoacidosis.]

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.



IX. ADIPOSE TISSUE IN FASTING

A. Carbohydrate metabolism

Glucose transport by insulin-sensitive GLUT-4 into the adipocyte and its subsequent metabolism are depressed due to low levels of circulating insulin. This results in decreased TAG synthesis.

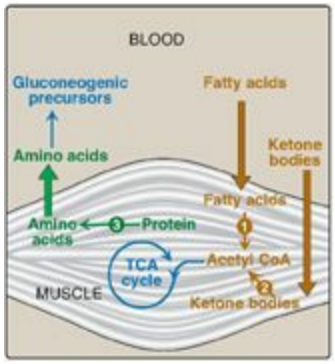
B. Fat metabolism

1. **Increased degradation of fat:** The PKA-mediated phosphorylation and activation of HSL (see p. 190) and subsequent hydrolysis of stored fat are enhanced by the elevated catecholamines norepinephrine and epinephrine. These hormones, which are released from the sympathetic nerve endings in adipose tissue and/or from the adrenal medulla, are physiologically important activators of HSL (Figure 24.14, ❶).

2. **Increased release of fatty acids:** FAs obtained from hydrolysis of stored TAG are primarily released into the blood (see Figure 24.14, ❷). 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. They also can be re-esterified to glycerol 3-phosphate (from glyceroneogenesis, see p. 190), generating TAG and reducing plasma FA concentration.]

3. **Decreased uptake of fatty acids:** In fasting, LPL activity of adipose tissue is low. Consequently, circulating TAG of lipoproteins is not available to adipose tissue.

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 FAs as its major fuel source in fasting. [Note: By contrast, exercising muscle initially uses its glycogen stores as a source of energy. During intense exercise, glucose 6-phosphate derived from glycogen is converted to lactate by anaerobic glycolysis (see p. 103). The lactate is used by liver for gluconeogenesis (Cori cycle; see p.118). As these glycogen reserves are depleted, free FAs provided by the mobilization of TAG from 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. 183).

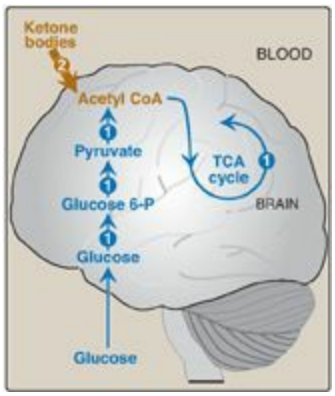
A. Carbohydrate metabolism

Glucose transport into skeletal muscle cells via insulin-sensitive GLUT-4 (see p. 97) and subsequent glucose metabolism are depressed because of low levels of circulating insulin. Therefore, the glucose from hepatic gluconeogenesis is unavailable to muscle (and adipose tissue).

B. Lipid metabolism

During the first 2 weeks of fasting, muscle uses FA from adipose tissue and ketone bodies from the liver as fuels (Figure 24.15, ❶ and ❷). After about 3 weeks of fasting, muscle decreases its use of ketone bodies (thus sparing them for brain) and oxidizes FA almost exclusively. [Note: The acetyl CoA from FA oxidation indirectly inhibits PDH (by activation of PDH kinase) and spares pyruvate, which is transaminated to alanine and used by liver for gluconeogenesis (glucose–alanine cycle; see p. 253).]

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

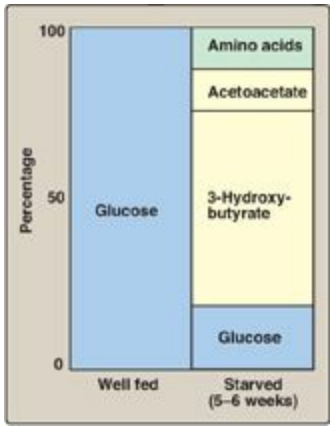
During the first few days of fasting, there is a rapid breakdown of muscle protein, providing amino acids that are used by the liver for gluconeogenesis (see Figure 24.15,

). 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 gluconeogenic amino acids released from muscle. They are produced by the catabolism of BCAAs (see p. 267).] The glutamine is used as a fuel by enterocytes, for example, which send out alanine that is used in hepatic gluconeogenesis. 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.

XI. BRAIN IN FASTING

During the early days of fasting, the brain continues to use only glucose as a fuel (Figure 24.16, ❶). Blood glucose is maintained by hepatic gluconeogenesis from glucogenic precursors, such as amino acids from proteolysis and glycerol from lipolysis. In prolonged fasting (beyond 2–3 weeks), plasma ketone bodies (see Figure 24.12) reach significantly elevated levels and replace glucose as the primary fuel for the brain (see Figure 24.16, ❷, and Figure 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 (65–70 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 24.19 (see below).

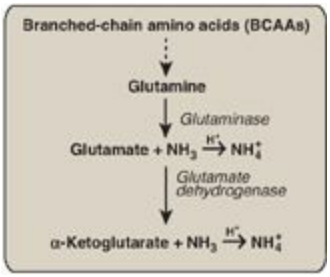
Figure 24.17 Fuel sources used by the brain to meet energy needs in the well fed and starved states.



XII. KIDNEY IN LONG-TERM FASTING

As fasting continues into early starvation and beyond, the kidney plays important roles. The kidney expresses the enzymes of gluconeogenesis, including glucose 6-phosphatase, and in late fasting about 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 BCAAs is taken up by the kidney and acted upon by renal glutaminase and glutamate dehydrogenase (see p. 256), producing α-ketoglutarate that 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 (Figure 24.18). In long-term fasting, then, there is a switch from nitrogen disposal in the form of urea to disposal in the form of ammonia. [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 24.18 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 kidney.



XIII. CHAPTER SUMMARY

The flow of intermediates through metabolic pathways is controlled by four 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 2–4-hour period after ingestion of a meal, these regulatory mechanisms ensure that available nutrients are captured as **glycogen**, **triacylglycerol** (**TAG**), and **protein** (Figure 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** by the **pancreas**. The elevated insulin-to-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 addition, the **liver** replenishes its **glycogen** stores, replaces any needed **hepatic proteins**, and increases **TAG** synthesis. The latter are packaged in **very-low-density lipoproteins**, which are exported to the peripheral tissues. **Adipose tissue** increases **TAG** synthesis and storage, whereas **muscle** increases **protein** synthesis to replace protein degraded since the previous meal. In the fed state, the **brain** uses glucose exclusively as a fuel. 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** and **epinephrine release**. The decreased insulin/counterregulatory hormone ratio and the decreased availability of circulating substrates make the fasting state a **catabolic period**. This sets into motion an **exchange of substrates** among 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 fatty acids (FAs) from adipose tissue and release ketone bodies from liver to supply energy to other tissues. To accomplish these goals, the **liver** degrades **glycogen** and initiates **gluconeogenesis**, using **increased fatty acid oxidation** as a source of the energy and reducing equivalents needed for gluconeogenesis and to supply the acetyl coenzyme A building blocks for **ketogenesis**. The **adipose tissue** degrades stored **TAG**, thus providing **FAs** and **glycerol** to the liver. The **muscle** can also use **FAs** as fuel as well as **ketone bodies** supplied by the liver. Muscle **protein** is **degraded** to supply **amino acids** for the liver to use in gluconeogenesis, but deceases 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 b y **synthesizing glucose** and **excreting the protons** from ketone body dissociation as ammonium (**NH_4^+**).

Figure 24.19 Intertissue relationships during starvation and the hormonal signals that promote them. P = phosphate; TCA = tricarboxylic acid; CoA = coenzyme A.

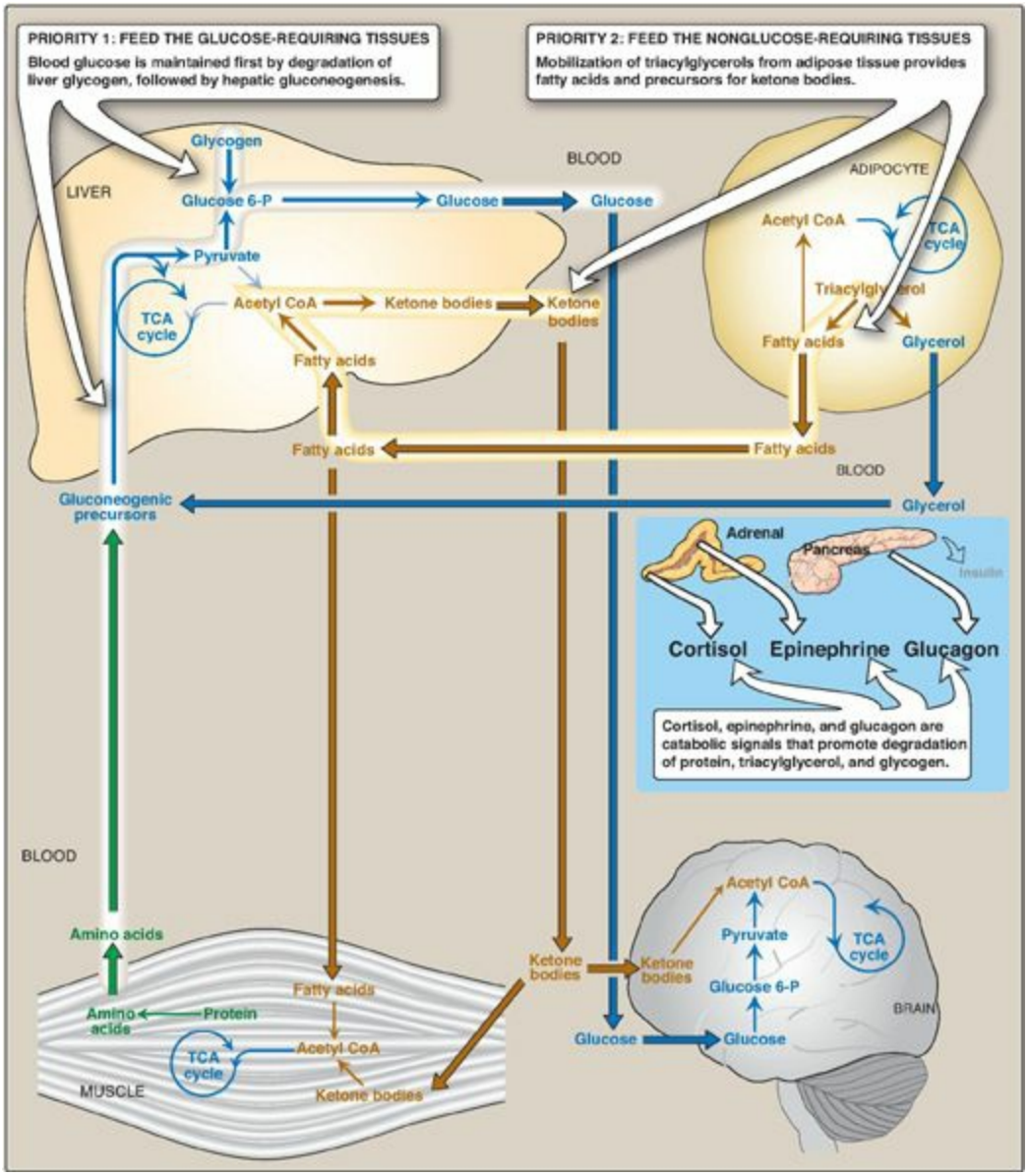
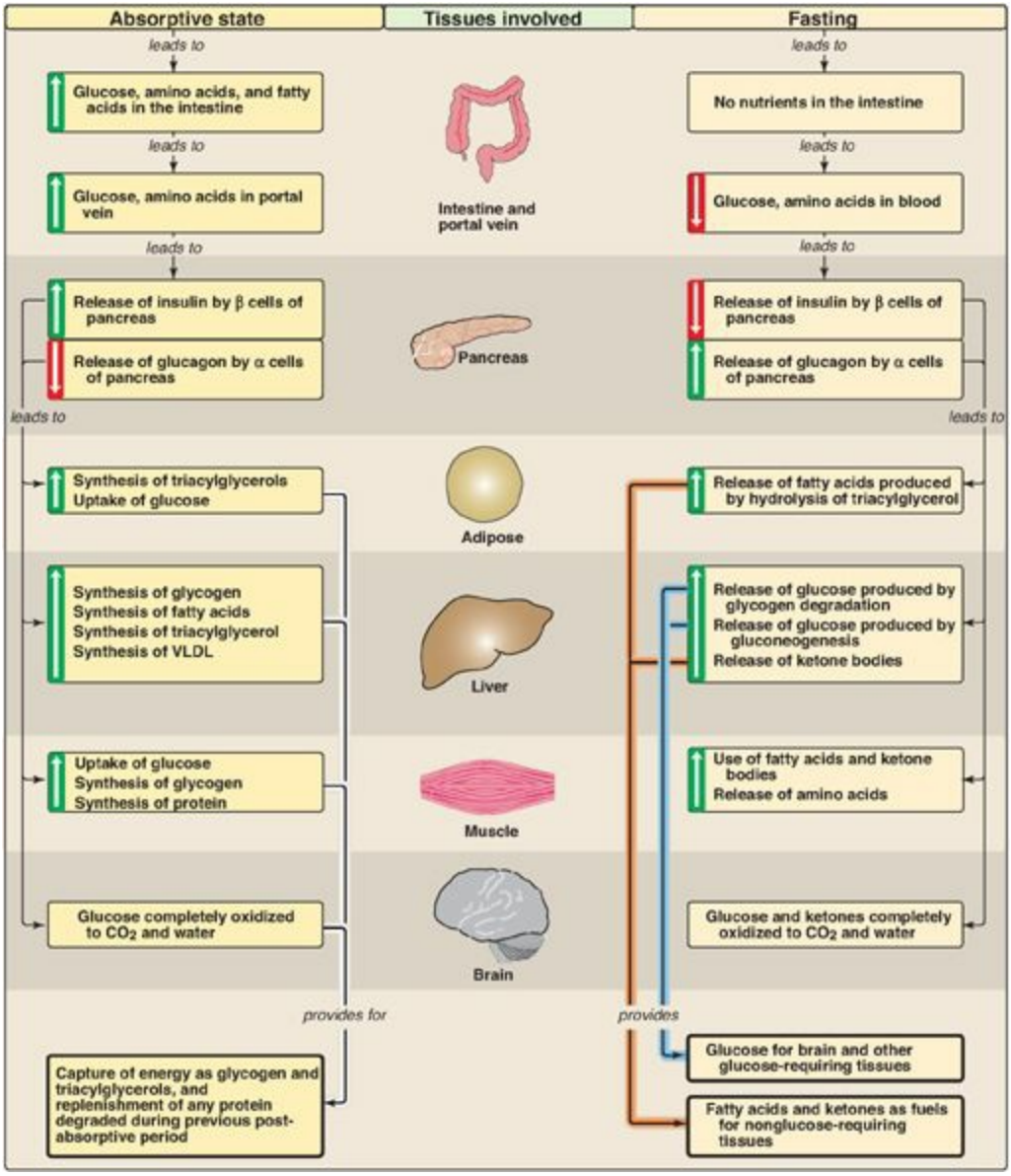


Figure 24.20 Key concept map for feed-fast cycle. VLDL = very-low-density lipoprotein.


Absorptive state
Tissues involved
Fasting
leads to
Glucose, amino acids, and fatty acids in the intestine
leads to
Glucose, amino acids in portal vein
leads to
Intestine and portal vein
leads to
No nutrients in the intestine
leads to
Glucose, amino acids in blood
leads to
Release of insulin by β cells of pancreas
Release of glucagon by α cells of pancreas
Pancreas
Release of insulin by β cells of pancreas
Release of glucagon by α cells of pancreas
leads to
leads to
Synthesis of triacylglycerols
Uptake of glucose
Adipose
Release of fatty acids produced by hydrolysis of triacylglycerol
Synthesis of glycogen
Synthesis of fatty acids
Synthesis of triacylglycerol
Synthesis of VLDL
Liver
Release of glucose produced by glycogen degradation
Release of glucose produced by gluconeogenesis
Release of ketone bodies
Uptake of glucose
Synthesis of glycogen
Synthesis of protein
Muscle
Use of fatty acids and ketone bodies
Release of amino acids
Glucose completely oxidized to CO_2 and water
Brain
Glucose and ketones completely oxidized to CO_2 and water
provides for
provides
Capture of energy as glycogen and triacylglycerols, and replenishment of any protein degraded during previous post-absorptive period
Glucose for brain and other glucose-requiring tissues
Fatty acids and ketones as fuels for nonglucose-requiring tissues

Study Questions

Choose the ONE best answer.

24.1 Which one of the following is elevated in plasma during the absorptive (fed) period as compared with the postabsorptive (fasted) state?

A. Acetoacetate
B. Chylomicrons
C. Free fatty acids
D. Glucagon

Correct answer = B. Triacylglycerol-rich chylomicrons are synthesized in (and released from) the intestine following ingestion of a meal. Acetoacetate, free fatty acids, and glucagon are elevated in the fasted state, not the absorptive state.

24.2 Which one of the following statements concerning liver in the fed state is correct?

A. Fructose 2,6-bisphosphate is elevated.
B. Insulin stimulates the uptake of glucose.
C. Most enzymes that are regulated by covalent modification 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 fed state promote the synthesis of fructose 2,6-bisphosphate, which allosterically activates phosphofructokinase-1 of glycolysis. Most covalently modified enzymes are in the dephosphorylated state and are active. Acetyl coenzyme A is not oxidized in the 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 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-1

C. Fatty acid synthase
D. Glycogen synthase
E. Hormone-sensitive lipase
F. Phosphofructokinase-1
G. Pyruvate dehydrogenase

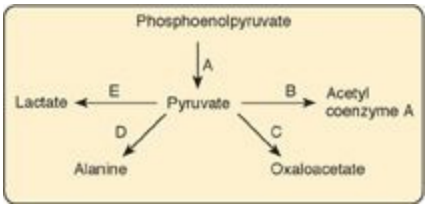
Correct answer = E. Hormone-sensitive lipase of adipocytes is phosphorylated and activated by protein kinase A in response to epinephrine. Choices A, B, C, and F are not regulated covalently. Choices D and G are regulated covalently but are inactive if phosphorylated.

24.4 For a 70-kg man, 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 man would not be able to supply his energy needs for 5 months.

24.5 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 adenosine triphosphate) and is allosterically activated by acetyl coenzyme A from fatty acid oxidation. None of the other choices meets these criteria.

Diabetes Mellitus 25

I. OVERVIEW OF DIABETES MELLITUS

Diabetes mellitus ("diabetes") is not one disease, but rather is a heterogeneous group of multifactorial, polygenic syndromes characterized by an elevated fasting blood glucose (FBG) caused by a relative or absolute deficiency in insulin. Nearly 26 million people in the United States (about 8% of the population) have diabetes. Of this number, approximately 7 million are as yet undiagnosed. Diabetes is the leading cause of adult blindness and amputation and a major cause of renal failure, nerve damage, heart attacks, and strokes. Most cases of diabetes mellitus can be separated into two groups (Figure 25.1), type 1 ([T1D] formerly called insulin-dependent diabetes mellitus) and type 2 ([T2D] formerly called noninsulin-dependent diabetes mellitus). The incidence and prevalence of T2D is increasing because of the aging of the U.S. population and the increasing prevalence of obesity and sedentary lifestyles (see p. 349). The increase in children with T2D is particularly disturbing.

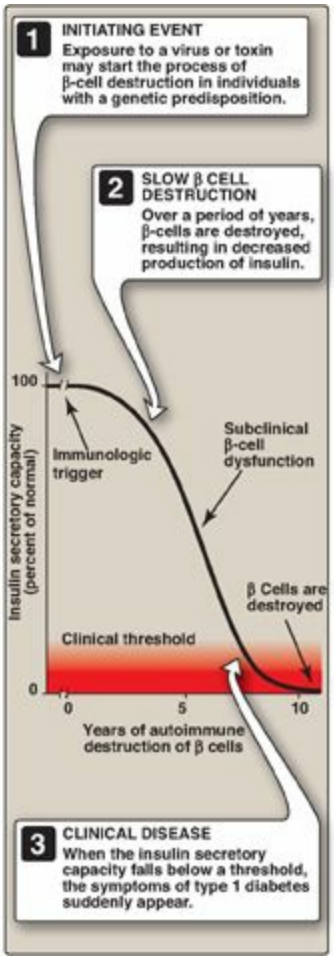
Figure 25.1 Comparison of type 1 and type 2 diabetes mellitus. [Note: The name of the disease reflects the clinical presentation of copious amounts of glucose-containing urine and is derived from the Greek word for siphon (diabetes) and the Latin word for honey-sweet (mellitus).]

	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 Cells are destroyed, 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 is 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) is essential to therapy

II. TYPE 1 DIABETES

Persons with T1D constitute less than 10% of the nearly 20 million known diabetics in the United States. The disease is characterized by an absolute deficiency of insulin caused by an autoimmune attack on the 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 on the β cells leads to gradual depletion of the β-cell population (Figure 25.2). However, symptoms appear abruptly when 80%–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 allows the β cells to be recognized as "nonself." [Note: Among monozygotic (identical) twins, if one sibling develops T1D, the other twin has only a 30%–50% chance of developing the disease. This contrasts with T2D (see p. 341), in which the genetic influence is stronger and, in virtually all monozygotic twinships, the disease eventually develops in both individuals.]

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 of type 1 diabetes

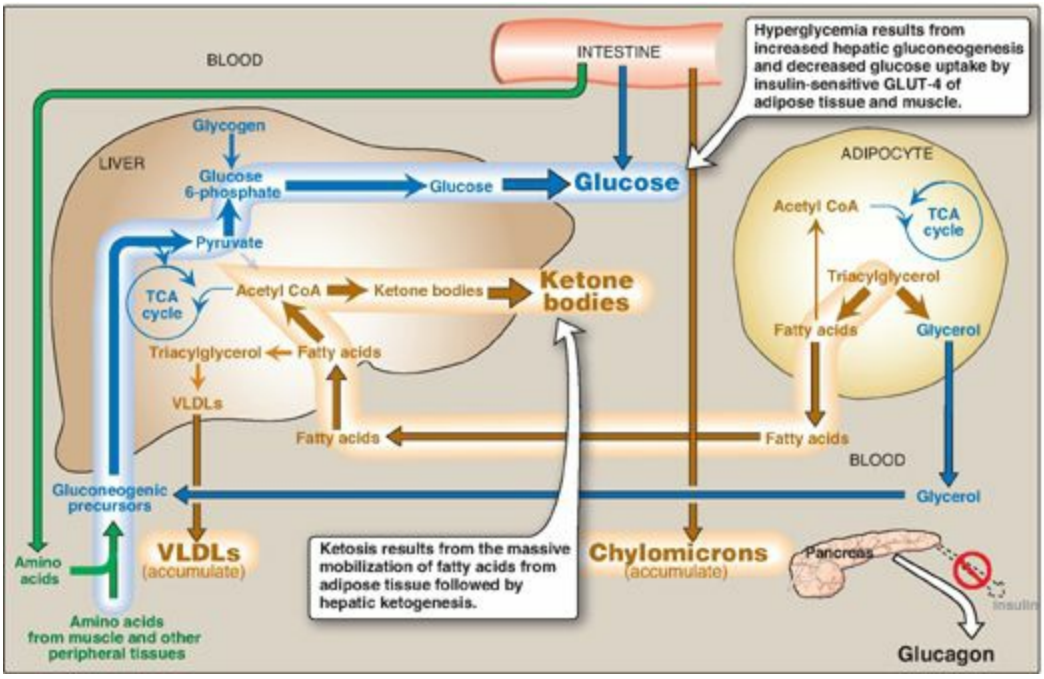
The onset of T1D is typically during childhood or puberty, and symptoms develop suddenly. Patients with T1D can usually be recognized by the abrupt appearance of polyuria (frequent urination), polydipsia (excessive thirst), and polyphagia (excessive hunger), often triggered by physiologic stress such as an illness. These symptoms are usually accompanied by fatigue and weight loss. The diagnosis is confirmed by a glycosylated hemoglobin (see p. 340) concentration ≥ 6.5 mg/dl (normal is less than 5.7), or a FBG ≥ 126 mg/dl (normal is 70–99). [Note: A FBG of 100–125 mg/dl is categorized as an impaired FBG. Individuals with impaired FBG are considered "prediabetic" and are at increased risk for developing T2D.] Fasting is defined as no caloric intake for at least 8 hours. Diagnosis can also be made on the basis of a nonfasting (random) blood glucose level greater than 200 mg/dl in an individual with symptoms of hyperglycemia. [Note: The oral glucose tolerance test, in which blood glucose is measured 2 hours after ingestion of a solution containing 75 g of glucose, also is used but is less convenient. It is most typically used to identify pregnant women with gestational diabetes (see p. 342).]

> When blood glucose is greater than 180 mg/dl, the ability of the kidneys to reclaim glucose is impaired. This results in glucose "spilling" into the 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 in type 1 diabetes

The metabolic abnormalities of T1D mellitus result from a deficiency of insulin that profoundly affects metabolism in three tissues: liver, muscle, and adipose (Figure 25.3).

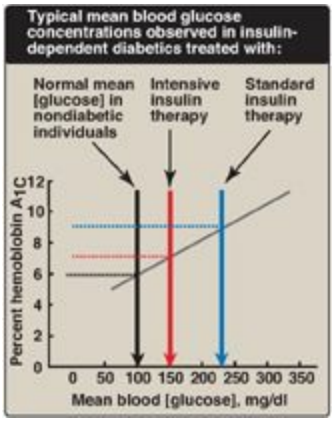
Figure 25.3 Intertissue relationships in type 1 diabetes. TCA = tricarboxylic acid; CoA = coenzyme A; VLDLs = very-low-density lipoproteins; GLUT = glucose transporter.



1. **Hyperglycemia and ketoacidosis:** Elevated levels of blood glucose and ketone bodies are the hallmarks of untreated T1D (see Figure 25.3). Hyperglycemia is caused by increased hepatic production of glucose via gluconeogenesis, combined with diminished peripheral utilization (muscle and adipose tissue have the insulin-sensitive glucose transporter GLUT-4; see p. 97). Ketosis results from increased mobilization of fatty acids (FAs) from adipose tissue, combined with accelerated hepatic FA β-oxidation and synthesis of 3-hydroxybutyrate and acetoacetate. [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, occurs in 25%–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 FAs flooding the liver can be disposed of through oxidation or ketone body synthesis. These excess fatty acids are converted to triacylglycerol (TAG), which is packaged and secreted in very-low-density lipoproteins ([VLDLs] see p. 231). Chylomicrons are synthesized from dietary lipids by the intestinal mucosal cells following a meal (see p. 178). Because lipoprotein degradation catalyzed by lipoprotein lipase in the capillary beds of adipose tissue (see. p. 228) is low in diabetics (synthesis of the enzyme is decreased when insulin levels are low), the plasma chylomicron and VLDL levels are elevated, resulting in hypertriacylglycerolemia (see Figure 25.3).

Figure 25.4 Correlation between mean blood glucose and hemoglobin A_{1C} in patients with type 1 diabetes.

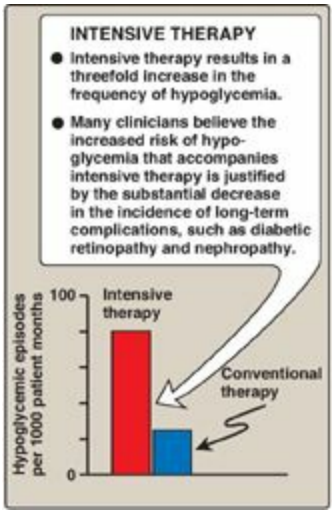


C. Treatment of type 1 diabetes

Individuals with T1D must rely on exogenous insulin delivered subcutaneously either by periodic injection or continuous pump-assisted infusion to control the hyperglycemia and ketoacidosis. Two therapeutic regimens are currently in use, standard and intensive insulin treatment.

1. **Standard treatment versus intensive treatment:** Standard treatment typically consists of one or two daily injections of recombinant human insulin. Mean blood glucose levels obtained are typically in the 225–275 mg/dl range, with a glycosylated hemoglobin (HbA_{1c}) level (see p. 34) of 8%–9% of the total hemoglobin (blue arrow in Figure 25.4). [Note: The rate of formation of HbA_{1c} is proportional to the average blood glucose concentration over the previous 3 months. Thus, HbA_{1c} provides a measure of how well treatment has normalized blood glucose in the diabetic patient over that time.] In contrast to standard therapy, intensive treatment seeks to more closely normalize blood glucose through more frequent monitoring and subsequent injections of insulin, typically three or more times a day. Mean blood glucose levels of 150 mg/dl can be achieved, with HbA_{1c} approximately 7% of the total hemoglobin (see red arrow in Figure 25.4). [Note: Normal mean blood glucose is approximately 100 mg/dl, and HbA_{1c} is 6% or less (see black arrow in Figure 25.4).] Therefore, normalization of glucose values (euglycemia) is not achieved even in intensively treated patients. Nonetheless, patients on intensive therapy show a 50% or more reduction in the long-term microvascular complications of diabetes (that is, 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.

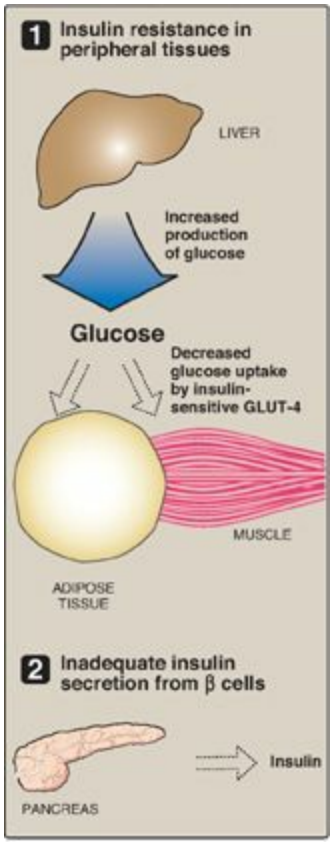
Figure 25.5 Effect of tight glucose control on hypoglycemic episodes in a population of patients on intensive therapy or conventional therapy.



2. **Hypoglycemia in type 1 diabetes:** 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. 344 for a discussion of the chronic complications of diabetes). However, appropriate dosage of insulin is difficult to achieve. Hypoglycemia caused by excess insulin is the most common complication of insulin therapy, occurring in over 90% of patients. The frequency of hypoglycemic episodes, coma, and seizures is particularly high with intensive treatment regimens designed to achieve tight control of blood glucose (Figure 25.5). Recall that in normal individuals, hypoglycemia triggers a compensatory secretion of counterregulatory hormones, most notably glucagon and epinephrine, which promote hepatic production of glucose. 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. These patients, therefore, 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. Exercise promotes glucose uptake into muscle and decreases the need for exogenous insulin. Patients are advised, therefore, to check blood glucose levels before or after intensive exercise to prevent or abort hypoglycemia.

3. **Contraindications for 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 over 90% of the diabetic population in the United States. [Note: American Indians, Alaskan Natives, Hispanic and Latino Americans, African Americans, and Asian Americans have the highest prevalence.] 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 (Figure 25.6) but do not require insulin to sustain life, although insulin eventually will be required to control hyperglycemia and keep HbA_{1c} below 7% in over 90% of patients. 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. (Recall that insulin suppresses the release of glucagon.) 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.]

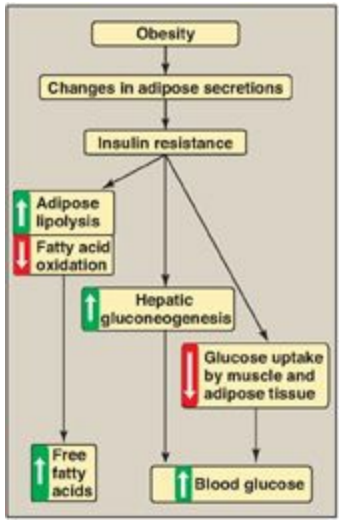
> T2D is characterized by hyperglycemia; insulin resistance; impaired insulin secretion; and, ultimately, β-cell failure. The eventual need for insulin therapy has eliminated the designation of T2D as "noninsulin-dependent" diabetes.

A. Insulin resistance

Insulin resistance is the decreased ability of target tissues, such as liver, adipose, and 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 ([FFAs] Figure 25.7).

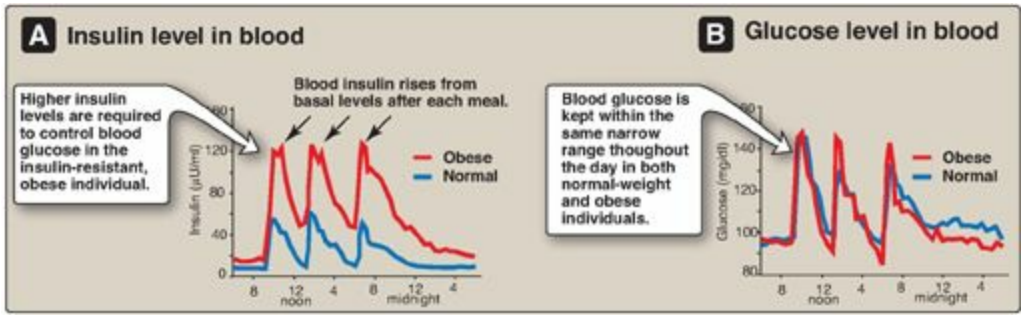
1. **Insulin resistance and obesity:** Although obesity is the most common cause of insulin resistance and T2D, most people with obesity and insulin resistance do not become diabetic. In the absence of a defect in β-cell function, nondiabetic, obese individuals can compensate for insulin resistance with elevated levels of insulin. For example, Figure 25.8A shows that insulin secretion is two to three times higher in obese subjects than it is in lean individuals. 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 (Figure 25.8B).

Figure 25.7 Obesity, insulin resistance, and hyperglycemia. [Note: Inflammation also is associated with insulin resistance.]



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 are obese, physically inactive, or elderly and in the 3%–5% of pregnant women who develop gestational diabetes. These patients are unable to sufficiently compensate for insulin resistance with increased insulin release. Figure 25.9 shows the time course for the development of hyperglycemia and the loss of β-cell function.

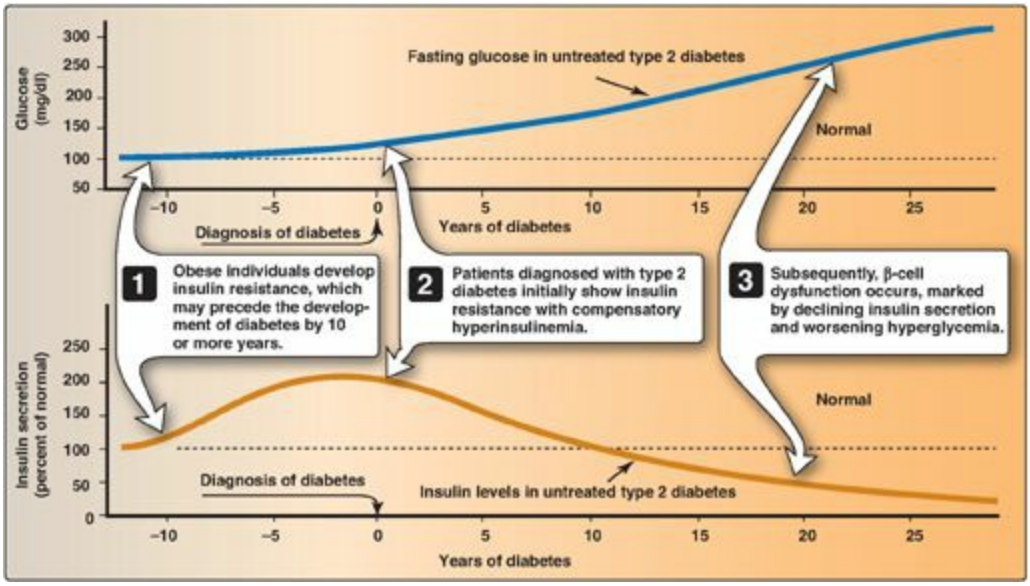
Figure 25.8 Blood insulin and glucose levels in normal-weight and obese subjects.



3. **Causes of insulin resistance:** Insulin resistance increases with weight gain and decreases with weight loss, and excess adipose tissue is key in the development of insulin resistance (see Figure 25.7). Adipose tissue is not simply an energy storage organ, but also a secretory organ. With obesity, there are changes in adipose secretions that result in insulin resistance. These include secretion of proinflammatory cytokines such as interleukin 6 (inflammation is associated with insulin resistance); increased synthesis of leptin, a protein with proinflammatory effects (see p. 353 for additional effects of leptin); and decreased secretion of adiponectin, an adipocyte protein with anti-inflammatory effects. One effect of insulin resistance is increased lipolysis and production of FFAs. FFA availability

decreases use of glucose, contributing to hyperglycemia, and increases deposition of TAG in liver (hepatic steatosis). FFAs also have a proinflammatory effect. In the long-term, FFAs suppress glucose-induced insulin release. [Note: Adiponectin increases FA β-oxidation (see p. 353). Consequently, a decrease in this adipocyte protein contributes to FFA availability.]

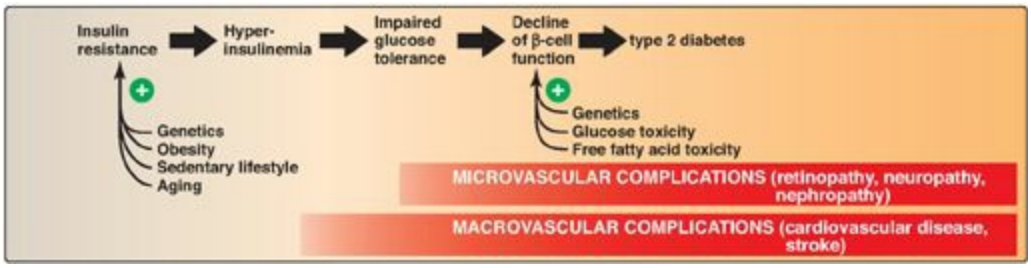
Figure 25.9 Progression of blood glucose and insulin levels in patients with type 2 diabetes.



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 typical, obese, T2D patients but not as high as in similarly obese individuals who are nondiabetic. Thus, the natural progression of the disease results in a declining ability to control hyperglycemia with endogenous secretion of insulin (Figure 25.10). Deterioration of β-cell function may be accelerated by the toxic effects of sustained hyperglycemia and elevated FFAs and a proinflammatory environment.

Figure 25.10 Typical progression of type 2 diabetes.



C. Metabolic changes in type 2 diabetes

The metabolic abnormalities of T2D are the result of insulin resistance expressed primarily in liver, muscle, and adipose tissue (Figure 25.11).

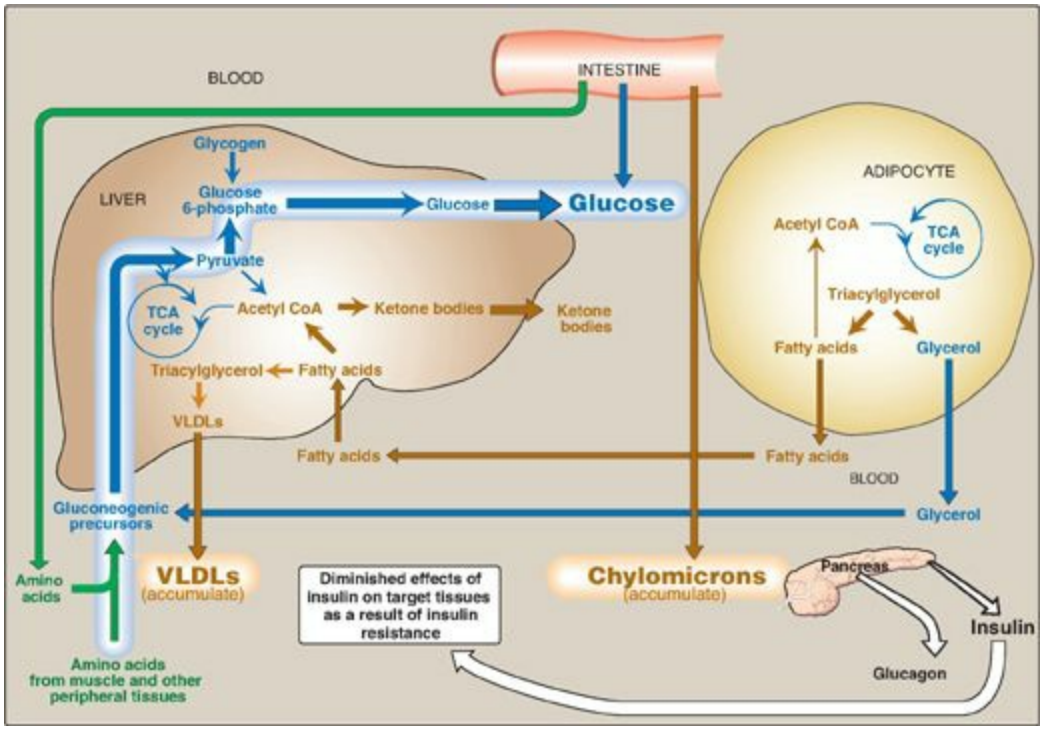
1. **Hyperglycemia:** Hyperglycemia is caused by increased hepatic production of glucose, combined with diminished peripheral use. Ketosis is usually minimal or absent in patients with T2D because the presence of insulin, even in the presence of insulin resistance, restrains hepatic ketogenesis.

2. **Dyslipidemia:** In the liver, FAs are converted to TAGs, which are packaged and secreted in VLDL. Chylomicrons are synthesized from dietary lipids by the intestinal mucosal cells following a meal (see p. 177). Because lipoprotein degradation catalyzed by lipoprotein lipase in adipose tissue is low in diabetics, the plasma chylomicron and VLDL levels are elevated, resulting in hypertriacylglycerolemia (see Figure 25.10). Low levels of high-density lipoproteins are also associated with T2D, likely as a result of increased degradation.

D. Treatment of type 2 diabetes

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) often correct the hyperglycemia of newly diagnosed T2D. Hypoglycemic agents (for example, metformin, which decreases hepatic output of glucose), sulfonylureas (increase insulin secretion; see p. 310), thiazolidinediones (increase peripheral insulin sensitivity), α-glucosidase inhibitors (decrease absorption of dietary carbohydrate) or insulin therapy may be required to achieve satisfactory plasma glucose levels. [Note: Bariatric surgery in morbidly obese individuals with T2D has been shown to result in disease remission in most patients. Remission may not be permanent.]

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; VLDL = very-low-density lipoprotein.


BLOOD
INTESTINE
Glycogen
LIVER
Glucose 6-phosphate
Glucose
Glucose
ADIPOCYTE
Acetyl CoA
TCA cycle
Pyruvate
Triacylglycerol
TCA cycle
Acetyl CoA
Ketone bodies
Ketone bodies
Fatty acids
Glycerol
Triacylglycerol
Fatty acids
VLDLs
Fatty acids
Fatty acids
BLOOD
Gluconeogenic precursors
Glycerol
Amino acids
VLDLs
(accumulate)
Diminished effects of insulin on target tissues as a result of insulin resistance
Chylomicrons
(accumulate)
Pancreas
Insulin
Glucagon
Amino acids from muscle and other peripheral tissues

IV. CHRONIC EFFECTS AND PREVENTION OF DIABETES

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 complications of diabetes including premature atherosclerosis as well as cardiovascular disease and stroke, retinopathy, nephropathy, and neuropathy. Intensive treatment with insulin (see p. 340) delays the onset and slows the progression of these long-term complications. For example, the incidence of retinopathy decreases as control of blood glucose improves and HbA_{1c} levels decrease (Figure 25.12). 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 entry of glucose is not dependent on insulin, elevated blood glucose leads to increased intracellular glucose and its metabolites. For example, increased intracellular sorbitol contributes to the formation of cataracts (see p. 140) in diabetics. Additionally, hyperglycemia promotes the nonenzymic condensation of glucose with cellular proteins in a reaction analogous to the formation of HbA_{1c} (see p. 34). 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. AGEs bind to their receptors (RAGEs), 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 25.12 shows the incidence of disease in normal and overweight individuals with varying degrees of exercise. The beneficial effect of intensive therapy on cardiovascular disease has not been shown in individuals with long-standing T2D. In contrast, initial intensive control in individuals with newly diagnosed diabetes has long-term benefit in decreasing the risk of myocardial infarction, diabetes-related death, and overall death. The clinical evidence, thus, supports initiating intensive therapy with the goal of lowering HbA_{1c} levels to below 7% as early as possible in the course of diabetes.

Figure 25.12 Relationship of glycemic control and diabetic retinopathy. Hb = hemoglobin.

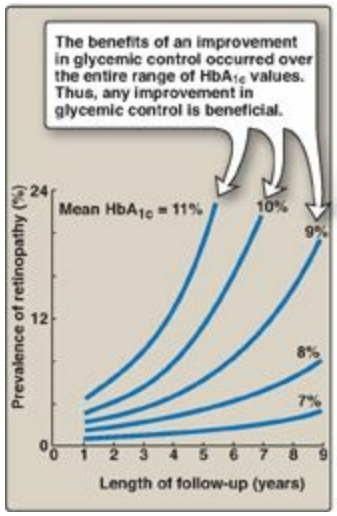
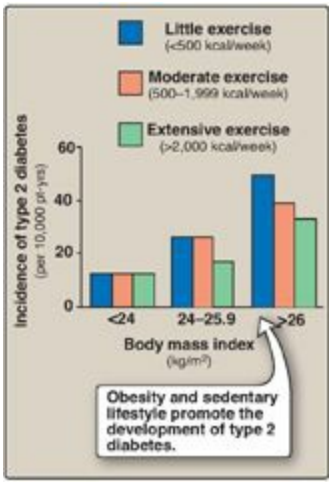


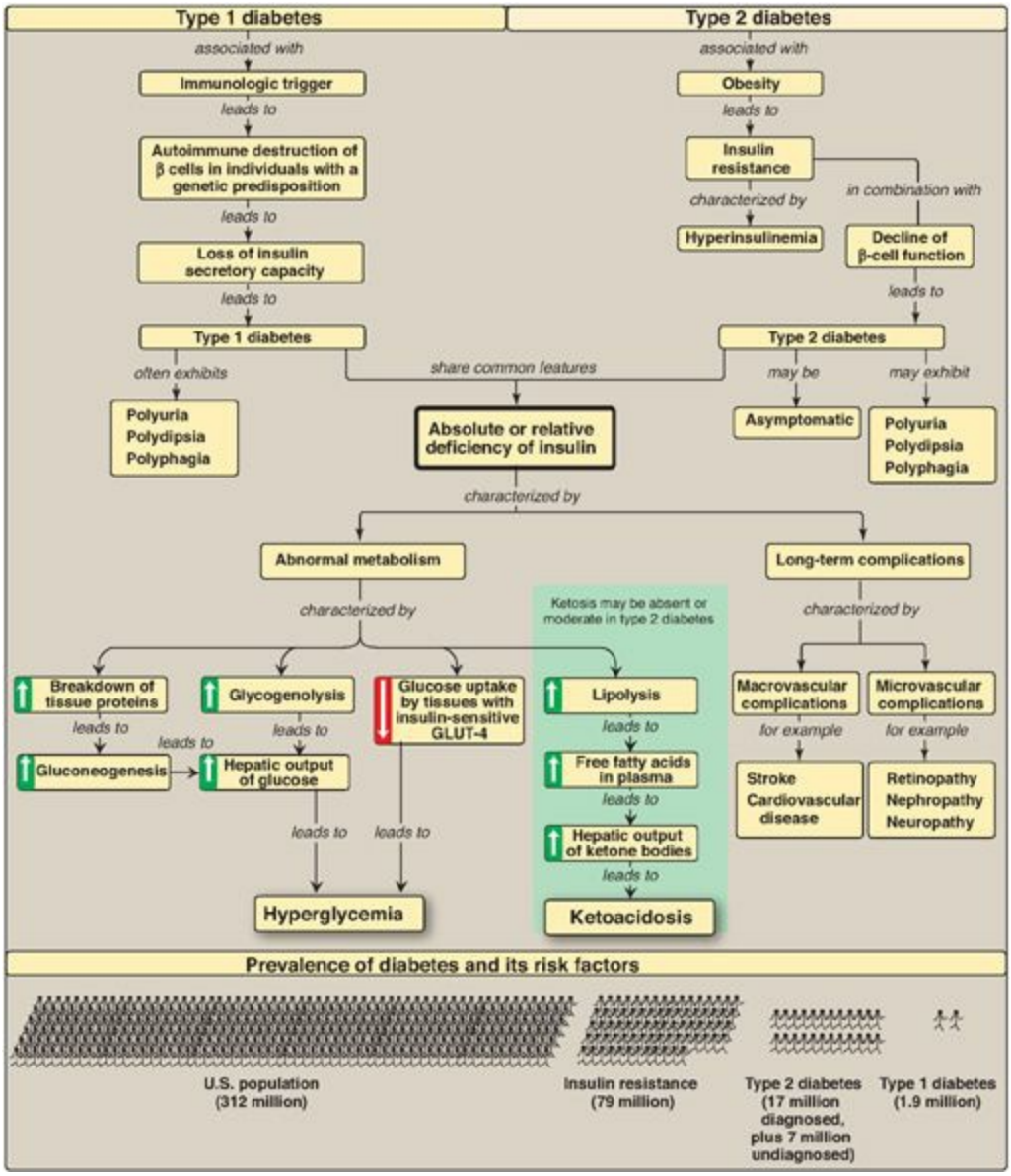
Figure 25.13 Effect of body weight and exercise on the development of type 2 diabetes.



V. CHAPTER SUMMARY

Diabetes mellitus is a heterogeneous group of syndromes characterized by an **elevation of fasting blood glucose** that is caused by a relative or absolute deficiency of insulin (Figure 25.14). 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, **type 1** (T1D) and **type 2** (T2D). **Type 1** diabetics constitute approximately 10% of the nearly 26 million diabetics in the United States. The disease is characterized by an **absolute deficiency of insulin** caused by an **autoimmune attack** on the β **cells of the pancreas**. This destruction requires a **stimulus from the environment** (such as a viral infection) and a **genetic determinant** that allows the β cell to be recognized as "nonself." The **metabolic abnormalities** of T1D mellitus include **hyperglycemia**, **ketoacidosis**, and **hypertriacylglycerolemia** that result from a deficiency of insulin. Type 1 diabetics 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, adipose, and 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 become diabetic. In the absence of a defect in β-cell function, **nondiabetic**, **obese individuals** 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 **metabolic alterations** observed in T2D are **milder** than those described for the insulin-dependent form of the disease, in part, because insulin secretion in T2D, although inadequate, does restrain ketogenesis and blunts the development of diabetic ketoacidosis. 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 **premature atherosclerosis** (**macrovascular**) as well as **retinopathy**, **nephropathy**, and **neuropathy** (**microvascular**).

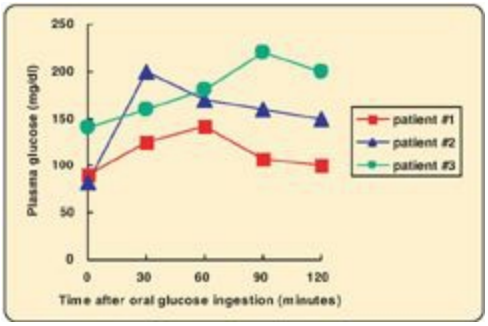
Figure 25.14 Key concept map for diabetes. GLUT = glucose transporter.


Type 1 diabetes
Type 2 diabetes
associated with
Immunologic trigger
leads to
Autoimmune destruction of β cells in individuals with a genetic predisposition
leads to
Loss of insulin secretory capacity
leads to
Type 1 diabetes
often exhibits
Polyuria
Polydipsia
Polyphagia
associated with
Obesity
leads to
Insulin resistance
characterized by
Hyperinsulinemia
in combination with
Decline of β-cell function
leads to
Type 2 diabetes
may be
Asymptomatic
may exhibit
Polyuria
Polydipsia
Polyphagia
share common features
Absolute or relative deficiency of insulin
characterized by
Abnormal metabolism
characterized by
Breakdown of tissue proteins
leads to
Gluconeogenesis
Glycogenolysis
leads to
Hepatic output of glucose
Glucose uptake by tissues with insulin-sensitive GLUT-4
leads to
leads to
Hyperglycemia
Ketosis may be absent or moderate in type 2 diabetes
Lipolysis
leads to
Free fatty acids in plasma
leads to
Hepatic output of ketone bodies
leads to
Ketoacidosis
Long-term complications
characterized by
Macrovascular complications
for example
Stroke
Cardiovascular disease
Microvascular complications
for example
Retinopathy
Nephropathy
Neuropathy
Prevalence of diabetes and its risk factors
U.S. population (312 million)
Insulin resistance (79 million)
Type 2 diabetes (17 million diagnosed, plus 7 million undiagnosed)
Type 1 diabetes (1.9 million)

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. 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 is diabetic.

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 adipose tissue. Low insulin also causes activation of hormone-sensitive lipase, 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 A1c levels. Ketoacidosis is rare in T2D, whereas obesity is rare in T1D. 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 obese individual with type 2 diabetes typically:

A. benefits from receiving insulin about 6 hours after a meal.
B. has a lower 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. Most type 2 diabetics 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.

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, 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 two decades. [Note: Approximately 17% of those ages 2-19 years are obese.] In the United States, the lifetime risk of becoming overweight or obese is approximately 50% and 25%, respectively. Obesity has increased globally. In fact, by some estimates, there are more obese than undernourished individuals worldwide.

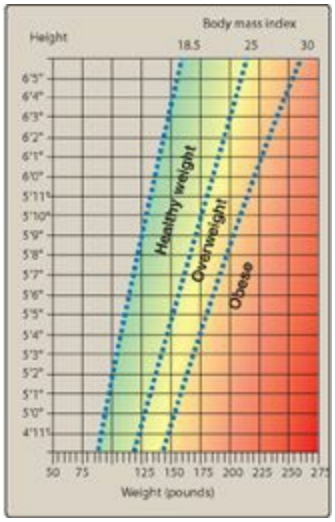
II. ASSESSMENT OF OBESITY

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 inches in men and ≥ 35 inches in women is considered a risk factor.]

A. Body mass index

The BMI (weight in kg)/(height in meters)^2 provides a measure of relative weight, adjusted for height. This allows comparisons both 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 equal to or greater than 30 are defined as obese, and a BMI over 40 is considered extremely obese. Anyone more than 100 pounds overweight is considered severely (morbidly) obese (Figure 26.1). These cutoffs are based on the studies examining the relationship of BMI to premature death and are similar in men and women. Nearly two thirds of American adults are overweight, and more than one third of those are obese.

Figure 26.1 Body mass index (BMI) Chart. To use the BMI Chart, find height in the lefthand column. Move across the row to weight. Height and weight intersect at the individual's BMI. [Note: To calculate BMI using inches and pounds, use BMI = [weight in pounds/ (height in inches)^2] x 703.



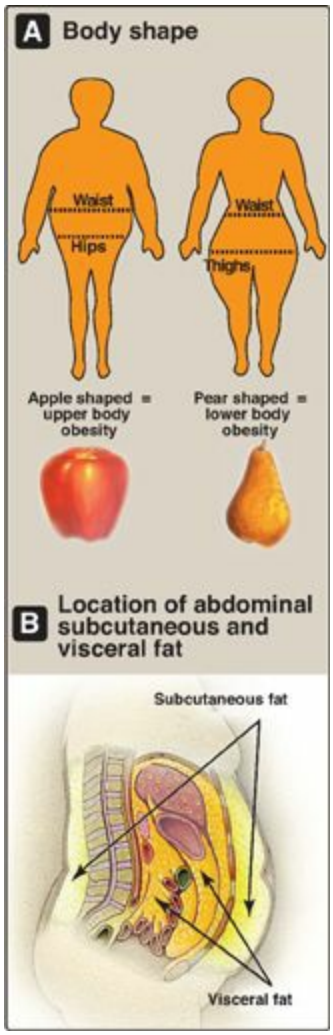
B. Anatomic differences in fat deposition

The anatomic distribution of body fat has a major influence on associated health risks. A waist-to-hip ratio of more than 0.8 for women and more than 1.0 for men is defined as android, "apple-shaped," or upper body obesity, and is associated with more fat

deposition in the trunk (Figure 26.2A). In contrast, a lower waist/hip ratio 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 waist/hip ratio of less than 0.8 for women and less than 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.

About 80%–90% of the fat stored in the human body is in subcutaneous depots, just under the skin, in the abdominal (upper body) and the gluteal-femoral (lower body) regions. In addition, 10%–20% of body fat is stored in so-called visceral depots (omental and mesenteric), which are located within the abdominal cavity in close association with the digestive tract (Figure 26.2B). Excess fat in visceral and abdominal subcutaneous stores increases health risks 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.



C. Biochemical differences in regional fat depots

The regional types of fat described above are biochemically different. Subcutaneous adipocytes from the lower body (gluteal-femoral), particularly in women, are larger, very efficient at fat (triacylglycerol [TAG]) deposition, and tend to mobilize fatty acids more slowly than those from the abdominal subcutaneous depots. Visceral adipocytes are the most metabolically active. Both abdominal subcutaneous and visceral depots of obese subjects have high rates of lipolysis and contribute to increased availability of free fatty acids (FFAs). These metabolic differences may contribute to the higher risk found in individuals with upper body obesity.

1. **Endocrine function:** White adipose tissue, once thought to be a passive reservoir of TAGs, 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, such as the hormones leptin and adiponectin. Leptin regulates appetite as well as metabolism (see p. 353). Adiponectin reduces levels of FFAs in the blood and has been associated with improved lipid profiles, increased insulin sensitivity resulting in better glycemic control, and reduced inflammation in diabetic patients. [Note: Adiponectin levels decrease as body weight increases, and leptin levels increase.]

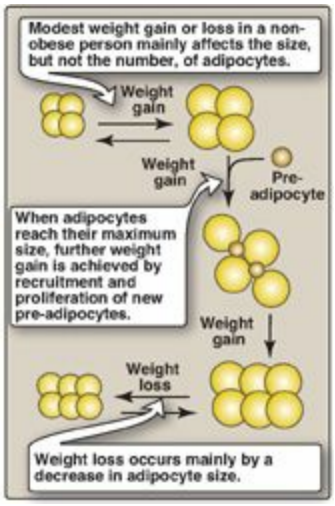
2. **Importance of portal circulation:** With obesity, there is increased release of FFAs and secretion of proinflammatory cytokines, such as interleukin 6 (IL-6), from adipose tissue. [Note: Cytokines are small proteins that regulate the immune system.] One reason that visceral and abdominal adipose depots may have such a large influence on metabolic dysfunction in obesity is that the FFAs 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. 342) and increased hepatic synthesis of TAGs, which are released as components of very-low-density lipoprotein particles and contribute to the hypertriacylglycerolemia associated with obesity. By contrast, FFAs from lower body subcutaneous adipose depots enter the general circulation, where they can be oxidized in muscle and, therefore, reach the liver in lower concentration.

D. Size and number of fat cells

As TAGs are stored, adipocytes can expand to an average of two to three times their normal volume. (Figure 26.3). However, the ability of a fat cell 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). 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.

Obese individuals can have up to five times the normal number of fat cells. If excess calories cannot be accommodated within adipose tissue, the excess fatty acids "spill over" into other tissues, such as muscle and liver. The amount of this so-called "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 of fat cells is not usually affected. Thus, a normal body fat is achieved by decreasing the size of the fat cell below normal. Small fat cells are very efficient at reaccumulating fat, and this may drive appetite and weight regain.

Figure 26.3 Hypertrophic (increased size) and hyperplastic (increased number) changes to adipocytes are thought to occur in severe obesity.



III. BODY WEIGHT REGULATION

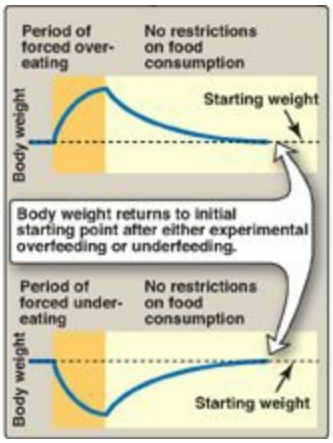
The body weight of most individuals tends to be relatively stable over time. This observation prompted the hypothesis that each individual has a biologically predetermined "set point" for body weight. The body attempts to add to adipose stores when the body weight falls below the set point and to lose adipose from stores when the body weight is higher than 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 (Figure 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 to obesity

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 BMIs (Figure 26.5), whether reared together or apart, and their BMIs are more similar than those of nonidentical, dizygotic twins.

Figure 26.4 Weight changes following episodes of overfeeding or underfeeding followed by feeding with no restrictions.



2. **Mutations:** Rare, single gene mutations can cause human obesity. For example, mutations in the gene for the adipocyte hormone leptin or its receptor produce hyperphagia (increased appetite for and consumption of food) and massive obesity (Figure 26.6), underscoring the importance of the leptin system in regulating human body weight (Section IV). Most obese humans have elevated leptin levels but appear to be resistant to the appetite-regulating effects of this hormone.

Figure 26.5 Identical twins with combined weight of 1,300 pounds. Note similarity in

body shape.

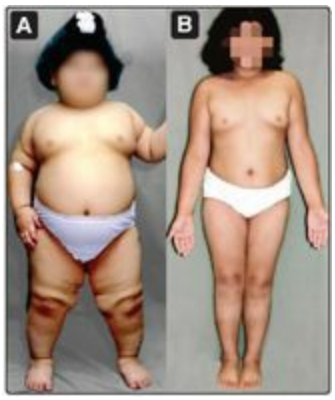
B. Environmental and behavioral contributions

The epidemic of obesity occurring over the last decade 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 in the increased prevalence of obesity. Furthermore, sedentary lifestyles encouraged by TV watching, automobiles, computer usage, and energy-sparing devices in the workplace and at home decrease physical activity and enhance the tendency to gain weight. Eating behaviors, such as snacking, portion size, variety of foods consumed, an individual's unique food preferences, and the number of people present during eating also influence food consumption. It is important to note, however, that in this same environment, many individuals 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. Epigenetic changes (see p. 409), therefore, likely influence the risk for obesity.

IV. MOLECULES THAT INFLUENCE OBESITY

The cause of obesity can be summarized in a deceptively simple statement 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 psychologic 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 (Figure 26.7).

Figure 26.6 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 injection of recombinant leptin.



A. Long-term signals

Long-term signals reflect the status of TAG stores.

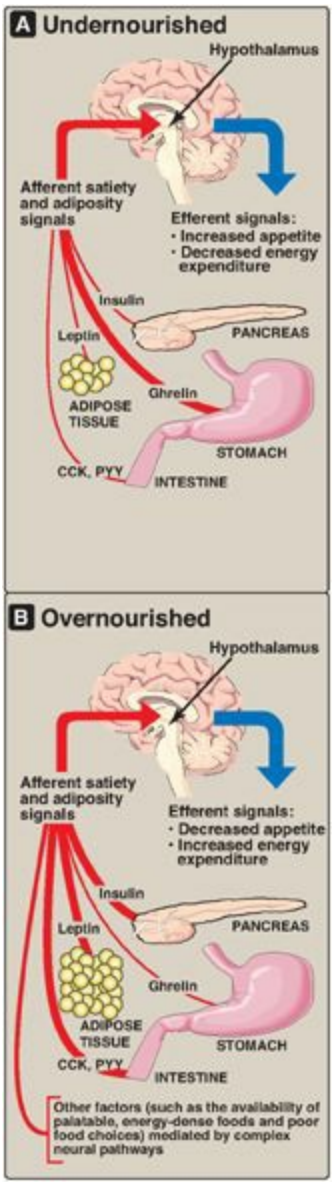
1. **Leptin:** Leptin is an adipocyte peptide hormone that is secreted in proportion to the size of fat stores. When we consume fewer calories than we need, body fat declines, and leptin production from the fat cell decreases. The body adapts by minimizing energy utilization (decreasing activity) and increasing appetite. Unfortunately, in many individuals, the leptin system may be better at preventing weight loss than preventing weight gain. Although a meal or overeating increases leptin and this should, in theory, also dampen appetite (an anorexigenic effect) and prevent overconsumption of calories, other cues that stimulate appetite can apparently overcome the leptin system in many individuals. [Note: Leptin's effects are mediated through binding to its receptors in the arcuate nucleus of the hypothalamus.]

2. **Insulin:** Obese individuals are also hyperinsulinemic. Like leptin, insulin acts on hypothalamic neurons to dampen appetite. (See Chapter 23 for the effects of insulin on metabolism.)

B. Short-term signals

Short-term signals from the gastrointestinal 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, gut hormones, including cholecystokinin (CCK) 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 neuropeptide Y (orexigenic) and α-melanocyte–stimulating hormone (α-MSH), which is anorexigenic, and neurotransmitters, such as serotonin and dopamine, are important in regulating hunger and satiety. Long-term and short-term signals interact, insofar as leptin can affect the sensitivity of hypothalamic neurons to short-term signals such as CCK. Thus, there are many and complex regulatory loops that control the size and number of meals in relationship to the status of body fat stores. [Note: α-MSH binds to the melanocortin-4 receptor (MC4R). Loss-of-function mutations to MC4R are associated with early-onset obesity.]

Figure 26.7 Some signals that influence appetite and satiety. CCK = cholecystokinin, PYY = peptide YY.



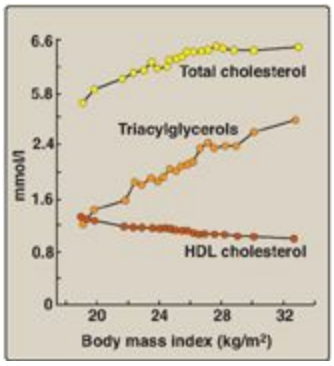
V. METABOLIC CHANGES IN OBESITY

The primary metabolic effects of obesity include dyslipidemias, glucose intolerance, and insulin resistance expressed primarily in the liver, muscle, and adipose tissue. These metabolic abnormalities reflect molecular signals originating from the increased mass of adipocytes. (see Figure 25.7 and Figure 26.7)

A. Metabolic syndrome

Abdominal obesity is associated with a cluster of metabolic abnormalities that is referred to as the metabolic syndrome and includes glucose intolerance (hyperglycemia below that classified as diabetes; see p. 338), insulin resistance, hyperinsulinemia, dyslipidemia (low levels of high-density lipoprotein and elevated TAGs), and hypertension (Figure 26.8). The metabolic syndrome is also associated with a state of low-grade, chronic, systemic inflammation that contributes to the pathogenesis of insulin resistance and atherosclerosis. In obesity, adipocytes release proinflammatory mediators such as IL-6. Additionally, low levels of the adipocyte hormone adiponectin that normally dampens inflammation and sensitizes tissues, especially the liver, to insulin, may contribute to the metabolic syndrome and, therefore, the risk of T2D and heart disease.

Figure 26.8 Body mass index and changes in blood lipids. HDL= high-density lipoprotein.



VI. OBESITY AND HEALTH

Obesity is correlated with an increased risk of death (Figure 26.9) and is a risk factor for a number of chronic conditions, including T2D, dyslipidemias, hypertension, heart disease, some cancers, gallstones, arthritis, gout, pelvic floor disorders (for example, urinary incontinence), nonalcoholic fatty liver disease, and sleep apnea. The relationship between obesity and associated morbidities is stronger among individuals younger than age 55 years. After age 74 years, there is no longer an association between increased BMI and mortality. Weight loss in obese individuals leads to decreased blood pressure, serum TAGs, and blood glucose levels. High-density lipoproteins increase.

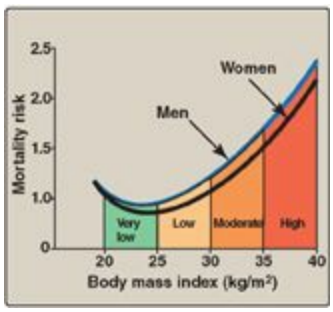
VII. WEIGHT REDUCTION

Weight reduction can help reduce the complications of obesity, including T2D and hypertension. 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 prescription for weight reduction combines dietary change; increased physical activity; and behavioral modification, which can include nutritional education and meal planning, recording and monitoring food intake through food diaries, modifying factors that lead to overeating, and relearning cues to satiety. 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. 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 cardiovascular disease, independent of weight loss. Persons who combine caloric restriction and exercise with behavioral treatment may expect to lose about 5%–10% of initial body weight over a period of 4–6 months. Studies show that individuals who maintain their exercise program regain less weight after their initial weight loss.

Figure 26.9 Body mass index and the relative risk of death.



B. Caloric restriction

Dieting is the most commonly practiced approach to weight control. Because 1 pound of adipose tissue corresponds to approximately 3,500 kcal, one can estimate the effect that caloric restriction will have on the amount of adipose tissue. Weight loss on calorie-restricted diets is determined primarily by energy intake and not nutrient composition. [Note: Compositional aspects can, however, affect glycemic control and the blood lipid profile.] Caloric restriction is ineffective over the long term for many individuals. More than 90% of people who attempt to lose weight regain the lost weight when dietary intervention is suspended. Nonetheless, it is important to recognize that, 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. The health benefits of even relatively small weight losses should, therefore, be emphasized to the patient.

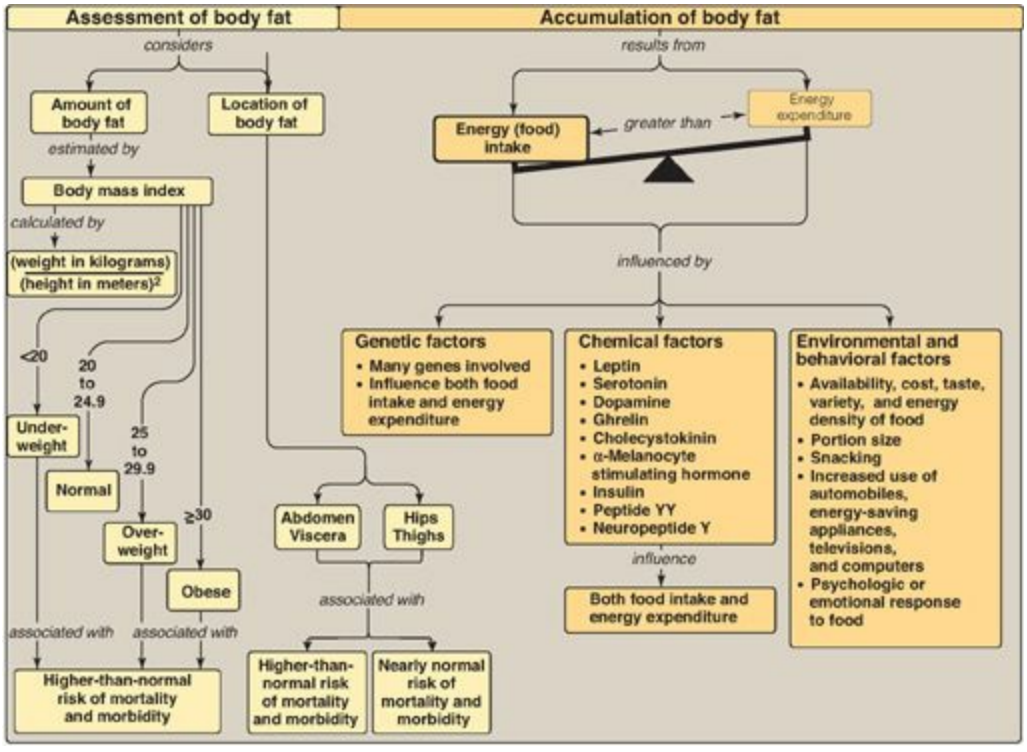
C. Pharmacologic treatment

Several weight-loss medications are currently approved by the U.S. Food and Drug Administration for use in adults with a BMI of 30 or higher. Three approved for long-term use are: 1) orlistat (decreases absorption of dietary fat), 2) lorcaserin (promotes satiety), and 3) a combination of phentermine (suppresses appetite) and extended-release topiramate (controls seizures). [Note: Phentermine monotherapy is approved for short-term use only.] Their effects on weight reduction tend to be modest, and weight regain upon termination of drug therapy is common.

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 poor blood sugar control in morbidly obese diabetic individuals.

Figure 26.10 Key concept map for obesity.



VIII. CHAPTER SUMMARY

Obesity, the accumulation of excess body fat, results when energy (caloric) intake exceeds energy expenditure. 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 **body mass index** (**BMI**) is easy to determine and highly correlated to body fat. Nearly two thirds of U.S. adults are **overweight** (BMI ≥ 25 kg/m^2) and more than one third of this group are **obese** (BMI ≥ 30 kg/m^2). The anatomic distribution of body fat has a major influence on associated health risks. Excess fat located in the **central abdominal** area 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. A person's weight is determined by genetic and environmental factors. **Appetite** is influenced by **afferent**, or incoming, **signals** (that is, neural signals, circulating hormones, and metabolites) that are integrated by the **hypothalamus**. These diverse signals prompt release of hypothalamic peptides 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**. 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.

Study Questions

Choose the ONE best answer.

For Questions 26.1 and 26.2:

A 40-year-old woman, 5 feet, 1 inch (155 cm) tall and weighing 188 pounds (85.5 kg), seeks your advice on how to lose weight. Her waist measured 41 inches and her hips 39 inches. The remainder of the physical examination and the blood laboratory data were all within the normal range. Her only child (who is 14 years old), 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 5–25 pounds each time. On discontinuation of the diets, she regained weight, returning to 185–190 pounds.

26.1 Calculate and interpret the body mass index for the patient.

Body mass index (BMI) = weight (kg)/height (m^2) = $85.5/(1.55)^2$ = 35.6 kg/m^2. Because her BMI is greater than 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-to-hip ratio is 41/39 = 1.05. Apple shape is defined as a waist/hip ratio of more than 0.8 for women and more than 1.0 for men. She has, therefore, 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 an adipose depot made up of too many adipocytes, each fully loaded with triacylglycerols (TAGs). Plasma leptin levels are proportional to fat mass, suggesting that resistance to leptin, rather than its deficiency,

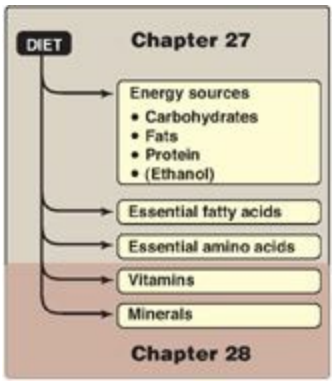
occurs in human obesity. Adiponectin levels decrease. The elevated circulating fatty acids characteristic of obesity are carried to the liver and converted to TAGs. The TAGs are released as components of very-low-density lipoproteins, resulting in elevated serum TAG levels.

Nutrition 27

I. OVERVIEW

Nutrients are the constituents of food necessary to sustain the normal functions of the body. All energy is provided by three classes of nutrients: fats, carbohydrates, and protein, with ethanol providing calories in some diets (Figure 27.1). Because the intake of these energy-rich molecules is larger than that of the other dietary nutrients, they are called macronutrients. This chapter focuses on the kinds and amounts of macronutrients that are needed to maintain optimal health and prevent chronic disease in adults. Those nutrients needed in lesser amounts, vitamins and minerals, are called micronutrients and are considered in Chapter 28.

Figure 27.1 Essential nutrients obtained from the diet. [Note: Ethanol is not considered an essential component of the diet but may provide a significant contribution to the daily caloric intake of some individuals.]



II. DIETARY REFERENCE INTAKES

Committees of U.S. 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 (DRIs), 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 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.

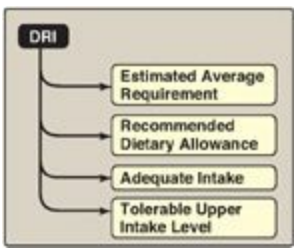
A. Definition of Dietary Reference Intake

The DRIs consist of four dietary reference standards for the intake of nutrients designated for specific life stage (age) groups, physiologic states, and gender (Figure 27.2).

1. **Estimated Average Requirement:** The average daily nutrient intake level estimated to meet the requirement of one half of the healthy individuals in a particular life stage and gender group is the Estimated Average Requirement (EAR). It is useful in estimating the actual requirements in groups and individuals.

2. **Recommended Dietary Allowance:** The RDA is the average daily dietary intake level that is sufficient to meet the nutrient requirements of nearly all (97%–98%) individuals in a life stage and gender group. 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 SDs above the EAR (that is, RDA = EAR + $2SD_{EAR}$).

Figure 27.2 Components of the Dietary Reference Intakes (DRI).



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 4–6 months, is based on the estimated daily mean

nutrient intake supplied by human milk for healthy, full-term infants who are exclusively breastfed.

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 (Figure 27.3). 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. 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% or less (Figure 27.4). Intakes between the EAR and RDA likely need to be improved because the probability of adequacy is less than 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: The DRI is designed to meet the nutritional needs of the healthy and, thus, does not include any special needs of the sick.]

Figure 27.3 Dietary Reference Intakes for vitamins and minerals in individuals age 1 year and older. EAR = Estimated Average Requirement; RDA = Recommended Dietary Allowance; AI = Adequate Intake; UL = Tolerable Upper Intake Level; – = no value established.

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

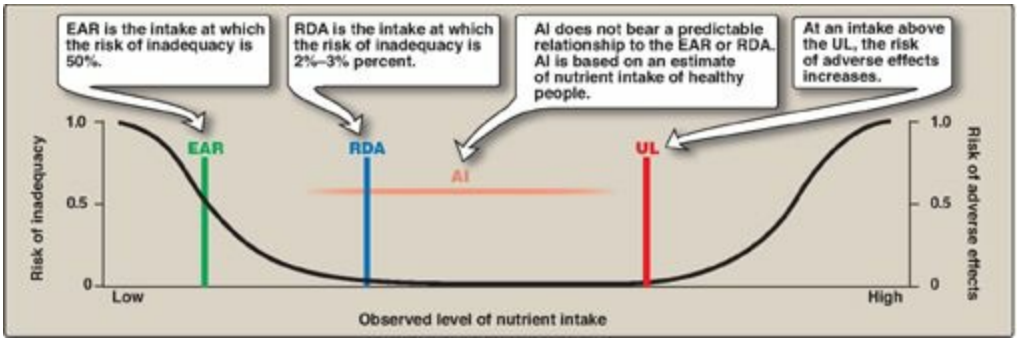
III. ENERGY REQUIREMENT IN HUMANS

The Estimated Energy Requirement (EER) is the average dietary energy intake predicted to maintain an energy balance (that is, when 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 about 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. [Note: The EER that is listed on food labels is either 2,000 or 2,500 kcal/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. 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 a unit of energy widely used in countries other than the United States. One cal = 4.2 J; 1 Cal (1 kcal, 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.]

Figure 27.4 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.

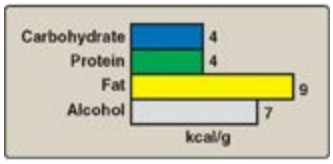


B. Use of food energy in the body

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 thermic effect of food (formerly termed specific dynamic action). The number of calories expended by these processes in a 24-hour period is the total

energy expenditure (TEE).

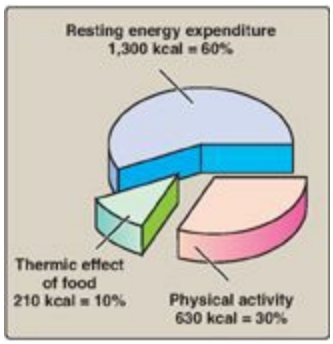
Figure 27.5 Average energy available from the major food components.



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. [Note: A basal metabolic rate (BMR) is determined under more stringent environmental conditions. BMR can be determined by measuring O_2 consumed or CO_2 produced (indirect calorimetry). It also can be estimated using equations that include sex and age (BMR 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/hour for men and 0.9 kcal/kg/hour for women. RMR is about 10% higher than the BMR.] In an adult, the 24-hour RMR, known as the resting energy expenditure (REE), is about 1,800 kcal for men (70 kg) and 1,300 kcal for women (50 kg). From 50%–70% of the TEE in sedentary individuals is attributable to the REE (Figure 27.6). [Note: Hospitalized individuals are commonly hypercatabolic, and an "injury factor" is included in calculating their TEE.]

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. The daily expenditure of energy can be estimated by carefully recording the type and duration of all activities to determine a physical activity factor. In general, a sedentary person requires about 30%–50% more calories than the RMR (see Figure 27.6), whereas a highly active individual may require 100% or more calories above the RMR.

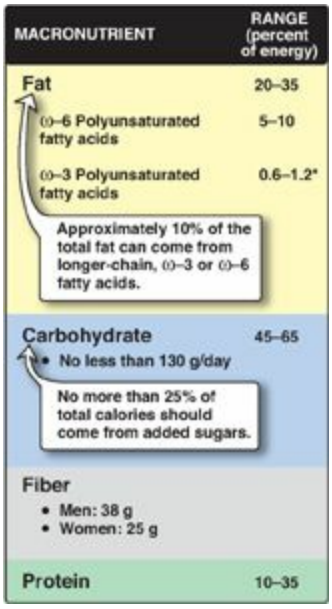
Figure 27.6 Estimated total energy expenditure in a healthy 20-year-old woman, 165 cm (5 feet, 4 inches) tall, weighing 50 kg (110 lb), and engaged in light activity.



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%–10% of the TEE.

Figure 27.7 Acceptable macronutrient distribution ranges in adults. *A growing body of evidence suggests that higher levels of ω-3 polyunsaturated fatty acids provide protection against coronary heart disease.



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%–65% of their total calories from carbohydrates, 20%–35% from fat, and 10%–35% from protein (Figure 27.7). 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 (Figure 27.8). 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.

> Although earlier recommendations emphasized decreasing the total amount of fat and cholesterol in the diet, data now show that the type of fat is a more important risk factor than the total amount of fat consumed.

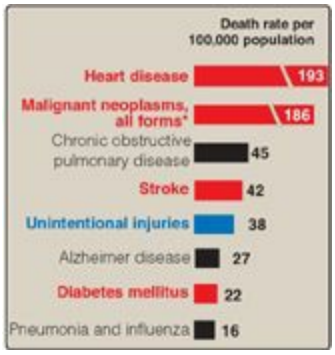
A. Plasma lipids and coronary heart disease

Plasma cholesterol may arise 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 lipoprotein and high-density lipoprotein:** The level of plasma cholesterol is not precisely regulated but, rather, varies in response to the diet. Elevated levels of total cholesterol (hypercholesterolemia) result in an increased risk for CHD (Figure 27.9). A much stronger correlation exists between CHD and the level of cholesterol in low-density lipoproteins ([LDL-C] see p. 232). As LDL-C increases, CHD increases. In contrast, high levels of high-density lipoprotein cholesterol (HDL-C) have been associated with a decreased risk for heart disease (see p. 235). [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 drug treatment 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 of plasma lipoprotein concentrations are modest, typically 10%–20%, whereas treatment with "statin" drugs decreases plasma cholesterol by 30%–60% (see p. 224). [Note: Dietary and drug treatment can lower TAGs.]

Figure 27.8 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. (*Diet plays a role in only some forms of cancer.)



B. Dietary fats and plasma lipids

TAGs are quantitatively the most important class of dietary fats. The influence of TAGs on blood lipids is determined by the chemical nature of their constituent fatty acids. The absence or presence and number of double bonds (saturated versus mono- and polyunsaturated), the location of the double bonds (w-6 versus w-3), and the cis versus trans configuration of the unsaturated fatty acids are the most important structural features that influence blood lipids.

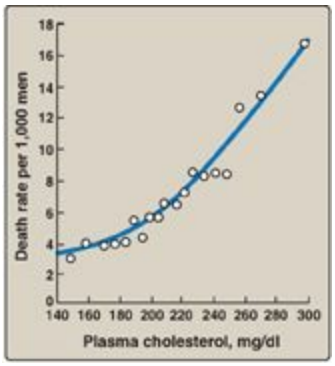
1. **Saturated fat:** TAGs composed primarily of fatty acids whose hydrocarbon chains do not contain any double bonds are referred to as saturated fats. Consumption of saturated fats is positively associated with high levels of total plasma cholesterol and LDL-C and an increased risk of CHD. The main 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; Figure 27.10). Most experts strongly advise limiting intake of saturated fats to less than 10% of total caloric intake.

> Saturated fatty acids with carbon chain lengths of 14 (myristic) and 16 (palmitic) are most potent in increasing the serum cholesterol. 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 fat. Unsaturated fatty acids are generally derived from vegetables and fish. 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. This ability of monounsaturated fats 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. It is recommended that monounsaturated fats account for about 50% of the total fat intake.

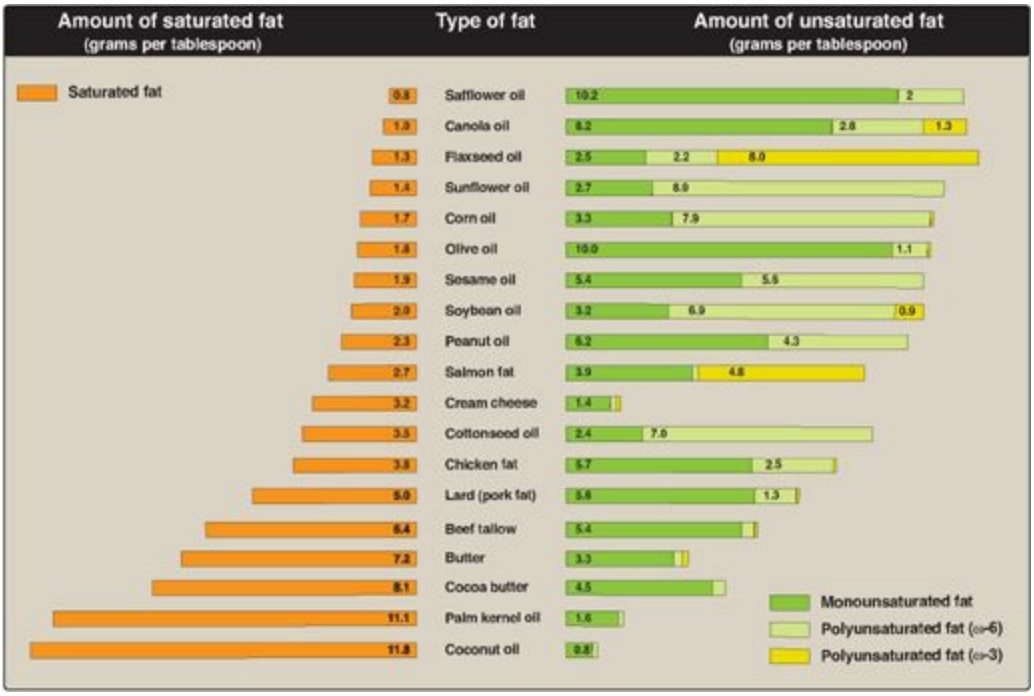
Figure 27.9 Correlation of the death rate from coronary heart disease with the

concentration of plasma cholesterol. [Note: The data were obtained from a 6-year study of men with the death rate adjusted for age.]



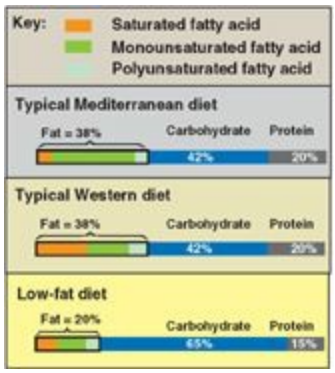
a. The Mediterranean diet: The Mediterranean diet is an example of a diet rich in monounsaturated fatty acids, or MUFAs (from olive oil), and polyunsaturated fatty acids, or PUFAs (from fish oils and some nuts), but low in saturated fat. For example, Figure 27.11 shows the composition of the Mediterranean 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 serum total cholesterol and LDL-C, decreased TAGs, and increased HDL-C when compared with a typical Western diet higher in saturated fats.

Figure 27.10 Compositions of commonly encountered dietary fats.

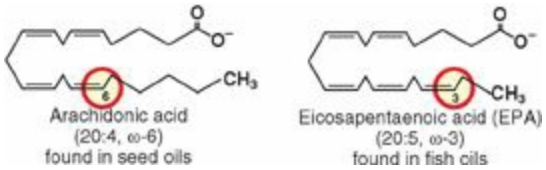


3. **Polyunsaturated fats:** TAGs containing primarily fatty acids with more than one double bond are referred to as polyunsaturated fats. The effects of PUFAs on cardiovascular disease is influenced by the location of the double bonds within the molecule.

Figure 27.11 Composition of typical Mediterranean, Western, and low-fat diets.



a. ω-6 Fatty acids: These are long-chain PUFAs, 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 p. 183).]

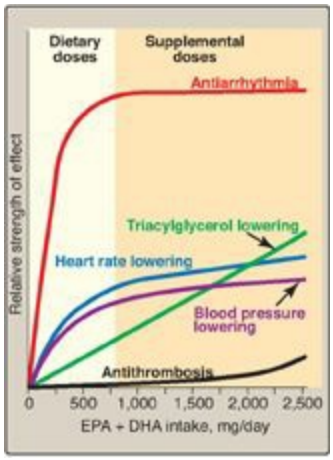


Consumption of fats containing w-6 PUFAs, principally linoleic acid (18:2 [9,12]), obtained from vegetable oils, lowers plasma cholesterol when substituted for saturated fats. Plasma LDL-C is lowered, but HDL-C, which protects against CHD, is also lowered. The powerful benefits of lowering LDLs are only partially offset because of the decreased HDL. Nuts, avocados, olives, soybeans, and various oils, including sunflower, cottonseed, and corn oil, are common sources of these fatty acids (see Figure 27.10). The AMDR for linoleic acid is 5%–10%. [Note: The lower recommendation for PUFAs relative to MUFAs is because of concern that free radical–mediated oxidation (peroxidation) of PUFAs may lead to deleterious products.]

b. ω-3 Fatty acids: These are long-chain PUFAs, with the first double bond beginning at the third bond position from the methyl (ω) end. Dietary w-3 PUFAs suppress cardiac arrhythmias, reduce serum TAGs, decrease the tendency for thrombosis, lower blood pressure, and substantially reduce risk of cardiovascular mortality (Figure 27.12), but they have little effect on LDL-C or HDL-C levels. Evidence suggests that they have anti-inflammatory effects. The w-3 PUFAs, principally α-linolenic acid, 18:3(9,12,15), are found in plant oils, such as flaxseed and canola, and some nuts. The AMDR for α-linolenic acid is 0.6%–1.2%. Fish oil contains the long-chain ω-3 docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Two fatty fish (for example, salmon) meals per week are recommended. For patients with documented CHD, 1 g/day of fish oils is recommended, while 2–4 g/day is prescribed to lower TAGs. [Note: The ω-3 long-chain PUFAs are 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 p. 213). EFA deficiency is characterized by scaly dermatitis as a result of the depletion of skin

ceramides with long-chain fatty acids (see p. 206).]

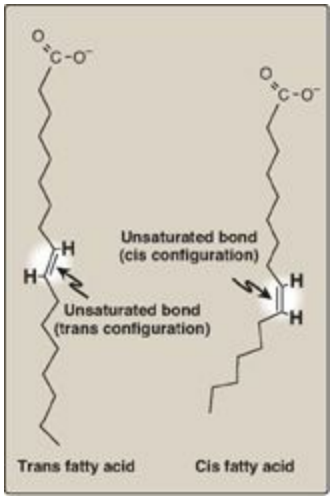
Figure 27.12 Dose responses of physiologic effects of fish oil intake. EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid.



4. **Trans fatty acids:** Trans fatty acids (Figure 27.13) are chemically classified as unsaturated fatty acids but behave more like saturated fatty acids in the body because they elevate serum LDL-C (but not HDL-C), and they increase 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 liquid vegetable oils (for example, 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 cakes, and most deep-fried foods. Many manufacturers have reformulated their products to be free of trans fats. Starting in 2006, the U.S. Food and Drug Administration requires that Nutrition Facts labels portray the trans fat content of packaged food. Some municipalities, such as New York City, have banned the use of trans fats in restaurants.

5. **Dietary cholesterol:** Cholesterol is found only in animal products. The effect of dietary cholesterol on plasma cholesterol (Figure 27.14) is less important than the amount and types of fatty acids consumed. Cholesterol consumption should be no more than 300 mg/day.

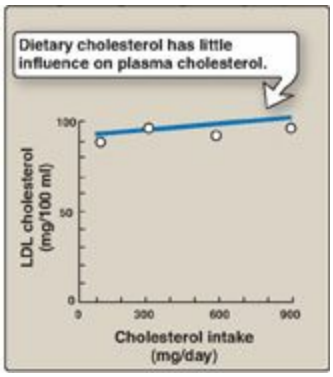
Figure 27.13 Structure of cis and trans fatty acids.



C. Other dietary factors affecting coronary heart disease

Moderate consumption of alcohol (up to 1 drink a day for women and up to 2 drinks a day for men) decreases the risk of CHD, because there is a positive correlation between moderate alcohol 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 (for example, red wine contains phenolic compounds that inhibit lipoprotein oxidation; see p. 235). [Note: These antioxidants are also present in raisins and grape juice.] Figure 27.15 summarizes the effects of dietary fats.

Figure 27.14 Response of plasma low-density lipoprotein (LDL) concentrations to an increase in dietary cholesterol intake.



VI. DIETARY CARBOHYDRATES

The primary role of dietary carbohydrate is to provide energy. Although caloric intake in the United States has shown a modest increase since 1971, the incidence of obesity has dramatically increased (see p. 349). 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.

A. Classification of carbohydrates

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 and sucrose in honey and fruits (for example, apples).

Figure 27.15 Effects of dietary fats. LDL = low-density lipoprotein; HDL = high-density lipoprotein.

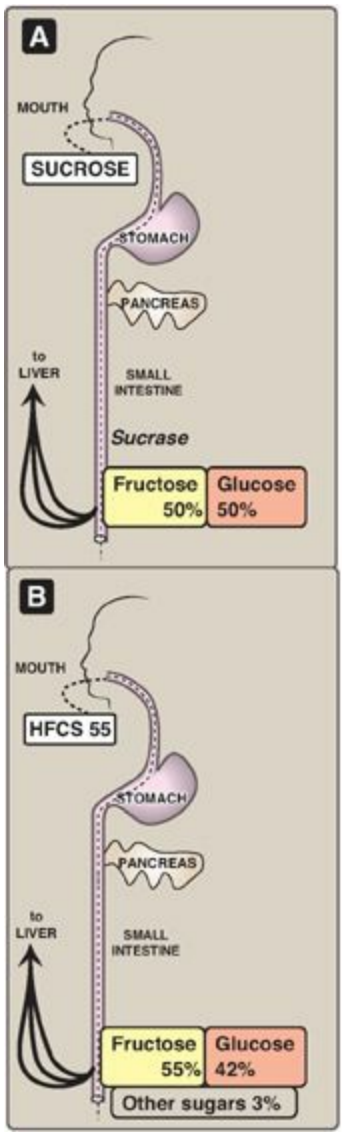
TYPE OF FAT	METABOLIC EFFECTS	EFFECTS ON DISEASE PREVENTION
Trans fatty acid	↑ LDL ↓ HDL	↑ Incidence of coronary heart disease
Saturated fatty acid	↑ LDL Little effect on HDL	↑ Incidence of coronary heart disease; may increase risk of prostate, colon cancer
Monounsaturated fatty acid	↓ LDL Maintain or increase HDL	↓ Incidence of coronary heart disease
Polyunsaturated fatty acids ω-6	↓ LDL ↓ HDL Provide arachidonic acid, which is an important precursor of prostaglandins and leukotrienes	↓ Incidence of coronary heart disease
Polyunsaturated fatty acids ω-3	Little effect on LDL Little effect on HDL Suppress cardiac arrhythmias, reduce serum triacylglycerols, decrease the tendency for thrombosis, lower blood pressure	↓ Incidence of coronary heart disease ↓ Risk of sudden cardiac death

   a. **High-fructose corn syrup:** High-fructose corn syrups (HFCSs) are corn syrups that have undergone enzymatic processing to convert their glucose into fructose and have then been mixed with pure corn syrup (100% glucose) 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 (Figure 27.16). Most studies have shown no significant difference between sucrose and HFCS syrup 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.]

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 polysaccharides. It is also found in significant quantities in beer and malt liquors. The term "sugar" refers to monosaccharides and disaccharides. "Added sugars" are those sugars and syrups (such as HFCSs) added to foods during processing or preparation. [Note: Fructose is 1.7 times sweeter than sucrose.]

Figure 27.16 Digestion of high-fructose corn syrup (HFCS) 55 or sucrose leads to absorption of glucose plus fructose.

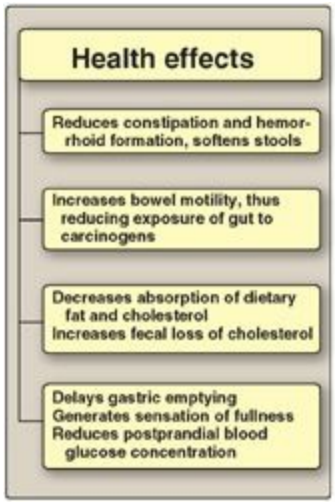


3. **Polysaccharides:** Complex carbohydrates are polysaccharides (most often polymers of glucose) that do not have a sweet taste. Starch is an example of a complex carbohydrate that is found in abundance in plants. Common sources include wheat and other grains, potatoes, dried peas and beans, and vegetables.

4. **Fiber:** Dietary fiber is defined as the nondigestible, nonstarch carbohydrates and

lignin (a noncarbohydrate polymer of aromatic alcohols) present intact in plants. Soluble fiber is the edible parts of plants that is resistant to digestion and absorption in the human small intestine but is completely or partially fermented by bacteria to short-chain fatty acids in the large intestine. Insoluble fiber passes through the digestive track largely intact. Dietary fiber provides little energy but has several beneficial effects. First, it adds bulk to the diet (Figure 27.17). Fiber can absorb 10–15 times its own weight in water, drawing fluid into the lumen of the intestine and increasing bowel motility and promoting normal laxation. Soluble fiber delays gastric emptying and can result in a sensation of fullness. This delayed emptying also results in reduced peaks of blood glucose following a meal. Second, consumption of soluble fiber has now been shown to lower LDL-C levels by increasing fecal bile acid excretion and interfering with bile acid absorption (see p. 225). For example, diets rich (25–50 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. Total fiber is the sum of dietary fiber and functional fiber.]

Figure 27.17 Actions of dietary fiber.



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. Thus, they differ in their glycemic response (GR). [Note: Fiber blunts the GR.] The glycemic index (GI) quantitates these differences in the time course of postprandial glucose concentrations (Figure 27.18). GI is defined as the area under the blood glucose curves seen after ingestion of a meal with carbohydrate-rich food, compared with the area under the blood glucose curve observed after a meal

consisting of the same amount (50 g) of carbohydrate either as glucose or white bread. The clinical importance of the GI is unresolved, but 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 (for example, carrots) can have a high GI and a low GL.]

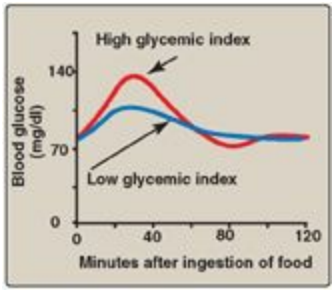
C. Requirements for carbohydrate

Carbohydrates are not essential nutrients, because the carbon skeletons of most amino acids can be converted into glucose (see p. 261). However, the absence of dietary carbohydrate leads to ketone body production (see p. 262) and degradation of body protein whose constituent amino acids provide carbon skeletons for gluconeogenesis (see p. 118). 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 to meet energy needs. Adults should consume 45%–65% of their total calories from carbohydrates. It is recommended that added sugar represent no more than 25% of total energy because of concerns that sugar may displace nutrient-rich foods from the diet, potentially leading to deficiencies of certain micronutrients.

D. Simple sugars and disease

There is no direct evidence that the consumption of simple sugars 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 Figure 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.

Figure 27.18 Blood glucose concentrations following ingestion of food with low or high glycemic index.



VII. DIETARY PROTEIN

Humans have no dietary requirement for protein per se, but the protein in food does provide essential amino acids (see Figure 20.2, p. 262). Nine of the 20 amino acids needed for the synthesis of body proteins are essential (that is, they cannot be synthesized in humans).

A. Quality of proteins

The quality of a dietary protein is a measure of its ability to provide the essential amino acids 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 essential amino acids 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.

1. **Proteins from animal sources:** Proteins from animal sources (meat, poultry, milk, and fish) have a high quality because they contain all the essential amino acids in proportions similar to those required for synthesis of human tissue proteins (Figure 27.19), 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 essential amino acids.]

2. **Proteins from plant sources:** Proteins from plant sources have a lower quality than do animal proteins. However, 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 an improved biologic value. Therefore, eating foods with different amino acids during the day can result in a dietary combination with a higher biologic value than either of the component proteins (Figure 27.20). [Note: Animal proteins can also complement the biologic value of plant proteins.]

Figure 27.19 Relative quality of some common dietary proteins. PDCAAS = protein digestibility-corrected amino acid score.

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

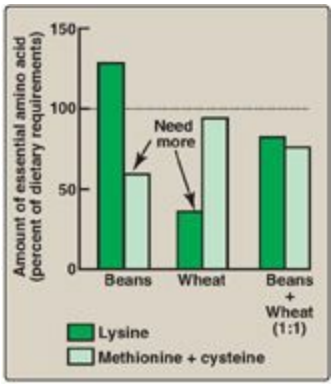
B. Nitrogen balance

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.

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 essential amino acid; or during physiologic stresses, such as trauma, burns, illness, or surgery.

> Nitrogen (N) balance can be determined by the formula, N balance = protein N intake (g/24 hrs) – (UUN + 4 g), where 4 g accounts for urinary loss in forms other than UUN plus loss in skin and feces. Alternatively, protein balance = protein intake (g/24 hrs) – [(UUN + 4 g) x 6.25] can be used because there are, on average, 6.25 g N in 1 g protein.

Figure 27.20 Combining two incomplete proteins that have complementary amino acid deficiencies results in a mixture with a higher biologic value.



C. Requirement for protein in humans

The amount of dietary protein required in the diet varies with its biologic value. The greater the proportion of animal protein included 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 about 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 about 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.

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 and osteoporosis.

2. **The protein-sparing effect of carbohydrate:** 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 less than 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.

Figure 27.21 Features of protein-energy malnutrition in children.

CHARACTERISTIC	KWASHIORKOR	MARASMUS
Weight for age (% expected)	60–80	<60
Weight for height	Normal or decreased	Markedly decreased
Edema	Present	Absent
Mood	Irritable when picked up; apathetic when left alone	Alert, irritable
Appetite	Poor	Good

D. Protein-energy (calorie) malnutrition

In developed countries, protein-energy malnutrition (PEM) 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 (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 energy 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 (Figure 27.21). [Note: PEM is also referred to as protein-energy undernutrition (PEU).]

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, enlarged fatty liver, 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 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 nonadapted malnutrition.]

> 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.

Figure 27.22 A. Listless child with kwashiorkor. Note the swollen belly.
B. Child suffering with marasmus.

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 with watery gruels of native cereals that are usually deficient in both protein and calories. Typical symptoms include arrested growth, extreme muscle wasting and loss of subcutaneous fat (emaciation), weakness, and anemia (Figure 27.22). Individuals with marasmus do not show the edema observed in kwashiorkor.

VIII. CHAPTER SUMMARY

The **Dietary Reference Intakes** (**DRIs**) provide estimates of the amounts of nutrients required to prevent deficiencies and maintain optimal health and growth. It consists of the **Estimated Average Requirement** (**EAR)**, 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; the **Recommended Dietary Allowance** (**RDA**), the average daily dietary intake level that is sufficient to meet the nutrient requirements of nearly all (97%–98%) individuals in a life stage and gender group; the **Adequate Intake** (**AI**), which is set instead of an RDA if sufficient scientific evidence is not available to calculate the RDA; and the **Tolerable Upper Intake Level** (**UL**), 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** is used for three energy-requiring processes that occur in the body: **resting metabolic rate**, **physical activity, and 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%–65%** of their **total calories** from **carbohydrates**, **20%–35%** from **fat**, and **10%–35%** from **protein** (Figure 27.23). Elevated levels of cholesterol in low-density lipoproteins (LDL-C) result in increased risk for **cardiovascular disease**. In contrast, high levels of cholesterol in high-density lipoproteins (HDL-C) have been associated with a decreased risk for heart disease. Dietary or drug treatment of **hypercholesterolemia** is effective in decreasing LDL-C, increasing HDL-C, and reducing the risk for cardiovascular events. 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 w**-6 polyunsaturated fatty acids** lowers plasma LDL-C, but HDL-C, which protects against coronary heart disease, is also lowered. Dietary w**-3 polyunsaturated fats** suppress cardiac arrhythmias and reduce serum 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**. The **quality of a protein** 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 balance** occurs when nitrogen intake exceeds nitrogen excretion. It is observed in situations in which tissue growth occurs, for example, in childhood,

pregnancy, or during recovery from an emaciating illness. **Negative nitrogen balance** occurs when nitrogen losses are greater than nitrogen 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. Both are extreme forms of protein-energy malnutrition (PEM).

Study Questions

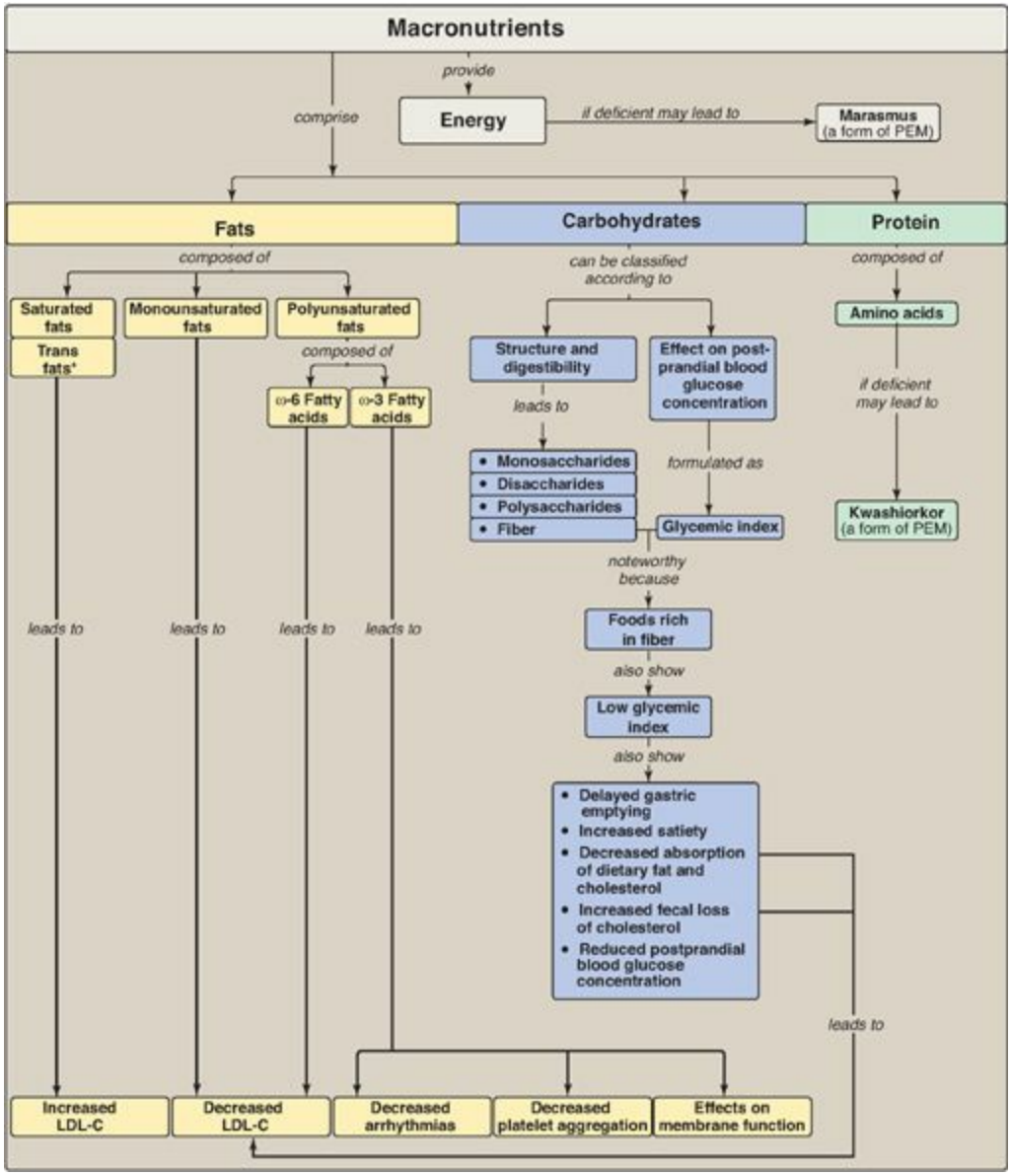
Choose the ONE best answer.

27.1 For the child shown at right, which of the statements is true and supports 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. Treatment includes a diet adequate in calories and protein.

Figure 27.23 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.



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 those from animals.

Correct answer = C. We 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 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 calories from carbohydrate is 1,100/1,985 = 55, percentage calories from protein is 300/1,985 = 15, and percentage calories derived 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.

For Questions 27.4 and 27.5:

A sedentary 50-year-old man weighing 80 kg (176 pounds) 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 less than 200 mg.) The man refuses drug therapy for his hypercholesterolemia. Analysis of a 1-day dietary recall showed the following:

Kilocalories	3,475 kcal
Protein	102 g
Carbohydrate	383 g
Fiber	6 g
Cholesterol	822 mg
Saturated fat	69 g
Total Fat	165 g

27.4 Decreasing which one of the following dietary components would have the greatest

effect in lowering the patient's plasma cholesterol?

A. Carbohydrate
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 40% of the fat as saturated fat. The most important dietary recommendations are 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.

27.5 What would you need to know to estimate the total energy expenditure of the patient?

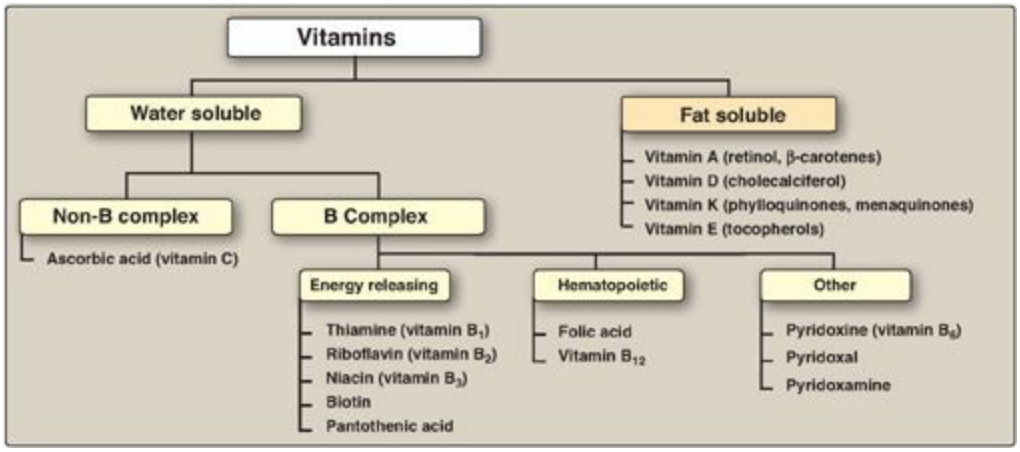
The daily basal energy expenditure (estimated resting metabolic rate/hour x 24 hours) and an activity factor (AF) based on the type and duration of physical activities are needed variables. An additional 10% would be added to account for the thermic effect of food. Note that if the patient were hospitalized, an injury factor (IF) would be included in the calculation, and the AF would be modified. Tables of AFs and IFs are available.

Vitamins 28

I. OVERVIEW

Vitamins are chemically unrelated organic compounds 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 occur quickly. Four vitamins (A, D, K, and E) are termed fat soluble (Figure 28.1). They are released, absorbed, and transported (in chylomicrons) with dietary fat. They are not readily excreted, and significant quantities are stored in the liver and adipose tissue. In fact, consumption of vitamins A and D in excess of the Dietary Reference Intakes 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.

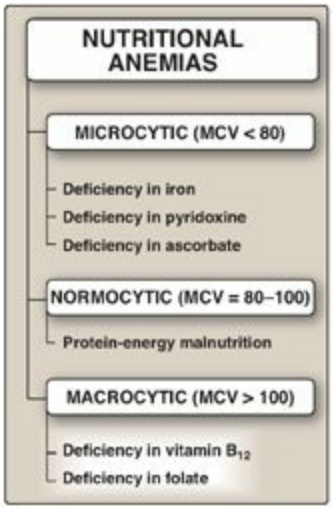
Figure 28.1 Classification of the vitamins.



II. FOLIC ACID

Folic acid (or folate), which plays a key role in one-carbon metabolism, 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 alcoholics. [Note: Leafy, dark green vegetables are a good source of folic acid.]

Figure 28.2 Classification of nutritional anemias by cell size. The normal mean corpuscular volume (MCV) for people older than age 18 is between 80 and 100 μ;m^3. [Note: Microcytic anemia is also seen with lead poisoning.]



A. Function of folic acid

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, purines, and thymidine monophosphate (TMP), a pyrimidine nucleotide found in DNA (Figure 28.3).

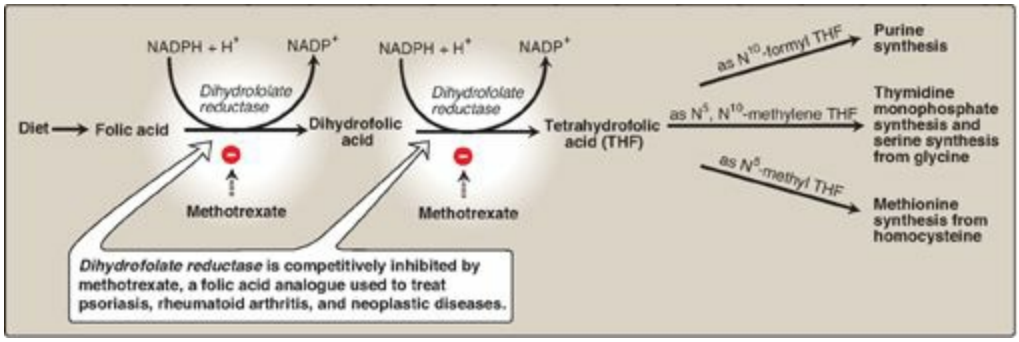
B. Nutritional anemias

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. Nutritional anemias (that is, 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 individual (Figure 28.2). 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.]

1. Folate and anemia: Inadequate serum levels of folate can be caused by increased

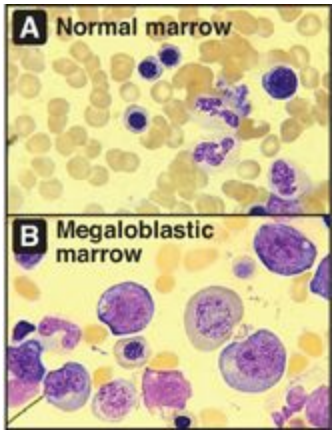
demand (for example, pregnancy and lactation), poor absorption caused by pathology of the small intestine, alcoholism, or treatment with drugs (for example, methotrexate) that are dihydrofolate reductase inhibitors (see Figure 28.3). A folate-free diet can cause a deficiency within a few weeks. A primary result of folic acid deficiency is megaloblastic anemia (Figure 28.4), caused by diminished synthesis of purines and TMP, which leads to an inability of cells (including RBC precursors) to make DNA and, therefore, an inability to divide.

Figure 28.3 Production and use of tetrahydrofolate. NADP(H) = nicotinamide adenine dinucleotide phosphate.



2. **Folate and neural tube defects in the fetus:** Spina bifida and anencephaly, the most common neural tube defects (NTDs), affect approximately 3,000 pregnancies in the United State annually. Folic acid supplementation before conception and during the first trimester has been shown to significantly reduce NTDs. Therefore, all women of childbearing age are advised to consume 0.4 mg/day of folic acid to reduce the risk of having a pregnancy affected by NTDs 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 addition of folic acid to enriched grain products, resulting in a dietary supplementation of about 0.1 mg/day. It is estimated that this supplementation allows approximately 50% of all reproductive-aged women to receive 0.4 mg of folate from all sources. [Note: High-dose folate supplementation can mask the symptoms of vitamin B_{12} deficiency (see blue box below) and is not recommended for most adults.]

Figure 28.4 Bone marrow histology in normal and folate-deficient individuals.


A Normal marrow
B Megaloblastic marrow

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 (FAs) with odd numbers of carbon atoms (Figure 28.5). When cobalamin is deficient, unusual (branched) FAs 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 of cobalamin and its coenzyme forms

Cobalamin contains a corrin ring system that resembles the porphyrin ring of heme (see p. 280), and but differs in that two of the pyrrole rings are linked directly rather than through a methene bridge. Cobalt 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 (Figure 28.6). The physiologic coenzyme forms of cobalamin are 5**I**-deoxyadenosylcobalamin and methylcobalamin, in which cyanide is replaced with 5-deoxyadenosine or a methyl group, respectively (see Figure 28.6).

Figure 28.5 Reactions requiring coenzyme forms of vitamin B_{12}. CoA = coenzyme A.

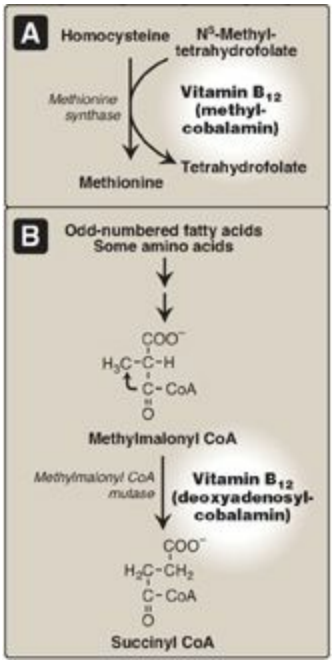
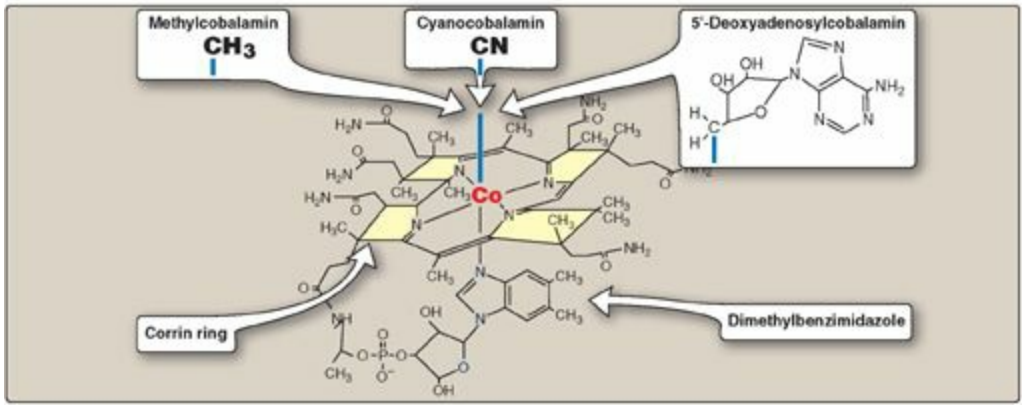


Figure 28.6 Structure of vitamin B_{12} (cyanocobalamin) and its coenzyme forms

(methylcobalamin and 5**I**-deoxyadenosylcobalamin).



B. Distribution of cobalamin

Vitamin B_{12} is synthesized only by microorganisms, and it is not present in plants. Animals obtain the vitamin preformed from their natural bacterial flora 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.

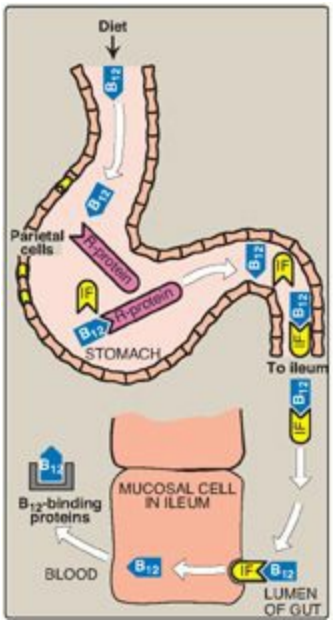
C. Folate trap hypothesis

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. 293 and 303). However, in vitamin B_{12} deficiency, the utilization of the N^5-methyl form of THF in the B_{12}-dependent methylation of homocysteine 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 vitamin B_{12}

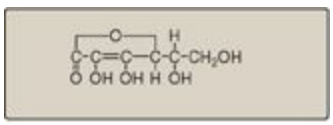
In contrast to other water-soluble vitamins, significant amounts (2–5 mg) of vitamin B_{12} are stored in the body. As a result, it may take several years for the clinical symptoms of B_{12} deficiency to develop as a result of decreased intake of the vitamin. [Note: Deficiency happens much more quickly if absorption is impaired (see below).] 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}. IF = intrinsic factor.



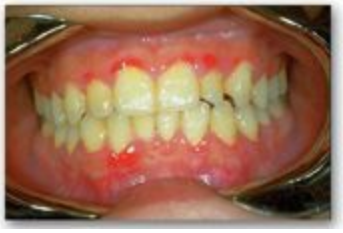
1. **Pernicious anemia:** Vitamin B_{12} deficiency is most commonly seen in patients who fail to absorb the vitamin from the intestine. B_{12} is released from food in the acidic environment of the stomach. [Note: Malabsorption of cobalamin in the elderly is most often due to reduced secretion of gastric acid (achlorhydria).] Free B_{12} then binds a glycoprotein (R-protein), 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 specific receptors 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, and they show neuropsychiatric symptoms later, as the disease develops. The CNS effects are irreversible and occur by mechanisms that appear to be different from those described for megaloblastic anemia. Pernicious anemia requires life-long treatment with either high-dose oral B_{12} or intramuscular injection of cyanocobalamin. [Note: Supplementation works even in the absence of IF because approximately 1% of B_{12} uptake is by IF-independent diffusion.]

Figure 28.8 Structure of ascorbic acid.



Folic acid supplementation can partially reverse the hematologic abnormalities of B_{12} deficiency and, therefore, can mask a cobalamin deficiency. Thus, to prevent the 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.

Figure 28.9 Structures of vitamin B6 and the antituberculosis drug isoniazid.



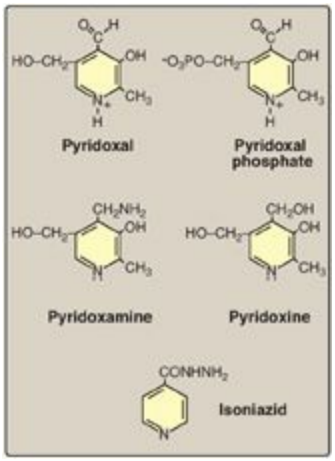
IV. ASCORBIC ACID (VITAMIN C)

The active form of vitamin C is ascorbic acid (Figure 28.8). The main function of ascorbate is as a reducing agent in several different reactions. Vitamin C has a well-documented role as a coenzyme in hydroxylation reactions (for example, hydroxylation of prolyl and lysyl residues of collagen; see p. 47). Vitamin C is, therefore, required for the maintenance of normal connective tissue as well as for wound healing. Vitamin C also reduces ferric iron to the ferrous form, thereby facilitating the absorption of dietary iron from the intestine.

A. Deficiency of ascorbic acid

A deficiency of ascorbic acid results in scurvy, a disease characterized by sore and spongy gums, loose teeth, fragile blood vessels, swollen joints, fatigue, and a microcytic anemia caused by decreased absorption of iron (Figure 28.9). Many of the deficiency symptoms can be explained by a deficiency in the hydroxylation of collagen, resulting in defective connective tissue.

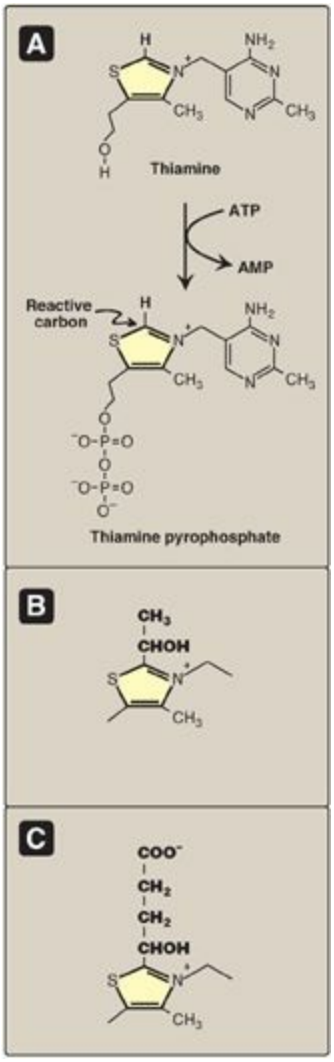
Figure 28.10 Structures of vitamin B_6 and the antituberculosis drug isoniazid.



B. Prevention of chronic disease

Vitamin C is one of a group of nutrients that includes vitamin E (see p. 391) and β-carotene (see p. 382), which are known as antioxidants. [Note: Ascorbate regenerates the functional, reduced form of vitamin E.] Consumption of diets rich in these compounds is associated with a decreased incidence of some chronic diseases, such as coronary heart disease and certain cancers. However, clinical trials involving supplementation with the isolated antioxidants have failed to demonstrate any convincing beneficial effects.

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.


A
Thiamine
ATP
AMP
Reactive carbon
Thiamine pyrophosphate
B
C

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 (Figure 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 synthesis of cysteine from Hcy (see p. 264). [Note: PLP is also required by glycogen phosphorylase (see p. 128).]

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. Dietary supplementation with B_6 is, thus, an adjunct to isoniazid treatment. 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 alcoholics.

B. Toxicity of pyridoxine

Pyridoxine 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 5 times the Tolerable Upper Limit (UL). Substantial improvement, but not complete recovery, occurs when the vitamin is discontinued.

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 adenosine triphosphate (ATP) to thiamine (Figure 28.11). TPP serves as a coenzyme in the formation or degradation of α-ketols by transketolase (Figure 28.12A), and in the oxidative decarboxylation of α-keto acids (Figure 28.12B).

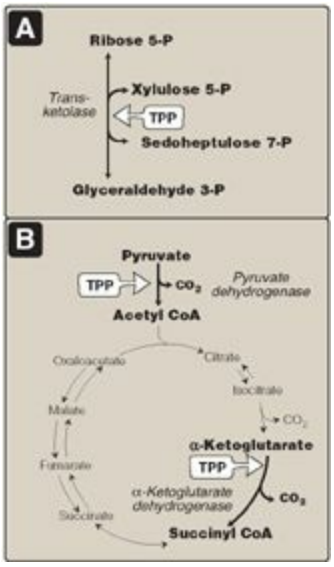
A. Clinical indications for thiamine

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. [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 on addition of TPP.]

1. **Beriberi:** This is a severe thiamine-deficiency syndrome found in areas where polished rice is the major component of the diet. Adult beriberi is classified as dry (characterized by peripheral neurologic deficits) or wet (characterized by edema due to cardiac dysfunction). Infantile beriberi is seen in nursing infants whose mothers are deficient in thiamine.

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 alcoholics develop Wernicke-Korsakoff syndrome, a thiamine deficiency state characterized by confusion, ataxia, and a rhythmic to-and-fro motion of the eyeballs (nystagmus) 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.

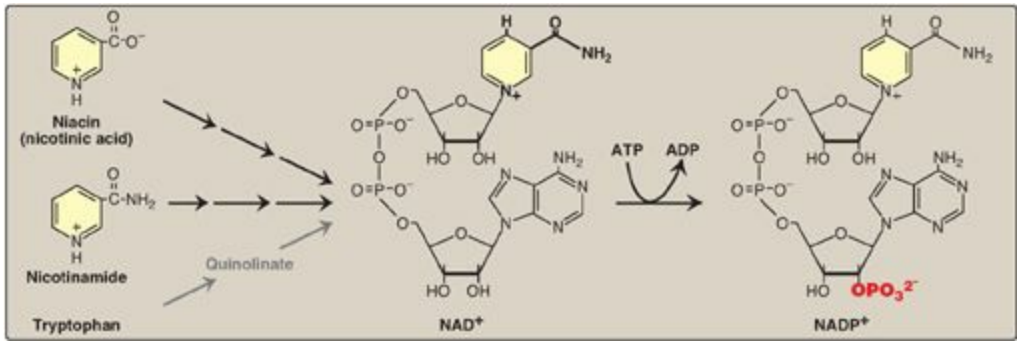
Figure 28.12 Reactions that use thiamine pyrophosphate (TPP) as coenzyme. A. Transketolase. B. Pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. Note that TPP is also used by branched-chain α-keto acid dehydrogenase. P = phosphate; CoA = coenzyme A.


A
Ribose 5-P
Trans-
ketolase
Xylulose 5-P
TPP
Sedoheptulose 7-P
Glyceraldehyde 3-P
B
Pyruvate
TPP
CO2
Pyruvate
dehydrogenase
Acetyl CoA
Oxaloacetate
Citrate
Isocitrate
Malate
CO2
α-Ketoglutarate
Fumarate
TPP
α-Ketoglutarate
dehydrogenase
CO2
Succinate
Succinyl CoA

VII. NIACIN

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 a hydride ion (hydrogen atom plus one electron) 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.]

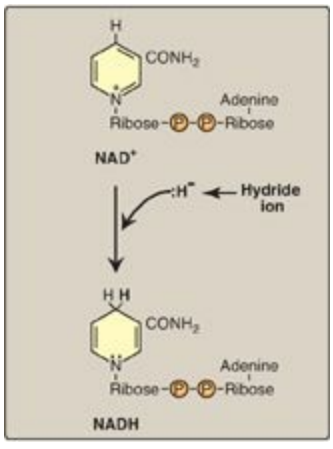
Figure 28.13 Structure and biosynthesis of oxidized nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$). ADP = adenosine diphosphate.



A. Distribution of niacin

Niacin is found in unrefined and enriched grains and cereal; milk; and lean meats, especially liver.

Figure 28.14 Reduction of oxidized nicotinamide adenine dinucleotide (NAD^+) to NADH. (P) = phosphate.

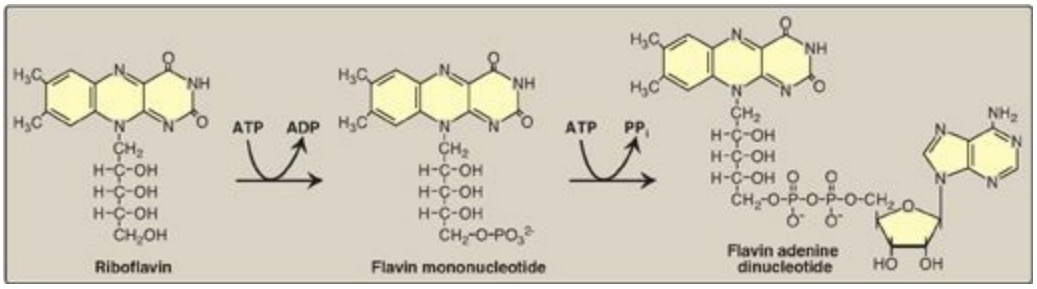


B. Clinical indications for niacin

1. **Deficiency of niacin:** 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; diarrhea; dementia; and, if untreated, death. 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. **Treatment of hyperlipidemia:** 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 FFAs 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. 231) 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. 214). [Note: Niacin raises high-density lipoprotein levels.]

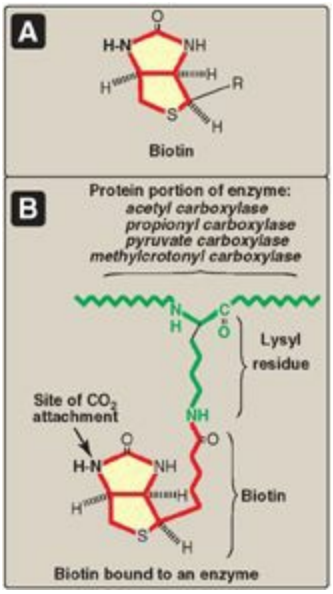
Figure 28.15 Structure and biosynthesis of the oxidized forms of flavin mononucleotide and flavin adenine dinucleotide. ADP = adenosine diphosphate; PP_i = pyrophosphate.



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 (Figure 28.15). FMN and FAD are each capable of reversibly accepting two hydrogen atoms, forming $FMNH_2$ or $FADH_2$. FMN and FAD are bound tightly, sometimes covalently, to flavoenzymes (for example, 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).

Figure 28.16 A. Structure of biotin. B. Biotin covalently bound to a lysyl residue of a biotin-dependent enzyme.

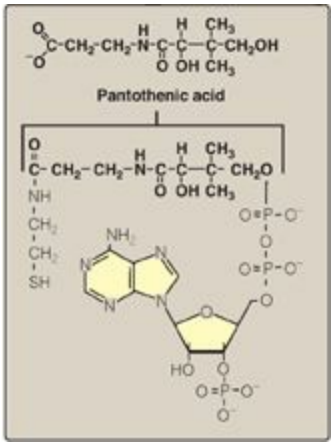


IX. BIOTIN

Biotin is a coenzyme in carboxylation reactions, in which it serves as a carrier of activated carbon dioxide (see Figure 10.3, p. 119, for the mechanism of biotin-dependent carboxylations). Biotin is covalently bound to the ε-amino group of lysine residues in biotin-dependent enzymes (Figure 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 induces symptoms of biotin deficiency, namely, dermatitis, glossitis, loss of appetite, and nausea. Raw egg white contains a 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. Thus, inclusion of an occasional raw egg in the diet does not lead to biotin deficiency, although eating raw eggs is generally not recommended due to the possibility of salmonella infection.

> Multiple carboxylase deficiency results from a defect in the ability to add biotin to carboxylases during their synthesis or to remove it from carboxylases during their degradation. Treatment is biotin supplementation.

Figure 28.17 Structure of coenzyme A.



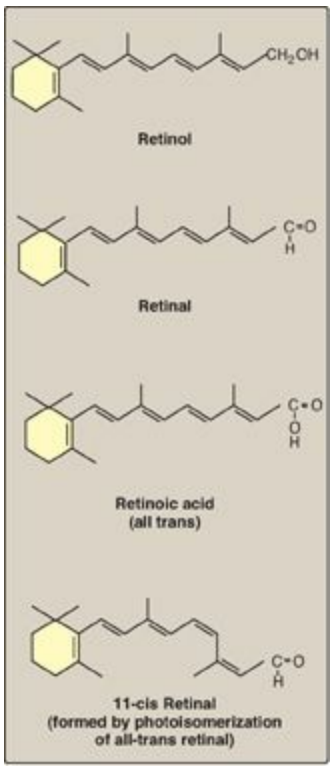
X. PANTOTHENIC ACID

Pantothenic acid is a component of CoA, which functions in the transfer of acyl groups (Figure 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 (see p. 184). 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

The retinoids, a family of molecules that are related to dietary retinol (vitamin A), 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 28.18 Structure of the retinoids.



A. Structure of vitamin A

Vitamin A is often used as a collective term for several related biologically active molecules (Figure 28.18). The term retinoids includes both natural and synthetic forms of vitamin A that may or may not show vitamin A activity.

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 FAs.

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, which can be oxidatively 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.

B. Absorption and transport of vitamin A

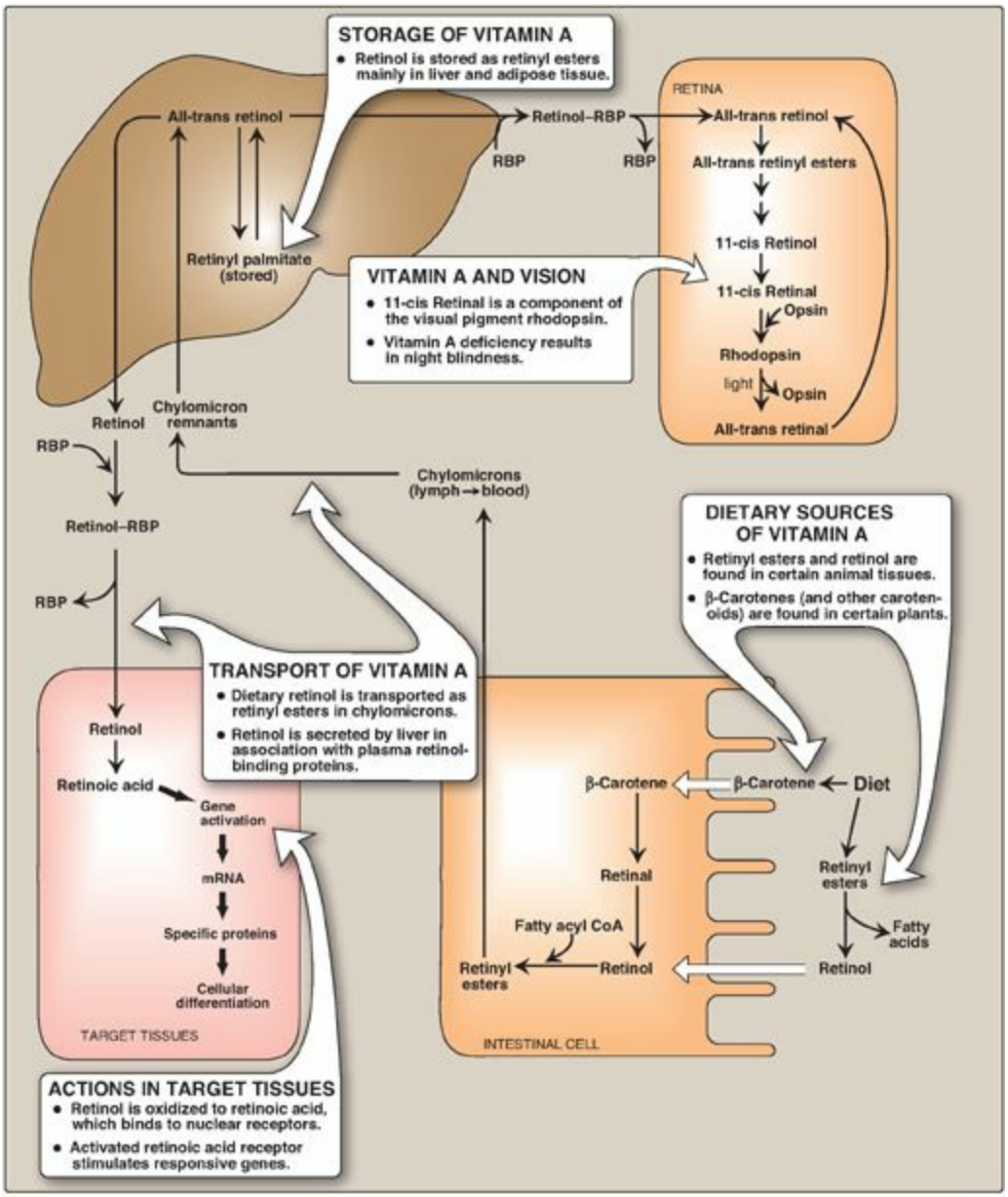
1. **Transport to the liver:** Retinyl esters present in the diet are hydrolyzed in the intestinal mucosa, releasing retinol and FFAs (Figure 28.19). Retinol derived from esters and from the cleavage and reduction of carotenes is re-esterified to long-chain FAs in the intestinal mucosa and secreted as a component of chylomicrons into the lymphatic system (see Figure 28.19). Retinyl esters contained in chylomicron remnants are taken up by, and stored in, the liver.

2. **Release from the liver:** When needed, retinol is released from the liver and transported to extrahepatic tissues by the plasma retinol-binding protein (RBP). The retinol–RBP complex binds to a transport protein on the surface of the cells of peripheral tissues, permitting retinol to enter. Many tissues contain a cellular retinol-binding protein that carries retinol to sites in the nucleus where the vitamin acts in a manner analogous to that of steroid hormones.

C. Mechanism of action of vitamin A

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 (Figure 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. The RAR proteins are part of the superfamily of transcriptional regulators that includes the nuclear receptors for steroid and thyroid hormones and 1,25-dihydroxycholecalciferol, all of which function in a similar way (see p. 240).

Figure 28.19 Absorption, transport, and storage of vitamin A and its derivatives. RBP = retinol-binding protein; CoA = coenzyme A; mRNA = messenger RNA.



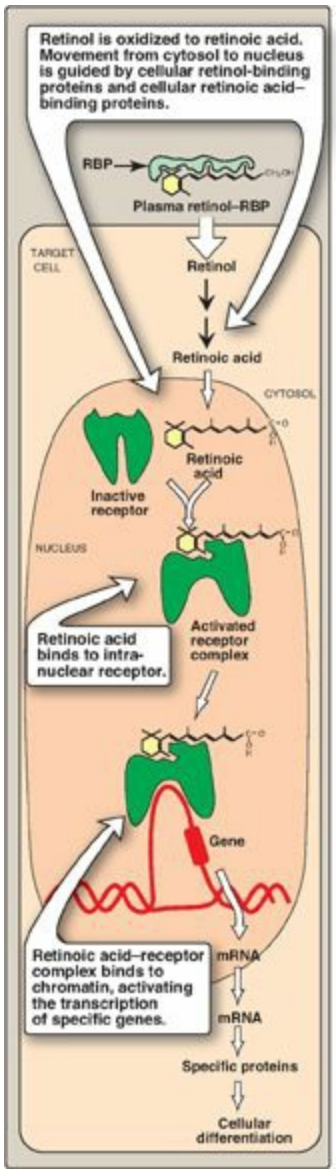
D. Functions of vitamin A

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 specifically bound to the protein opsin. When rhodopsin is exposed to light, a series of photochemical isomerizations occurs, which results in the bleaching of the visual pigment and release of all-trans retinal and opsin. This process triggers 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, after being released from rhodopsin, is reduced to all-trans retinol, esterfied, 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.

2. **Maintenance of epithelial cells:** 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. Therefore, animals given vitamin A only as retinoic acid from birth are blind and sterile.

Figure 28.20 Action of the retinoids. [Note: Retinoic acid-receptor complex forms a dimer, but is shown as monomer for simplicity.] RBP = retinol-binding protein; mRNA = messenger RNA.



E. Distribution of vitamin A

Liver, kidney, cream, butter, and egg yolk are good sources of preformed vitamin A. Yellow, orange, and dark green vegetables and fruits are good dietary sources of the carotenes, which serve as precursors of vitamin A.

F. Requirement for vitamin A

The RDA for adults is 900 retinol activity equivalents (RAEs) for males and 700 RAE for

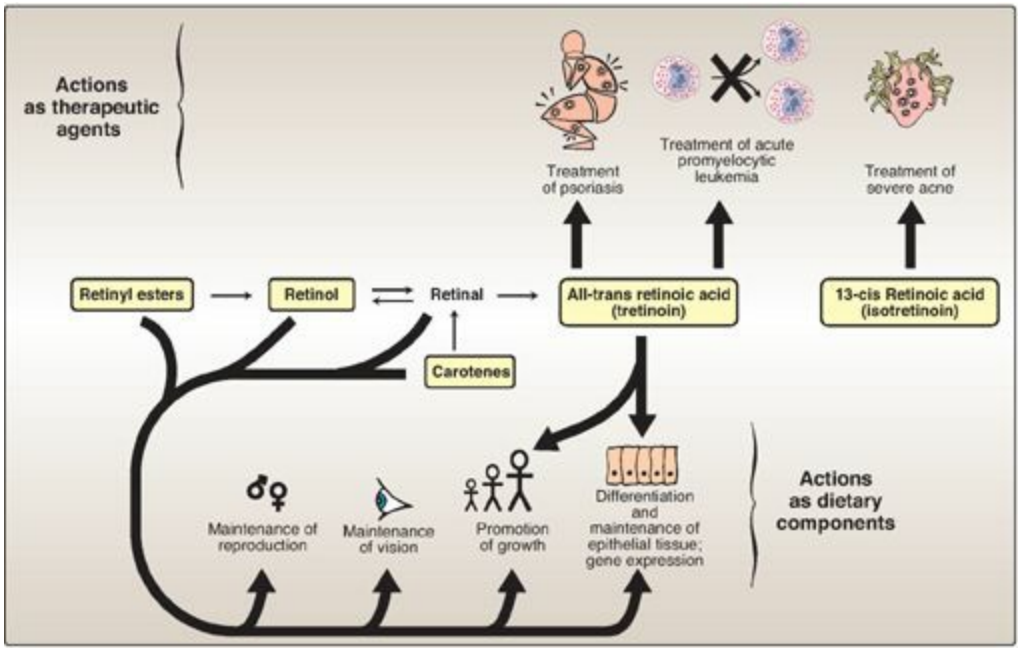
females. In comparison, 1 RAE = 1 mg of retinol, 12 mg of β-carotene, or 24 mg of other carotenoids.

G. Clinical indications

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.

1. **Dietary deficiency:** Vitamin A, administered as retinol or retinyl esters, is used to treat patients who are deficient in the vitamin (Figure 28.21). Night blindness 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 vitamin A 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.

Figure 28.21 Summary of actions of retinoids. Compounds in boxes are available as dietary components or as pharmacologic agents.



2. **Acne and psoriasis:** Dermatologic problems such as acne and psoriasis are effectively treated with retinoic acid or its derivatives (see Figure 28.21). Mild cases of acne, Darier disease (keratosis follicularis), and skin aging are treated with topical application of tretinoin (all-trans retinoic acid), as well as benzoyl peroxide and antibiotics. [Note: Tretinoin is too toxic for systemic administration and is confined to topical application.] In patients with severe cystic acne unresponsive to

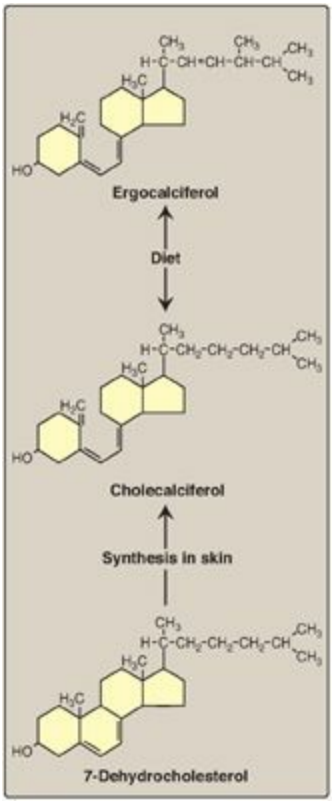
conventional therapies, the drug of choice is isotretinoin (13-cis retinoic acid) administered orally. Retinoic acid is also used in the treatment of acute promyelocytic leukemia.

H. Toxicity of retinoids

1. **Vitamin A:** Excessive intake of vitamin A 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 (due to decreased keratin synthesis); 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 particularly 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 mg/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 initiation of treatment, and adequate birth control must be used. Prolonged treatment with isotretinoin leads to hyperlipidemia with an increase in TAGs and cholesterol, providing some concern for an increased risk of cardiovascular disease.

Figure 28.22 Sources of vitamin D.



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] calcitriol), binds to intracellular receptor proteins. The 1,25-diOH-D_3–receptor complex interacts with DNA in the nucleus of target cells in a manner similar to that of vitamin A (see Figure 28.20) and either selectively stimulates or represses gene transcription. The most prominent actions of 1,25-diOH-D_3 are to regulate the plasma levels of calcium and phosphorus.

A. Distribution of vitamin D

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 (Figure 28.22). Ergocalciferol and cholecalciferol 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.]

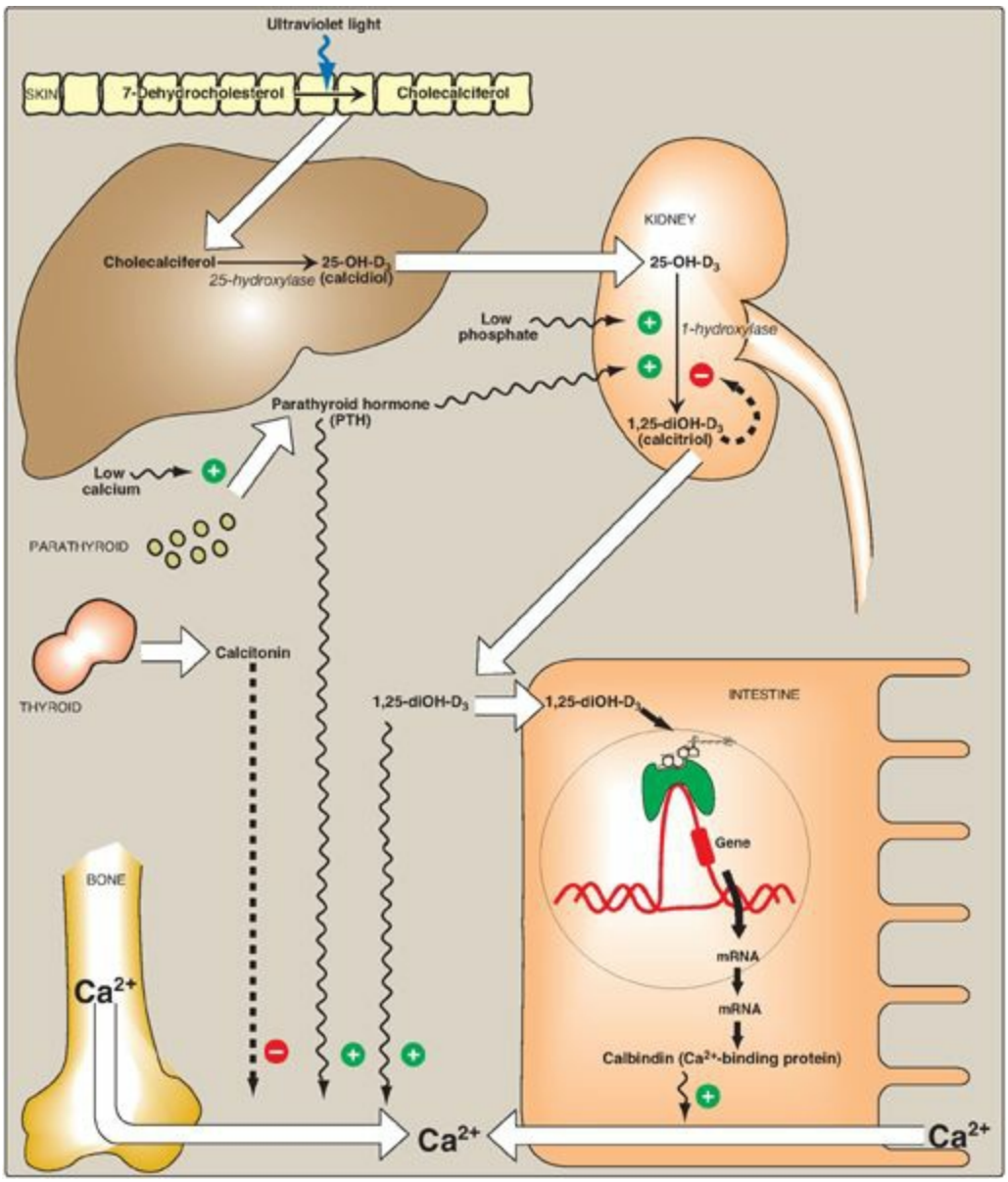
B. Metabolism of vitamin D

1. **Formation of 1,25-dihydroxycholecalciferol:** Vitamins D_2 and D_3 are not biologically active but are converted in vivo to the active form of the D vitamin by two sequential hydroxylation reactions (Figure 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 plasma and the major storage form of the vitamin. 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: This 1-hydroxylase, as well as the liver 25-hydroxylase, are cytochrome P450 (CYP) proteins (see p. 149).]

2. **Regulation of 25-hydroxycholecalciferol 1-hydroxylase:** 1,25-diOH-D_3 is the most potent vitamin D metabolite. Its formation is tightly regulated by the level of plasma phosphate and calcium ions (Figure 28.24) . 25-Hydroxycholecalciferol 1-hydroxylase activity is increased directly by low plasma phosphate or indirectly by low plasma calcium, 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 calcium results in elevated levels of plasma 1,25-diOH-D_3. [Note: 1,25-diOH-D_3 inhibits synthesis 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 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.



C. Function of vitamin D

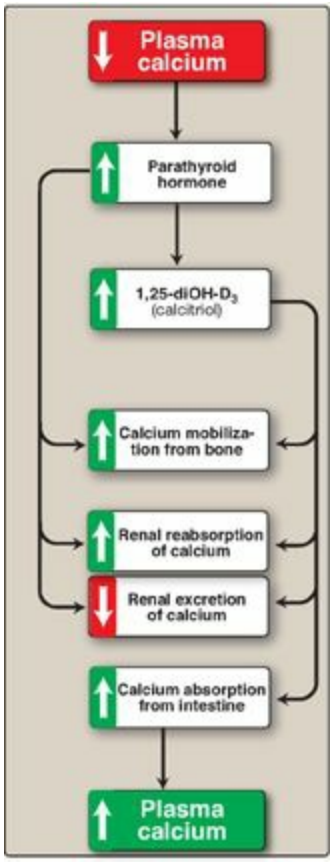
The overall function of 1,25-diOH-D_3 is to maintain adequate plasma levels of calcium. It performs this function by: 1) increasing uptake of calcium by the intestine, 2) minimizing loss of calcium by the kidney by increasing reabsorption, and 3) stimulating resorption (demineralization) of bone when blood calcium is low (see Figure 28.23).

1. **Effect of vitamin D on the intestine:** 1,25-diOH-D_3 stimulates intestinal absorption of calcium. 1,25-diOH-D_3 enters the intestinal cell and binds 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, calcium uptake is enhanced by an increased synthesis of a specific calcium-binding protein, calbindin. Thus, the mechanism of action of 1,25-diOH-D_3 is typical of steroid hormones (see p. 240).

2. **Effect of vitamin D on bone:** 1,25-diOH-D_3 stimulates the mobilization of calcium from bone by a process that requires protein synthesis and the presence of PTH. The result is an increase in plasma calcium and phosphate. Therefore, bone is an important reservoir of calcium that can be mobilized to maintain plasma levels. [Note: PTH and calcitriol also work together to prevent renal loss of calcium.]

Figure 28.24 Response to low plasma calcium. 1,25-diOH-D_3 = 1,25-dihydroxycholecalciferol.



D. Distribution and requirement of vitamin D

Vitamin D occurs naturally in fatty fish, liver, and egg yolk. Milk, unless it is artificially fortified, is not a good source of the vitamin. The RDA for individuals ages 1 to 70 years is 15 mg/day and 20 mg/day if over age 70 years. Experts disagree, however, on the optimal level of vitamin D needed to maintain health. [Note: 1 mg = 40 international units (IUs).] Because breast milk is a poor source of vitamin D, supplementation is recommended for breastfed babies.

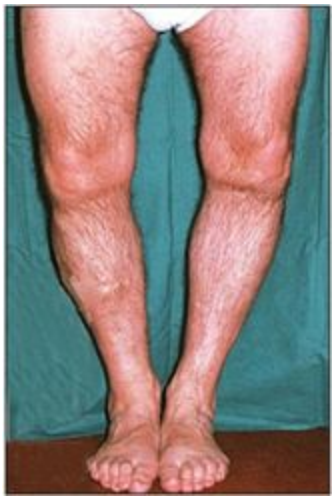
E. Clinical indications

1. **Nutritional rickets:** Vitamin D deficiency causes a net demineralization of bone, resulting in rickets in children and osteomalacia in adults (Figure 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 occur 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.

2. **Renal osteodystrophy:** Chronic kidney disease causes decreased ability to form active vitamin D as well as increased retention of phosphate, resulting in hyperphosphatemia and hypocalcemia. The low blood calcium causes a rise in PTH and associated bone demineralization with release of calcium and phosphate. Supplementation with calcitriol is an effective therapy. However, supplementation must be accompanied by phosphate reduction therapy to prevent further bone loss and precipitation of calcium phosphate crystals.

3. **Hypoparathyroidism:** Lack of PTH causes hypocalcemia and hyperphosphatemia. These patients may be treated with calcitriol and calcium supplementation.

Figure 28.25 Bowed legs of middle-aged man with osteomalacia, a nutritional vitamin D deficiency that results in demineralization of the skeleton.



F. Toxicity of vitamin D

Like all fat-soluble vitamins, vitamin D can be stored in the body and is only slowly metabolized. High doses (100,000 IUs for weeks or months) can cause loss of appetite, nausea, thirst, and stupor. Enhanced calcium absorption and bone resorption results in hypercalcemia, which can lead to deposition of calcium in many organs, particularly the arteries and kidneys. The UL is 100 mg/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.]

XIII. VITAMIN K

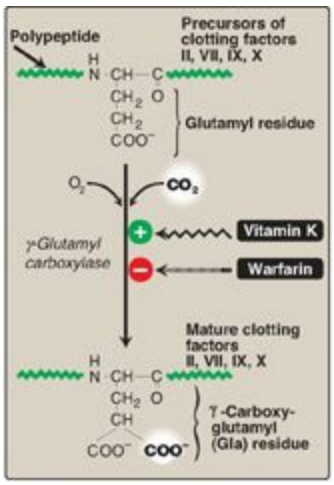
The principal role of vitamin K is in the posttranslational modification of a number of proteins (most of which are involved with blood clotting), in which it serves as a coenzyme in the carboxylation of certain glutamic acid residues present in these proteins. Vitamin K exists in several forms, for example, in plants as phylloquinone (or vitamin K_1), and in intestinal bacterial flora as menaquinone (or vitamin K_2). A synthetic form of vitamin K, menadione, is able to be converted to K_2.

A. Function of vitamin K

1. **Formation of γ-carboxyglutamate:** Vitamin K is required in the hepatic synthesis of the blood clotting proteins, prothrombin (factor II) and factors VII, IX, and X. (See online Chapter 34.) Formation of the functional clotting factors requires the vitamin K–dependent carboxylation of several glutamic acid residues to γ-carboxyglutamate (Gla) residues (Figure 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. **Interaction of prothrombin 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 (Figure 28.27).

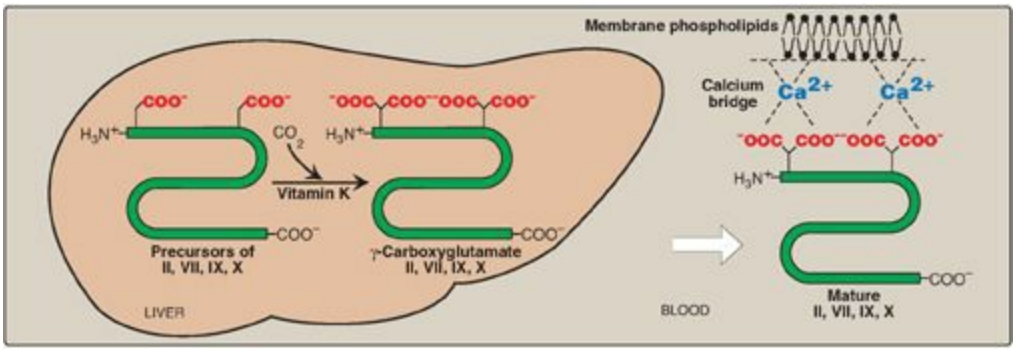
Figure 28.26 Carboxylation of glutamate to form γ-carboxyglutamate.



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 of bone and proteins C and S (involved in limiting the formation of blood clots) also undergo γ-carboxylation.

Figure 28.27 Role of vitamin K in blood coagulation.



B. Distribution and requirement of vitamin K

Vitamin K is found in cabbage, kale, spinach, egg yolk, and liver. There is also extensive synthesis of the vitamin by the bacteria in the gut. The adequate intake for vitamin K is 120 mg/day for adult males and 90 mg for adult females.

C. Clinical indications

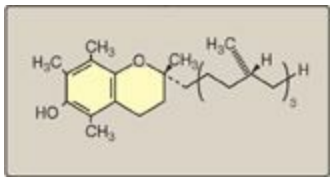
1. **Deficiency of vitamin K:** A true vitamin K deficiency is unusual because adequate amounts are generally produced by intestinal bacteria or obtained from the diet. If the bacterial population in the gut is decreased (for example, by antibiotics), the amount of endogenously formed vitamin is depressed, and this can lead to hypoprothrombinemia in the marginally malnourished individual (for example, a debilitated geriatric patient). This condition may require supplementation with vitamin K to correct the bleeding tendency. In addition, certain second-generation cephalosporin antibiotics (for example, cefamandole) cause hypoprothrombinemia, apparently by a warfarin-like mechanism that inhibits VKOR. Consequently, their use in treatment is usually supplemented with vitamin K.

2. **Deficiency of vitamin K in the newborn:** Newborns have sterile intestines and, so, 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.

D. Toxicity of vitamin K

Prolonged administration of large doses of synthetic vitamin K (menadione) can produce hemolytic anemia and jaundice in the infant, due to toxic effects on the membrane of RBCs. Therefore, it is no longer used to treat vitamin K deficiency. No UL has been set for vitamin K.

Figure 28.28 Structure of vitamin E.



XIV. VITAMIN E

The E vitamins consist of eight naturally occurring tocopherols, of which α-tocopherol is the most active (Figure 28.28). The primary function of vitamin E is as an antioxidant in prevention of the nonenzymic oxidation of cell components (for example, peroxidation of polyunsaturated FAs by molecular oxygen and free radicals).

A. Distribution and requirements of vitamin E

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 of vitamin E

Newborns have low reserves of vitamin E, but breast milk (and formulas) contain the vitamin. Very-low-birth-weight infants may be given supplements to prevent the hemolysis and retinopathy associated with deficiency of vitamin E. 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. 231).]

C. Clinical indications

Vitamin E is not recommended for the prevention of chronic disease, such as coronary heart disease 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.

D. Toxicity of vitamin E

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 vitamins A, C, or E; multivitamins with folic acid; or antioxidant combinations for the prevention of cancer or cardiovascular disease.

The vitamins are summarized in Figure 28.29.

Figure 28.29 Summary of vitamins. P = phosphate; NAD(P) = nicotinamide adenine dinucleotide (phosphate); FMN = flavin mononucleotide; FAD = flavin adenine dinucleotide; CoA = coenzyme A. Summary of vitamins.

VITAMIN	OTHER NAMES	ACTIVE FORM	FUNCTION
Folic acid	–	Tetrahydro-folic acid	Transfer one-carbon units; synthesis of methionine, purines, 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	Pridoxine 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
Niacin	Nicotinic acid Nicotinamide	NAD^+, $NADP^+$	Electron transfer
Vitamin B_2	Riboflavin	FMN, FAD	Electron transfer
Biotin	–	Enzyme-bound biotin	Carboxylation reactions
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-Dihydroxy-cholecalciferol	Calcium uptake Gene expression
Vitamin K	Menadione Menaquinone Phylloquinone	Menadione Menaquinone Phylloquinon	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	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 alcoholics)	Tachycardia, vomiting, convulsions Apathy, loss of memory, dysregulated eye movements	None	–
Pellagra	Dermatitis Diarrhea Dementia	None	High doses of niacin used to treat hyperlipidemia
Rare	Dermatitis Angular stomatitis	None	–
Rare	–	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
infertility Night blindness Retardation of growth Xerophthalmia	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 not established in controlled trials

Study Questions

Choose the ONE best answer.

For Questions 28.1–28.5, match the vitamin deficiency to the clinical consequence.

A. Folic acid
E. Vitamin C
B. Niacin
F. Vitamin D
C. Vitamin A
G. Vitamin E
D. Vitamin B_{12}
H. Vitamin K

28.1 Bleeding

28.2 Diarrhea and dermatitis

28.3 Neural tube defects

28.4 Night blindness

28.5 Sore, spongy gums and loose teeth

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 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.

28.6 A 52-year-old woman 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 woman?

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 to methionine, which also requires folic acid (as tetrahydrofolate [THF]), and the conversion of methymalonyl coenzyme A to succinyl coenzyme A, which does not require THF. The elevated homocysteine 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 girl, 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 vitamin D–deficient rickets. 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.

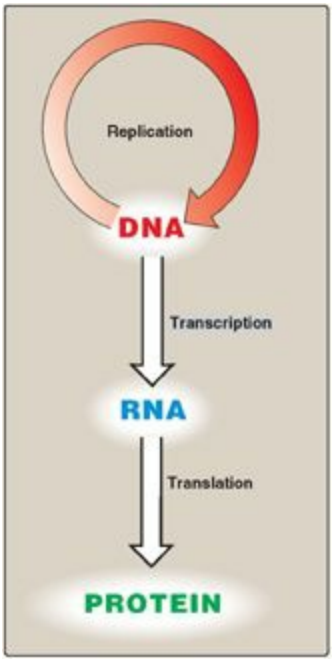
DNA Structure, Replication, and Repair

29

I. OVERVIEW

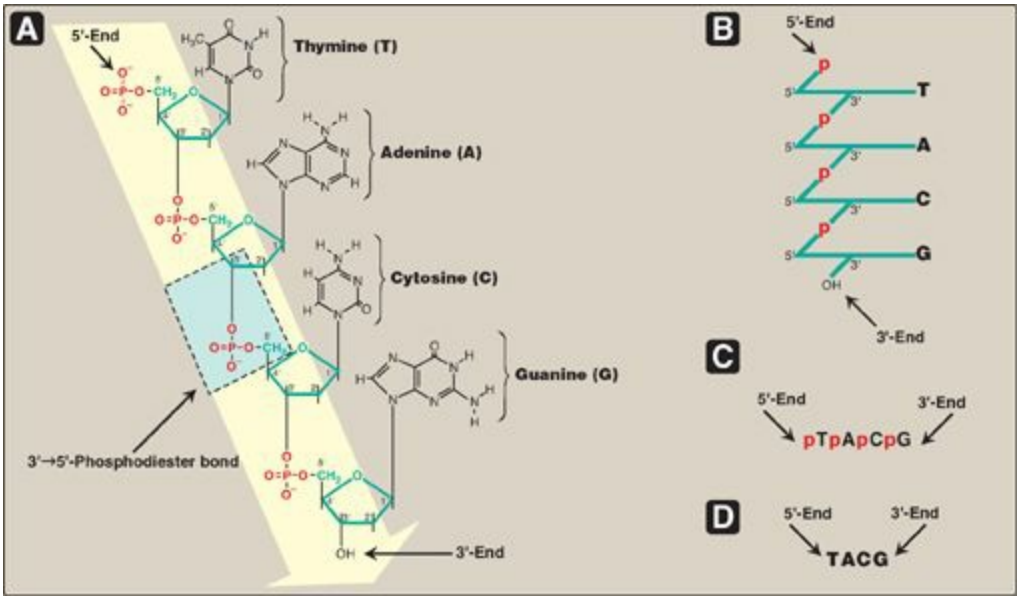
Nucleic acids are required for the storage and expression of genetic information. There are two chemically distinct types of nucleic acids: deoxyribonucleic acid (DNA) and ribonucleic acid ([RNA], see Chapter 30). DNA, the repository of genetic information, is present not only in chromosomes in the nucleus 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. 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. Each cell is specialized, expressing only those functions that are required for it to perform its role in maintaining the organism. Therefore, DNA must be able to not only replicate precisely each time a cell divides, but also to have the information that it contains be selectively expressed. Transcription (RNA synthesis) is the first stage in the expression of genetic information (see Chapter 30). Next, the code contained in the nucleotide sequence of messenger RNA molecules is translated (protein synthesis; see Chapter 31), thus completing gene expression. The regulation of gene expression is discussed in Chapter 32.

Figure 29.1 The "central dogma" of molecular biology.



> The flow of information from DNA to RNA to protein is termed the "central dogma" of molecular biology (Figure 29.1) and is descriptive of all organisms, with the exception of some viruses that have RNA as the repository of their genetic information.

Figure 29.2 A. DNA chain with the nucleotide sequence shown written in the 5′→3′ direction. A 3′→5′-phosphodiester bond is shown highlighted in the blue box, and the deoxyribose-phosphate backbone is shaded in yellow. B. The DNA chain written in a more stylized form, emphasizing the deoxyribose-phosphate backbone. C. A simpler representation of the nucleotide sequence. D. The simplest (and most common) representation, with the abbreviations for the bases written in the conventional 5′→3′ direction.



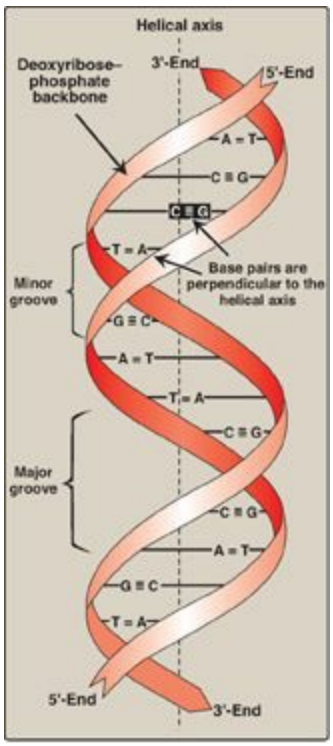
II. STRUCTURE OF DNA

DNA is a polymer of deoxyribonucleoside monophosphates (dNMPs) covalently linked by 3→5–phosphodiester bonds. With the exception of a few viruses that contain single-stranded (ss) DNA, DNA exists as a double-stranded (ds) molecule, in which the two strands wind around each other, forming a double helix. [Note: The sequence of the linked dNMPs 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 nonmembrane-bound region known as the nucleoid.

A. 3′→5′-Phosphodiester bonds

Phosphodiester bonds join the 3-hydroxyl group of the deoxypentose of one nucleotide to the 5-hydroxyl group of the deoxypentose of an adjacent nucleotide through a phosphoryl group (Figure 29.2). 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. The bases located along the resulting deoxyribose–phosphate backbone are, by convention, always written in sequence from the 5-end of the chain to the 3-end. For example, the sequence of bases in the DNA shown in Figure 29.2D (5′-TACG-3′) 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 29.3 DNA double helix, illustrating some of its major structural features.



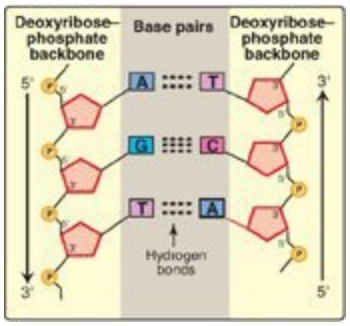
B. Double helix

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 (that is, the 5′-end of one strand is paired with the 3′-end of the other strand) as shown in Figure 29.3. 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 narrow 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 are perpendicular to the helical axis (see Figure 29.3).] 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 (Figure 29.4). [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 equals the total amount of pyrimidines.] The base pairs are held together by hydrogen bonds: two between A and T and three between G and C (Figure 29.5). These hydrogen bonds, plus the hydrophobic interactions between the stacked bases, stabilize the structure of the double helix.

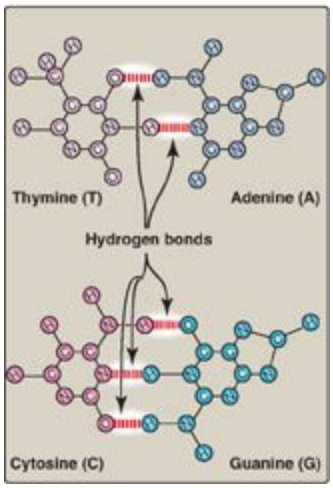
2. **Separation of the two DNA strands in the double helix:** The two strands of the double helix separate when hydrogen bonds between the paired bases are disrupted. Disruption can occur in the laboratory if the pH of the DNA solution is altered so that the nucleotide bases ionize, or if the solution is heated. [Note: Phosphodiester bonds are not broken by such treatment.] When DNA is heated, the temperature at which one half of the helical structure is lost 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 G- and C-rich DNA (Figure 29.6). Under appropriate conditions, 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 29.4 Two complementary DNA sequences. T= thymine; A = adenine; C = cytosine; G = guanine.



3. **Structural forms of the double helix:** 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 base pairs per 360° 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 (Figure 29.7 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 base pairs per turn, and the planes of the base pairs are tilted 20° away from the perpendicular to the helical axis. The conformation found in DNA–RNA hybrids or RNA–RNA double-stranded regions is probably very close to the A form. Z-DNA is a left-handed helix that contains 12 base pairs per turn (see Figure 29.7). [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 (for example, poly GC). Transitions between the B and Z helical forms of DNA may play a role in regulating gene expression.

Figure 29.5 Hydrogen bonds between complementary bases.



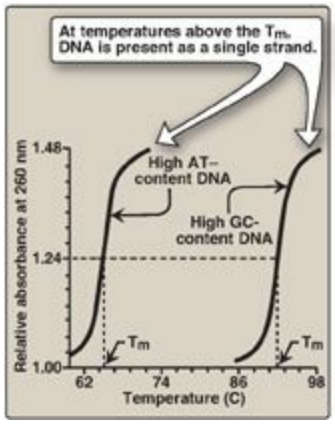
C. Linear and circular DNA molecules

Each chromosome in the nucleus of a eukaryote contains one long, linear molecule of dsDNA, which is bound to a complex mixture of proteins (histone and nonhistone, see

p. 409) 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. [Note: Circular DNA is "supercoiled", that is, the double helix crosses over on itself one or more times. Supercoiling can result in overwinding (positive supercoiling) or underwinding (negative supercoiling) of DNA. Supercoiling, a type of tertiary structure, compacts DNA.] 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 33.]

> 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.

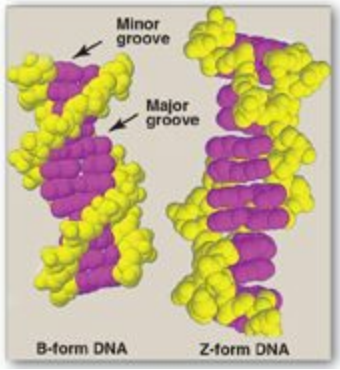
Figure 29.6 Melting temperatures (T_m) of DNA molecules with different nucleotide compositions.



III. STEPS IN PROKARYOTIC DNA SYNTHESIS

When the two strands of the DNA double helix are separated, each can serve as a template for the replication of a new complementary strand. This produces two daughter molecules, each of which contains two DNA strands with an antiparallel orientation (see Figure 29.3). 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 individual parental strands remains intact in one of the two new duplexes (Figure 29.8). The enzymes involved in the DNA replication process are template-directed 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 29.7 Structures of B-DNA and Z-DNA.



A. Separation of the two complementary DNA strands

In order for the two 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 (Figure 29.9A). [Note: This sequence is referred to as a consensus sequence, because the order of nucleotides is essentially the same at each site.] The ori includes short, AT-rich segments that facilitate melting. In eukaryotes, replication begins at multiple sites along the DNA helix (Figure 29.9B). Having multiple origins of replication provides a mechanism for rapidly replicating the great length of eukaryotic DNA molecules.

B. Formation of the replication fork

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 Figure 29.9). [Note: The term "replication fork" derives from the Y-shaped structure in which the tines of the fork represent the separated strands

(Figure 29.10).]

1. **Proteins required for DNA strand separation:** Initiation of DNA replication requires the recognition of the origin by a group of proteins that form the prepriming complex. These proteins are responsible for maintaining the separation of the parental strands, and for unwinding the double helix ahead of the advancing replication fork. These proteins include the following.

Figure 29.8 Semiconservative replication of DNA. T= thymine; A = adenine; C = cytosine; G = guanine.

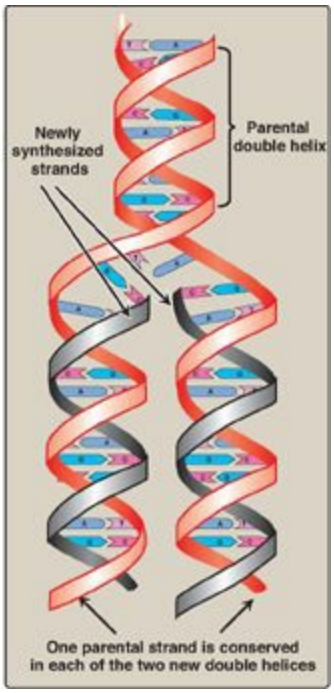
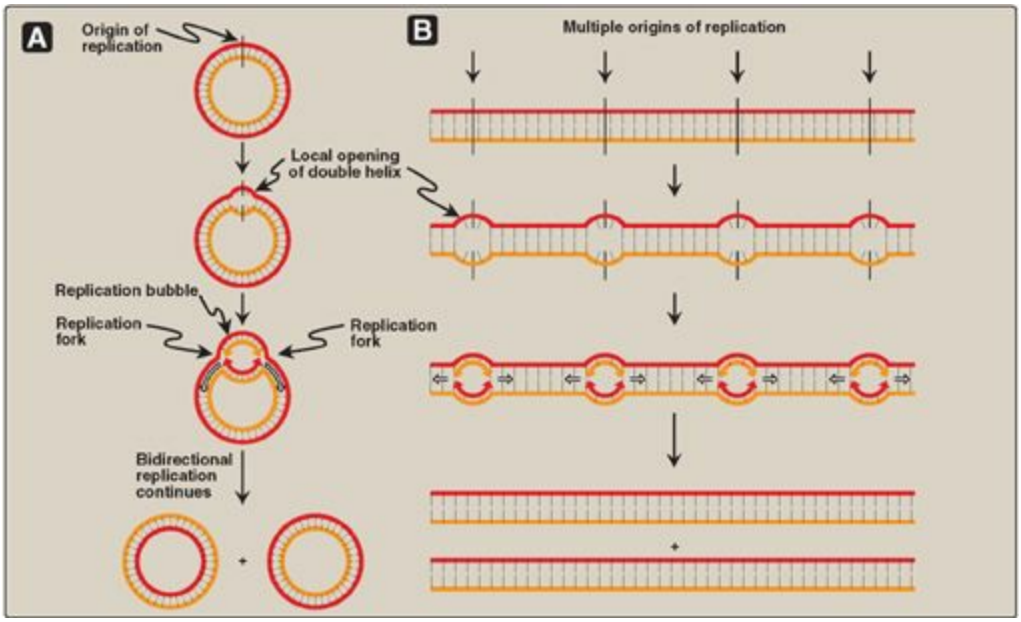


Figure 29.9 Replication of DNA: origins and replication forks. A. Small prokaryotic circular DNA. B. Very long eukaryotic DNA.

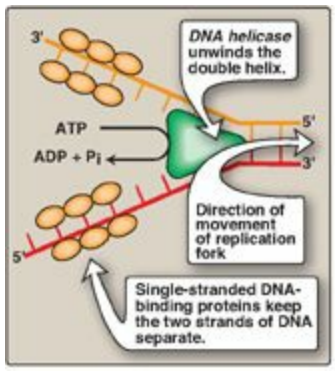


a. DnaA protein: DnaA protein binds to specific nucleotide sequences (DnaA boxes) within the origin of replication, causing the short, tandemly arranged (one after the other) AT-rich regions in the origin to melt. Melting is adenosine triphosphate (ATP) dependent, and results in strand separation with the formation of localized regions 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 (see Figure 29.10). 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. Its binding to DNA requires DnaC.]

c. Single-stranded DNA-binding protein: This protein binds to the ssDNA generated by helicases (see Figure 29.10). Binding is cooperative (that is, 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.

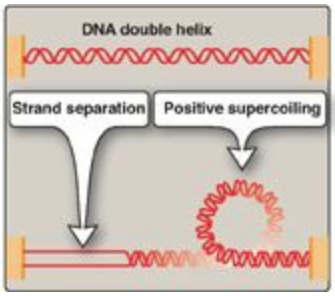
Figure 29.10 Proteins responsible for maintaining the separation of the parental strands and unwinding the double helix ahead of the advancing replication fork (➢). ADP = adenosine diphosphate; P_i = inorganic phosphate.



2. Solving the problem of supercoils: 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 as a result of overwinding (Figure 29.11), and negative supercoils in the region behind the fork. The accumulating positive supercoils interfere with further unwinding of the double helix. [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

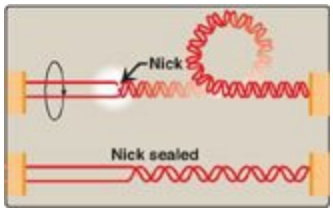
the coils, the cord will wrap around itself in space to form positive supercoils. If the cord is twisted in the direction of loosening the coils, the cord will wrap around itself in the opposite direction to form negative supercoils.] To solve this problem, there is a group of enzymes called DNA topoisomerases, which are responsible for removing supercoils in the helix by transiently cleaving one or both of the DNA strands.

Figure 29.11 Positive supercoiling resulting from DNA strand separation.



a. **Type I DNA topoisomerases:** These enzymes reversibly cleave one strand of the double helix. 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 (Figure 29.12). Each time a transient "nick" is created in one DNA strand, the intact DNA strand is passed through the break before it is resealed, thus relieving ("relaxing") accumulated supercoils. Type I topoisomerases relax negative supercoils (that is, those that contain fewer turns of the helix than relaxed DNA) in E. coli, and both negative and positive supercoils (that is, those that contain fewer or more turns of the helix than relaxed DNA) in many prokaryotic cells (but not E. coli) and in eukaryotic cells.

Figure 29.12 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 causes a second stretch of the DNA double helix to pass through the break and, finally, reseals the break (Figure 29.13). 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. 420).

> 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 (for example, ciprofloxacin).

Figure 29.13 Action of type II DNA topoisomerase.

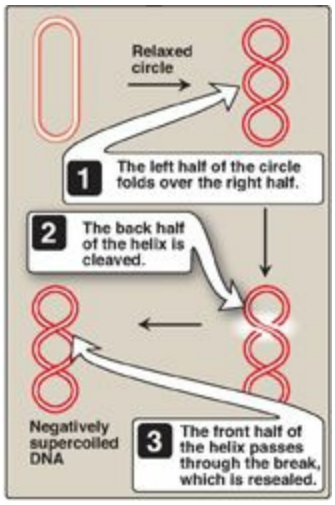
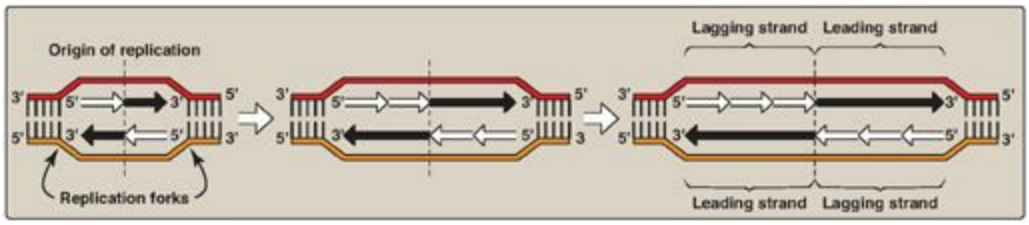


Figure 29.14 Discontinuous synthesis of DNA.



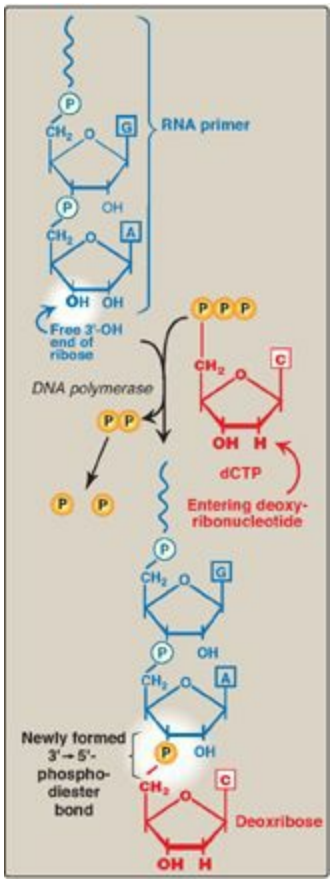
C. Direction of DNA replication

The DNA polymerases 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 (Figure 29.14). 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 called the leading strand and is synthesized continuously.

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) to become a single, continuous strand. The new strand of DNA produced by this mechanism is termed the lagging strand.

Figure 29.15 Use of an RNA primer to initiate DNA synthesis. P = phosphate.



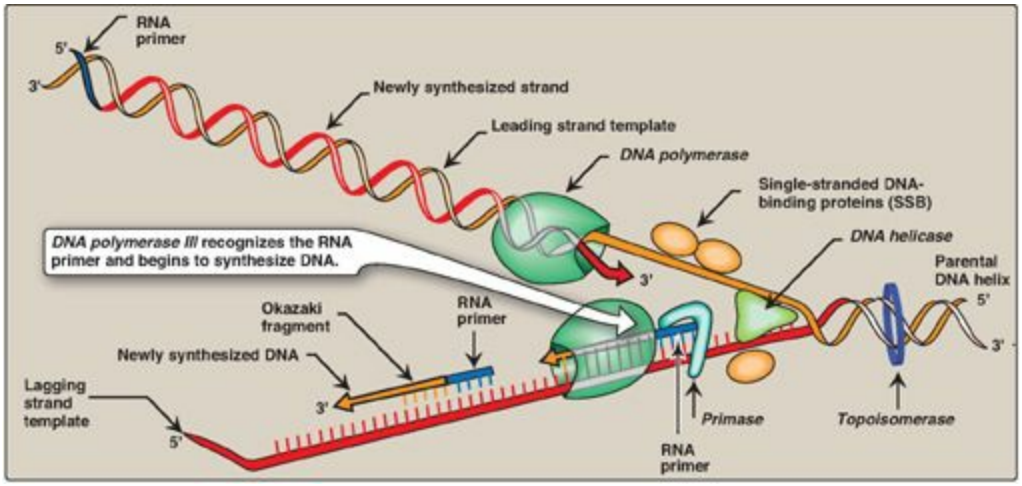
D. RNA primer

DNA polymerases 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, double-stranded region consisting of RNA base-paired to the DNA template, with a free hydroxyl group on the 3I-end of the RNA strand (Figure 29.15). This hydroxyl group serves as the first acceptor of a deoxynucleotide by action of a DNA polymerase. [Note: Recall that glycogen synthase also requires a primer (see p. 126).]

1. **Primase:** A specific RNA polymerase, called primase (DnaG), synthesizes the short stretches of RNA (approximately ten nucleotides long) that are complementary and antiparallel to the DNA template. In the resulting hybrid duplex, the U (uracil) in RNA pairs with A in DNA. As shown in Figure 29.16, 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 5I-ribonucleoside triphosphates, and pyrophosphate is

released as each ribonucleoside monophosphate is added through formation of a 3′→5′ phosphodiester bond. [Note: The RNA primer is later removed as described on p. 405.]

Figure 29.16 Elongation of the leading and lagging strands. [Note: The DNA sliding clamp is not shown.]



2. **Primosome:** The addition of primase converts the prepriming complex of proteins required for DNA strand separation (see p. 399) to a primosome. The primosome makes the RNA primer required for leading strand synthesis and initiates Okazaki fragment formation in lagging strand synthesis. As with DNA synthesis, the direction of synthesis of the primer is 5′→3′.

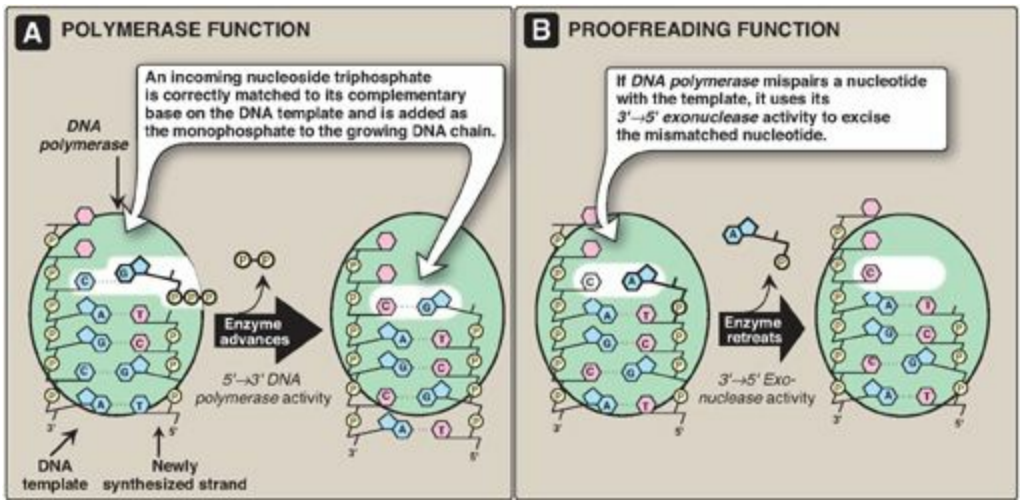
E. Chain elongation

Prokaryotic (and eukaryotic) DNA polymerases (DNA pols) elongate a new DNA strand by adding deoxyribonucleotides, one at a time, to the 3′-end of the growing chain (see Figure 29.16). The sequence of nucleotides that are added is dictated by the base sequence of the template strand with which the incoming nucleotides are paired.

1. **DNA polymerase III:** DNA chain elongation is catalyzed by the multisubunit enzyme, DNA polymerase III (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 (that is, 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 its β subunit 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 strand grows in the 5′→3′ direction, antiparallel to the parental strand (see Figure 29.16). The nucleotide substrates are 5′-deoxyribonucleoside triphosphates. Pyrophosphate (PP_i) is released when each new deoxynucleoside monophosphate is

added to the growing chain (see Figure 29.15). Hydrolysis of PP_i to $2P_i$ means that a total of two high-energy bonds are used to drive the addition of each deoxynucleotide.

Figure 29.17 3′→5′ Exonuclease activity enables DNA polymerase III to "proofread" the newly synthesized DNA strand.



> The production of PP_i with subsequent hydrolysis to $2P_i$, as seen in DNA replication, is a common theme in biochemistry. Removal of the PP_i product drives a reaction 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. If one of the four is in short supply, DNA synthesis stops when that nucleotide is depleted.

2. **Proofreading of 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, Figure 29.17) in addition to its 5′→3′ polymerase activity. As each nucleotide is added to the chain, DNA pol III checks to make certain the added nucleotide is, in fact, correctly matched to its complementary base on the template. If it is not, the 3′→5′ exonuclease activity removes the error. [Note: The enzyme requires an improperly base-paired 3′-hydroxy terminus and, therefore, does not degrade correctly paired nucleotide sequences.] For example, if the template base is C and the enzyme mistakenly 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 replaces it with the correct nucleotide containing G (see Figure 29.17). [Note: The proofreading exonuclease activity requires movement

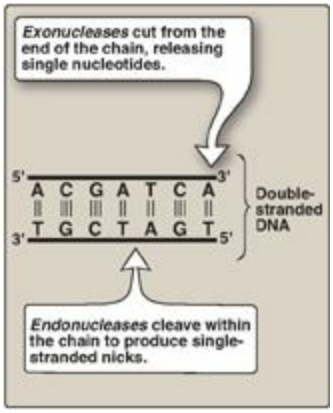
in the 3′→5′ direction, not 5′→3′ like the polymerase activity. This is because the excision must be done in the reverse direction from that of synthesis.]

F. Excision of RNA primers and their replacement by DNA

DNA pol III continues to synthesize DNA on the lagging strand until it is blocked by proximity to an RNA primer. When this occurs, the RNA is excised and the gap filled by DNA pol I.

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 chain like DNA pol III, DNA pol I also has a 5′→3′ exonuclease activity that is able to hydrolytically remove the RNA primer. [Note: These activities are exonucleases because they remove nucleotides from the end of the DNA chain, rather than cleaving the chain internally as do the endonucleases (Figure 29.18).] 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 the RNA, DNA pol I replaces it with deoxyribonucleotides, synthesizing DNA in the 5′→3′ direction (5′→3′ polymerase activity). As it synthesizes the DNA, it also "proofreads" the new chain using its 3′→5′ exonuclease activity to remove errors. This removal/synthesis/proofreading continues, one nucleotide at a time, until the RNA primer is totally degraded, and the gap is filled with DNA (Figure 29.19). [Note: DNA pol I uses its 5′→3′ polymerase activity to fill in gaps generated during DNA repair (see p.410).]

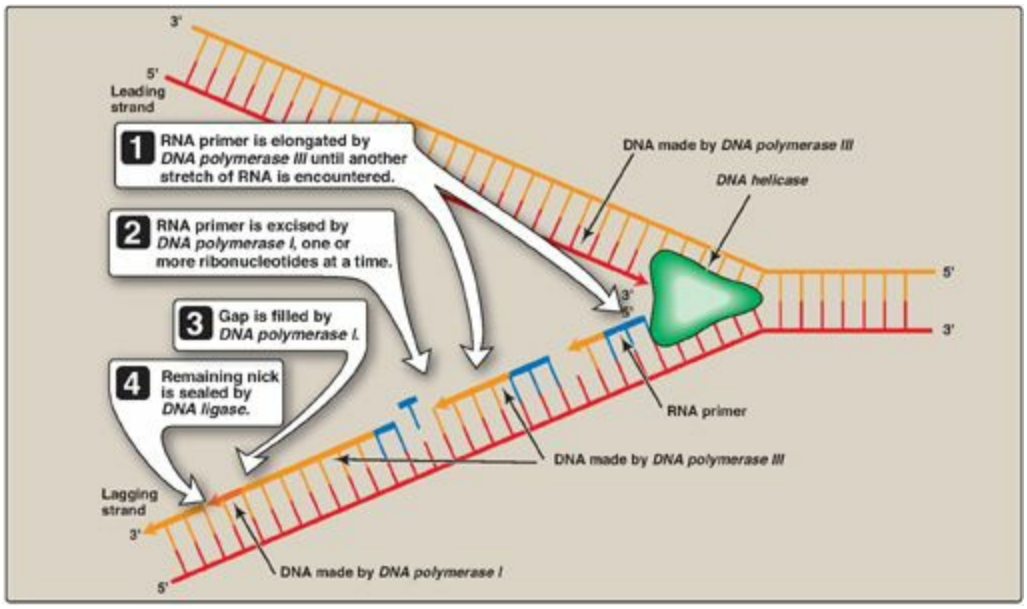
Figure 29.18 Endonuclease versus exonuclease activity. [Note: Restriction endonucleases (see p. 465) cleave both strands.] T= thymine; A = adenine; C = cytosine; G = guanine.



2. **Comparison of 5′→3′ and 3′→5′ exonucleases:** 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 20 to 30 nucleotide–long RNA primer. In contrast, the 3′→5′ exonuclease activity of DNA pol I, as well as DNA pol

II and 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 one error per 10^7 nucleotides.

Figure 29.19 Removal of RNA primer and filling of the resulting "gaps" by DNA polymerase I.



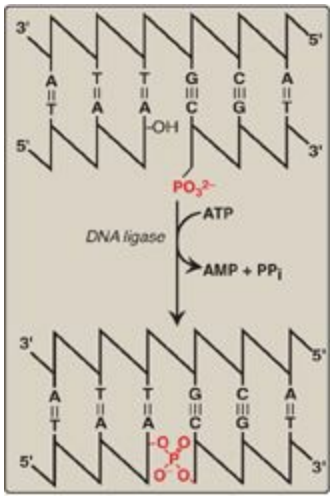
G. DNA ligase

The final phosphodiester linkage between the 5′-phosphate group on the DNA chain synthesized by DNA pol III and the 3′-hydroxyl group on the chain made by DNA pol I is catalyzed by DNA ligase (Figure 29.20). The joining of these two stretches of DNA requires energy, which in most organisms is provided by the cleavage of ATP to AMP + PP_i.

H. Termination

Replication termination in E. coli is mediated by sequence-specific binding of the protein, Tus (terminus utilization substance) to replication termination sites (ter sites) on the DNA, stopping the movement of DNA polymerase.

Figure 29.20 Formation of a phosphodiester bond by DNA ligase. [Note: AMP is first linked to ligase, then to the 5′ phosphate, and then released.]


3'
5'
A
T
T
A
T
A
G
C
C
G
A
T
OH
5'
3'
PO_3^{2-}
ATP
DNA ligase
AMP + PP_i
3'
5'
A
T
T
A
T
A
G
C
C
G
A
T
O
P
O
O
O
5'
3'

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 single-stranded DNA-binding proteins and ATP-dependent DNA helicases have been identified, whose functions are analogous to those of the prokaryotic enzymes previously discussed. In contrast, RNA primers are removed by RNase H and flap endonuclease-1 (FEN1) rather than by a DNA polymerase (Figure 29.21).

A. Eukaryotic cell cycle

The events surrounding eukaryotic DNA replication and cell division (mitosis) are coordinated to produce the cell cycle (Figure 29.22). The period preceding replication is called the G_1 phase (Gap 1). DNA replication occurs during the S (synthesis) phase. Following DNA synthesis, there is another period (G_2 phase, 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 29.21 Proteins and their function in eukaryotic replication. ORC = origin recognition complex, MCM = minichromosome maintenance (complex), RPA = replication protein A, PCNA = proliferating cell nuclear antigen.

FUNCTION	PROTEIN (s)
Origin recognition	ORC
Helicase activity	*MCM*
ssDNA protection	RPA
Primer synthesis	*Pol α/primase*
Sliding clamp	PCNA
Primer removal	*RNase H, FEN 1*

B. Eukaryotic DNA polymerases

At least five high-fidelity eukaryotic DNA polymerases (pol) 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 (Figure 29.23).

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. [Note: Pol α is also referred to as pol α/primase.]

2. **Pol ε and pol** d: Pol ε is recruited to complete DNA synthesis on the leading strand, whereas pol d 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 subunit of DNA pol III does in E. coli, thus ensuring high processivity.]

3. **Pol β and pol** γ: Pol β is involved in "gap filling" in DNA repair (see below). Pol γ replicates mitochondrial DNA.

Figure 29.22 The eukaryotic cell cycle. [Note: Cells can leave the cell cycle and enter a reversible quiescent state called G_0.]

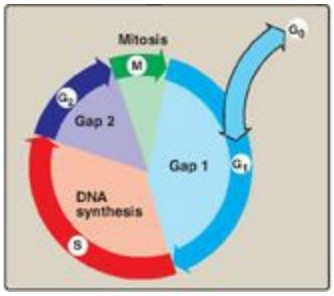


Figure 29.23 Activities of eukaryotic DNA polymerase (pol) *3′→5′ exonuclease activity.

POLY-MERASE	FUNCTION	PROOF-READING*
Pol α (alpha)	• Contains primase • Initiates DNA synthesis	–
Pol β (beta)	• Repair	–
Pol γ (gamma)	• Replicates mitochondrial DNA	+
Pol δ (delta)	• Elongates Okazaki fragments of the lagging strand	+
Pol ε (epsilon)	• Elongates the leading strand	+

C. Telomeres

Telomeres are complexes of noncoding DNA plus 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 a complementary region of Cs and As. The GT-rich strand is longer than its CA complement, 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.

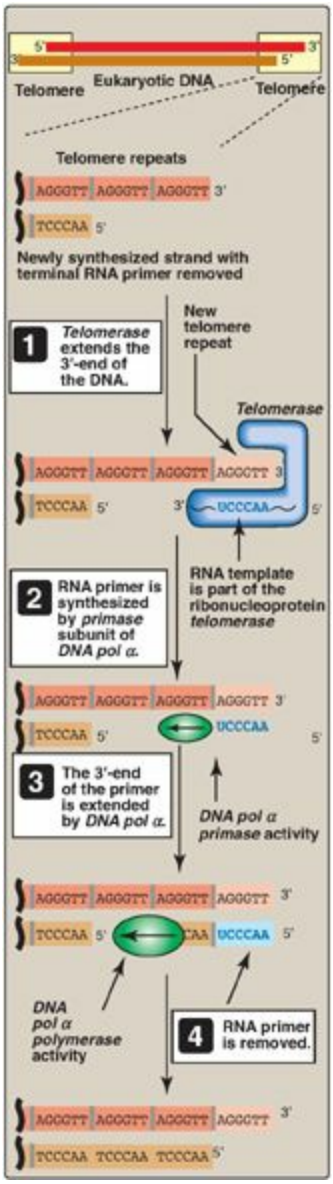
1. **Telomere shortening:** Eukaryotic cells face a special problem in replicating the ends of their linear DNA molecules. Following removal of the RNA primer from the

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 other stem cells, as well as in cancer cells, telomeres do not shorten and the cells do not senesce. This is a result of the presence of a 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 CA-rich RNA template base-pairs with the GT-rich, single-stranded 3′-end of telomeric DNA (Figure 29.24). 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 GT-rich strand has been lengthened, primase activity of DNA pol α can use it as a template to synthesize an RNA primer. The RNA primer is extended by DNA pol α, and then removed.

> 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 aging and cancer.

Figure 29.24 Mechanism of action of telomerase. T= thymine; A = adenine; C = cytosine; G = guanine; pol = polymerase.



D. Reverse transcriptases

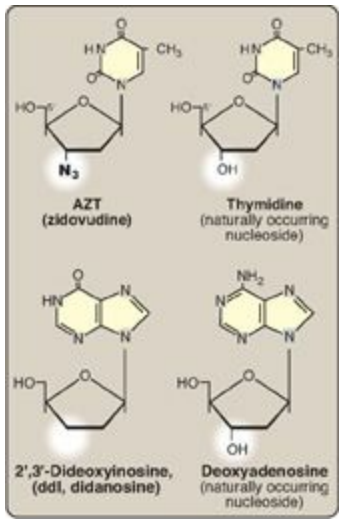
As seen with telomerase, reverse transcriptases are RNA-directed DNA polymerases. A reverse transcriptase is involved in the replication of retroviruses, such as human immunodeficiency virus (HIV). These viruses carry their genome in the form of ssRNA molecules. Following infection of a host cell, the 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. 461). In eukaryotes, such elements 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.]

E. Inhibition of DNA synthesis by nucleoside analogs

DNA chain growth can be blocked by the incorporation of certain nucleoside analogs that have been modified on the sugar portion (Figure 29.25). For example, removal of

the hydroxyl group from the 3**I**-carbon of the deoxyribose ring as in 2**I**,3**I**-dideoxyinosine ([ddI] also known as didanosine), or conversion of the deoxyribose to another sugar, such as arabinose, prevents further chain elongation. By blocking DNA replication, these compounds slow the division of rapidly growing cells and 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 zidovudine (AZT, ZDV), also terminates DNA chain elongation. [Note: These drugs are generally supplied as nucleosides, which are then converted to the active nucleotides by cellular kinases.

Figure 29.25 Examples of nucleoside analogs that lack a 3**I**-hydroxyl group. [Note: ddI is converted to its active form (ddATP).]



V. ORGANIZATION OF EUKARYOTIC DNA

A typical (diploid) human cell contains 46 chromosomes, whose total DNA is approximately 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 the formation of nucleosomes

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, causing supercoiling (Figure 29.26). [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 (see p. 422). Histone modification is an example of "epigenetics" or heritable changes in gene expression without alteration of the nucleotide sequence.] Neighboring nucleosomes are joined by "linker" DNA approximately 50 base pairs long. H1, of which there are several related species, 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 29.26 Organization of human DNA, illustrating the structure of nucleosomes. H = histone.

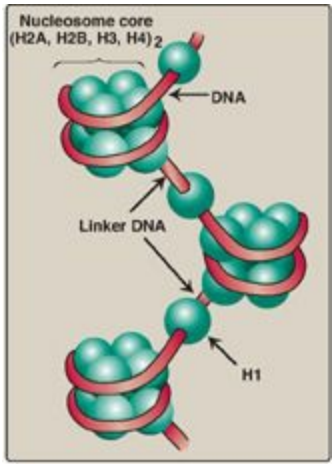
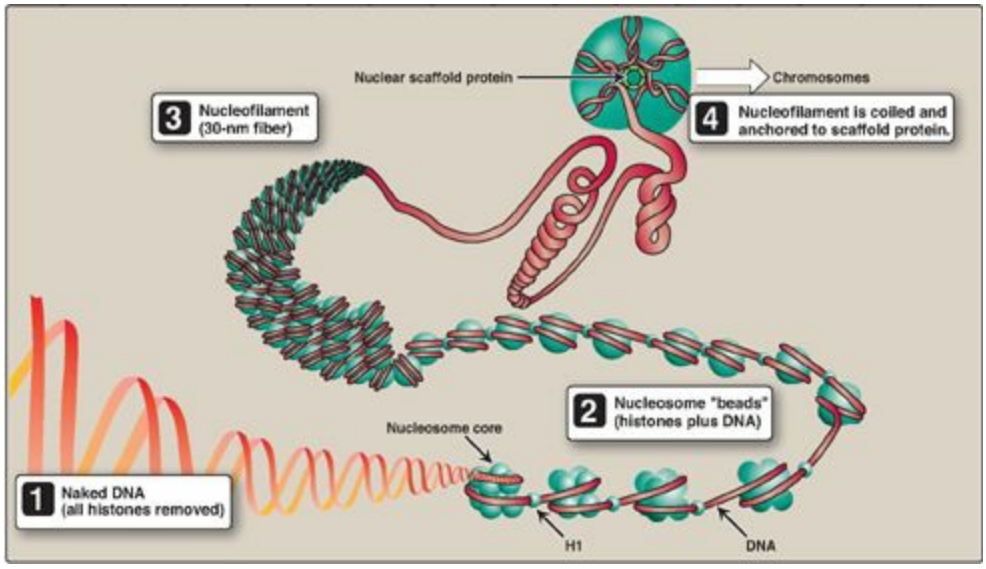


Figure 29.27 Structural organization of eukaryotic DNA. [Note: A 10^4 compaction is seen from 1 to 4.] H = histone.



2. **Higher levels of organization:** Nucleosomes can be packed more tightly to form a polynucleosome (also called 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 (Figure 29.27).

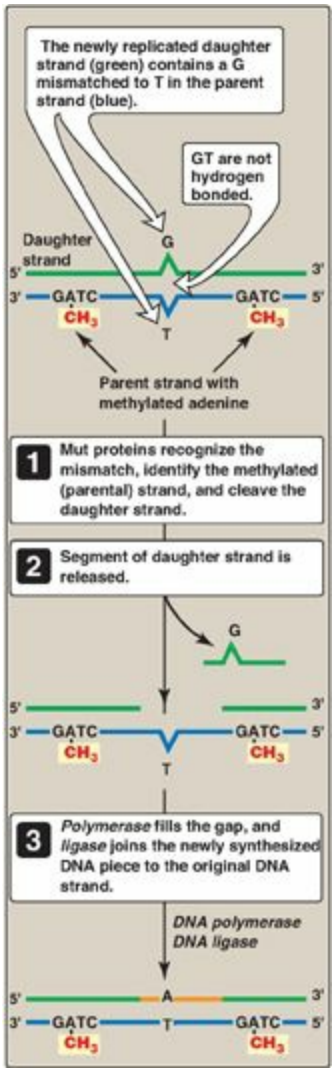
B. Fate of nucleosomes during DNA replication

Parental nucleosomes are disassembled to allow access to DNA during replication. Once DNA is synthesized, nucleosomes form rapidly. Their histone proteins come both from new synthesis and from the transfer of intact parental histone octamers.

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 (for example, nitrous acid, which can deaminate bases), or radiation (for example, non-ionizing ultraviolet light, 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. Most of the repair systems involve recognition of the damage (lesion) on the DNA, removal or excision of the damage, replacement or filling the gap left by excision using the sister strand as a template for DNA synthesis, and ligation. These excision repair systems remove one to tens of nucleotides. [Note: Repair synthesis of DNA can occur outside of the S phase.]

Figure 29.28 Methyl-directed mismatch repair in E. coli. [Note: The Mut proteins, S and L, recognize the mismatch and identify the parental (methylated) strand, and Mut H cleaves the daughter strand.] A = adenine; C= cytosine; G = guanine; T = thymine.



A. Repair of mismatched bases (mismatch repair)

Sometimes replication errors escape the proofreading function 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 (Figure 29.28). Homologous proteins are present in humans. [Note: MMR reduces the error rate of replication from one in ten million to one in a billion.]

1. **Identification of the mismatched strand:** 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. Discrimination is based on the degree of methylation. GATC sequences, which are found approximately once every thousand nucleotides, are methylated on the adenine (A) residue. This methylation is not done immediately after synthesis, so the newly synthesized DNA is hemimethylated (that is, 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.]

2. **Repair of damaged DNA:** 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 polymerase, typically DNA pol I. 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 (see p. 406).

> Mutation to the proteins involved in mismatch repair in humans is associated with hereditary nonpolyposis colorectal cancer (HNPCC), also known as Lynch syndrome. With HNPCC, there is an increased risk for developing colon cancer (as well as other cancers); however, only about 5% of all colon cancer is the result of mutations in mismatch repair.

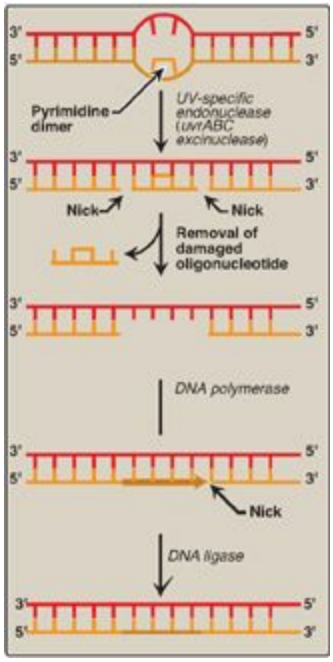
B. Repair of damage caused by ultraviolet light (nucleotide excision repair)

Exposure of a cell to ultrtaviolet (UV) light can result in the covalent joining of two adjacent pyrimidines (usually thymines), producing a dimer. These thymine dimers prevent DNA pol from replicating the DNA strand beyond the site of dimer formation. Thymine dimers are excised in bacteria by UvrABC proteins in a process known as nucleotide excision repair (NER) as illustrated in Figure 29.29. A related pathway is present in humans (see below).

1. **Recognition and excision of dimers by UV-specific endonuclease:** First, a UV-specific endonuclease (called uvrABC excinuclease) recognizes the dimer and cleaves the damaged strand on both the 5′-side and 3′-side of the dimer. A short oligonucleotide containing the dimer is released, leaving a gap in the DNA strand that formerly contained the dimer. This gap is filled in using a DNA polymerase and DNA ligase.

2. **UV radiation and cancer:** Pyrimidine dimers can be formed in the skin cells of humans exposed to unfiltered sunlight. In the rare genetic disease xeroderma pigmentosum (XP), the cells cannot repair the damaged DNA, resulting in extensive accumulation of mutations and, consequently, early and numerous skin cancers (Figure 29.30). XP can be caused by defects in any of the several genes that code for the XP proteins required for nucleotide excision repair of UV damage in humans.

Figure 29.29 Nucleotide excision repair of pyrimidine dimers in E. coli DNA. UV = ultraviolet.



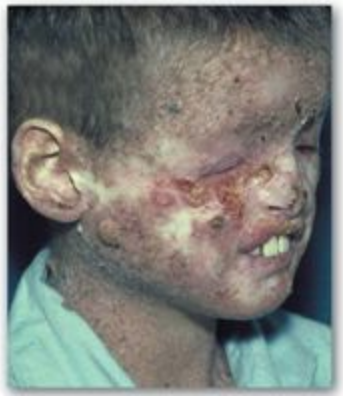
C. Repair of base alterations (base excision repair)

The bases of DNA can be altered, either spontaneously, as is the case with cytosine, which slowly undergoes deamination (the loss of its amino group) to form uracil, or by the action of deaminating or alkylating compounds. For example, nitrous acid, which is formed by the cell from precursors such as the nitrosamines, nitrites, and nitrates, is a potent compound that deaminates cytosine, adenine (to hypoxanthine), and guanine (to xanthine). Bases can also be lost spontaneously. For example, approximately 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] Figure 29.31).

1. **Removal of abnormal bases:** In BER, abnormal bases, such as uracil, which can occur in DNA either by deamination of cytosine or improper use of dUTP instead of dTTP during DNA synthesis, are recognized by specific 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. **Recognition and repair of an AP site:** 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**I**-side of the AP site. A deoxyribose phosphate lyase removes the single, base-free, sugar phosphate residue. DNA polymerase and DNA ligase complete the repair process.

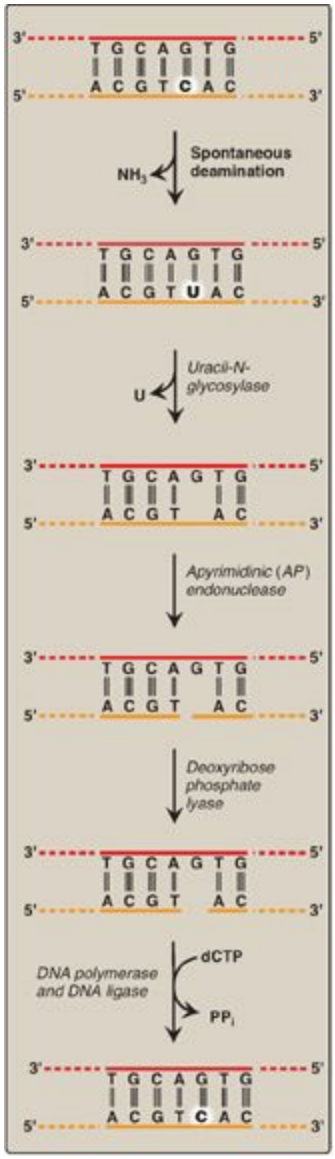
Figure 29.30 Patient with xeroderma pigmentosum.



D. Repair of double-strand breaks

Ionizing radiation or oxidative free radicals (see p. 148) can cause double-strand breaks in DNA that are potentially lethal to the cell. Such breaks also occur naturally during gene rearrangements. dsDNA breaks cannot be corrected by the previously described strategy of excising the damage on one strand and using the remaining 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, this mechanism of repair is error prone and mutagenic. Defects in this repair system 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 than NHEJ because any DNA that was lost is replaced using homologous DNA as a template. [Note: Mutations to the proteins, BRCA1 or 2 (breast cancer 1 or 2), which are involved in HR, increase the risk for developing breast cancer.]

Figure 29.31 Correction of base alterations by base excision repair. T= thymine; A = adenine; C = cytosine; G = guanine; PP_i = pyrophosphate.

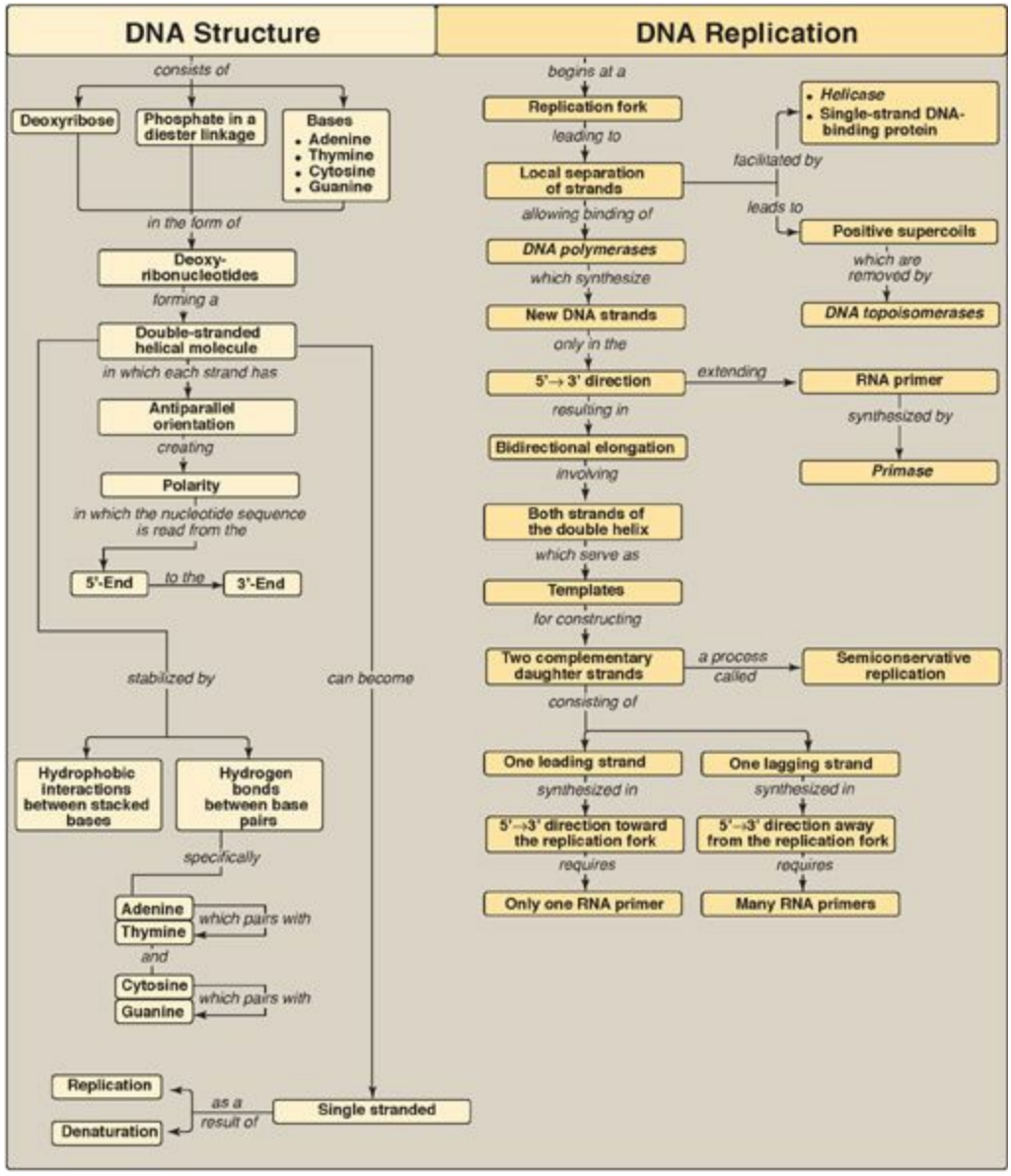

3' T G C A G T G 5'
5' A C G T C A C 3'
Spontaneous deamination
NH3
3' T G C A G T G 5'
5' A C G T U A C 3'
Uracil-N-glycosylase
U
3' T G C A G T G 5'
5' A C G T A C 3'
Apyrimidinic (AP) endonuclease
3' T G C A G T G 5'
5' A C G T A C 3'
Deoxyribose phosphate lyase
3' T G C A G T G 5'
5' A C G T A C 3'
DNA polymerase and DNA ligase
dCTP
PPi
3' T G C A G T G 5'
5' A C G T C A C 3'

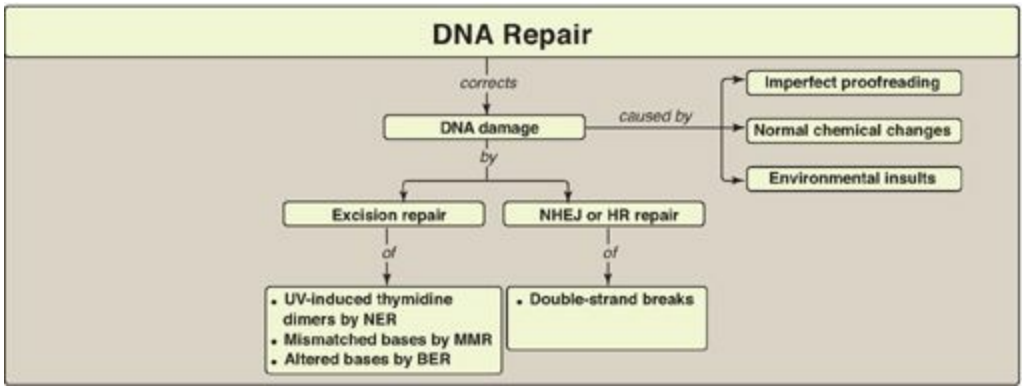
VII. CHAPTER SUMMARY

DNA is a polymer of deoxyribonucleoside monophosphates covalently linked by **3′→5′-phosphodiester bonds** (Figure 29.32). The resulting long, unbranched chain has **polarity**, with both a 5′-end and a 3′-end. The sequence of nucleotides is read 5′→3′. DNA exists as a **double-stranded** molecule, in which the two chains are paired in an **antiparallel** manner, and wind around each other, forming a **double helix**. **Adenine** pairs with **thymine**, and **cytosine** pairs with **guanine**. Each strand of the double helix serves as a **template** for constructing a **complementary** daughter strand (**semiconservative replication**). DNA replication occurs in the **S phase** of the cell cycle and begins at the **origin of replication**. As the two strands unwind and separate, synthesis occurs at two replication forks that move away from the origin in opposite directions (bidirectionally). **Helicase** unwinds the double helix. As the two strands of the double helix are separated, **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 polymerases (pol)** synthesize **n**ew DNA strands only in the **5′→3′** direction. Therefore, one of the newly synthesized stretches of nucleotide chains must grow in the 5′→3′ direction toward the replication fork (**leading strand**) and one in the 5′→3′ direction away from the replication fork (**lagging strand**). DNA pols require a **primer**. The primer for de novo DNA synthesis is a short stretch of RNA synthesized by **primase**. The leading strand only needs one RNA primer, whereas the lagging strand needs many. 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 polymerases**. **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** complexed with protein that 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 (for example, germline and cancer cells), **telomerase** employs its enzyme component **reverse transcriptase to** extend the telomeres, using its **RNA** as a **template**. There are five classes of **positively charged histone** (**H**) **proteins**. Two each of histones 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 can be packed more tightly to form a nucleofilament. Additional levels of organization create a chromosome. Most DNA damage can be corrected by **excision repair** involving recognition and removal of the damage by repair proteins, followed by replacement in E. coli by DNA pol and joining by ligase. **Ultraviolet light** can cause thymine dimers that are recognized and removed by **uvrABC proteins of nucleotide excision repair.** Defects in the **XP proteins** needed for thymine dimer repair in humans result in **xeroderma pigmentosum**. **Mismatched** bases are repaired by a similar process of recognition and removal by **Mut proteins** in E. coli. The extent of **methylation** is used for strand identification in prokaryotes. Defective mismatch repair by homologous proteins in humans is associated with **hereditary nonpolyposis colorectal cancer**. Abnormal bases (such as uracil) are removed by **glycosylases** in **base excision repair**, and the sugar phosphate at the apyrimidinic or apurinic (AP) site is cut out. Double-strand breaks in DNA are repaired by **nonhomologous end-joining** (error prone) and **homologous recombination**.

Figure 29.32 Key concept map for DNA structure, replication, and repair. Key concept map for DNA structure, replication, and repair. NHEJ = nonhomologous end-joining; HR = homologous recombination; NER = nucleotide excision repair; MMR = mismatch repair; BER = base excision repair; UV = ultraviolet light.


DNA Structure
consists of
Deoxyribose
Phosphate in a diester linkage
Bases
Adenine
Thymine
Cytosine
Guanine
in the form of
Deoxy-ribonucleotides
forming a
Double-stranded helical molecule
in which each strand has
Antiparallel orientation
creating
Polarity
in which the nucleotide sequence is read from the
5'-End
to the
3'-End
stabilized by
can become
Hydrophobic interactions between stacked bases
Hydrogen bonds between base pairs
specifically
Adenine
Thymine
which pairs with
and
Cytosine
Guanine
which pairs with
Replication
Denaturation
as a result of
Single stranded
DNA Replication
begins at a
Replication fork
leading to
Local separation of strands
facilitated by
Helicase
Single-strand DNA-binding protein
allowing binding of
DNA polymerases
leads to
Positive supercoils
which synthesize
New DNA strands
which are removed by
DNA topoisomerases
only in the
5'→ 3' direction
extending
RNA primer
resulting in
Bidirectional elongation
synthesized by
Primase
involving
Both strands of the double helix
which serve as
Templates
for constructing
Two complementary daughter strands
a process called
Semiconservative replication
consisting of
One leading strand
One lagging strand
synthesized in
synthesized in
5'→3' direction toward the replication fork
5'→3' direction away from the replication fork
requires
requires
Only one RNA primer
Many RNA primers


DNA Repair
corrects
DNA damage
caused by
Imperfect proofreading
Normal chemical changes
Environmental insults
by
Excision repair
NHEJ or HR repair
of
of
UV-induced thymidine dimers by NER
Mismatched bases by MMR
Altered bases by BER
Double-strand breaks

Study Questions

Choose the ONE best answer.

29.1 A 10-year-old girl 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

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 light–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 here does not cause XP.

29.2 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 in 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 3′→5′ 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 5′→3′ strand that gets extended.

29.3 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 As, 25 Gs, 30 Cs, and 22 Ts. How many of each base is found in the complete double-stranded molecule?

A. A = 40, G = 50, C = 60, T = 44
B. A = 44, G = 60, C = 50, T = 40
C. A = 45, G = 45, C = 52, T = 52
D. A = 50, G = 47, C = 50, T = 47
E. A = 42, G = 55, C = 55, T = 42

Correct answer = E. 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 As on the first strand would be paired with 20 Ts on the second strand, the 25 Gs on the first strand would be paired with 25 Cs on the second strand, and so forth. When these are all added together, the correct numbers of each base are indicated in choice E. Notice that, in the correct answer, A = T and G = C.

29.4 List the order in which the following enzymes participate in 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 III extends the primer with DNA (and proofreads);

polymerase 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′→3′ phosphodiester bond that links the DNA made by polymerase I and polymerase III.

29.5 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 → 5′-phosphate bond that links one nucleotide to the next in DNA.

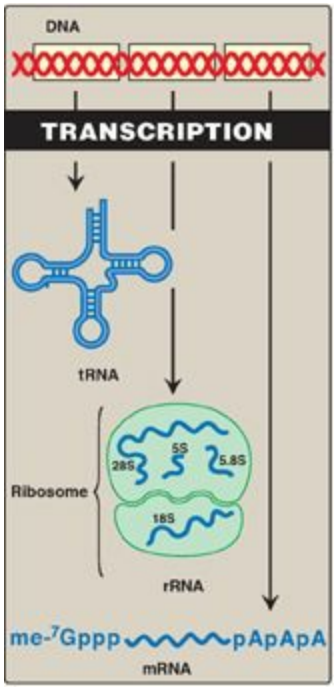
RNA Structure, Synthesis, and Processing

30

I. OVERVIEW

The genetic master plan of an organism is contained in the sequence of deoxyribonucleotides in its deoxyribonucleic acid (DNA). However, it is through the ribonucleic acid (RNA), the "working copies" of the DNA, that the master plan is expressed (Figure 30.1). The copying process, during which a DNA strand serves as a template for the synthesis of RNA, is called transcription. Transcription produces messenger RNAs (mRNAs) that are translated into sequences of amino acids (polypeptide chains or proteins) and ribosomal RNAs (rRNAs), transfer RNAs (tRNAs), and additional small RNA molecules that perform specialized structural, catalytic, and regulatory functions and are not translated. That is, they are noncoding RNAs (ncRNAs). [Note: Only about 2% of the genome codes for proteins.] The final product of gene expression, therefore, can be RNA or protein, depending upon the gene. 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 where to start, how often to start, and where to stop transcription. A variety of regulatory proteins is 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.

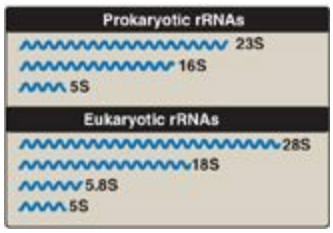
Figure 30.1 Expression of genetic information by transcription. [Note: RNAs shown are eukaryotic.] tRNA = transfer RNA; rRNA = ribosomal RNA; mRNA = messenger RNA; me-7Gppp = 7-methylguanosine triphosphate "cap;" AAA = poly-A "tail," each described on p. 418.


DNA
TRANSCRIPTION
tRNA
Ribosome
28S
5S
5.8S
18S
rRNA
me-7Gppp
pApApA
mRNA

II. STRUCTURE OF RNA

There are three major types of RNA that participate in the process of protein synthesis: rRNA, tRNA, and mRNA. Like DNA, these three types of RNA are unbranched polymeric molecules composed of nucleoside monophosphates joined together by 3′→5′-phosphodiester bonds (see p. 396). However, they differ from DNA in several ways. For example, they are considerably smaller than DNA, contain ribose instead of deoxyribose and uracil instead of thymine, 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 (snoRNAs), nucleus (snRNA), and cytoplasm (miRNA) perform specialized functions as described on pp. 425, 426, and 459.]

Figure 30.2 Prokaryotic and eukaryotic ribosomal RNAs (rRNAs). S = Svedberg unit.



A. Ribosomal RNA

rRNAs 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. 436). There are three distinct size species of rRNA (23S, 16S, and 5S) in prokaryotic cells (Figure 30.2). In the eukaryotic cytosol, there are four rRNA species (28S, 18S, 5.8S, and 5S, where "S" is the Svedberg unit for sedimentation rate, which is determined by the size and shape of the particle.) Together, rRNAs make up about 80% of the total RNA in the cell. [Note: Some RNAs function as catalysts, for example, an rRNA in protein synthesis (see p. 439). RNA with catalytic activity is termed a "ribozyme."]

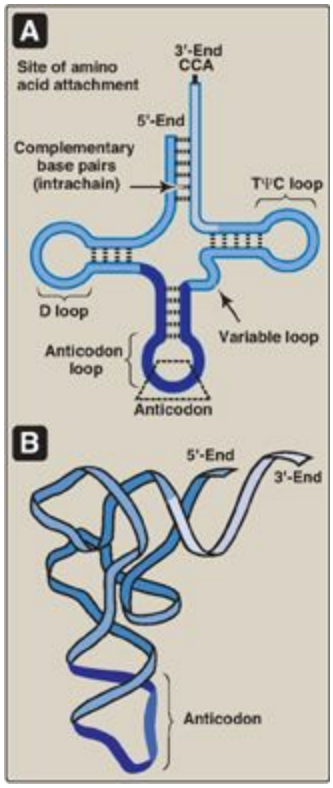
B. Transfer RNA

tRNAs 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, tRNAs make up about 15% of the total RNA in the cell. The tRNA molecules contain a high percentage of unusual bases, for example, dihydrouracil (see Figure 22.2, p. 292) and have extensive intrachain base-pairing (Figure 30.3) that leads to characteristic secondary 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 its amino acid to the growing peptide chain (see p. 432).

C. Messenger RNA

mRNA comprises only about 5% of the RNA in the cell, yet is by far the most heterogeneous type of RNA in size and base sequence. mRNA carries genetic information from DNA for use in protein synthesis. In eukaryotes, this involves transfer of mRNA out of the nucleus and into the cytosol. If the mRNA carries information from more than one gene, it is said to be polycistronic (cistron = gene). Polycistronic mRNA is characteristic of prokaryotes. If the mRNA carries information from just one gene, it is said to be 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 (Figure 30.4). Special structural characteristics of eukaryotic (but not prokaryotic) mRNA include a long sequence of adenine nucleotides (a poly-A "tail") on the 3′-end of the RNA chain, plus a "cap" on the 5′-end consisting of a molecule of 7-methylguanosine attached through an unusual (5′→5′) triphosphate linkage. The mechanisms for modifying mRNA to create these special structural characteristics are discussed on p. 425.

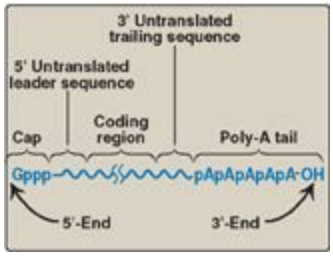
Figure 30.3 A. Characteristic transfer RNA (tRNA) secondary structure (cloverleaf). B. Folded (tertiary) tRNA structure found in cells. D = dihydrouracil; Ψ = pseudouracil; T = thymine; C = cytosine; A = adenine.



III. TRANSCRIPTION OF PROKARYOTIC GENES

The structure of RNA polymerase (RNA pol), the signals that control transcription, and the varieties of modification that RNA transcripts can undergo differ among organisms, and particularly from prokaryotes to eukaryotes. Therefore, the discussions of prokaryotic and eukaryotic transcription are presented separately.

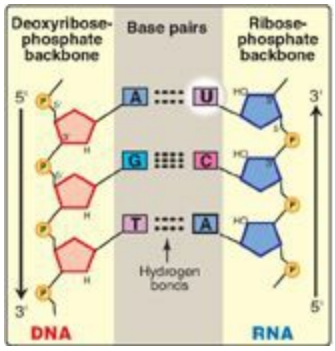
Figure 30.4 Structure of eukaryotic messenger RNA.



A. Properties of 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 a specialized enzyme, primase (see p. 402).] 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. 397). 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 thymine (T) specifies an adenine (A), but an A specifies a uracil (U) instead of a T (Figure 30.5). 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:

Figure 30.5 Antiparallel, complementary base pairs between DNA and RNA. T= thymine; A = adenine; C = cytosine; G = guanine; U = uracil.



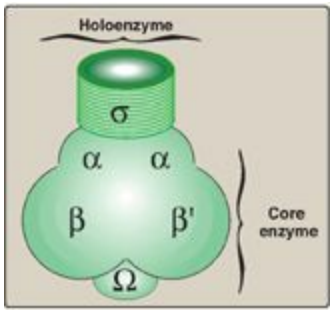
1. **Core enzyme:** Five of the enzyme's peptide subunits, 2α, 1β, 1β′, and 1Ω, are required for enzyme assembly (α, Ω) template binding (β′), and the 5′→3′ RNA polymerase activity (β), and are referred to as the core enzyme (Figure 30.6). However, this enzyme lacks specificity (that is, it cannot recognize the promoter region on the DNA template).

2. **Holoenzyme:** The s subunit ("sigma factor") enables RNA pol to recognize promoter regions on the DNA. The s subunit plus the core enzyme make up the holoenzyme. [Note: Different s factors recognize different groups of genes.]

B. Steps in RNA synthesis

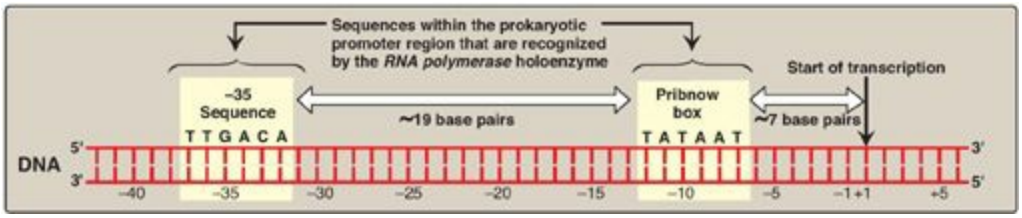
The process of transcription of a typical gene of Escherichia coli (E. coli) can be divided into three phases: initiation, elongation, and termination. A transcription unit extends from the promoter to the termination region, and the initial product of transcription by RNA pol is termed the primary transcript.

Figure 30.6 Components of prokaryotic RNA polymerase.



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 (Figure 30.7). [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 polymerase s factors include:

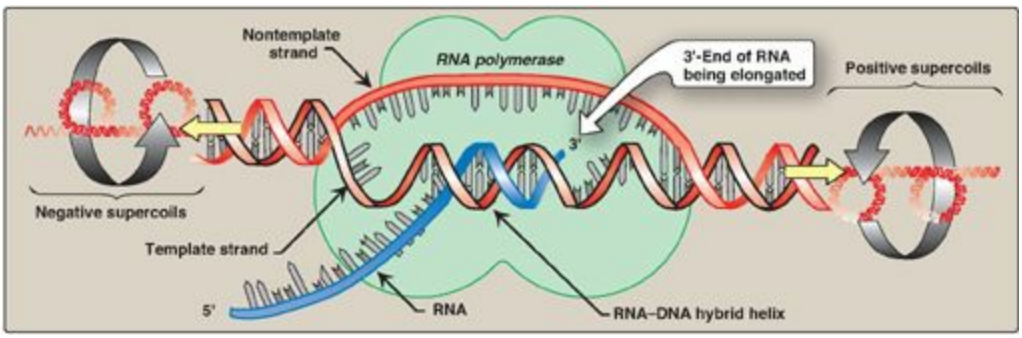
Figure 30.7 Structure of the prokaryotic promoter region. T = thymine; G = guanine; A = adenine; C = cytosine.



a. –35 Sequence: A consensus sequence (5-TTGACA-3), centered about 35 bases to the left of the transcription start site (see Figure 30.7), is the initial point of contact for the holoenzyme, and a closed complex is formed. [Note: The regulatory sequences that control transcription are, by convention, 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**I**-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**I**-TATAAT-3**I**), centered at about –10 (see Figure 30.7), which is the site of initial DNA melting (unwinding). Melting of a short stretch (about 14 bases) converts the closed 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 30.8 Local unwinding of DNA caused by RNA polymerase and formation of an open initiation complex.



2. Elongation: Once the promoter region has been recognized and bound by the holoenzyme, local unwinding of the DNA helix continues (Figure 30.8), mediated by the polymerase. [Note: Unwinding generates supercoils in the DNA that can be relieved by DNA topoisomerases (see p. 401.] RNA pol begins to synthesize a transcript of the DNA sequence, and several short pieces of RNA are made and discarded. The elongation phase is said to begin when the transcript (typically starting with a purine) exceeds ten nucleotides in length. Sigma 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

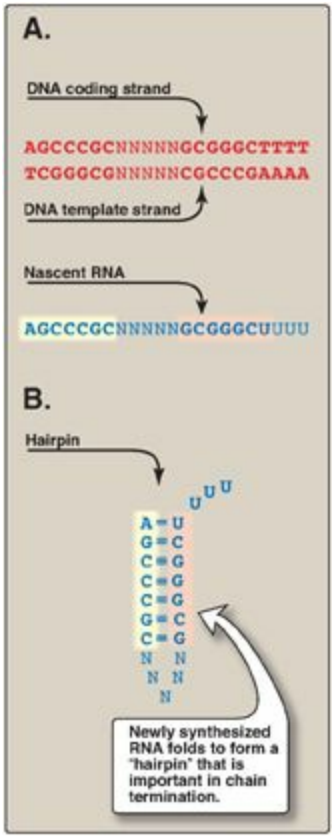
transcription, a short DNA–RNA hybrid helix is formed (see Figure 30.8). Like DNA pol, RNA pol uses nucleoside triphosphates as substrates and releases pyrophosphate each time a nucleoside monophosphate is added to the growing chain. As with replication, transcription is always in the 5′→3′ direction. In contrast to DNA pol, RNA pol does not require a primer and does not appear to have 3′→5′ exonuclease (proofreading) activity.

3. **Termination:** The elongation of the single-stranded RNA chain continues until a termination signal is reached. Termination can be intrinsic (spontaneous) or dependent upon the participation of a protein known as the ρ (rho) factor.

   a. **ρ-Independent termination**: Seen with most prokaryotic genes, this requires that a sequence in the DNA template generates a sequence in the nascent (newly made) RNA that is self-complementary (Figure 30.9). 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 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 polymerase.

   b. **r-Dependent termination:** This requires the participation of an additional protein, rho (r), which is a hexameric ATPase with helicase activity. Rho binds a C-rich "rho recognition 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 r separates the RNA–DNA hybrid helix, causing the release of the RNA.

Figure 30.9 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. [Note: Termination of eukaryotic transcription is not well understood.]



4. **Action of antibiotics:** Some antibiotics prevent bacterial cell growth by inhibiting RNA synthesis. For example, rifampin (rifampicin) inhibits transcription by binding to the β subunit of prokaryotic RNA pol, and preventing chain extension beyond three nucleotides (Figure 30.10). Rifampin is important in the treatment of tuberculosis. Dactinomycin (known to biochemists as actinomycin D) was the first antibiotic to find therapeutic application in tumor chemotherapy. It binds to the DNA template and interferes with the movement of RNA pol along the DNA.

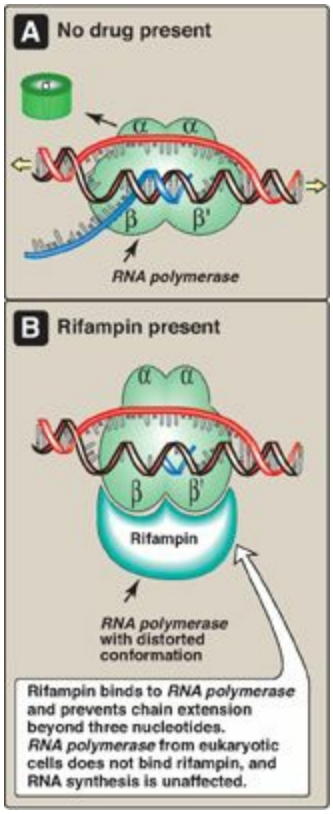
IV. TRANSCRIPTION OF EUKARYOTIC GENES

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 either within the core promoter region, close (proximal) to it, or some distance away (distal). They are required both for the assembly of a transcription 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 TFs to recognize and bind to their specific DNA sequences, the chromatin structure in that region must be altered (relaxed) to allow access to the DNA. The role of transcription in the regulation of gene expression is discussed in Chapter 32.

A. Chromatin structure and gene expression

The association of DNA with histones to form nucleosomes (see p. 409) affects the ability of the transcription machinery to access the DNA to be transcribed. Most actively transcribed genes are found in a relatively relaxed form of chromatin called euchromatin, whereas most inactive segments of DNA are found in highly condensed heterochromatin. [Note: The interconversion of these forms is called chromatin remodeling.] A major component of chromatin remodeling is the covalent modification of histones (for example, the acetylation of lysine residues at the amino terminus of histone proteins) as shown in Figure 30.11. 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.]

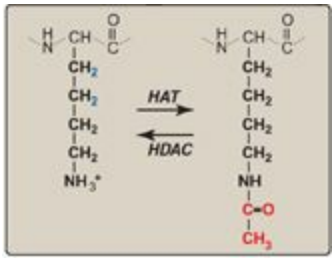
Figure 30.10 Inhibition of prokaryotic RNA polymerase by rifampin.



B. Nuclear RNA polymerases of eukaryotic cells

There are three distinct classes of RNA pol in the nucleus of eukaryotic cells. All are large enzymes with multiple subunits. Each class of RNA pol recognizes particular types of genes.

Figure 30.11 Acetylation/deacetylation of a lysine residue in a histone protein. HAT = histone acetyltransferase; HDAC = histone deacetylase.



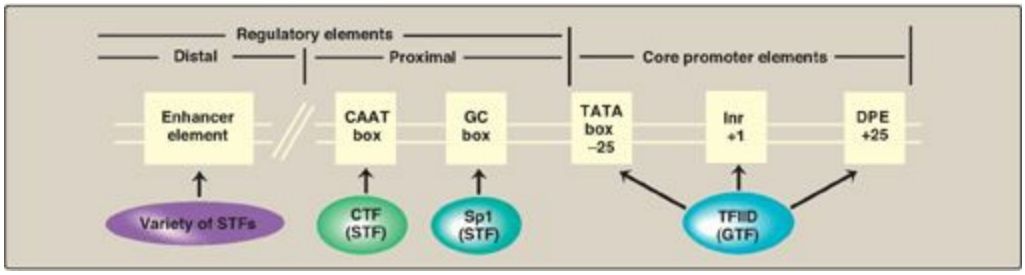
1. **RNA polymerase I:** This enzyme synthesizes the precursor of the 28S, 18S, and 5.8S rRNA in the nucleolus.

2. **RNA polymerase II:** This enzyme synthesizes the nuclear precursors of mRNA that are subsequently translated to produce proteins. RNA pol II also synthesizes certain small ncRNAs, such as snoRNA (see p. 425), snRNA (see p. 426) and miRNA (see p. 459).

   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. 420) is found centered about 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 Inr (initiator) or DPE (downstream promoter element) are present (Figure 30.12). [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.

Figure 30.12 Eukaryotic gene cis-acting promoter and regulatory elements and their trans-acting general and specific transcription factors (GTF and STF, respectively). Inr = initiator; DPE = downstream promoter element.

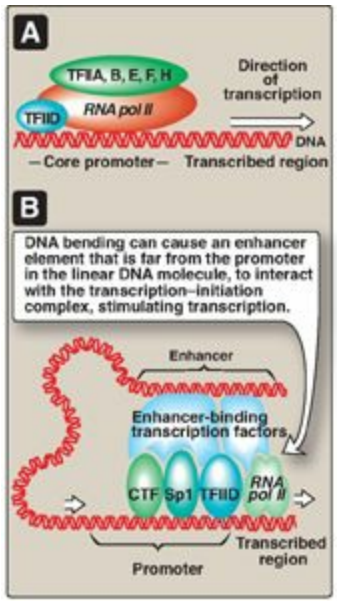


b. General transcription factors: These are the minimal requirements for recognition of the promoter, recruitment of RNA pol II to the promoter, and initiation of transcription at a basal level (Figure 30.13A). GTFs are encoded by different genes, synthesized in the cytosol, and transit to their sites 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, 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: Upstream of the core promoter are additional consensus sequences (see Figure 30.12). 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 p. 424). 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. 456) and regulate which genes are expressed at a given point in time. A typical protein-coding eukaryotic gene has binding sites for

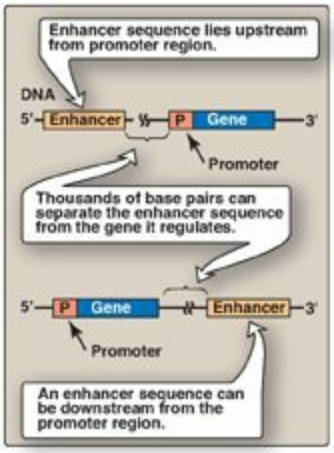
many such factors. [Note: STFs have two binding domains. One is a DNA-binding domain, the other is a transcription activation domain that recruits the GTFs to the core promoter as well as "coactivator" proteins such as the HAT enzymes involved in chromatin modification.]

Figure 30.13 A. Association of the general transcription factors (TFIIs) and RNA polymerase II (RNA pol II) at the core promoters. [Note: The Roman numeral II denotes the TFs for RNA pol II.] B. Enhancer stimulation of transcription. CTF = CAAT box transcription factor; Sp1 = specificity factor-1.



Transcriptional activators bind DNA through a variety of motifs, such as the helix-loop-helix, zinc finger, and leucine zipper (see p. 18).

Figure 30.14 Some possible locations of enhancer sequences.



d. Role of enhancers in eukaryotic gene regulation: 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 (Figure 30.13B). 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 (Figure 30.14), and 3) occur on either strand of the DNA. Enhancers contain DNA sequences called "response elements" that bind STFs (transcriptional activators). By bending or looping the DNA, these enhancer-binding proteins can interact with other transcription factors bound to a promoter and with RNA pol II, thereby stimulating transcription (see Figure 30.13B). [Note: Although silencers are similar to enhancers in that they also can act over long distances, they reduce gene expression.]

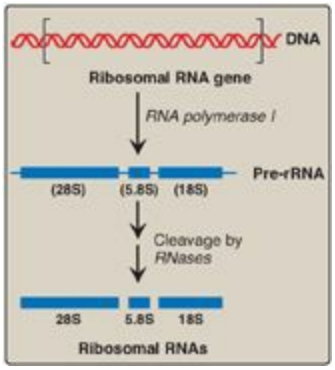
e. Inhibitors of RNA polymerase II: α-Amanitin, a potent toxin produced by the poisonous mushroom Amanita phalloides (sometimes called "the death cap"), forms a tight complex with RNA pol II, thereby inhibiting mRNA synthesis.

3. RNA polymerase III: This enzyme synthesizes tRNA, 5S rRNA, and some snRNA and snoRNA.

C. Mitochondrial RNA polymerase

Mitochondria contain a single RNA pol that more closely resembles bacterial RNA pol than the eukaryotic nuclear enzymes.

Figure 30.15 Posttranscriptional processing of eukaryotic ribosomal RNA by ribonucleases (RNases). S = Svedberg unit.



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. tRNAs are then 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.

A. Ribosomal RNA

rRNAs of both prokaryotic and eukaryotic cells are generated from long precursor molecules called pre-rRNAs. 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 (Figure 30.15). [Note: Eukaryotic 5S rRNA is synthesized by RNA pol III and modified separately.] The pre-rRNAs 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. Some of the proteins destined to become components of the ribosome associate with pre-rRNA prior to and during its modification.]

B. Transfer RNA

Both eukaryotic and prokaryotic tRNAs are also made from longer precursor molecules that must be modified (Figure 30.16). Sequences at both ends of the molecule are removed, and, if present, an 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. 292).

C. Eukaryotic mRNA

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. These modifications usually include the following.

1. **5′ "Capping":** This is the first of the processing reactions for pre-mRNA (Figure 30.17). The cap is a 7-methylguanosine attached to the 5′-terminal end of the mRNA through an unusual 5′→5′ triphosphate linkage that is resistant to most nucleases. Creation of the cap requires removal of the g phosphoryl group from the 5′-triphosphate of the pre-mRNA, followed by addition of guanosine monophosphate

(GMP) (from GTP) by the nuclear enzyme guanylyltransferase. Methylation of this terminal guanine occurs in the cytosol and is catalyzed by guanine-7-methyltransferase. S-adenosylmethionine is the source of the methyl group (see p. 263). Additional methylation steps may occur. The addition of this 7-methylguanosine cap helps stabilize the mRNA and permits efficient initiation of translation (see p. 439).

Figure 30.16 A. Primary transfer RNA (tRNA) transcript. B. Functional tRNA after posttranscriptional modification. Modified bases include D (dihydrouracil); Ψ (pseudouracil); and m, which means that the base has been methylated.

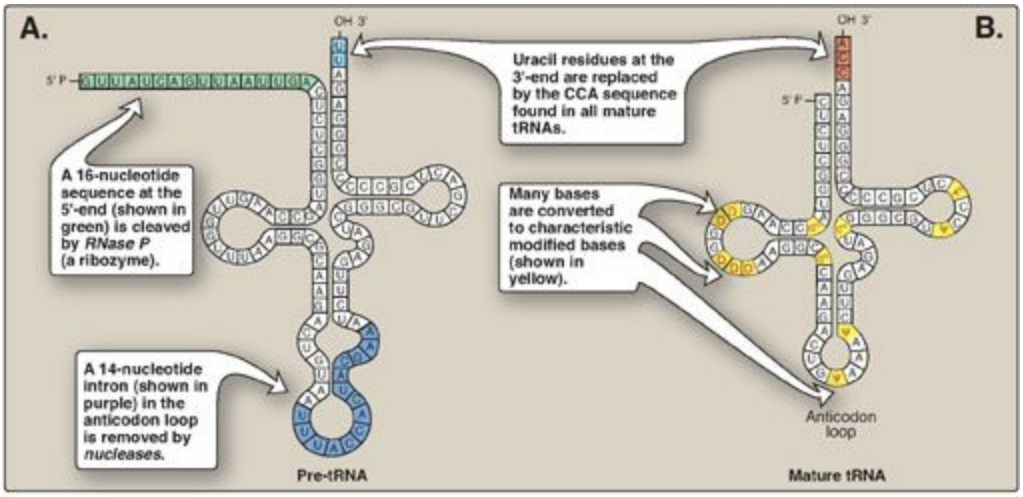
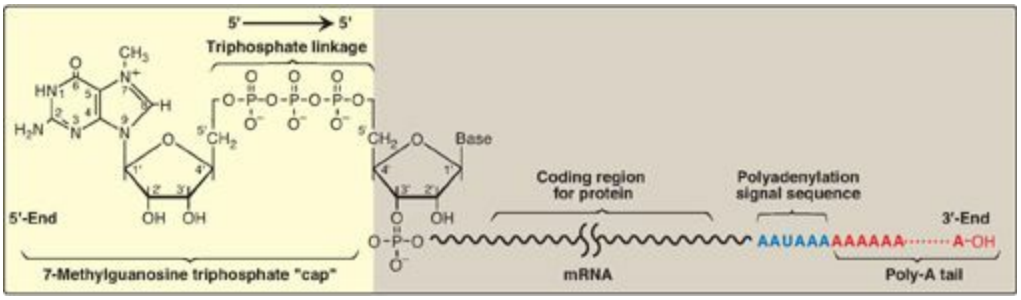


Figure 30.17 Posttranscriptional modification of messenger RNA (mRNA) showing the 7-methylguanosine cap and poly-A tail.



2. **Addition of a poly-A tail:** Most eukaryotic mRNA (with several notable exceptions, including those coding for the histones) have a chain of 40–250 adenine nucleotides attached to the 3′-end (see Figure 30.17). This poly-A tail is not transcribed from the DNA, but rather is added after transcription 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. These tails help stabilize the mRNA, facilitate its exit from the nucleus, and aid in translation. After the mRNA enters the cytosol, the poly-A tail is gradually shortened.
3. **Removal of introns:** 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 (for example, those from histone genes). Others contain a few introns, whereas some, such as the primary transcripts for the a chains of collagen, contain more than 50 intervening sequences that must be removed before mature mRNA is ready for translation.

a. Role of small nuclear RNAs: In association with multiple proteins, uracil-rich snRNAs form small nuclear ribonucleoprotein particles (snRNPs, or "snurps," 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 (Figure 30.18). [Note: In systemic lupus erythematosus, an autoimmune disease, patients produce antibodies against their own nuclear proteins such as snRNPs.]

b. Mechanism of splicing: The binding of snRNPs brings the sequences of the neighboring exons into the correct alignment for splicing, allowing two transesterification reactions to occur. The 2′-OH group of an adenine 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 Figure 30.18). 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. [Note: The GU and AG sequences at the beginning and end, respectively, of introns are invariant.] After introns have been removed and exons joined, the mature mRNA molecules leave the nucleus and pass into the cytosol through pores in the nuclear membrane. [Note: The introns in tRNA (see Figure 30.16) 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 over 15% 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. 38). 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 aren't normally used.

4. Alternative splicing of mRNA molecules: The pre-mRNA molecules from over

50% of human genes can be spliced in alternative ways in different tissues. This produces multiple variations of the mRNA and, therefore, of its protein product (Figure 30.19), and thus is a mechanism for producing a large, diverse set of proteins from a limited set of genes. For example, in eukaryotic cells, the mRNA for tropomyosin, 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 tropomyosin protein.

Figure 30.18 Splicing. snRNP = small nuclear ribonucleoprotein particle; mRNA = messenger RNA. [Note: U1 binds the 5**I** donor site, U2 binds the branch A, and addition of U4-U6 completes the complex.]

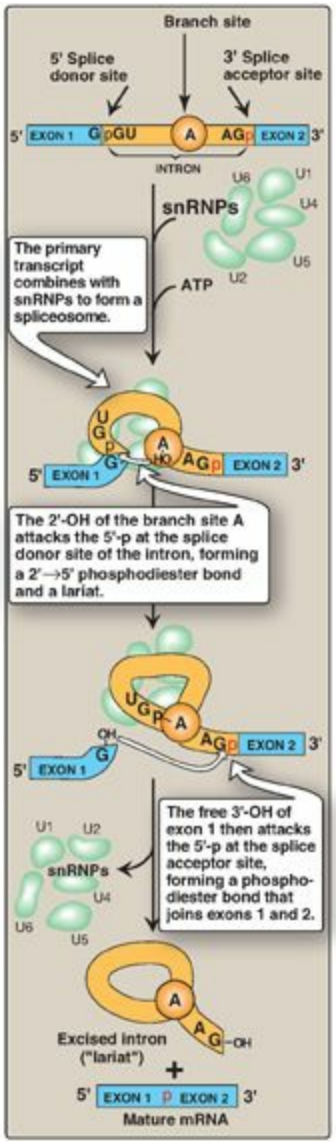
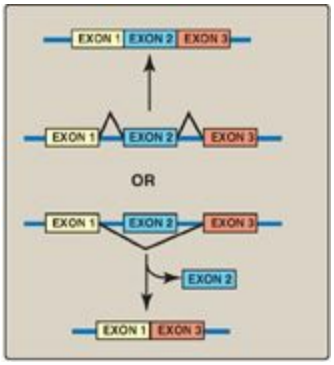


Figure 30.19 Alternative splicing patterns in eukaryotic messenger RNA.

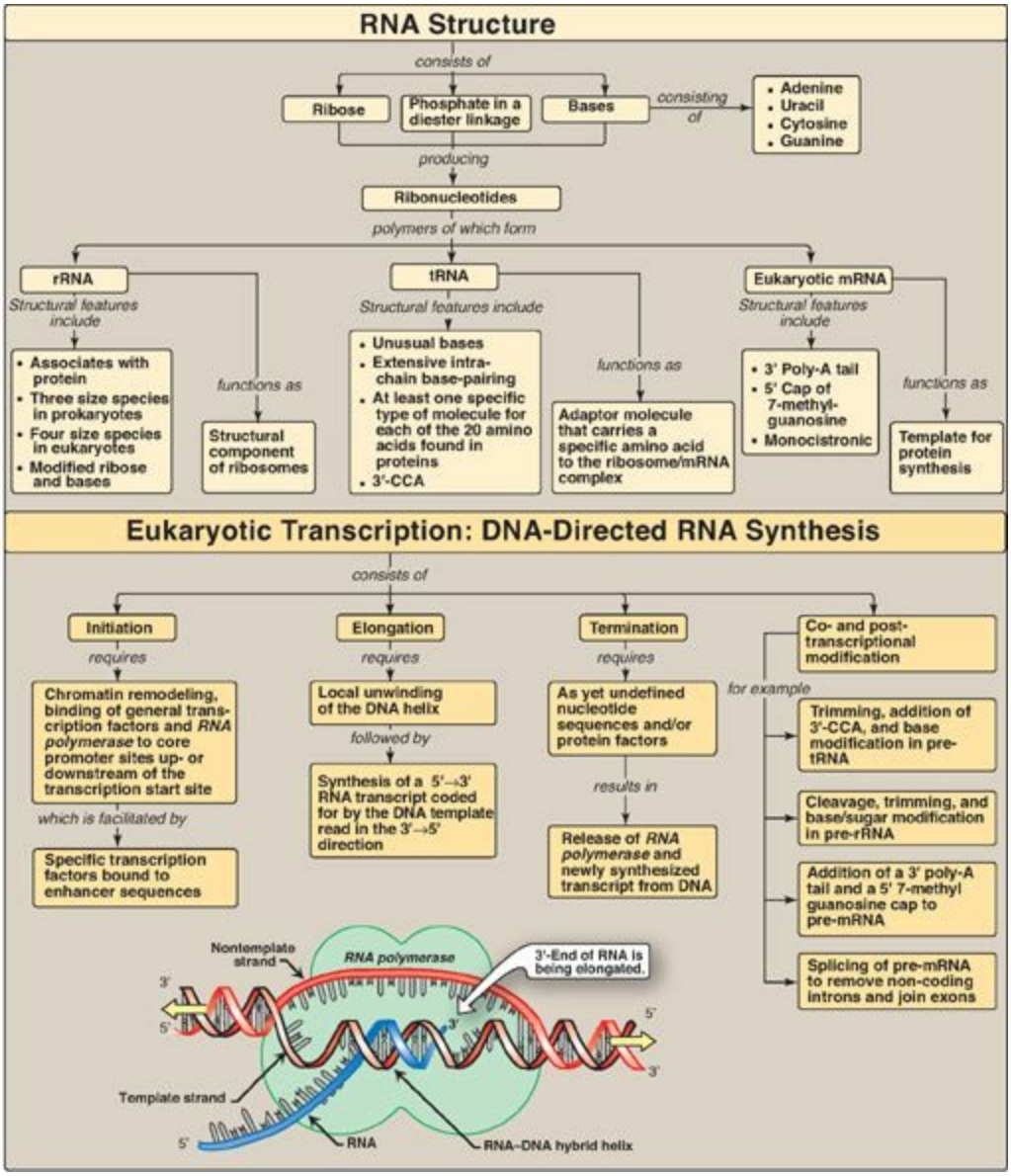

EXON 1
EXON 2
EXON 3
EXON 1
EXON 2
EXON 3
OR
EXON 1
EXON 2
EXON 3
EXON 2
EXON 1
EXON 3

VI. CHAPTER SUMMARY

There are three major types of RNA that participate in the process of protein synthesis: **ribosomal RNA** (**rRNA**), **transfer RNA** (**tRNA**), and **messenger RNA** (**mRNA**) (Figure 30.20). They are unbranched polymers of nucleotides but differ from DNA by containing **ribose** instead of deoxyribose and **uracil** instead of thymine. **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** carries genetic information from DNA for use in protein synthesis. The process of RNA synthesis is called **transcription**, and the substrates are **ribonucleoside triphosphates**. The enzyme that synthesizes RNA is **RNA polymerase** (**RNA pol**). In **prokaryotic** cells, the **core enzyme** has five subunits (2α, 1β, 1β′, and 1Ω) and possesses **5′→3′ polymerase activity** that **elongates** the growing RNA strand. This enzyme requires an additional subunit, sigma (**s**) **factor**, that recognizes the nucleotide sequence (**promoter** region) at the beginning of a length of DNA that is to be transcribed. This region contains **consensus sequences** that are highly conserved and include the **TATA** (**Pribnow**) **box** and the **–35 sequence**. Another protein—rho (**r**) **factor**—is required for **termination** of transcription of some genes. There are three distinct classes of RNA pol in the nucleus of eukaryotic cells. **RNA pol I** synthesizes the precursor of rRNAs in the nucleolus. In the nucleoplasm, **RNA pol II** synthesizes the precursors for mRNA and some noncoding RNAs, and **RNA pol III** produces the precursors of **tRNA**. In both prokaryotes and eukaryotes, RNA pol does not require a primer and has no 3′→5′ exonuclease (proofreading) activity. Core **promoters** for genes transcribed by **RNA pol II** contain cis-acting consensus sequences, such as the TATA-like **Hogness box**, that serve as binding sites for trans-acting general transcription factors. Upstream of these are proximal regulatory elements, such as the CAAT and GC boxes, and distal regulatory elements such as enhancers. Transcriptional activators (specific transcription factors) bind these elements and regulate the frequency of transcription initiation, the response to signals such as hormones, and which genes are expressed at a given point in time. Eukaryotic transcription requires that the **chromatin** be accessible. **A primary transcript** is a linear 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. **rRNAs** of both prokaryotic and eukaryotic cells are synthesized from long precursor molecules called pre-rRNA. These precursors are cleaved and trimmed by ribonucleases, producing the three largest rRNA, and bases and sugars are modified. Eukaryotic 5S rRNA is synthesized by RNA pol III and is modified separately. Prokaryotic mRNA is generally identical to its primary transcript, whereas eukaryotic mRNA is extensively modified co- and posttranscriptionally. For example, a **7-methylguanosine cap** is attached to the 5′-terminal end of the mRNA through a 5′→5′ linkage. A **long poly-A tail**, not transcribed from the DNA, is attached to the 3′-end of most mRNAs. Most eukaryotic

mRNAs also contain i**ntervening sequences** (**introns**) that must be removed to make the mRNA functional. Their removal, as well as the **joining of expressed sequences** (**exons**), requires a **spliceosome** composed of **small, nuclear ribonucleoprotein particles** (**snurps**) that mediate the process of **splicing**. Eukaryotic mRNA is monocistronic, containing information from just one gene. Prokaryotic and eukaryotic tRNAs are also made from longer precursor molecules. If present, an intron is removed by nucleases, and both ends of the molecule are trimmed by ribonucleases. A **3I-CCA** sequence is added, and bases at specific positions are modified, producing "unusual" bases.

Figure 30.20 Key concept map for RNA structure and synthesis. rRNA = ribosomal RNA; tRNA = transfer RNA; mRNA = messenger RNA.



Study Questions

Choose the ONE correct answer.

30.1 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. Exon 2 can, therefore, have these extra 19 nucleotides at its 5′-end. 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 mRNAs 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.

30.2 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 would be the most likely effect of this mutation?

A. Initiation of dystrophin transcription will be defective.
B. Termination of dystrophin transcription will be defective.
C. Capping of dystrophin messenger RNA will be defective.
D. Splicing of dystrophin messenger RNA will be defective.
E. Tailing of dystrophin messenger RNA will be defective.

Correct answer = A. Mutations in the promoter typically prevent

formation of the RNA polymerase II transcription 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 a mRNA in which too many or too few introns have been removed (splicing defects).

30.3 A mutation to this sequence in eukaryotic messenger RNA (RNA) will affect the process by which the 3**I**-end 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 messenger RNA just downstream of this polyadenylation signal, creating a new 3**I**-end to which the poly A 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**I**-end of transfer RNA by nucleotidyltransferase. GU…A…AG denotes an intron.

30.4. This protein factor 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-messenger RNA.

30.5 What is the sequence (conventionally written) of the RNA product of the DNA template sequence, GATCTAC?

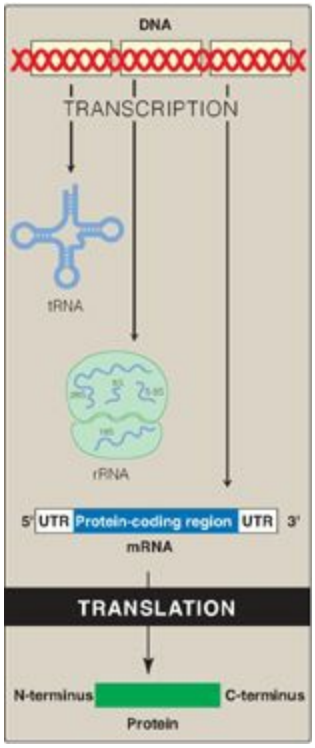
Correct answer = 5′-GUAGAUC-3′. The RNA product has a sequence that is complementary to the template strand, with U replacing T.

Protein Synthesis 31

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 (polypeptide chains) as shown in Figure 31.1. 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 nucleic acid sequence is expressed to produce a specific sequence of amino acids. Any alteration in the nucleic acid sequence may result in an incorrect amino acid being inserted into the polypeptide chain, potentially causing disease or even death of the organism. Newly made (nascent) proteins undergo a number of processes to achieve their functional form. They must fold properly, and misfolding can result in aggregation or degradation of the protein. Many proteins are covalently modified to activate them or alter their activities. Finally, proteins are targeted to their final intra- or extracellular destinations by signals present in the proteins themselves.

Figure 31.1 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.

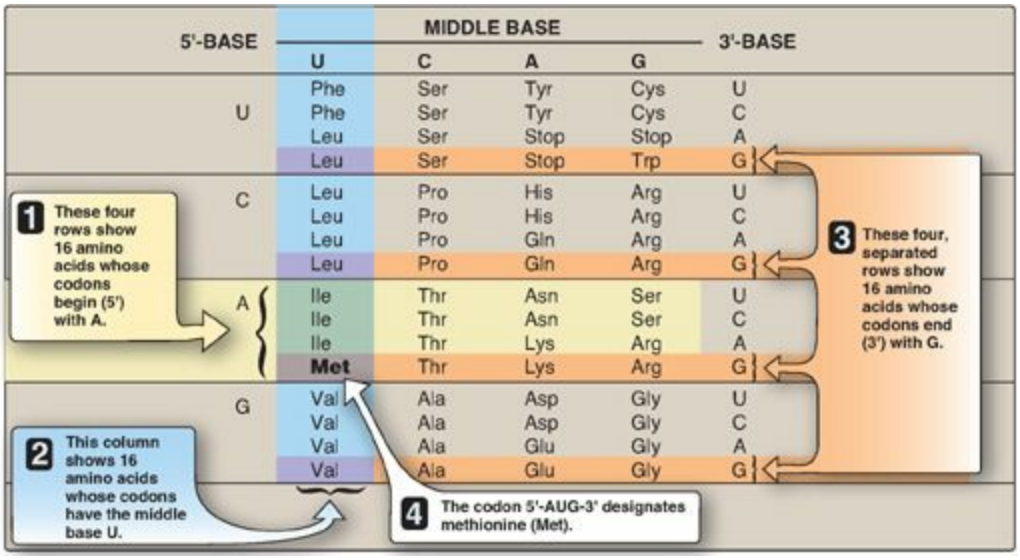
A. Codons

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. There are, therefore, 64 different combinations of bases, taken three at a time (a triplet code) as shown in the table in Figure 31.2.

1. **How to translate a codon:** This table (or "dictionary") 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 5′-AUG-3′ codes for methionine ([Met] see Figure 31.2). [Note: AUG is the initiation (start) codon for translation.] Sixty-one of the 64 codons code for the 20 common amino acids.

2. **Termination ("stop," or "nonsense") codons:** Three of the codons, UAA, UAG, and UGA, do not code for amino acids but, rather, are termination codons. When one of these codons appears in an mRNA sequence, synthesis of the polypeptide coded for by that mRNA stops.

Figure 31.2 Use of the genetic code table to translate the codon AUG. A = adenine; G = guanine; C = cytosine; U = uracil. The abbreviations for many common amino acids are shown as examples.



B. Characteristics of the genetic code

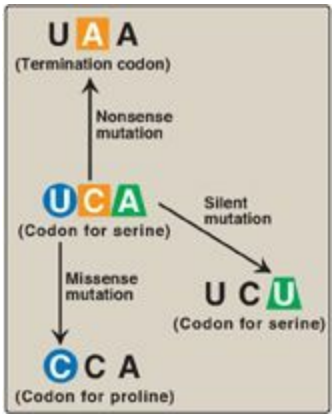
Usage of the genetic code is remarkably consistent throughout all living organisms. It is assumed that once the standard genetic code evolved in primitive organisms, any

mutation 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 31.2. For example, UGA codes for tryptophan (Trp).]

Figure 31.3 Possible effects of changing a single nucleotide base in the coding region of a messenger RNA chain. A = adenine; C = cytosine; U = uracil.



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 Figure 31.2). Only Met and Trp have just one coding 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.

C. Consequences of altering the nucleotide sequence

Changing a single nucleotide base on the mRNA chain (a "point mutation") can lead to any one of three results (Figure 31.3).

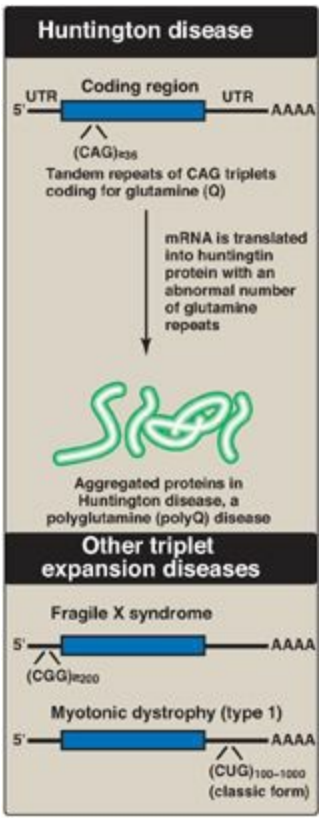
1. **Silent mutation:** The codon containing the changed base may code for the same amino acid. For example, if the serine (Ser) codon UCA is given a different third base, U, to become UCU, it still codes for Ser. This is termed a "silent" mutation.

2. **Missense mutation:** The codon containing the changed base may code for a

different amino acid. For example, if the Ser codon UCA is given a different first base, C, to become CCA, it will code for a different amino acid (in this case, proline [Pro]). The substitution of an incorrect amino acid is called 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 given a different second base, A, to become UAA, the new codon causes termination of translation at that point and the production of a shortened (truncated) protein. The creation of a termination (stop) codon at an inappropriate place is called a "nonsense" mutation.

Figure 31.4 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 fewer than 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.



4. **Other mutations:** These can alter the amount or structure of the protein produced by translation.

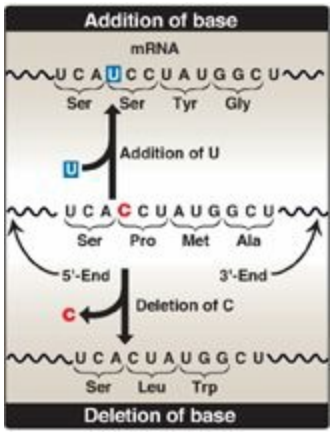
   a. **Trinucleotide repeat expansion:** Occasionally, a sequence of three bases that is repeated in tandem will become amplified in number so that too many copies of the triplet occur. If this happens within the coding region of a gene, the protein will contain many extra copies of one amino acid. For example, amplification of the CAG codon leads to the insertion of many extra glutamine residues in the huntingtin protein, causing the neurodegenerative disorder

Huntington disease (Figure 31.4). The additional glutamines result in changes in secondary structure that cause the accumulation of protein aggregates. 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, the expansion results in gene silencing through DNA hypermethylation (see p. 460).]

b. Splice-site mutations: Mutations at splice sites (see p. 427) 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. Frame-shift mutations: If one or two nucleotides are either deleted from or added to the coding region of a mRNA, a frame-shift 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 creation of a termination codon (Figure 31.5). If three nucleotides are added, a new amino acid is added to the peptide, or, if three are deleted, an amino acid is lost. Loss of three nucleotides maintains 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 at the 508th position (DF508) in the protein encoded by that gene. This DF508 mutation prevents normal folding of the protein, CF transmembrane conductance regulator (CFTR), leading to its destruction by the proteasome (see p. 247). 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 digestive deficiencies (see p. 248). The incidence of CF is highest (1 in 3300) in those of Northern European origin. In over 70% of individuals with CF, the DF508 mutation is the cause of the disease.

Figure 31.5 Frame-shift mutations as a result of addition or deletion of a base can cause an alteration in the reading frame of messenger RNA (mRNA). A = adenine; C = cytosine; G = guanine; U = uracil.


Addition of base
mRNA
U C A U C C U A U G G C U
Ser Ser Tyr Gly
U
Addition of U
U C A C C U A U G G C U
Ser Pro Met Ala
5'-End
3'-End
C
Deletion of C
U C A C U A U G G C U
Ser Leu Trp
Deletion of base

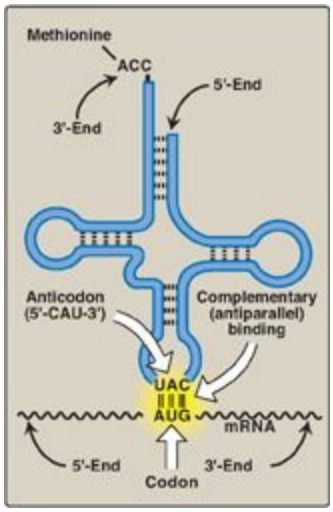
III. COMPONENTS REQUIRED FOR TRANSLATION

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. 262) in sufficient quantities in the diet to ensure continued protein synthesis.]

Figure 31.6 Complementary, antiparallel binding of the anticodon for methionyl-tRNA (CAU) to the messenger RNA (mRNA) codon for methionine (AUG), the initiation codon for translation.



B. Transfer RNA

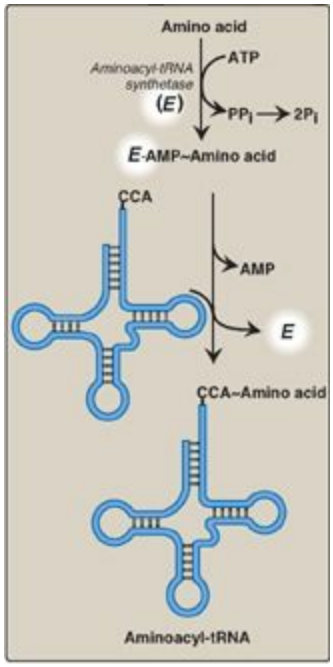
At least one specific type of tRNA is required for each amino acid. In humans, there are at least 50 species of tRNA, whereas bacteria contain at least 30 species. Because there are only 20 different amino acids commonly carried by tRNA, some amino acids have more than one specific tRNA molecule. This 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**I**-end (Figure 31.6). The carboxyl group of the amino acid is in an ester linkage with the 3-hydroxyl of the ribose portion of the adenine (A) nucleotide in the —CCA sequence at the 3**I**-end of the tRNA. [Note: When a tRNA has a covalently attached amino acid, it is said to be charged, and

when it does not, it is said to be uncharged. The amino acid attached to the tRNA molecule is said to be activated.]

2. **Anticodon:** Each tRNA molecule also contains a three-base nucleotide sequence, the anticodon, that pairs with a specific codon on the mRNA (see Figure 31.6). This codon specifies the insertion into the growing peptide chain of the amino acid carried by that tRNA.

Figure 31.7 Attachment of a specific amino acid to its corresponding tRNA by aminoacyl-tRNA synthetase. PP_i = pyrophosphate; P_i = inorganic phosphate; A = adenine; C = cytosine; ATP = adenosine triphosphate; AMP = adenosine monophosphate.



C. Aminoacyl-tRNA synthetases

This family of enzymes is required for attachment of amino acids to their corresponding tRNAs. Each member of this family recognizes a specific amino acid and all the tRNAs that correspond to that amino acid (isoaccepting tRNAs, up to five per amino acid). Aminoacyl-tRNA synthetases catalyze a two-step reaction that results in the covalent attachment of the carboxyl group of an amino acid to the 3**I**-end of its corresponding tRNA. The overall reaction requires adenosine triphosphate (ATP), which is cleaved to adenosine monophosphate (AMP) and inorganic pyrophosphate (PP_i) as shown in Figure 31.7. 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.

D. Messenger RNA

The specific mRNA required as a template for the synthesis of the desired polypeptide

chain must be present. [Note: In eukaryotes, mRNA is circularized for use in translation.]

E. Functionally competent ribosomes

Ribosomes are large complexes of protein and ribosomal RNA ([rRNA], Figure 31.8), 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 S (Svedberg) values. [Note: Because the S values are determined both by shape as well as molecular mass, their numeric values are not strictly additive. For example, 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.

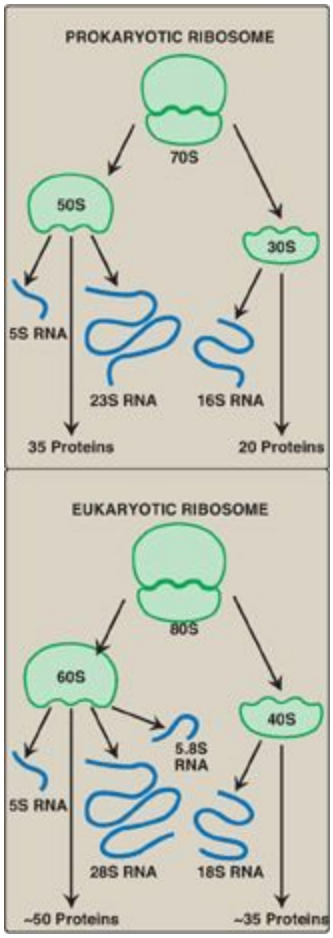
> The small ribosomal subunit binds mRNA and is responsible for the accuracy of translation by ensuring correct base-pairing between the codon in the mRNA and the anticodon in the tRNA. The large ribosomal subunit catalyzes formation of the peptide bonds that link amino acid residues in a protein.

1. **Ribosomal RNA:** As discussed on p. 418, prokaryotic ribosomes contain three size species of rRNA, whereas eukaryotic ribosomes contain four (see Figure 31.8). The rRNAs are generated from a single pre-rRNA by the action of ribonucleases, and some bases and riboses 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 on the ribosome:** 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 codon 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 Figure 31.13 for an illustration of the role of the A, P, and E sites in translation.)

Figure 31.8 Ribosomal composition. [Note: The number of proteins in the eukaryotic ribosomal subunits varies somewhat from species to species.] S = Svedberg unit.



4. **Cellular location of ribosomes:** In eukaryotic cells, the ribosomes are either "free" in the cytosol or are in close association with the endoplasmic reticulum (which is then known as the "rough" endoplasmic reticulum, or RER). The RER-associated ribosomes are responsible for synthesizing proteins that are to be exported from the cell as well as those that are destined to become incorporated into plasma, endoplasmic reticulum, or Golgi membranes or imported into lysosomes (see p. 169 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 set of ribosomes and their own unique, circular DNA. Most mitochondrial proteins, however, are encoded by nuclear DNA, synthesized in the cytosol, and posttranslationally targeted to mitochondria.]

F. Protein factors

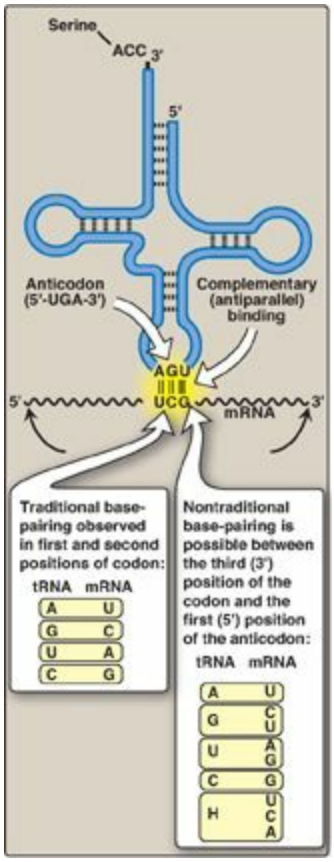
Initiation, elongation, and termination (or release) factors are required for peptide synthesis. Some of these protein factors perform a catalytic function, whereas others appear to stabilize the synthetic machinery. [Note: A number of the factors are monomeric G proteins, and thus are active when bound to guanosine triphosphate (GTP) and inactive when bound to guanosine diphosphate (GDP) (see p. 95 for a discussion of the heterotrimeric G proteins).]

G. ATP and GTP are required as sources of energy

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 inorganic phosphate by pyrophosphatase —and two from GTP—one for binding the aminoacyl-tRNA to the A site and one for the translocation step (see Figure 31.13, p. 440). [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.]

Figure 31.9 Wobble: Nontraditional base-pairing between the 5**I**-nucleotide (first nucleotide) of the anticodon with the 3**I**-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.



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 Figure 31.6). Some tRNAs (isoaccepting tRNAs) recognize more than one codon for a given amino acid.

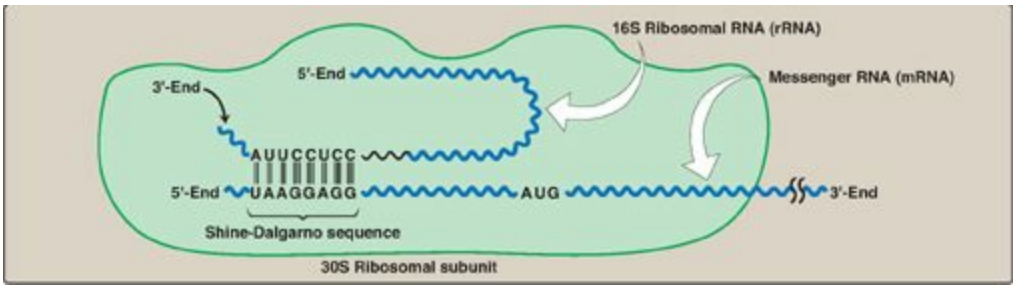
A. Antiparallel binding between codon and anticodon

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 "flipped" (3→5) orientation (Figure 31.9). [Note: Nucleotide sequences are always assumed to be 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 tRNAs 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 (C pairs with G 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 31.9. The result of wobble is that there need not be 61 tRNA species to read the 61 codons that code for amino acids.

Figure 31.10 Complementary binding between prokaryotic mRNA Shine-Dalgarno sequence and 16S rRNA. S = Svedberg unit.

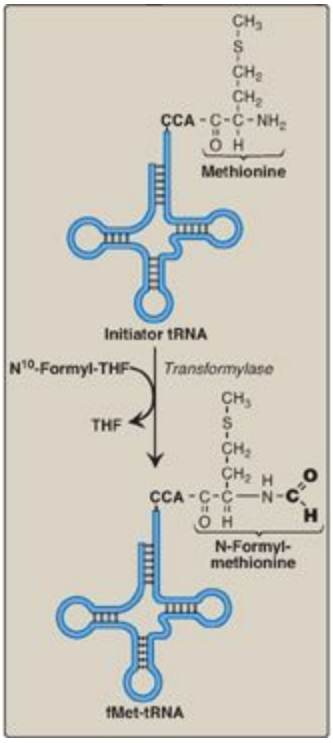


V. STEPS IN PROTEIN SYNTHESIS

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 mRNAs often have several coding regions (that is, they are polycistronic; see p. 418). 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 (that is, it is monocistronic). The process of translation is divided into three separate steps: initiation, elongation, and termination. Eukaryotic protein synthesis 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.

Figure 31.11 Generation of the initiator N-formylmethionyl-tRNA (fMet-tRNA). THF = tetrahydrofolate; C = cytosine; A = adenine.



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 (which provides energy for the process), and initiation factors that facilitate the assembly of this initiation complex (see Figure 31.13). [Note: In prokaryotes, three initiation factors 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.

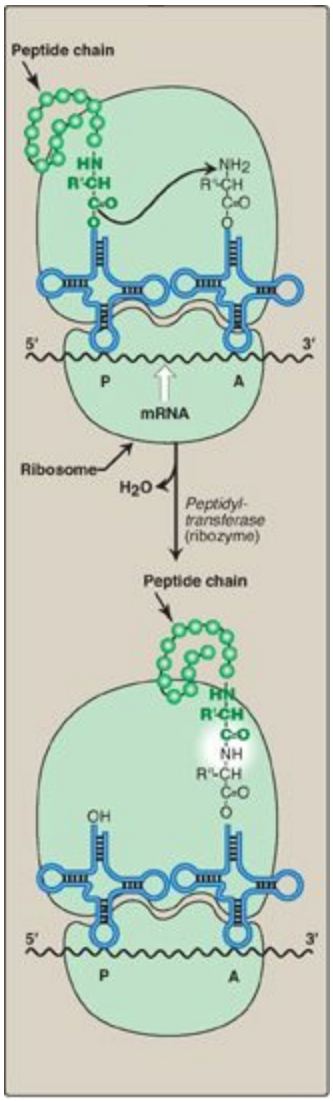
1. **Shine-Dalgarno sequence:** In Escherichia coli (E. coli), a purine-rich sequence of nucleotide bases, known as the Shine-Dalgarno (SD) sequence, is located six to ten bases upstream of the initiating AUG codon on the mRNA molecule (that is, near its 5**I**-end). The 16S rRNA component of the small (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 small ribosomal subunit on the mRNA in close proximity to the initiating AUG codon (Figure 31.10).

2. **5I Cap:** Eukaryotic mRNAs 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 down the mRNA until it encounters the initiator AUG. This "scanning" process requires ATP. [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.]

3. **Initiation codon:** The initiating AUG is recognized by a special initiator tRNA. Recognition is facilitated by IF-2-GTP in prokaryotes and eIF-2-GTP (plus additional eIFs) in eukaryotes. The charged initiator tRNA enters the P site on the small subunit. The initiator tRNA is the only tRNA recognized by (e)IF-2 and the only tRNA to go directly to the P site. In bacteria and in mitochondria, the initiator tRNA carries an N-formylated methionine (fMet, Figure 31.11). After Met is attached to the initiator tRNA, the formyl group is added by the enzyme transformylase, which uses N^{10}-formyl tetrahydrofolate (see p. 267) as the carbon donor. In eukaryotes, the initiator tRNA 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 initiating tRNA in the P site. The A site is empty. [Note: Specific (e)IFs function as anti-association factors and prevent premature addition of the large subunit.] The GTP on (e)IF-2 gets hydrolyzed to GDP. A guanine nucleotide exchange factor facilitates the reactivation of (e)IF-2-GDP through replacement of GDP by GTP.

Figure 31.12 Formation of a peptide bond. Peptide bond formation involves 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.

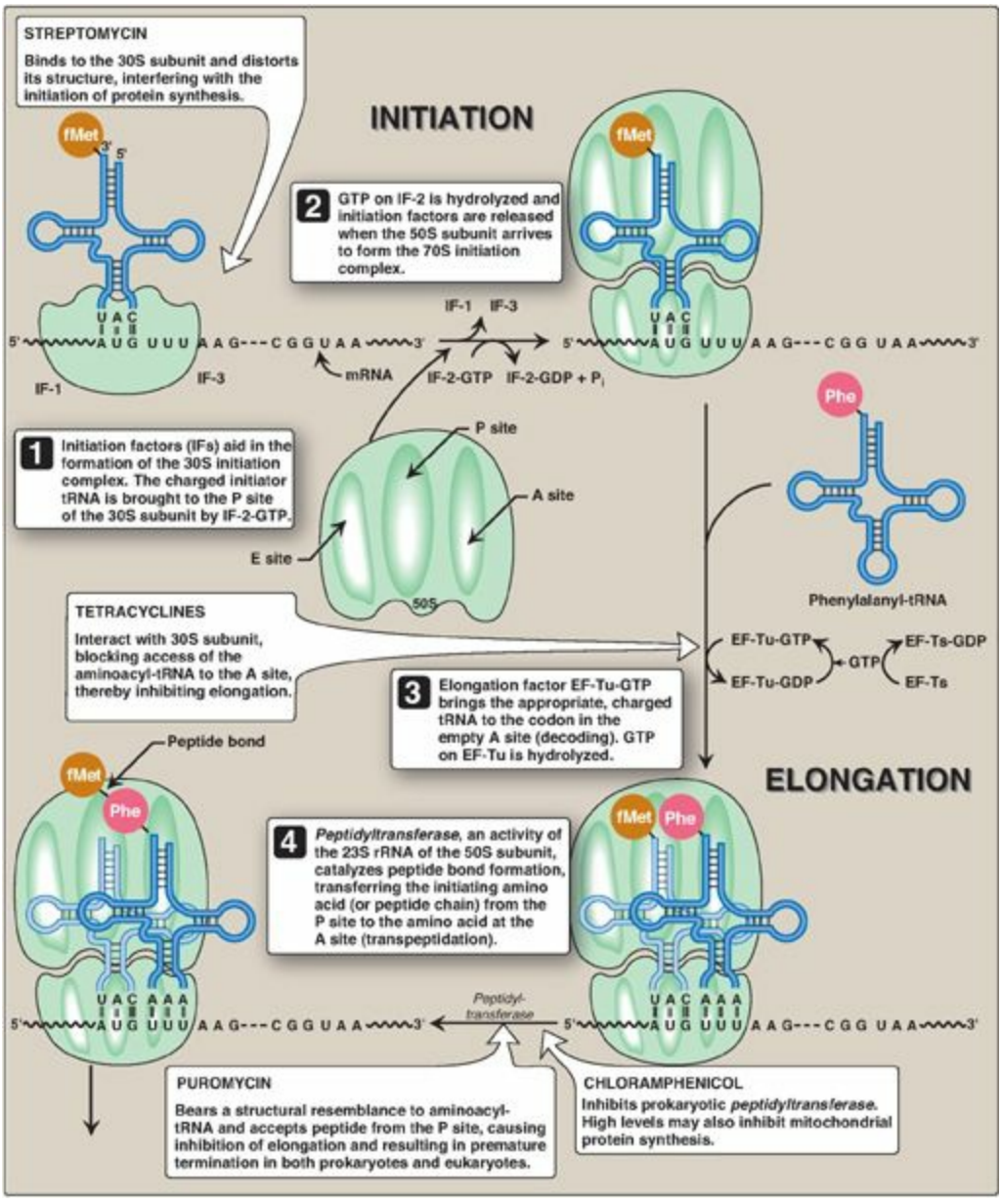


B. Elongation

Elongation of the polypeptide chain involves the addition of amino acids to the carboxyl end of the growing chain. During elongation, the ribosome moves from the 5′-end to the 3′-end of the mRNA that is being translated. 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-1a-GTP and EF-1bg. Both EF-Ts and EF-1bg function in guanine nucleotide exchange.] The formation of the peptide bond is catalyzed by peptidyltransferase, an activity intrinsic to the 23S rRNA found in the large (50S) ribosomal subunit (Figure 31.12). [Note: Because this rRNA catalyzes the reaction, it is referred to as a ribozyme.] After the peptide bond has been formed, what was attached to the tRNA at the P site is now linked to the amino acid on the tRNA at the A site. 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 (eukaryotic cells 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.

Figure 31.13 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 GDP, allowing its replacement by GTP. The eukaryotic equivalent is EF-1βγ.] fMet = formylated methionine; S = Svedberg unit; GTP = guanine nucleoside triphosphate; Phe = phenylalanine.
[Note: Ricin, a toxin from castor beans, removes an A from the 28 S rRNA in the large subunit of eukaryotic ribosomes, thereby inhibiting their function.] Lys = lysine; Arg = arginine.



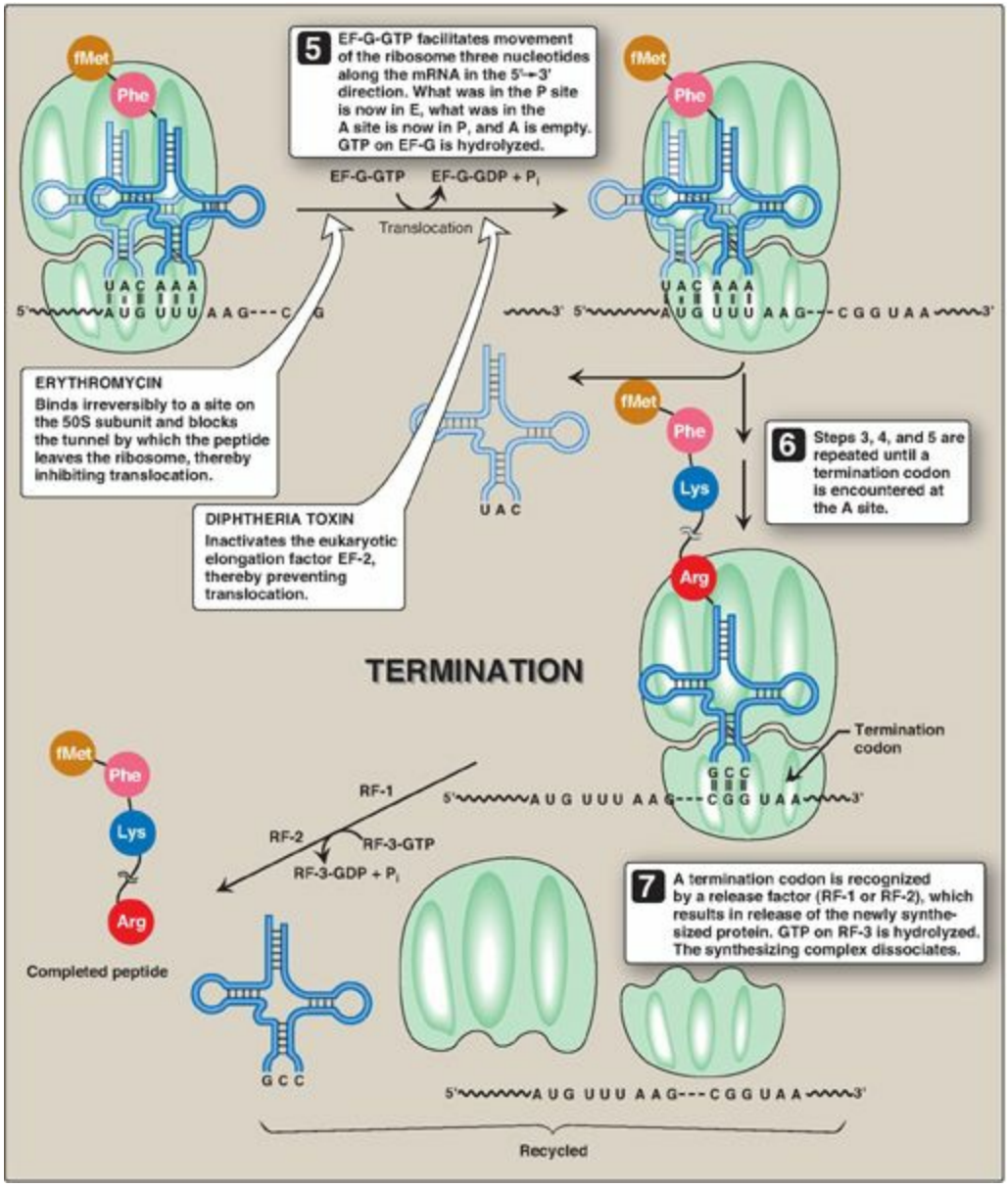
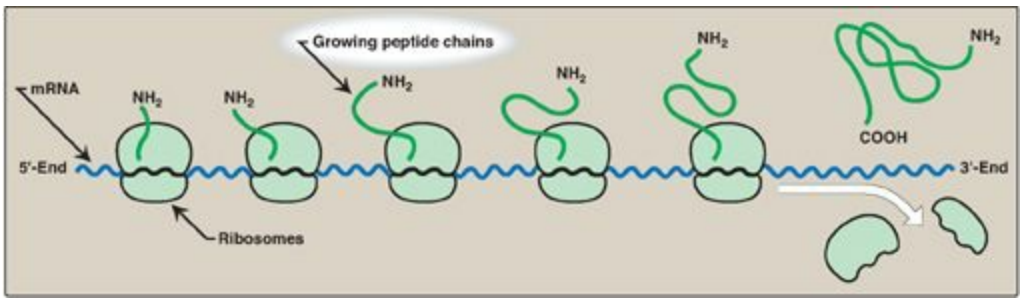


Figure 31.14 A polyribosome consists of several ribosomes simultaneously translating one messenger RNA (mRNA). [Note: Eukaryotic mRNA is circularized for translation.]



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 the termination codons 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 Figure 31.13). [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 31.15 for a summary of the factors used in translation.] The steps in prokaryotic protein synthesis are summarized in Figure 31.13. 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.] Some antibiotic inhibitors of protein synthesis are illustrated in Figure 31.13, as is diphtheria toxin.

Figure 31.15 Protein factors in the three stages of translation. P = prokaryotes; E = eukaryotes; tRNA = transfer RNA; IF = initiation factor; EF = elongation factor; RF = release factor.

Cell	Factor	Function
Initiation		
P E	IF-2-GTP eIF-2-GTP	Bring charged initiating tRNA to P site
P E	IF-3 eIF-3	Prevent association of subunits
Elongation		
P E	EF-Tu-GTP EF1α-GTP	Bring all other charged tRNAs to A site
P E	EF-Ts EF-1βγ	Guanine nucleotide exchange factor
P E	EF-G-GTP EF-2-GTP	Translocation
Termination		
P E	RF-1, 2 eRF	Recognize "stop" codons
P E	RF-3-GTP eRF-3-GTP	Release of other RFs

D. Polysomes

Translation begins at the 5′-end of the mRNA, with the ribosome proceeding along the RNA molecule. Because of the length of most mRNAs, more than one ribosome at a time can translate a message (Figure 31.14). Such a complex of one mRNA and a number of ribosomes is called a polysome or polyribosome.

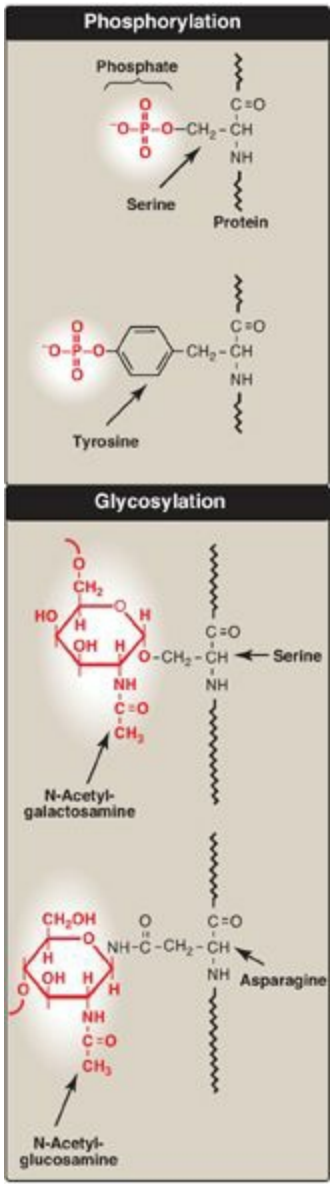
E. Regulation of translation

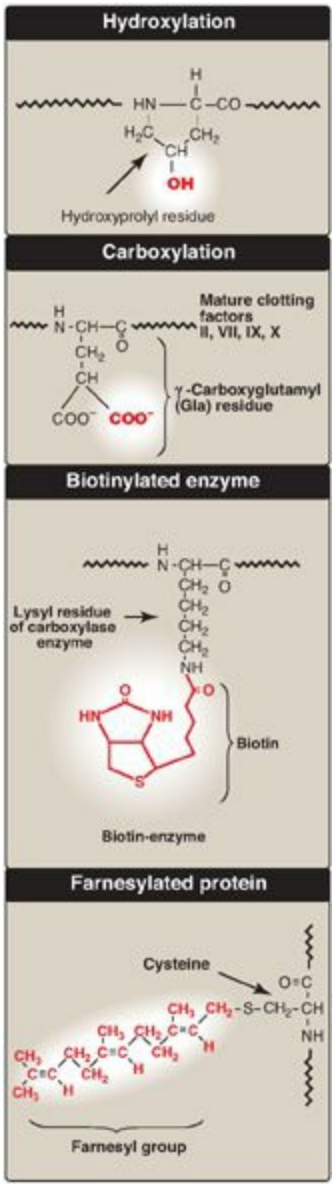
Gene expression is most commonly regulated at the transcriptional level, but translation may also be regulated. An important mechanism by which this is achieved in eukaryotes is by covalent modification of eIF-2: phosphorylated eIF-2 is inactive. In both eukaryotes and prokaryotes, regulation can also be achieved through proteins that bind mRNA and inhibit its use by blocking translation or extend its use by protecting it from degradation. For a more detailed discussion of the regulation of translation, see p. 454.

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 usually contain amino acid sequences that direct the proteins to their final locations. For example, proteins destined for secretion from the cell are targeted during their synthesis (cotranslational targeting) to the RER (see p. 436) by the presence of an N-terminal hydrophobic signal sequence. [Note: The sequence is recognized and bound by the signal recognition particle, which facilitates transport to the RER.] Proteins targeted after synthesis (posttranslational) include nuclear proteins that contain an internal, short, basic "nuclear localization signal" and mitochondrial matrix proteins that contain an N-terminal, amphipathic, α-helical "mitochondrial entry sequence."

Figure 31.16 Covalent modifications of some amino acid residues.




Hydroxylation
Hydroxyprolyl residue
Carboxylation
Mature clotting factors II, VII, IX, X
γ-Carboxyglutamyl (Gla) residue
Biotinylated enzyme
Lysyl residue of carboxylase enzyme
Biotin
Biotin-enzyme
Farnesylated protein
Cysteine
Farnesyl group

VI. CO- AND POSTTRANSLATIONAL MODIFICATION OF POLYPEPTIDE CHAINS

Many polypeptide chains 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. Examples of such modifications are listed below.

A. Trimming

Many proteins destined for secretion from the cell are initially made as large, precursor molecules that are not functionally active. Portions of the protein chain 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 endoplasmic reticulum or the Golgi apparatus; others are cleaved in developing secretory vesicles (for example, insulin; see Figure 23.4, p. 309); and still others, such as collagen (see p. 47), are cleaved after secretion.

B. Covalent attachments

Proteins may be activated or inactivated by the covalent attachment of a variety of chemical groups (Figure 31.16). Examples include the following.

1. **Phosphorylation:** Phosphorylation occurs on the hydroxyl groups of serine; threonine; or, less frequently, tyrosine residues in a protein. This phosphorylation is catalyzed by one of a family of protein kinases and may be reversed by the action of cellular protein phosphatases. The phosphorylation may increase or decrease the functional activity of the protein. Several examples of phosphorylation reactions have been previously discussed (for example, see Chapter 11, p. 131, for the regulation of glycogen synthesis and degradation).

2. **Glycosylation:** Many of the proteins that are destined to become part of a plasma membrane or to be secreted from a cell have carbohydrate chains added en bloc to the amide nitrogen of asparagine (N-linked) or built sequentially on the hydroxyl groups of serine, threonine, or hydroxylysine (O-linked). N-glycosylation occurs in the endoplasmic reticulum and O-glycosyation in the Golgi. (The process of producing such glycoproteins was discussed on p. 165.) Glycosylation is also used to target proteins to the matrix of lysosomes. Lysosomal acid hydrolases are modified by the phosphorylation of mannose residues at carbon 6 (see p. 169).

3. **Hydroxylation:** Proline and lysine residues of the a chains of collagen are extensively hydroxylated by vitamin C–dependent hydroxylases in the endoplasmic reticulum (see p. 47).

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. 389). The resulting g-carboxyglutamate (Gla) residues are essential for the activity of several of the blood-clotting proteins. (See online Chapter 34.) Biotin is covalently bound to the e-amino groups of lysine residues of biotin-dependent enzymes that catalyze carboxylation reactions such as pyruvate carboxylase (see p. 119). Attachment of lipids, such as farnesyl groups, can help anchor proteins to membranes. Many eukaryotic proteins are cotranslationally acetylated at the N-end. [Note: Reversible acetylation of histone proteins influences gene expression (see p. 409).]

C. 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. 20).

D. Protein degradation

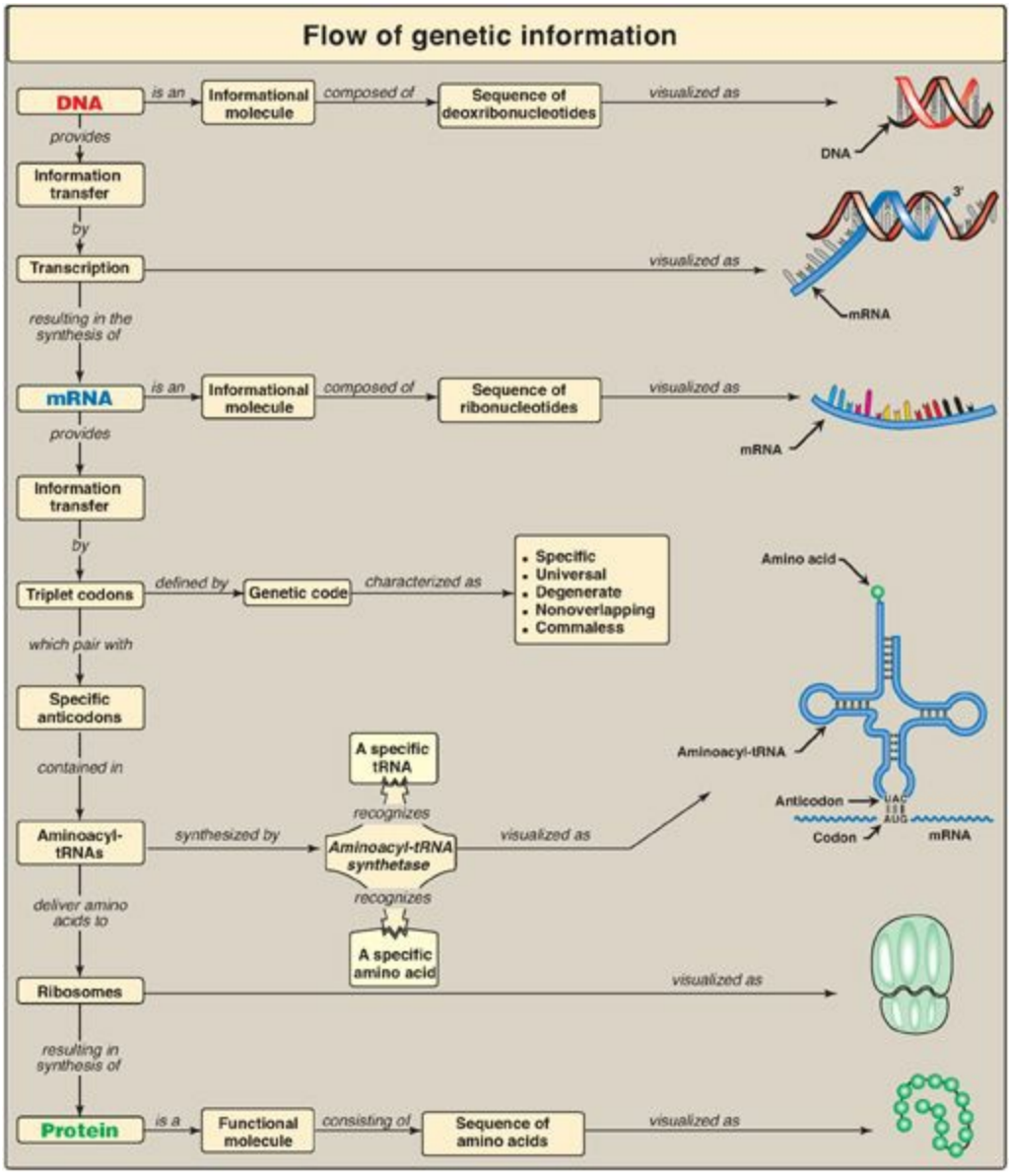
Proteins that are defective (for example, misfolded) or destined for rapid turnover are often marked for destruction by ubiquitination, the attachment of chains of a small, highly conserved protein, called ubiquitin (see Figure 19.3 on p. 247). Proteins marked in this way are rapidly degraded by a cellular component known as the proteasome, which is a macromolecular, ATP-dependent, proteolytic system located in the cytosol. [Note: The DF508 mutation seen in CF causes misfolding of the CFTR protein, resulting in its proteasomal degradation.]

VII. CHAPTER SUMMARY

Codons are composed of three nucleotide bases presented in the messenger (mRNA) language of **adenine** (**A**), **guanine** (**G**), **cytosine** (**C**), and **uracil** (**U**). They are always written **5′→3′**. Of the 64 possible three-base combinations, 61 code for the 20 common amino acids and 3 signal termination of protein synthesis (**translation**). 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). Characteristics of the genetic code include **specificity**, **universality**, and **degeneracy**, and it is **nonoverlapping** and **commaless** (Figure 31.17). Requirements for protein synthesis include all the amino acids that eventually appear in the finished protein, at least one specific type of transfer RNA (tRNA) for each amino acid, one **aminoacyl-tRNA synthetase** for each amino acid, the mRNA coding for the protein to be synthesized, fully competent **ribosomes**, **protein factors** needed for initiation, elongation, and termination of protein synthesis, and **ATP** and **GTP** as energy sources. tRNA has an attachment site for a specific amino acid at its 3′-end, and an anticodon region that can recognize the codon specifying the amino acid the tRNA is carrying. **Ribosomes** are large complexes of protein and ribosomal (rRNA). They consist of **two subunits**. Each ribosome has three binding sites for tRNA molecules: the A, P, and E sites that cover three neighboring codons. The **A-site** codon binds an **incoming aminoacyl-tRNA**, the **P-site** codon is occupied by **peptidyl-tRNA**, and the **E site** is occupied by the **empty tRNA** as it is about to exit the ribosome. Recognition of an mRNA codon is accomplished by the tRNA **anticodon**. The anticodon binds to the codon following the rules of **complementarity** and **antiparallel** binding. (Nucleotide sequences are always assumed to be written in the 5′ to 3′ direction unless otherwise noted.) The **"wobble" hypothesis** states that the first (5′) 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 last (3′) base of the codon, thus allowing a single tRNA to recognize more than one codon for a specific amino acid. For **initiation of protein synthesis**, the components of the translation system are assembled, and mRNA associates with the small ribosomal subunit. The process requires i**nitiation factors**. In **prokaryotes**, a purine-rich region of the mRNA (the **Shine-Dalgarno sequence**) base-pairs with a complementary sequence on 16S rRNA, resulting in the positioning of the small subunit on the mRNA so that translation can begin. The **5′-cap** (bound by proteins of the eIF-4 family) on **eukaryotic mRNA** is used to position the small subunit on the mRNA. The **initiation codon** is **AUG**, and **N-formylmethionine** is the initiating amino acid in prokaryotes, whereas methionine is used in eukaryotes. The polypeptide chain is elongated by the addition of amino acids to the carboxyl end of its growing chain. The process requires **elongation factors** that 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 **peptidyltransferase**, 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 mRNAs, more than one ribosome at a time can translate a message, forming a **polysome**. Termination begins when one of the three termination codons moves into the A site. These codons are recognized by **release factors**. The newly synthesized protein is released from the ribosomal complex, and the ribosome is dissociated from the mRNA. Initiation, elongation, and termination are driven by the hydrolysis of **GTP**. Initiation in eukaryotes also requires **ATP** for scanning. Numerous **antibiotics** interfere with the process of protein synthesis. Many polypeptide chains are covalently modified during or after translation. Such modifications include **removal of amino acids**; **phosphorylation,** which may activate or inactivate the protein; **glycosylation**, which plays a role in protein targeting; and **hydroxylation** such as that seen in collagen. Proteins must fold to achieve their functional form. Folding can be spontaneous or facilitated by **chaperones**. Proteins that are defective (for example 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**.

Figure 31.17 Key concept map for protein synthesis. mRNA = messenger RNA; tRNA = transfer RNA; A = adenine; G = guanine; C = cytosine; U = uracil.


Flow of genetic information
DNA
is an
Informational molecule
composed of
Sequence of deoxribonucleotides
visualized as
DNA
provides
Information transfer
by
Transcription
visualized as
3'
mRNA
resulting in the synthesis of
mRNA
is an
Informational molecule
composed of
Sequence of ribonucleotides
visualized as
mRNA
provides
Information transfer
by
Triplet codons
defined by
Genetic code
characterized as
Specific
Universal
Degenerate
Nonoverlapping
Commaless
which pair with
Specific anticodons
contained in
Aminoacyl-tRNAs
synthesized by
A specific tRNA
recognizes
Aminoacyl-tRNA synthetase
recognizes
A specific amino acid
visualized as
Amino acid
Aminoacyl-tRNA
Anticodon
UAC
AUG
Codon
mRNA
deliver amino acids to
Ribosomes
visualized as
resulting in synthesis of
Protein
is a
Functional molecule
consisting of
Sequence of amino acids
visualized as

Study Questions

Choose the ONE best answer.

31.1 A 20-year-old man 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?

A. CGA → UGA
B. GAU → GAC
C. GCA → GAA
D. UAA → CAA
E. UAA → UAG

Correct answer = D. Mutating the normal termination (stop) codon for β-globin from UAA to CAA 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 further 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 encode 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

31.2 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 the messenger RNA 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 charged transfer RNA to the ribosomal A site
C. Peptidyltransferase activity
D. Ribosomal translocation
E. Termination

Correct answer = D. Because fMet-Phe is made, the ribosomes must be able to complete initiation, bind Phe-tRNA to the A site, and use peptidyltransferase 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. The ribosome is, therefore, frozen before it reaches the termination codon of this message.

31.3 A transfer RNA (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 will be the fate of this alanine residue during protein synthesis?

A. It will be incorporated into a protein in response to a codon for alanine.
B. It will be incorporated into a protein in response to a codon for cysteine.
C. It will be incorporated randomly at any codon.
D. It will remain attached to the tRNA because it cannot be used for protein synthesis.
E. It will be chemically converted to cysteine by cellular enzymes.

Correct answer = B. Once an amino acid is attached to a transfer (tRNA) molecule, only the anticodon of that tRNA determines the specificity of incorporation. The mischarged alanine will, therefore, be incorporated into the protein at a position determined by a cysteine codon.

31.4 In a patient with cystic fibrosis caused by the ΔF508 mutation, the mutant cystic fibrosis 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 performs its normal function because the ubiquitin largely corrects for the effect of the mutation.
B. It is secreted from the cell.
C. It is placed into storage vesicles.
D. It is degraded by the proteasome.
E. It is repaired by cellular enzymes.

Correct answer = D. 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.

31.5 Many antimicrobials inhibit protein translation. Which of the following antimicrobials is correctly paired with its mechanism of action?

A. Erythromycin binds to the 60S ribosomal subunit.
B. Puromycin inactivates EF-2.
C. Streptomycin binds to the 30S ribosomal subunit.
D. Tetracyclines inhibit peptidyltransferase.

Correct answer = C. Streptomycin binds the 30S subunit and inhibits translation initiation. 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-tRNA. 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.

31.6 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. 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.

31.7 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. fMet-tRNA
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 fMet, and only eukaryotes have a nucleus and co- and posttranscriptionally process their mRNA.

31.8 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 apparatus.

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 removed 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.

31.9 Why is the genetic code described both as 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).

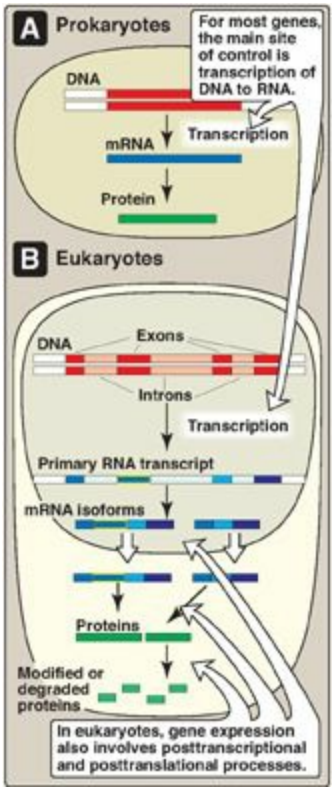
Regulation of Gene Expression 32

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 regulated. For example, genes described as constitutive encode products required for basic cellular functions and so are continually expressed. They are also known as "housekeeping" genes. Regulated genes, however, are expressed only under certain conditions. They may be expressed in all cells or in only a subset of cells, for example, hepatocytes. The ability to regulate gene expression (that is, 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 32.1 lists some common strategies employed in gene regulation.

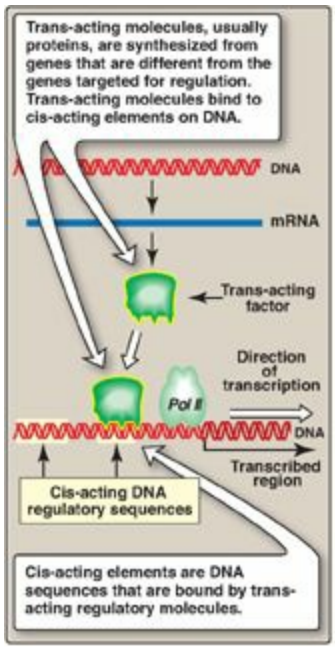
Figure 32.1 Control of gene expression. mRNA = messenger RNA.



II. REGULATORY SEQUENCES AND MOLECULES

Regulation of transcription, the initial step in all gene expression, is controlled by regulatory sequences of DNA, usually embedded in the noncoding regions of the genome. The interaction between these DNA segments and regulatory molecules, such as transcription factors, can engage or repress the transcriptional machinery, influencing the kinds and amounts of products that are produced. These DNA sequences flanking a gene are called cis-acting because they influence expression of genes only on the same chromosome (see p. 423). A trans-acting factor is the regulatory molecule itself, which can transit (diffuse) through the cell from its site of synthesis to its DNA-binding site (Figure 32.2). For example, a protein transcription factor (a trans-acting molecule) that regulates a gene on chromosome 6 might itself have been produced from a gene on chromosome 11. The binding of proteins to DNA is through structural motifs such as the zinc finger (Figure 32.3), leucine zipper, or helix-turn-helix in the protein. [Note: Some trans-acting factors can negatively affect gene expression.]

Figure 32.2 Cis-acting elements and trans-acting molecules. mRNA = messenger RNA; Pol II = RNA polymerase II.



III. REGULATION OF PROKARYOTIC GENE EXPRESSION

In prokaryotes such as 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. Transcription of messenger RNA from bacterial operons

In bacteria, the structural genes that code for proteins involved in a particular metabolic pathway are often found sequentially grouped on the chromosome along with the cis-acting regulatory elements that determine the transcription of these genes. The transcription product is a single polycistronic messenger RNA (mRNA) (see p. 418). The genes are, thus, coordinately controlled (that is, turned on or off as a unit). This entire package is referred to as an operon.

B. Role of operators in prokaryotic transcription

Prokaryotic operons contain an operator, a segment of DNA that regulates the activity of the structural genes of the operon. If the operator is not bound by a repressor molecule, RNA polymerase passes over the operator and reaches the protein-coding genes which it transcribes to mRNA. If a repressor molecule 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 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, the RNA polymerase can proceed with transcription. One of the best-understood examples is the inducible lactose operon of E. coli that illustrates both positive and negative regulation (Figure 32.4).

C. The lactose operon

The lactose (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 thiogalactoside transacetylase, 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 polymerase binds and two additional sites, the operator (O) site and the CAP site, where regulatory proteins bind. The lacZ, lacY, and lacA

genes are expressed only when the O site is empty, and the CAP site is bound by a complex of cyclic adenosine monophosphate ([cAMP] see p. 94) and the catabolite activator protein (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.]

Figure 32.3 Zinc (Zn) finger is a common motif in proteins that bind DNA. Cys = cysteine; His = histidine.

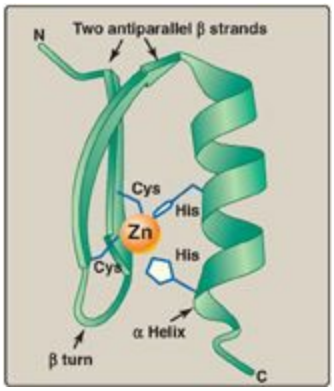
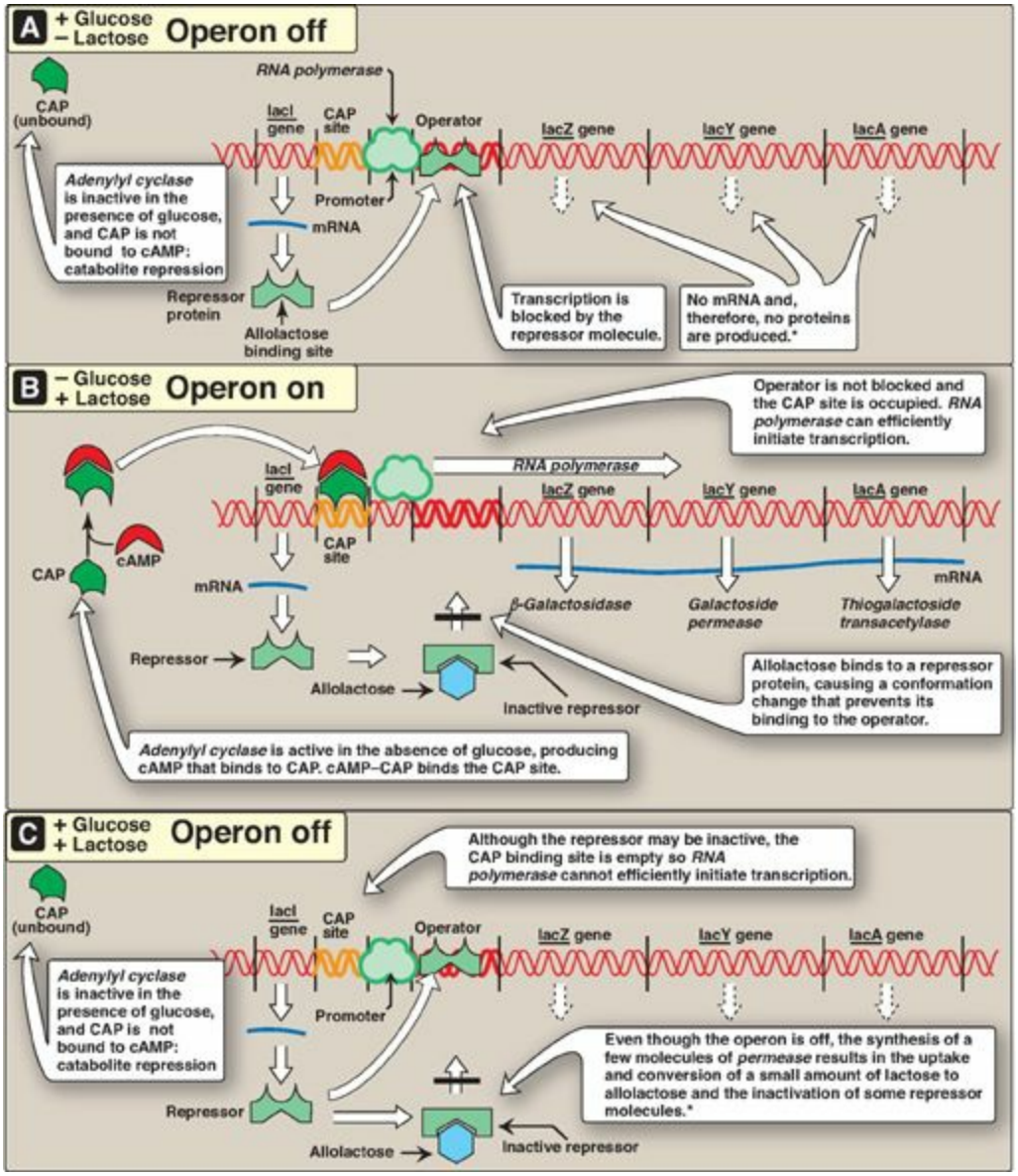


Figure 32.4 The lactose operon of E. coli. *[Note: Even when the operon has been turned off by catabolite repression, the repressor transiently dissociates from the operator at a slow rate, allowing a very low level of expression. The synthesis of a few molecules of permease (and β-galactosidase) allows the organism to respond rapidly should glucose become unavailable.] CAP = catabolite activator protein; cAMP = cyclic adenosine monophosphate; mRNA = messenger RNA.



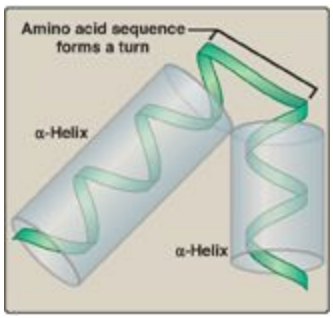
1. **When only glucose is available:** In this case, the lac operon is repressed (turned off). Repression is mediated by the repressor protein binding via a helix-turn-helix motif (Figure 32.5) to the operator site, which is downstream of the promoter region (see Figure 32.4A). Binding of the repressor interferes with the progress of RNA polymerase and blocks transcription of the structural genes. This is an example of negative regulation.

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 operator. In the absence of glucose, adenylyl cyclase is active, and sufficient quantities of cAMP are made and bind to the CAP protein. The cAMP–CAP trans-acting complex binds to the CAP site, causing RNA polymerase to more efficiently initiate transcription at the promoter site (see Figure 32.4B). 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, transcription of the lac operon 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. RNA polymerase is, therefore, unable to effectively initiate transcription, even though the repressor may not be bound to the operator region. Consequently, the three structural genes of the operon are not expressed (see Figure 32.4C).

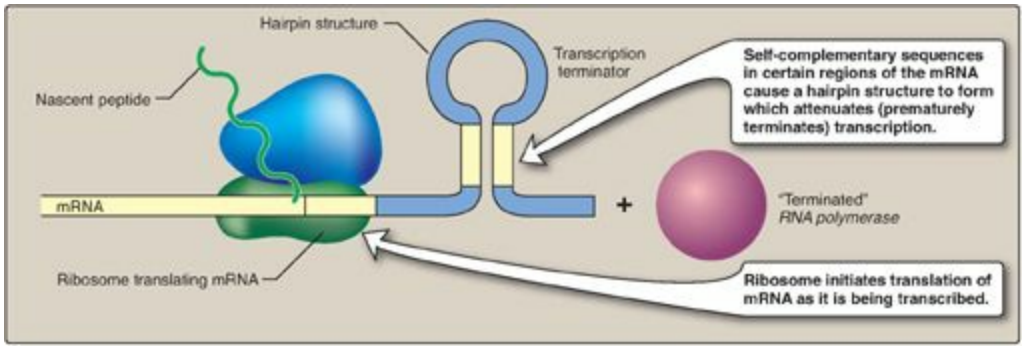
Figure 32.5 Helix-turn-helix motif of the lac repressor protein.



D. Tryptophan operon

The tryptophan (trp) operon contains five structural genes that code for enzymes required for the synthesis of the amino acid, tryptophan. 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, unlike the lac operon, the trp operon is also regulated by a process known as attenuation. With attenuation, transcription is initiated but is terminated well before completion (Figure 32.6). If Trp is plentiful, transcription initiation that escaped repression by Trp is attenuated (stopped) by the formation at the 5′-end of the mRNA of a hairpin (stem–loop) structure like that seen in rho-independent termination (see p. 421). [Note: Transcription and translation are temporally linked in prokaryotes (see p. 438), and, therefore, 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 32.6 Attenuation of transcription of the trp operon when tryptophan is plentiful. mRNA = messenger RNA.



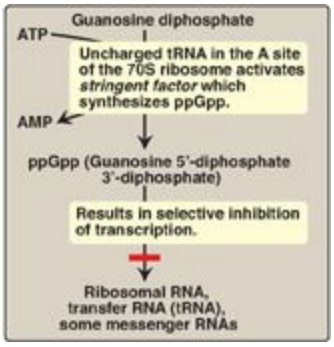
Transcriptional attenuation can occur in prokaryotes because translation of an mRNA begins before its synthesis is complete. This does not occur in eukaryotes because the presence of a membrane-bound nucleus spatially and temporally separates transcription and translation.

E. Coordination of transcription and translation in prokaryotes

Whereas transcriptional regulation of mRNA production is primary in bacteria, regulation at the level of ribosomal RNA (rRNA) and protein synthesis also occurs and plays important roles in the microbe's ability to adapt to environmental stress.

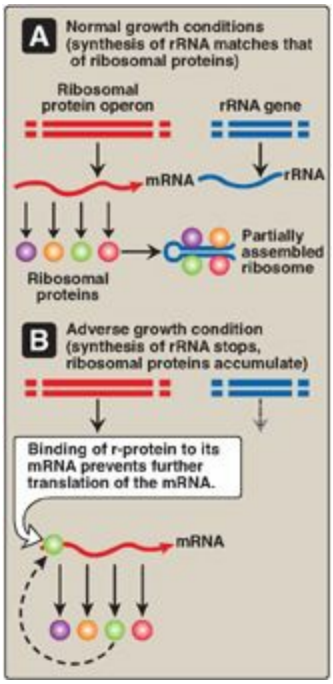
1. **Stringent response:** E. coli has seven operons that synthesize the rRNA needed for ribosome assembly, and each is regulated in response to changes in environmental conditions. Regulation in response to amino acid starvation is known as the stringent response. The binding of an uncharged transfer RNA (tRNA) to the A site of a ribosome (see p. 436) triggers a series of events that leads to the production of a polyphosphorylated guanosine, 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 (Figure 32.7). [Note: In addition to rRNA, tRNA synthesis and some mRNA synthesis (for example, for ribosomal proteins) are also inhibited. However, synthesis of mRNAs for enzymes required for amino acid biosynthesis is not inhibited. ppGpp appears to alter promoter selection through use of different sigma-factors for RNA polymerase (see p.419.]

Figure 32.7 Regulation of transcription by the stringent response to amino acid starvation. S = Svedberg unit.



2. **Regulatory ribosomal proteins:** Operons for ribosomal proteins (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 (Figure 32.8). 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. 439) and acting as a physical impediment to the binding of the small ribosomal subunit to the SD sequence. One r-protein thus 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.

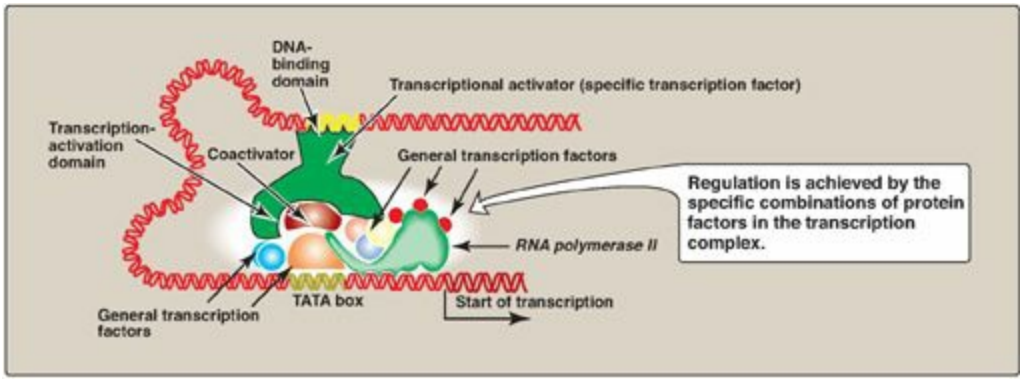
Figure 32.8 Regulation of translation by an excess of ribosomal proteins. mRNA = messenger RNA; rRNA = ribosomal RNA; r-protein = ribosomal protein.



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, the primary site of regulation is at the level of transcription. Again, the theme of trans-acting molecules binding to cis-acting elements is seen. Operons, however, are generally not found in eukaryotes, which must use alternative 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, 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 32.9 Combinatorial control of transcription.



A. Trans-acting molecules

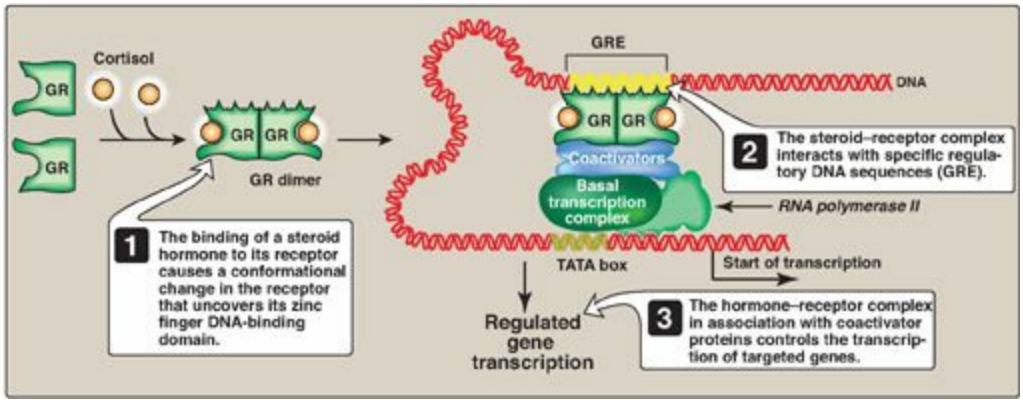
Specific transcription factors are trans-acting DNA-binding proteins that function as transcriptional activators. They have at least two binding domains: the DNA-binding domain, and the transcription-activation domain. The DNA-binding domain contains specific structural motifs, such as zinc fingers (see p. 450), that bind consensus sequences in DNA. The transcription-activation domain recruits other proteins, such as the general transcription factors ([GTFs] see p.423) and coactivators (for example, histone acetyltransferases [HATs]; see p. 422). These facilitate formation of the transcription initiation complex (RNA polymerase II plus the GTFs) at the promoter, and, thus, activate transcription (Figure 32.9). Regulation is achieved by the formation of a multiprotein complex bound to DNA, with protein–protein and protein–DNA interactions controlling assembly of the complex. Although activation domains recruit 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. [Note: DNA-binding proteins can also inhibit transcription.]

B. Cis-acting regulatory elements

The need to coordinately regulate a group of genes to cause a particular response is of

key importance in multicellular organisms including humans. An underlying theme occurs repeatedly: A protein binds to a regulatory consensus element on each of the genes in the group and coordinately affects the expression of those genes, even if they are on different chromosomes. For example, hormone-response elements (HREs) are cis-acting DNA sequences that bind trans-acting protein factors and regulate gene expression in response to hormonal signals. In general, hormones bind either to intracellular receptors (steroid hormones are an example; see p. 240) or to cell-surface receptors (the peptide hormone glucagon is an example; see p. 314).

Figure 32.10 Transcriptional regulation by intracellular steroid hormone receptors. GRE = glucocorticoid-response element (an example of a hormone-response element); GR = glucocorticoid receptor.



1. **Regulatory signals mediated by 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, all directly influence gene expression by functioning as specific transcription factors. These receptors, therefore, contain a DNA-binding domain and an activation domain. They also contain a ligand-binding domain. For example, steroid hormones such as cortisol (a glucocorticoid) bind to soluble, intracellular receptors at the ligand-binding domain (Figure 32.10). Binding causes a conformational change in the receptor that activates it. The receptor–ligand complex enters the nucleus, dimerizes, and binds via a zinc finger motif to nuclear DNA at a cis-acting regulatory element, the glucocorticoid-response element (GRE), an example of an HRE. Binding allows recruitement of coactivators to the activation domain and results in increased 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 when these genes are located on different chromosomes. The GRE can be located upstream or downstream of the genes it regulates and is able to function at great distances from those genes. The GRE, then, can function as a true enhancer (see p. 424). [Note: If associated with corepressors, hormone–receptor complexes inhibit transcription.]

2. **Regulatory signals mediated by cell-surface receptors:** Cell-surface 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 (Figure 32.11; also see Figure 8.7 on p. 95), 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 CREs in their promoters. [Note: The genes for phosphoenolpyruvate carboxykinase and glucose 6-phosphatase, key enzymes of gluconeogenesis (see p. 117), are examples of genes upregulated by the cAMP/CRE/CREB system.]

Figure 32.11 Transcriptional regulation by receptors located in the cell membrane. [Note: Cyclic AMP activates protein kinase A that phosphorylates cAMP response element-binding (CREB) protein.] CRE = cAMP response element.

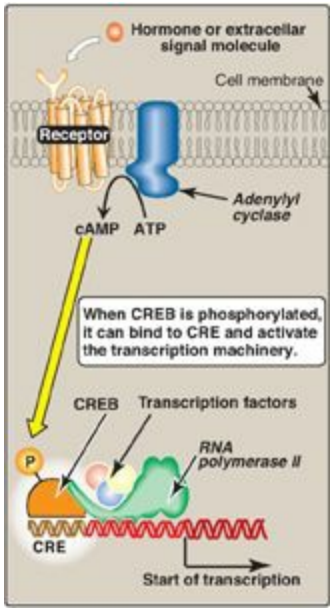
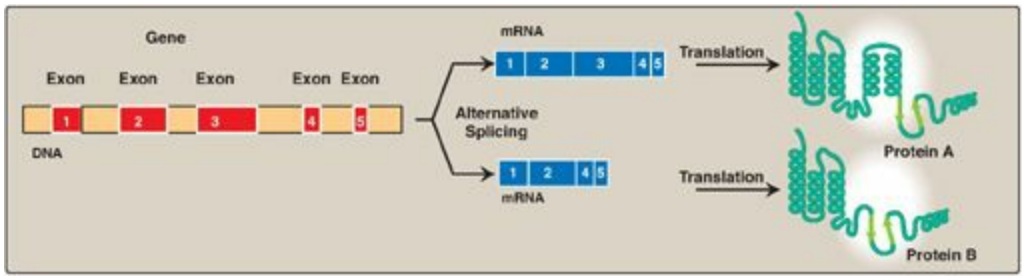


Figure 32.12 Tissue-specific alternative splicing produces multiple related proteins, or isoforms, from a single gene.



C. Regulation by processing of messenger RNA

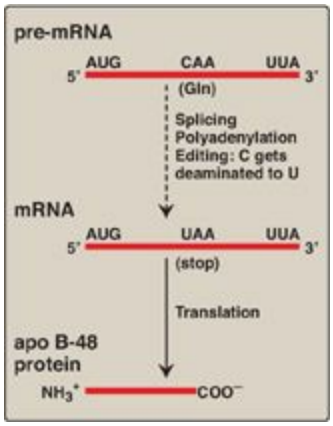
Eukaryotic mRNA undergoes several modifications before it is exported from the nucleus to the cytoplasm for use in protein synthesis (see p. 418). Capping at the 5′-end, polyadenylation at the 3′-end, and splicing are essential processing events for the production of a functional eukaryotic messenger from most pre-mRNA (see p. 425), and variations in these events can affect gene expression. In addition, messenger stability also affects gene expression.

1. **Splice-site choice:** Tissue-specific protein isoforms can be made from the same pre-mRNA through differential cotranscriptional processing, particularly the use of alternative splice sites (Figure 32.12). For example, tropomyosin (TM) is an actin filament–binding protein that regulates the functions of actin in both muscle and nonmuscle cells. Its pre-mRNA undergoes tissue-specific differential splicing to yield a number of TM isoforms (see p. 427).

> Over 60% percent of the approximately 25,000 genes in the human genome undergo differential splicing. The use of alternative polyadenylation and transcription start sites is also seen in many genes. This explains, at least in part, how 25,000 genes can give rise to hundreds of thousands of proteins.

2. **Messenger RNA editing:** Even after mRNA has been fully processed, it may undergo 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. 228) and very low density lipoproteins ([VLDL] see p. 231). Apo B mRNA is made in the liver and the small intestine. However, in the intestine only, the C residue in the CAA codon for glutamine is deaminated to U, changing the sense codon to a nonsense or stop codon (UAA), as shown in Figure 32.13. 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).

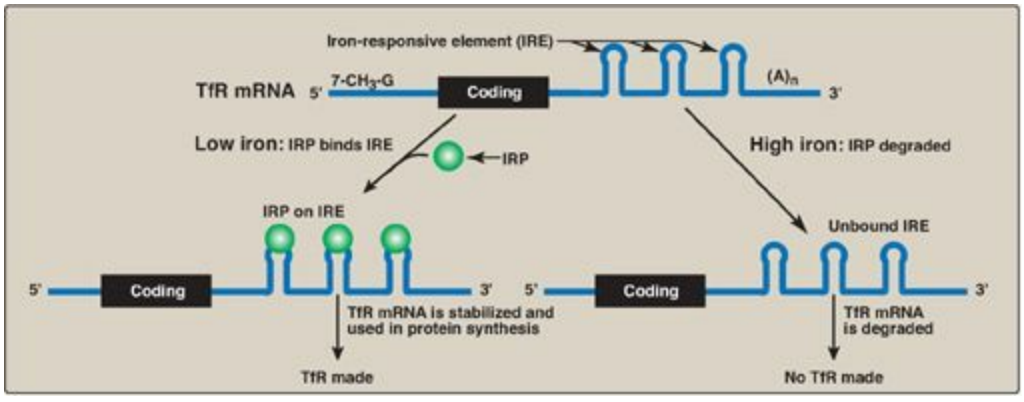
Figure 32.13 RNA editing of apolipoprotein (apo) B in the intestine and generation of the apo B-48 protein needed for chylomicron synthesis. Gln = glutamine; mRNA = messenger RNA.



3. **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 illustrate the importance of mRNA stability in the regulation of gene expression.

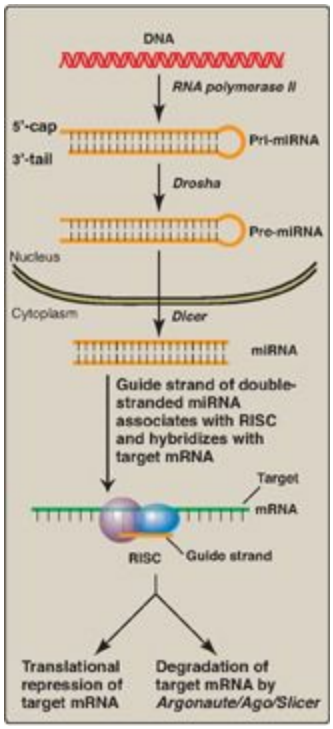
   a. **Iron metabolism:** Transferrin is a plasma protein that transports iron. Transferrin binds to cell-surface receptors (transferrin receptors [TfRs]) that get internalized and provide cells, such as erythroblasts, with iron. The mRNA for the TfR has several cis-acting iron-responsive elements (IREs) at its 3-end. IREs have a short stem–loop structure that can be bound by trans-acting iron regulatory proteins ([IRPs] Figure 32.14). When the iron concentration in the cell is low, the IRPs bind to the 3-IREs and stabilize the mRNA for TfR, allowing TfR synthesis. When intracellular iron levels are high, the IRPs are degraded. The lack of IRPs bound to the mRNA hastens its destruction, resulting in decreased TfR synthesis. [Note: The mRNA for apoferritin, an intracellular protein of iron storage, has a single IRE at its 5**I**-end. When iron levels in the cell are low, IRPs bind the 5**I**-IRE and prevent the use of the mRNA, and less apoferritin is made. When iron accumulates in the cell, the IRP is degraded, allowing synthesis of apoferritin molecules to store the excess iron. ALAS2, the regulated enzyme of heme synthesis (see p. 279) in erythroblasts, also contains a 5-IRE.]

Figure 32.14 Regulation of transferrin receptor (TfR) synthesis. IRP = iron regulatory protein. [Note: The IREs are located in the 3**I** UTR (untranslated region) of the TfR mRNA.]



b. RNA interference: RNA interference (RNAi) is a mechanism of gene silencing through decreased expression of mRNA, either by repression of translation or by increased degradation. It is thought to play a key role in such fundamental processes as cell proliferation, differentiation, and apoptosis. RNAi is mediated by short (~22 bp), noncoding RNAs called microRNAs (miRNAs), which 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, an 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 on 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 (Figure 32.15). RNAi can also be triggered by double-stranded short interfering RNAs (siRNAs) introduced into a cell from exogenous sources. [Note: In vertebrates, the function of siRNAs that may arise from endogenous sources is unclear.]

Figure 32.15 Biogenesis and actions of miRNA. [Note: The extent of complementarity between the target messenger RNA (mRNA) and the microRNA (miRNA) determines the final outcome.] RISC = RNA-induced silencing complex.

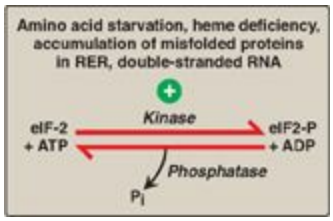


1) RNA-interference–based therapeutics: Modulation of gene expression by providing siRNA to trigger RNAi has enormous therapeutic potential. The first clinical trial of RNAi-based therapy involved patients with the neovascular form of age-related macular degeneration (AMD), a leading form of adult blindness. Neovascular

AMD is triggered by overproduction of vascular endothelial growth factor (VEGF), leading to the sprouting of excess blood vessels behind the retina. The vessels leak, clouding and often entirely destroying vision (therefore, neovascular AMD is also referred to as "wet" macular degeneration). An siRNA designed to target the mRNA of VEGF and promote its degradation went to clinical trials. Although considerable effort and resources have been expended to develop RNAi-based therapeutics, especially for the treatment of cancer, no products have gone from trials to the market. Development has been hindered by the problems of targeted delivery and stability. The use of nano-sized vectors such as liposomes may eliminate these issues. The research applications of RNAi, however, have grown rapidly.

4. **Translation of messenger RNA:** Regulation of gene expression can also occur at the level of translation. One mechanism by which translation is regulated is through phosphorylation of the eukaryotic translation initiation factor, eIF-2 (Figure 32.16). Phosphorylation of eIF-2 inhibits its function and so inhibits translation at the initiation step (see p. 443). [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.

Figure 32.16 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.



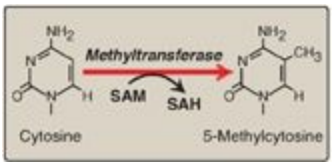
D. Regulation through modifications to DNA

Gene expression in eukaryotes is also influenced by the availability of DNA to the transcriptional apparatus, the amount of DNA, and the arrangement of DNA. [Note: Localized transitions between the B and Z forms of DNA (see p. 398) 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. 409). 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 acetylation or phosphorylation (see p. 422 for a discussion of histone

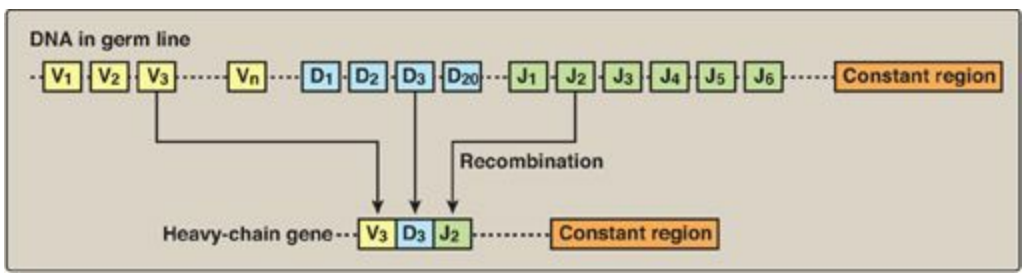
acetylation/deacetylation by the histone acetyltransferase and histone deacetylase enzymes). Such modifications decrease the positive charge of these basic proteins, thereby decreasing the strength of their association with negatively charged DNA. This relaxes the nucleosome (see p. 409), allowing transcription factors access to specific regions on the DNA. Nucleosomes can also be repositioned, an ATP-requiring process called chromatin remodeling. Another difference between transcriptionally active and inactive chromatin is the extent of methylation of cytosine 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 (Figure 32.17). Transcriptionally active genes are less methylated (hypomethylated) than their inactive counterparts, suggesting that DNA hypermethylation silences gene expression. [Note: Modification of histones and methylation of DNA are epigenetic. They are heritable changes in DNA that alter gene expression without altering the base sequence.]

Figure 32.17 The methylation of cytosine in eukaryotic DNA. SAM = S-adenosylmethionine; SAH = S-adenosylhomocysteine.



2. **Amount of DNA:** 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 seen with some diseases and in response to particular chemotherapeutic drugs such as methotrexate, an inhibitor of the enzyme dihydrofolate reductase (DHFR), required for the synthesis of thymidine triphosphate (TTP) in the pyrimidine biosynthetic pathway (see p. 304). TTP is essential for DNA synthesis. Gene amplification results in an increase in the number of DHFR genes and resistance to the drug, allowing TTP to be made.

Figure 32.18 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 (for example, 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 brought together through gene rearrangement to form a unique variable region (Figure 32.18). This process allows the generation of 10^9–10^{11} different immunoglobulins from a single gene, providing the diversity needed for the recognition of an enormous number of antigens. [Note: The shift from the membrane-bound form to the secreted form of immunoglobulins involves poly-A site choice (see p. 426).]

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, in which case the Tn is called a retrotransposon (see p. 408). Transposition has contributed to structural variation in the genome but also has the potential to alter gene expression and even to cause disease. Although the vast majority of retrotransposons in the human genome 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 Tns carrying antibiotic resistance genes, the recipient bacteria gain resistance to one or more antimicrobial drugs.]

V. CHAPTER SUMMARY

Gene expression results in the production of a functional gene product (either RNA or protein) through the processes of replication, transcription, and translation (Figure 32.19). **Genes** can be either **constitutive** (always expressed, housekeeping genes) or **regulated** (expressed only under certain conditions in all cells or in a subset of cells). The ability to appropriately **express** (**positive regulation**) or **repress** (**negative regulation**) genes is essential in all organisms. **Regulation** of gene expression occurs primarily at the level of **transcription** in both **prokaryotes** and **eukaryotes** and is mediated through the **binding** of **trans-acting proteins to cis-acting regulatory elements on the DNA**. In **eukaryotes**, regulation also occurs through **modifications to the DNA** as well as through **posttranscriptional** and **posttranslational events**. In **prokaryotes**, such as E. coli, the coordinate regulation of genes whose protein products are required for a particular process is achieved through **operons** (groups of genes sequentially arranged on the chromosome along with the regulatory elements that determine their transcription). The **lac operon** contains the Z, Y, and A structural genes, the protein products of which are needed for the catabolism of lactose. It is subject to negative and positive regulation. When **glucose is available**, the **operon is repressed** by the binding of the **repressor protein** (the product of the lacI gene) to the **operator**, thus preventing transcription. When **only lactose is present**, the **operon is induced** by an isomer of lactose (**allolactose**) that binds the repressor protein, preventing it from binding to the operator. In addition, **cyclic AMP binds the catabolite-activator protein (CAP)**, and the complex **binds the DNA** at the **CAP site**. This **increases promoter efficiency** and results in the expression of the structural genes through the production of a **polycistronic messenger RNA (mRNA)**. When both **glucose and lactose are present**, glucose prevents formation of cAMP and **transcription** of these genes is **negligible**. The **trp operon** contains genes needed for the synthesis of tryptophan (Trp), and, like the lac operon, it is regulated by **negative control**. Unlike the lac operon, it is **also regulated by attenuation**, in which mRNA synthesis that escaped repression by Trp is terminated before completion. **Transcription** of **ribosomal RNA** and **transfer RNA** is selectively **inhibited** in prokaryotes by the **stringent response to amino acid starvation**. **Translation** is also a site of **prokaryotic gene regulation**: Excess ribosomal proteins bind the Shine-Dalgarno sequence on their own polycistronic mRNA, preventing ribosomes from binding. **Gene regulation** is **more complex in eukaryotes**. **Operons** typically are **not present**, but **coordinate regulation** of the transcription of genes located on different chromosomes can be achieved through the **binding of trans-acting proteins to cis-acting elements**. In **multicellular organisms**, **hormones** can **cause coordinated regulation**, either through the **binding** of the **hormone receptor–hormone complex to the DNA** (as with steroid hormones) or through the **binding of a protein** that is activated in **response to a second messenger** (as with glucagon). In each case, binding to

DNA is mediated through structural motifs such as the **zinc finger. Co- and posttranscriptional regulation** is also seen in **eukaryotes** and includes **splice-site choice**, **polyA-site choice**, **mRNA editing**, and variations in **mRNA stability** as seen with transferrin receptor synthesis and with **RNA interference**. **Regulation at the translational level** can be caused by the **phosphorylation and inhibition of eukaryotic initiation factor, eIF-2**. Gene expression in eukaryotes is also influenced by availability of DNA to the transcriptional apparatus, the amount of DNA, and the arrangement of the DNA. **Epigenetic changes** to histone proteins and DNA also influence gene expression.

Study Questions

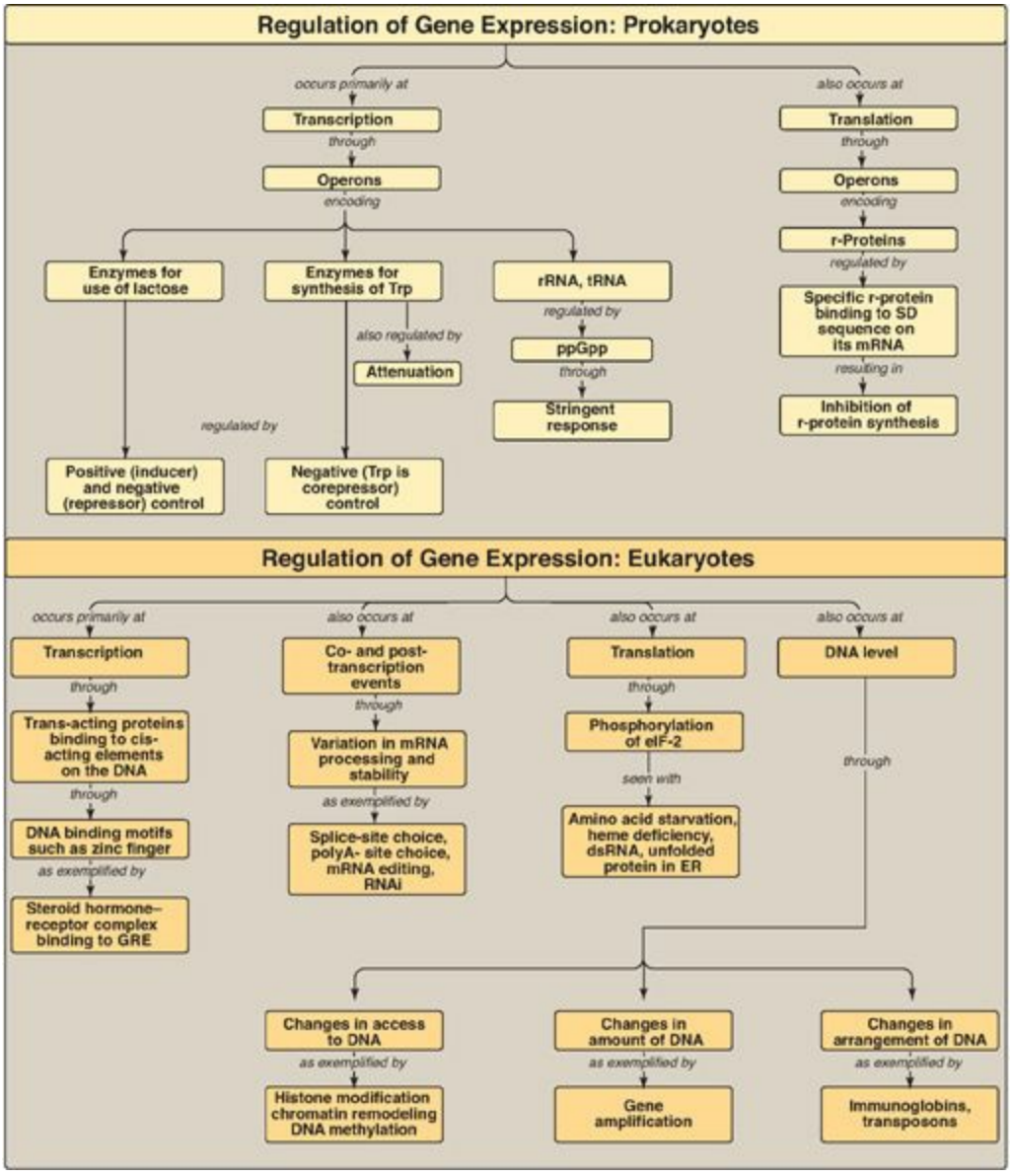
Choose the one best answer.

32.1 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 functionally impaired glucose transport

> Correct answer = A. In the absence of glucose, adenylyl cyclase makes cyclic AMP, 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 turned on 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 (constant) expression of the lac operon.

Figure 32.19 Summary of key concepts for the regulation of gene expression. GRE = glucocorticoid-response element; ppGpp = polyphosphorylated guanosine; r-protein = ribosomal protein; SD sequence = Shine-Dalgarno sequence; RNAi = RNA interference; eIF-2 = eukaryotic initiation factor 2.



32.2 Which of the following is best described as cis acting?

A. Cyclic AMP response element-binding protein
B. Operator
C. Repressor protein
D. Thyroid hormone nuclear receptor

Correct answer = B. The operator is part of the DNA itself, and so is cis acting. The cAMP response element-binding protein, repressor protein, and thyroid hormone nuclear receptor protein are molecules that transit to the DNA, bind, and affect the expression of that DNA and so are trans acting.

32.3 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 apoB-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 alternate splicing, do alter gene expression but are not the basis of apoB-48 tissue-specific production.

32.4 Which of the following is most likely to be true in hemochromatosis, a disease of iron accumulation?

A. The mRNA for the transferrin receptor (TfR) is stabilized by the binding of iron regulatory proteins to 3 iron-responsive elements.
B. The mRNA for the transferrin receptor is not bound by iron regulatory proteins and is degraded.
C. The mRNA for apoferritin is not bound by iron regulatory proteins at its 5 iron-responsive element and is translated.
D. The mRNA for apoferritin 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, apoferritin, and decreased synthesis of the transferrin receptor (TfR) that mediates iron uptake by cells. These effects are the result of trans-acting iron regulatory proteins binding cis-acting iron-responsive elements, resulting in degradation of the mRNA for TfR, and increased translation of the mRNA for apoferritin.

32.5 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 AMP
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 AMP is a regulatory signal mediated by cell-surface rather than nuclear receptors. Mammalian cells do not have operons.

32.6 The ZYA region of the lac operon will be maximally expressed if:

A. cyclic AMP levels are low.
B. glucose and lactose are both available.
C. the attenuation stem–loop is able to form.
D. the CAP site is occupied.

Correct answer = D. It is only when glucose is gone, cyclic AMP 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 decreasing transcription in some operons such as the tryptophan operon.

32.7 X chromosome inactivation is a process by which one of two X chromosomes in human 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?

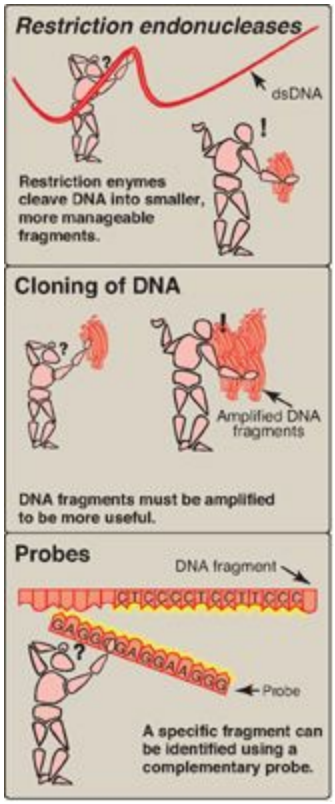
Cytosines in CG islands would be hypermethylated and histone proteins would be deacetylated. Both conditions are associated with decreased gene expression, and both are important in maintaining X inactivation.

Biotechnology and Human Disease 33

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 approximately three billion (10^9) base pairs (bp) 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 techniques that have already contributed to our understanding of many genetic diseases (Figure 33.1). These include, first, the discovery of restriction endonucleases that permit the cleavage of huge DNA molecules into defined fragments. Second, the development of cloning techniques that provide a mechanism for amplification of specific nucleotide sequences. Finally, 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 33.1 Three techniques that facilitate analysis of human DNA. dsDNA = double-stranded DNA.



II. RESTRICTION ENDONUCLEASES

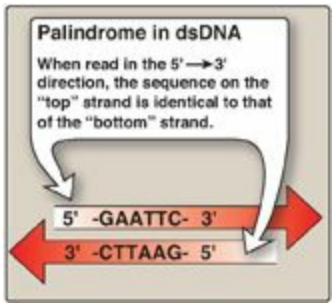
One of the major obstacles to molecular analysis of genomic DNA is the immense size of the molecules involved. The discovery of a special group of bacterial enzymes, called restriction endonucleases (restriction enzymes), which cleave double-stranded (ds) DNA into smaller, more manageable fragments, opened the way for DNA analysis. Because each enzyme cleaves DNA at a specific nucleotide sequence (restriction site), restriction enzymes are used experimentally to obtain precisely defined DNA segments called restriction fragments.

A. Specificity of restriction endonucleases

Restriction endonucleases recognize short stretches of dsDNA (four to eight bp) that contain specific nucleotide sequences. These sequences, which differ for each restriction enzyme, are palindromes, that is, they exhibit twofold rotational symmetry (Figure 33.2). This means that, within a short region of the double helix, the nucleotide sequence on the two strands is identical if each is read in the 5→3 direction. Therefore, if you turn the page upside down (that is, rotate it 180° around its axis of symmetry) the sequence remains the same.

> In bacteria, restriction endonucleases "restrict" the expression of nonbacterial (foreign) DNA through cleavage. Bacterial DNA is protected by methylation of bases at the restriction site.

Figure 33.2 Recognition sequence of restriction endonuclease EcoRI shows twofold rotational symmetry. dsDNA = double-stranded DNA; A = adenine; C = cytosine; G = guanine; T = thymine.



B. Nomenclature

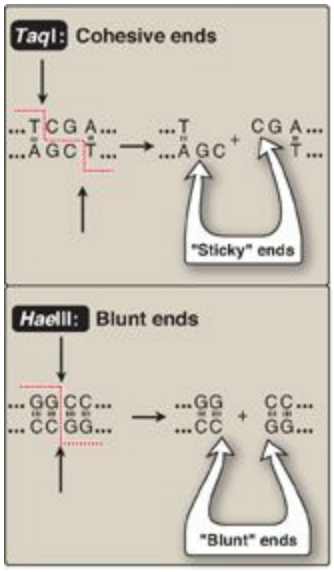
A restriction enzyme is named according to the organism from which it was isolated. The first letter of the name is from the genus of the bacterium. The next two letters are from the name of the species. An additional letter indicates the type or strain, and a number (Roman numeral) is appended to indicate 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**I**-hydroxyl group on one end and a 5**I**-phosphate group on the other. Some restriction endonucleases, such as TaqI, form staggered cuts that produce "sticky" or cohesive ends (that is, the resulting DNA fragments have single-stranded sequences that are complementary to each other) as shown in Figure 33.3. Other restriction endonucleases, such as HaeIII, produce fragments that have "blunt" ends that are double stranded and, therefore, do not form hydrogen bonds with each other. Using the enzyme DNA ligase (see p. 406), 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 (Figure 33.4). [Note: A ligase encoded by bacteriophage T4 can covalently join blunt-ended fragments.]

Figure 33.3 Specificity of TaqI and HaeIII restriction endonucleases; A = adenine; C = cytosine; G = guanine; T = thymine.



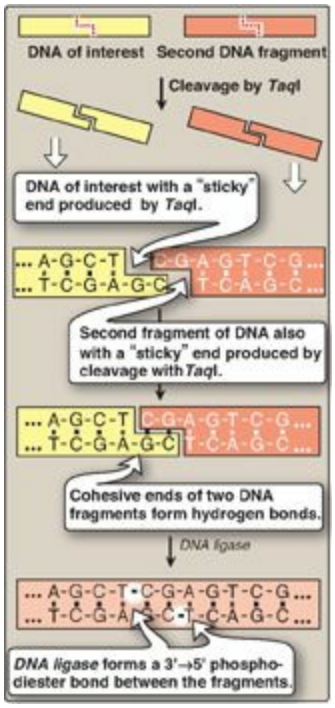
D. Restriction sites

A DNA sequence that is recognized and cut by a restriction enzyme is called a restriction site. Restriction endonucleases cleave dsDNA into fragments of different sizes depending upon the size of the sequence recognized. For example, an enzyme that recognizes a specific 4-bp sequence produces many cuts in the DNA molecule, one every 4^4 bp. In contrast, an enzyme requiring a unique sequence of 6 bp produces fewer cuts (one every 4^6 bp) and, therefore, longer pieces. 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 (that is, the production of many identical copies) of that DNA. [Note: Human DNA for cloning can be obtained from blood, saliva, and solid tissue.] 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 carries its inserted DNA fragment into a single host cell (for example, 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 forms a clone in which every bacterium contains copies of the same inserted DNA fragment, hence the name "cloning." The cloned DNA can be released from its vector by cleavage (using the appropriate restriction endonuclease) and isolated. By this mechanism, many identical copies of the DNA of interest can be produced. [Note: An alternative to amplification by biologic cloning, the polymerase chain reaction (PCR), is described on p. 479.]

Figure 33.4 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: 1) it must be capable of autonomous replication within a host cell, 2) it must contain at least one specific nucleotide

sequence recognized by a restriction endonuclease, and 3) it must carry at least one gene that confers the ability to select for the vector such as an antibiotic resistance gene. Commonly used vectors include plasmids and viruses.

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 (Figure 33.5). 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, their circular DNA cleaved at specific sites by restriction endonucleases, and up to 10 kb (kilobases) of foreign DNA (cut with the same restriction enzyme) inserted. The recombinant plasmid can be introduced into a bacterium, producing large numbers of copies of the plasmid. The bacteria are grown in the presence of antibiotics, thus selecting for cells containing the hybrid plasmids, which provide antibiotic resistance (Figure 33.6). Artificial plasmids are routinely constructed. An example is pRB322 (Figure 33.5), which contains an origin of replication, two antibiotic resistance genes, and over 40 unique restriction sites. Use of plasmids is limited by the size of the DNA that can be inserted.

Figure 33.5 A restriction map of plasmid pBR322 indicating the positions of its antibiotic resistance genes and 6 of the over 40 unique sites recognized by specific restriction endonucleases.

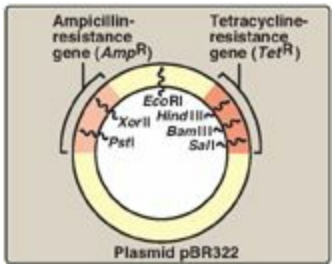
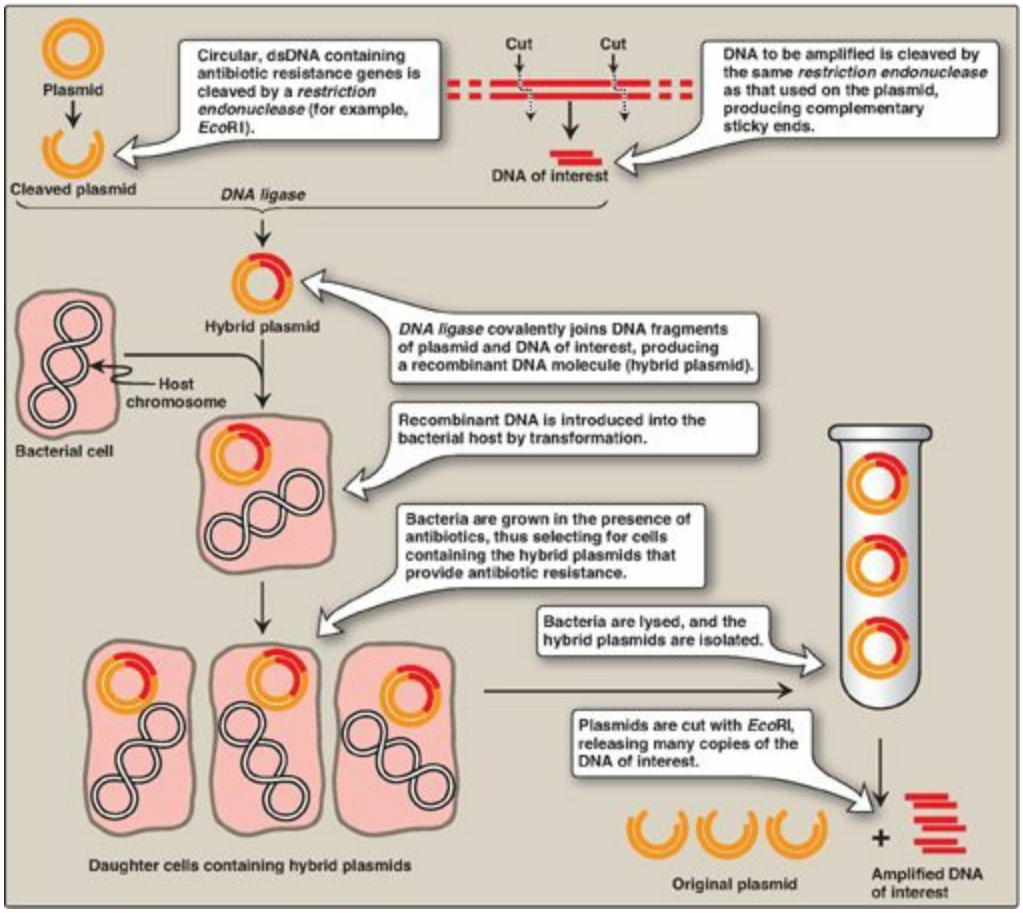


Figure 33.6 Summary of gene cloning. dsDNA = double-stranded DNA.



2. **Other vectors:** The development of improved vectors that can more efficiently accommodate larger DNA segments, or express the passenger genes in different cell types, has aided molecular genetics research. In addition to the prokaryotic plasmids described above, naturally occurring viruses that infect bacteria (bacteriophage l, for example) or mammalian cells (retroviruses, for example), as well as artificial constructs such as cosmids and bacterial or yeast artificial chromosomes (BACs or YACs, respectively), are currently used as cloning vectors. [Note: BACs and YACs can accept DNA inserts of 100–250 kb and 250–1000 kb, respectively.]

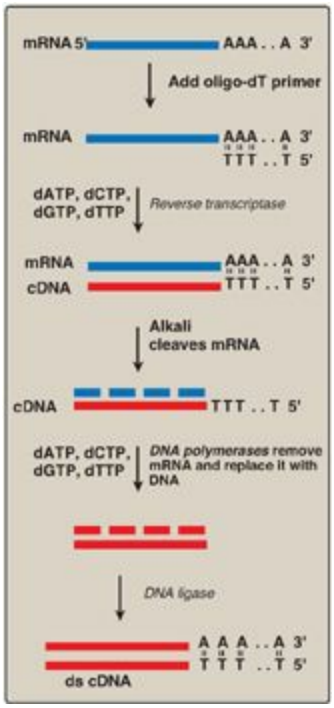
B. DNA libraries

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 from one cell type to another. [Note: cDNA lacks introns and the control regions of the genes, whereas these are present in genomic DNA.]

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 to an appropriate vector. The recombinant DNA molecules replicate within 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 rather 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 (that is, 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 about 20 kb. Enzymes that cut very frequently (that is, those that recognize four-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.

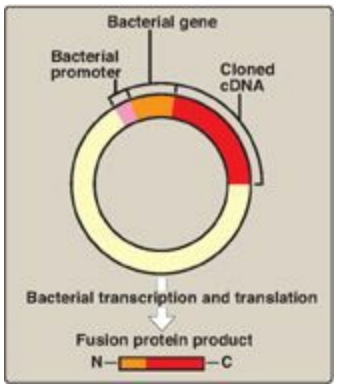
Figure 33.7 Synthesis of cDNA from messenger RNA (mRNA) using reverse transcriptase. Additional steps (not shown) are required to clone the cDNA. [Note: Recall that DNA is resistant to alkaline hydrolysis.]



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 encoding the α-globin and b-globin chains of hemoglobin. This mRNA can be used as a template to make a cDNA molecule using the enzyme reverse transcriptase (Figure 33.7). The resulting cDNA is, therefore, 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 cloning or by PCR. It can be used as a probe to locate the gene that coded for 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 size species, the resulting cDNA is heterogeneous. These

mixtures can be cloned to form a cDNA library. Because cDNA has no intervening sequences, it can be cloned into an expression vector for the synthesis of eukaryotic proteins by bacteria (Figure 33.8). These special plasmids contain a bacterial promoter for transcription of the cDNA and a Shine-Dalgarno (SD) sequence (see p. 438) 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 (for example, lacZ; see p.450), such that the mRNA produced contains an SD sequence, a few codons for the bacterial protein, and all 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 (see p. 443) required for most other human proteins (for example, blood clotting factors) necessitates the use of eukaryotic, even mammalian, hosts.]

Figure 33.8 An expression vector. The complementary DNA (cDNA) is inserted within a bacterial gene, downstream of the promoter sequence, and the sequences for the messenger RNA Shine-Dalgarno sequence, start codon, and codons for the first few amino acids of the bacterial protein. The product is a fusion protein that contains just some amino acids of the bacterial protein ■ and all the amino acids of the cDNA-encoded protein ■.



C. Sequencing of 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 dideoxy method illustrated in Figure 33.9. In this method, the single-stranded DNA (ssDNA) to be sequenced is used as the template for DNA synthesis by DNA polymerase. A radiolabeled primer complementary to the 3′-end of the target DNA is added, 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 using polyacrylamide gel

electrophoresis, followed by autoradiography, yields a pattern of bands from which the DNA base sequence can be read. [Note: The shorter the fragment, the farther it travels on the gel, with the shortest fragment representing that which was made first (that is, the 5**I**-end).] In place of a labeled primer, a mixture of the four ddNTPs linked to different fluorescent dyes and in a single reaction tube is now commonly used. [Note: The Human Genome Project used highly automated variations of this technique to determine the base sequence of the human genome.] Advances in sequencing technology, so-called next generation, or deep sequencing, now allow the sequencing of longer segments in a shorter time with increased fidelity and decreased cost through the simultaneous (parallel) sequencing of many DNA pieces.

IV. PROBES

Cleavage of large DNA molecules by restriction enzymes produces a bewildering array of fragments. How can the DNA sequence of interest be picked out of a mixture of thousands or even millions of irrelevant DNA fragments? 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 to identify which band on a gel or which clone in a library contains the target DNA, a process called screening.

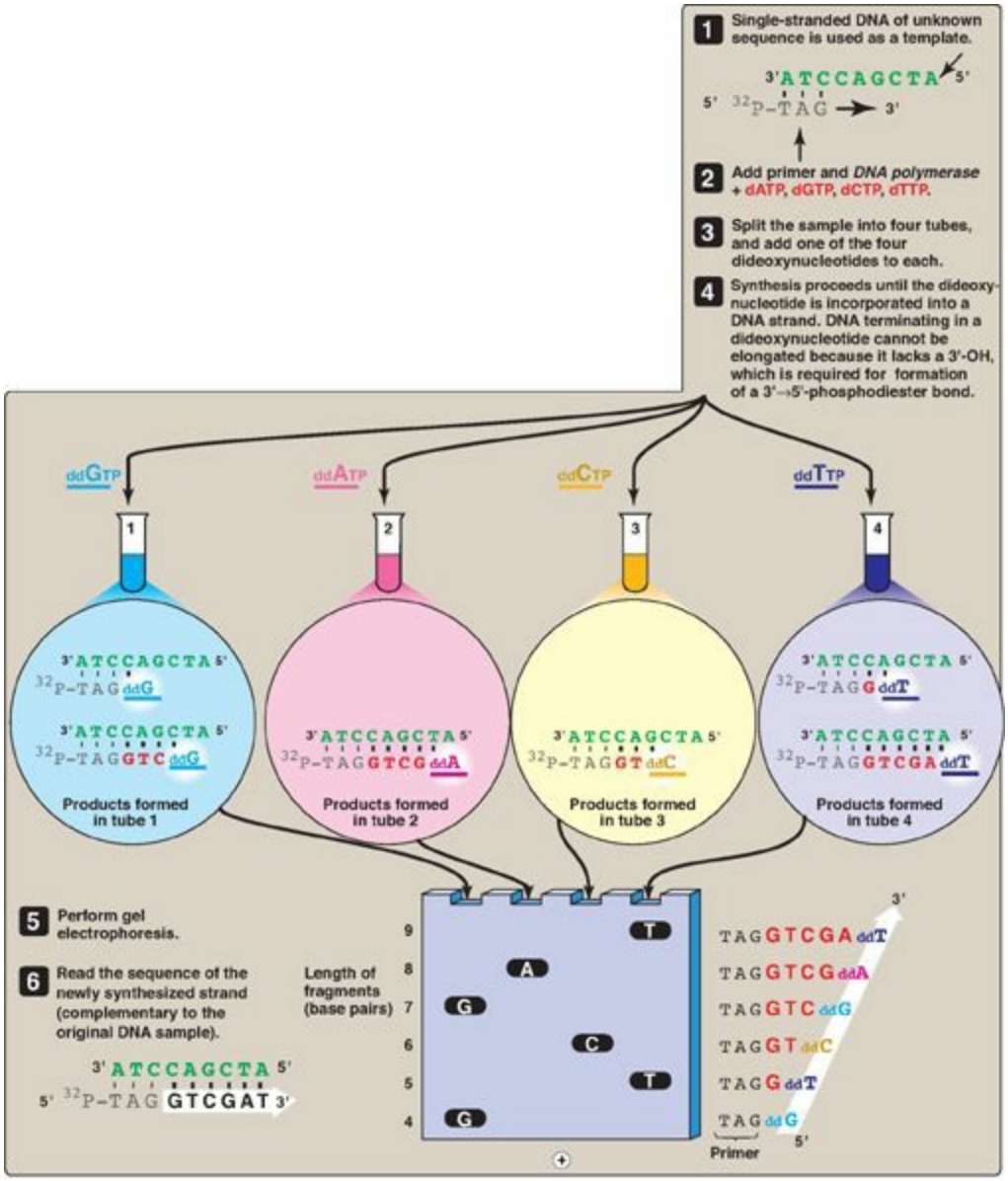
A. Hybridization of a probe to DNA fragments

The utility of probes hinges on the phenomenon 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, ssDNA 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, it is necessary to synthesize several oligonucleotides. [Note: Oligonucleotides can be used to detect single-base changes in the sequence to which they are complementary. In contrast, cDNA probes contain many thousands of bases, and their binding to a target DNA with a single-base change is unaffected.]

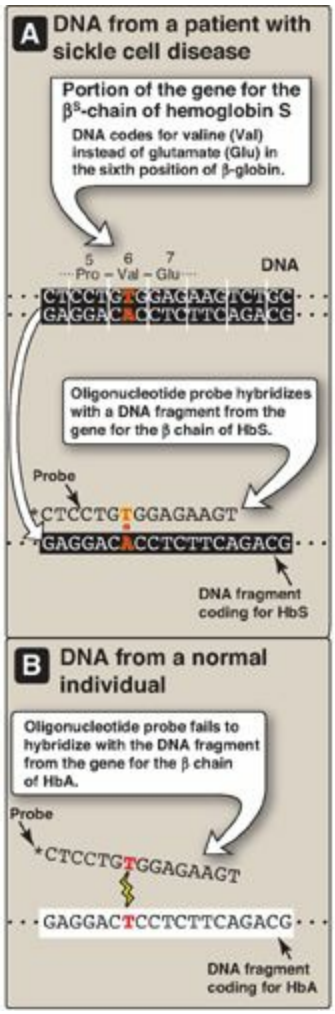
Figure 33.9 DNA sequencing by the Sanger dideoxy method. [Note: The original method utilized a radiolabeled primer. Fluorescent dye-labeled ddNTPs are now commonly used.] A = adenine; C = cytosine; G = guanine; T = thymine; d = deoxy; dd = dideoxy.



1. **Detecting the β^S-globin mutation:** A synthetic allele-specific oligonucleotide (ASO) probe can be used to detect the presence of the sickle cell mutation in the β-globin gene (Figure 33.10). DNA, isolated from leukocytes and amplified, is denatured and applied to a membrane. A radiolabeled oligonucleotide probe, complementary to the point mutation (GAG → GTG, glutamate → valine) at codon 6 in patients with the b^S gene, is applied to the membrane. DNA isolated from a heterozygous individual (sickle cell trait) or a homozygous patient (sickle cell disease) contains a sequence that is complementary to the probe, and a double-stranded hybrid forms that can be detected by electrophoresis. In contrast, DNA obtained from normal individuals is not complementary at this positon and, therefore, does not form a hybrid (see Figure 33.10). 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 (Figure 33.11). [Note: ASO probes are useful only if the mutation and its location are known.]

Figure 33.10 Allele-specific oligonucleotide probe detects hemoglobin (Hb) S allele.

[Note: * indicates ^{32}P radiolabel.] A = adenine; C = cytosine; G = guanine; T = thymine; Pro = proline.



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. 381), 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 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 binds 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, 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 is used to identify which bacterial colony produces the protein and, therefore, contains the cDNA of interest.

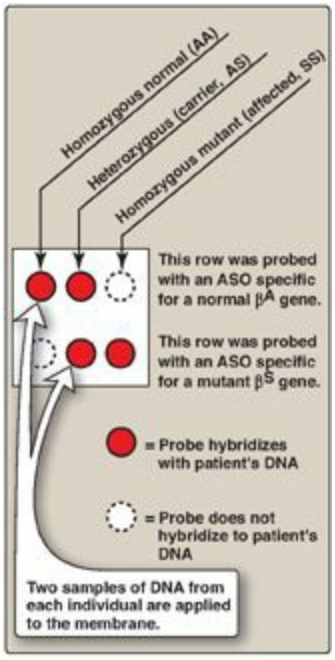
V. SOUTHERN BLOTTING

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. Experimental procedure

This method, named after its inventor, Edward Southern, involves the following steps (Figure 33.12). First, DNA is extracted from cells, for example, a patient's leukocytes. Second, the DNA is cleaved into many fragments using a restriction enzyme. Third, the resulting fragments 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 base pairs, can be calculated from comparison of the position of the band relative to standard fragments of known size.] The DNA fragments in the gel are denatured and transferred (blotted) to a nitrocellulose membrane for analysis. If the original DNA represents the individual's entire genome, the enzymic digest contains a million or more 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 segments 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" (electrophoresis of mRNA followed by hybridization with a specific probe), and "Western" (electrophoresis of protein followed by detection with an antibody directed against the protein of interest), neither of which relates to anyone's name or to points of the compass.]

Figure 33.11 Allele-specific oligonucleotide (ASO) probes used to detect the sickle cell mutation and differentiate between sickle cell trait and disease.



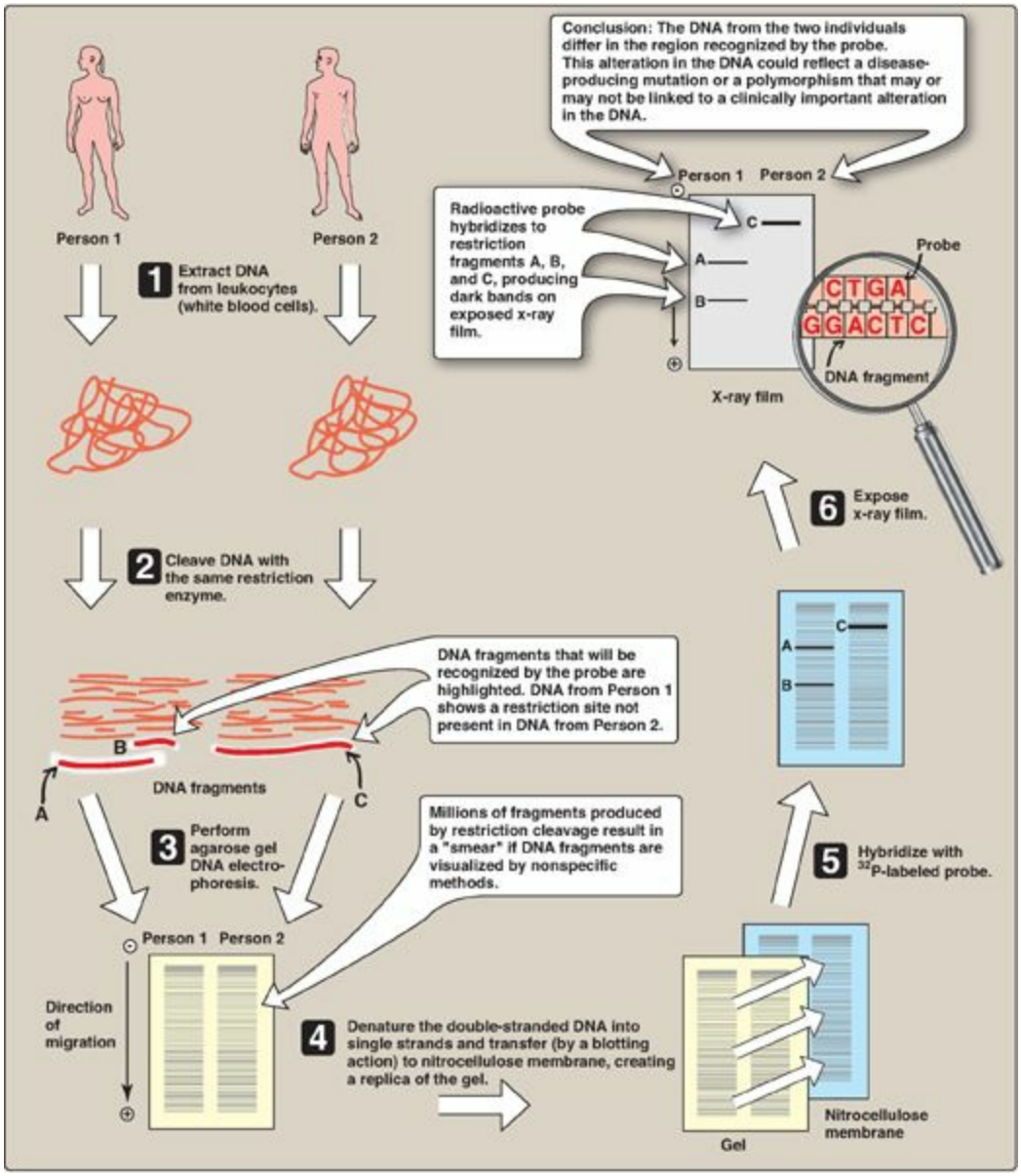
B. Detection of mutations

Southern blotting can detect DNA mutations such as large insertions, deletions, or rearrangements of nucleotides. It can also detect point mutations (replacement of one nucleotide by another; see p. 433) 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 33.12, 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.]

VI. RESTRICTION FRAGMENT LENGTH POLYMORPHISM

It has been estimated that the genomes of any two unrelated people are 99.5% identical. With 6 billion bp in the diploid human genome, that represents variation in about 30 million bp. These genome variations are the result of mutations that lead to polymorphisms. A polymorphism is a change in genotype that can result in no change in phenotype or a change in phenotype that is harmless; causes increased susceptibility to a disease; or, rarely, causes the disease. It is traditionally defined as a sequence variation at a given locus (allele) in more than 1% of a population. Polymorphisms primarily occur in the 98% of the genome that does not encode proteins (that is, 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 enzyme. The length of the restriction fragments is altered if the variant alters the DNA so as to create or abolish a site of restriction endonuclease cleavage (a restriction site). RFLP can be used to detect human genetic variations, for example, in prospective parents or in fetal tissue.

Figure 33.12 Southern blotting procedure. [Note: Nonradiolabeled probes are now commonly used.]



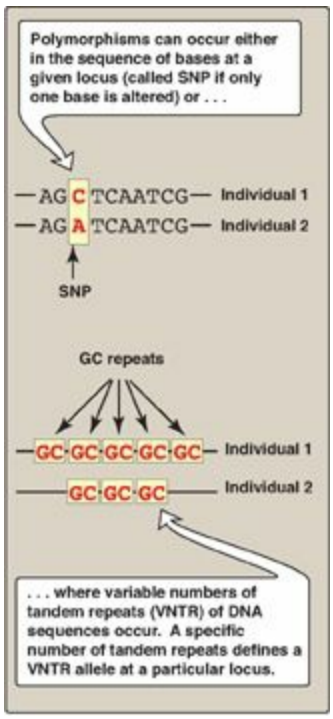
A. DNA variations resulting in restriction fragment length polymorphism

Two types of DNA variation commonly result in RFLP: single-base changes in the DNA sequence and tandem repeats of DNA sequences.

1. **Single-base changes in DNA:** 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 (Figure 33.13). 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 differing from the normal that can be detected by DNA hybridization (see Figure 33.12). 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 SNPs in the human genome. The data are being used in genome-wide association studies (GWAS) to identify those alleles that affect health and disease.]

2. **Tandem repeats:** Polymorphism in chromosomal DNA can also arise from the presence of a variable number of tandem repeats [VNTR] see Figure 33.14). 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 (Figure 33.14). 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.

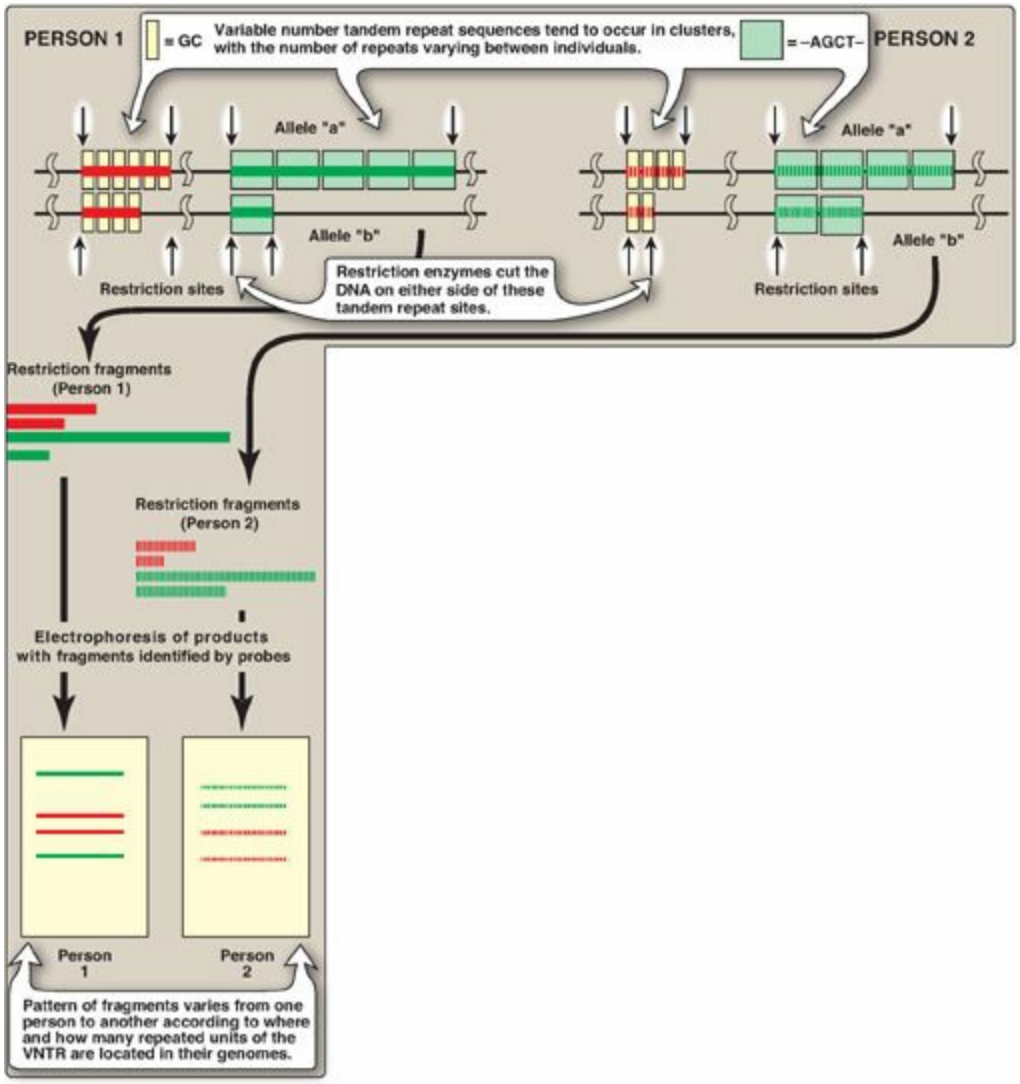
Figure 33.13 Common forms of genetic polymorphism. SNP = single-nucleotide polymorphism. A = adenine; C = cytosine; G = guanine; T = thymine.



B. Tracing chromosomes from parent to offspring

If the DNA of an individual has gained a restriction site by base substitution, then enzymic cleavage yields at least one additional fragment. Conversely, if a mutation results in loss of a restriction site, fewer fragments are produced by enzymic 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. In such individuals, each chromosome can be traced from parent to offspring by determining the presence or absence of the polymorphism.

Figure 33.14 Restriction fragment length polymorphism of variable number tandem repeats (VNTR). For each person, a pair of homologous chromosomes is shown.



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. Prenatal diagnosis, in association with genetic counseling, allows for an informed reproductive decision if the fetus is affected.

1. **Methods available:** The available 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 (for example, neural tube defects). 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 neural tube defects. 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.

2. **Sources of DNA:** DNA may be obtained from white blood cells, amniotic fluid, or chorionic villi (Figure 33.15). For amniotic fluid, it used to be necessary to grow cells

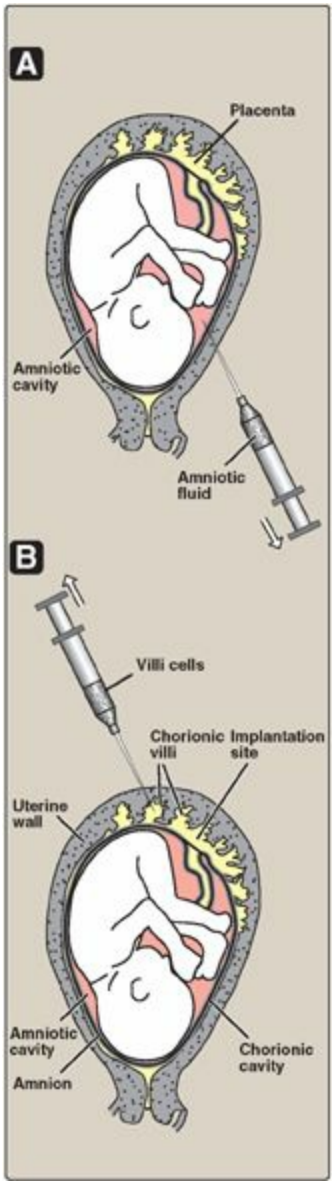
in culture for two to three weeks in order to have sufficient DNA for analysis. The ability to amplify DNA by PCR (see p. 479) has dramatically shortened the time needed for a DNA analysis.

3. **Direct diagnosis of sickle cell anemia using RFLP:** The genetic disorders of hemoglobin (Hb) are the most common genetic diseases in humans. In the case of sickle cell anemia (Figure 33.16), the mutation that gives rise to the disease is actually one and the same mutation that gives rise to the polymorphism. Direct detection by RFLP of diseases that result from point mutations is, however, limited to only a few genetic diseases.

   a. **Early efforts to diagnose sickle cell anemia:** In the past, prenatal diagnosis of hemoglobinopathies involved the determination of the amount and kinds of Hb synthesized in red cells obtained from fetal blood. However, the invasive procedures to obtain fetal blood have a high mortality rate (approximately 5%), and diagnosis cannot be carried out until late in the second trimester of pregnancy when HbS begins to be produced.

   b. **RFLP analysis:** Sickle cell anemia is an example of a genetic disease caused by a point mutation (see p. 35). The sequence altered by the mutation abolishes the recognition site of the restriction endonuclease MstII: CCTNAGG (where N is any nucleotide; see Figure 33.16). Thus, the A-to-T mutation in codon 6 of the b^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 b^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.]

Figure 33.15 Sampling of fetal cells. A. Amniotic fluid. B. Chorionic villus.

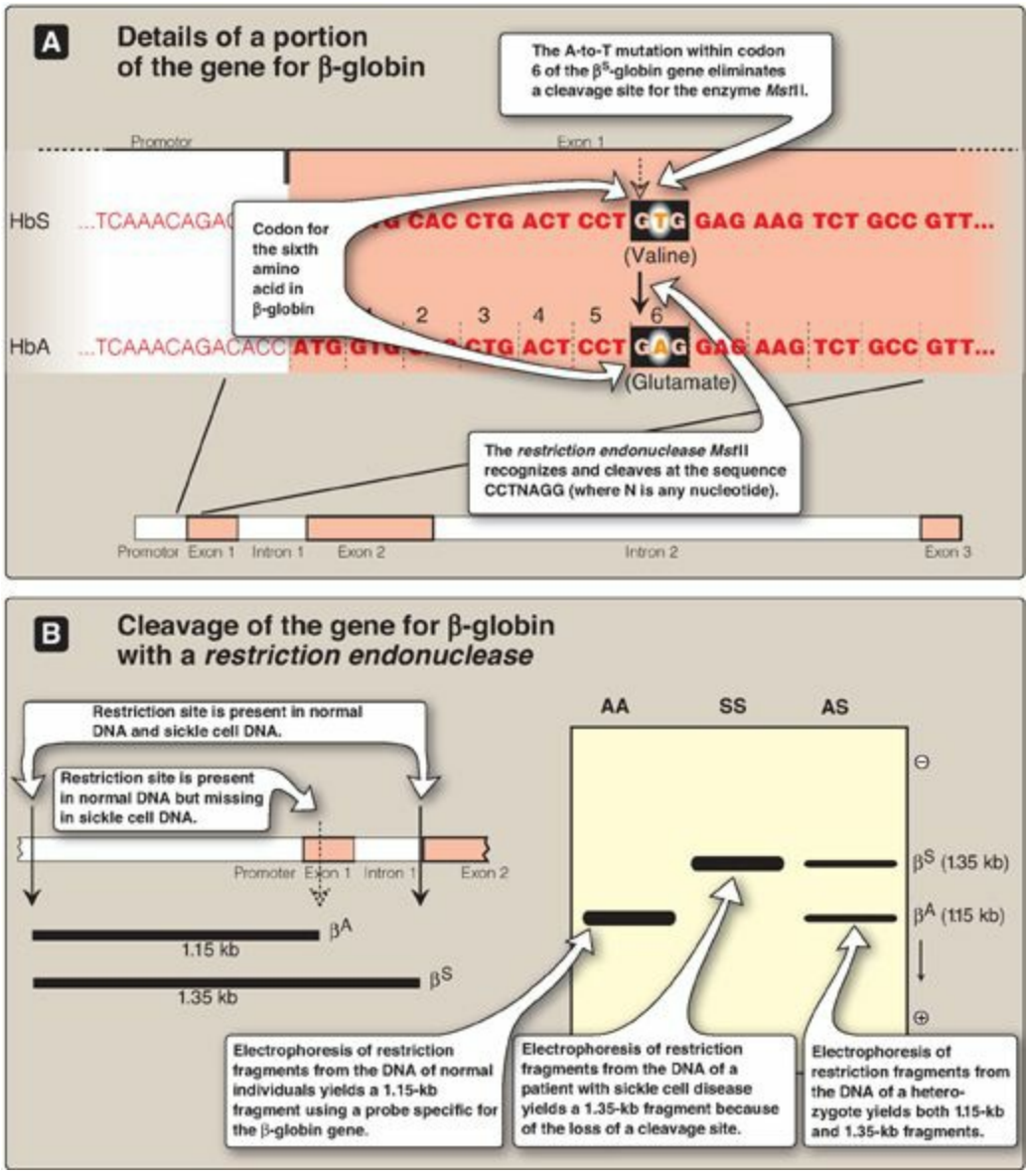


4. **Indirect, prenatal diagnosis of phenylketonuria using RFLP:** The gene for phenylalanine hydroxylase (PAH), deficient in phenylketonuria ([PKU] see p. 270), is located on chromosome 12. It spans about 90 kb of genomic DNA and contains 13 exons separated by introns (Figure 33.17; see p. 426 for a description of exons and introns). Mutations in PAH usually do not directly affect any restriction endonuclease recognition site. To establish a diagnostic protocol for this disease, DNA of family members of the affected individual must be analyzed. The goal is to identify genetic markers (RFLPs) that are tightly linked to the disease trait. Once these markers are identified, RFLP analysis can be used to carry out prenatal diagnosis.

    a. **Identification of the gene:** Determinining 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 (that is, 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 33.16 Detection of β^S-globin mutation. kb = kilobase (1 kb = 1000 base pairs in double-stranded DNA); Hb = hemoglobin.

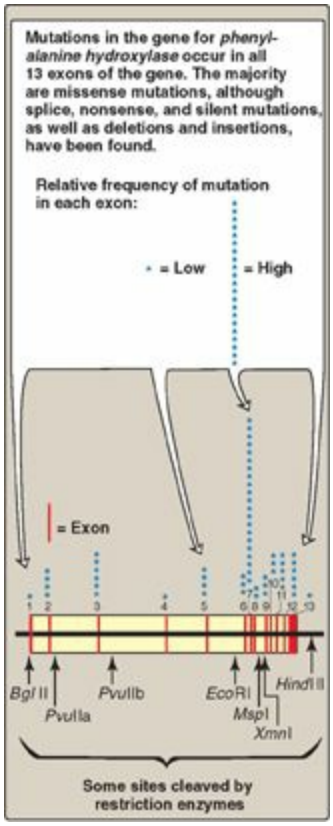


b. RFLP analysis: The presence of abnormal genes for PAH can be shown using DNA polymorphisms as markers to distinguish between normal and mutant genes. For example, Figure 33.18 shows a typical pattern obtained when DNA from the white blood cells of a family is cleaved with an appropriate restriction 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 33.18, examination of fetal DNA shows that the fetus inherited two abnormal genes from its parents and, therefore, has PKU.

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 33.17 The gene for phenylalanine hydroxylase showing 13 exons, restriction sites, and some of the mutations resulting in phenylketonuria.



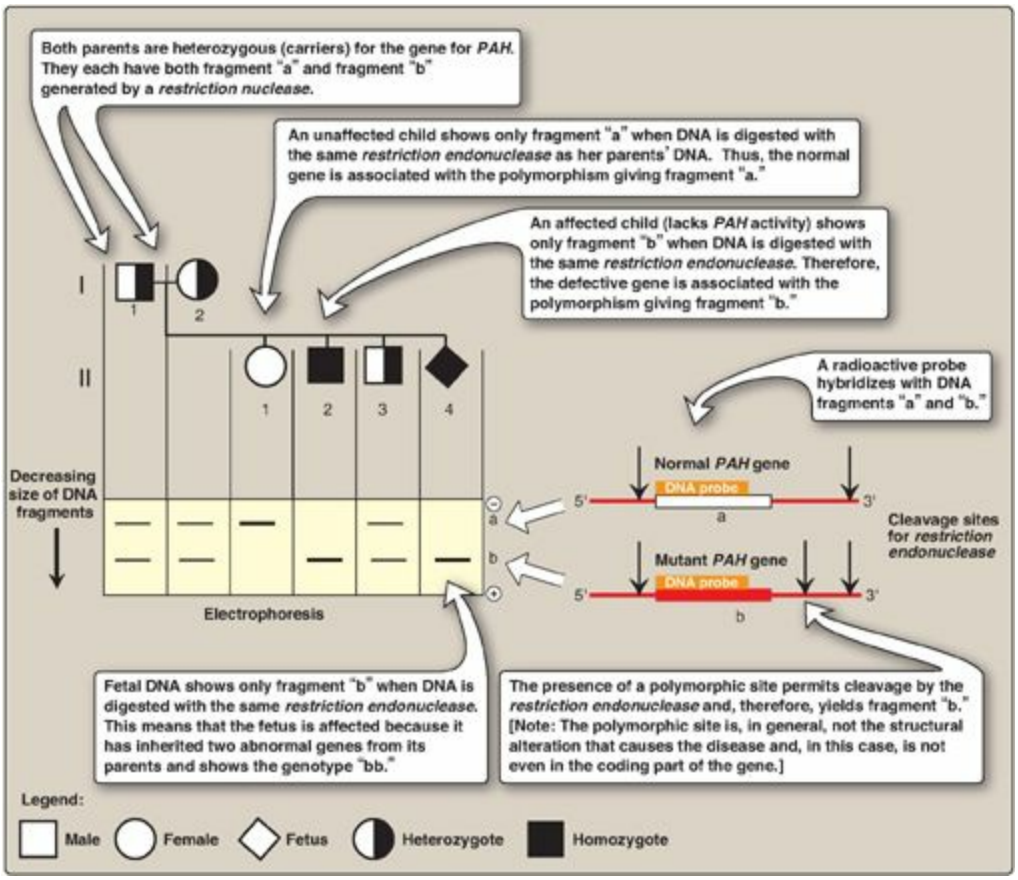
VII. POLYMERASE CHAIN REACTION

PCR is a test tube method for amplifying a selected DNA sequence that does not rely on the biologic cloning method described on p. 467. 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 33.19 and 33.20.

A. Steps of a polymerase chain reaction

PCR uses DNA polymerase 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.

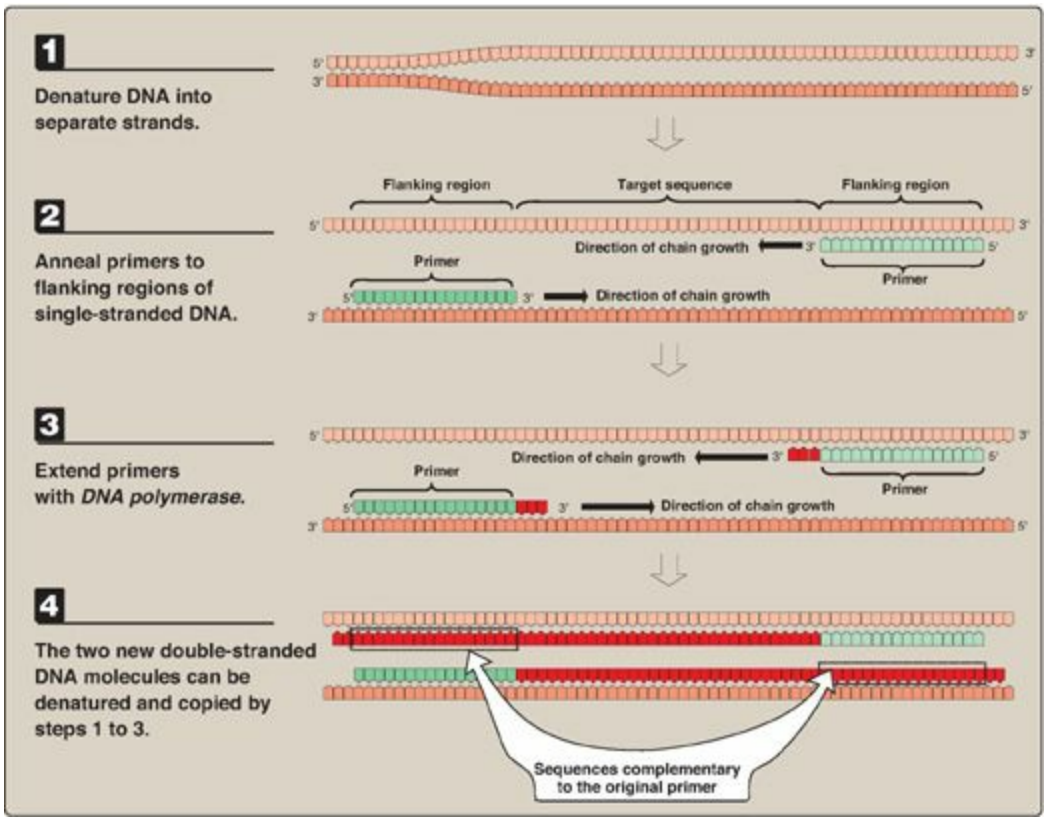
Figure 33.18 Analysis of restriction fragment length polymorphism in a family with a child affected by phenylketonuria (PKU), an autosomal recessive disease. The molecular defect in the gene for phenylalanine hydroxylase (PAH) in the family is not known. The family wanted to know if the current pregnancy would be affected by PKU.



1. **Primer construction:** 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 20–35 nucleotides long, which are complementary to the respective flanking sequences. The 3**I**-hydroxyl end of each oligonucleotide points toward the target sequence (see Figure 33.19). These synthetic oligonucleotides function as primers in PCR reactions.

Figure 33.19 Steps in one cycle of the polymerase chain reaction.



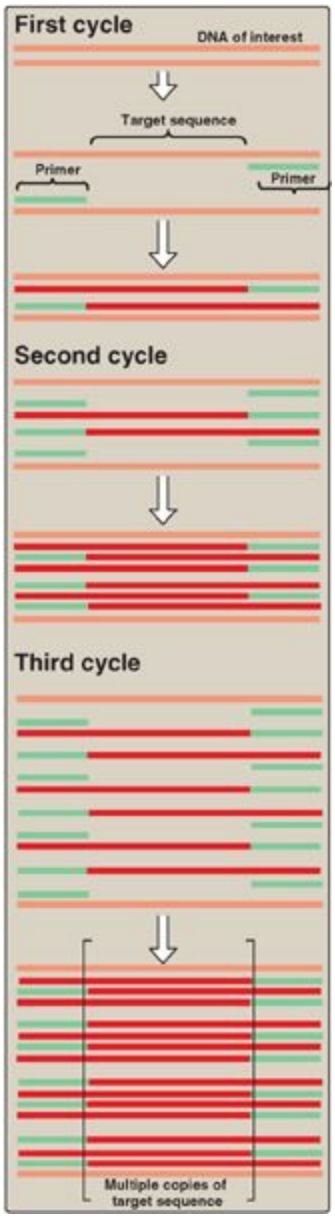
2. **Denature the DNA:** The DNA to be amplified is heated to separate the double-stranded target DNA into single strands.

3. **Annealing of primers to single-stranded DNA:** The separated strands are cooled and allowed to anneal to the two primers (one for each strand).

4. **Chain extension:** DNA polymerase and deoxyribonucleoside triphosphates (in excess) are added to the mixture to initiate the synthesis of two new strands complementary to the original DNA strands. DNA polymerase adds nucleotides to the 3**I**-hydroxyl end of the primer, and strand growth extends across the target DNA, making complementary copies of the target. [Note: PCR products can be several thousand bp 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 binds a complementary primer, and the cycle of chain extension is repeated. By using a heat-stable DNA polymerase (for example, Taq polymerase 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. Typically 20–30 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**I**-end that is complementary to the primer (see Figure 33.19). Thus, each newly synthesized strand can act as a template for the successive cycles (see Figure 33.20). 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 last few cycles.

Figure 33.20 Multiple cycles of polymerase chain reaction.



B. Advantages of polymerase chain reaction

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 using recombinant DNA techniques.

C. Applications

PCR has become a very common tool for a large number of applications.

1. **Comparison of a normal gene with a mutant form of the gene:** PCR allows the synthesis of mutant DNA in sufficient quantities for a sequencing protocol without laboriously cloning the altered DNA.

2. **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 real time PCR (qRT-PCR) allows quantification of starting amounts of the target nucleic acid as PCR progresses (in real time) rather than at the end and is useful in determining viral load (the amount of virus).]

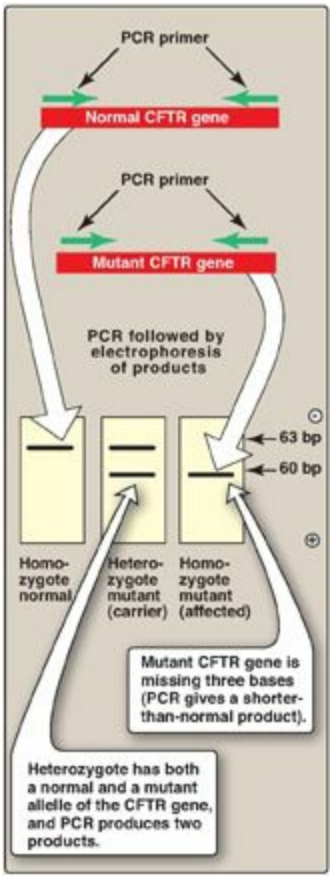
3. **Forensic analysis of DNA samples:** DNA fingerprinting by means of PCR has revolutionized the analysis of evidence from crime scenes. DNA isolated from a single human hair, a tiny spot of blood, or a sample of semen is sufficient to determine whether the sample comes from a specific individual. The DNA markers analyzed for such fingerprinting are most commonly short tandem repeat polymorphisms. These are very similar to the VNTRs described previously (see p. 475) but are smaller in size. [Note: Determination of paternity uses the same techniques.]

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. 434). 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 33.21 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 or more exons in a gene with many exons such as the gene for CFTR,

which has 27 exons.

Figure 33.21 Genetic testing for cystic fibrosis using the polymerase chain reaction (PCR). CFTR = cystic fibrosis transmembrane conductance regulator; bp = base pairs.



VIII. ANALYSIS OF GENE EXPRESSION

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.

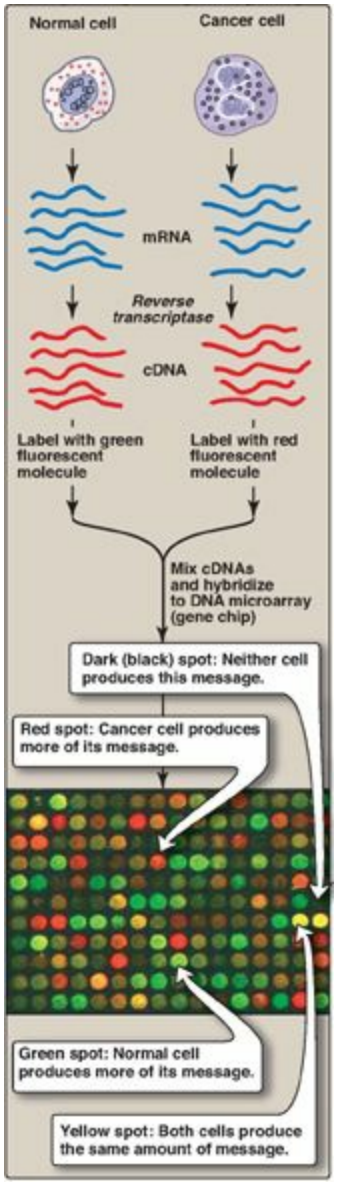
A. Determination of messenger RNA levels

mRNA levels are usually determined by the hybridization of labeled probes to either mRNA itself or to cDNA produced from mRNA. [Note: Amplification of cDNA made from mRNA by retroviral reverse transcriptase (RT) is referred to as RT-PCR.]

1. **Northern blots:** Northern blots are very similar to Southern blots (see Figure 33.12, p. 474), except that the original sample contains a mixture of mRNA molecules that are separated by electrophoresis, then transferred to a membrane and hybridized to a radiolabeled probe. The bands obtained by autoradiography give a measure of the amount and size of particular 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 (Figure 33.22). This mixture is then exposed to a gene (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 cell (for example, normal and cancer cells; see Figure 33.22). They can also be used to subclassify cancers, such as breast cancer, to optimize treatment. [Note: Microarrays involving proteins and the antibodies 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 medicine.]

Figure 33.22 Microarray analysis of gene expression using DNA (gene) chips. [Note: Protein chips are also used.] mRNA = messenger RNA; cDNA = complementary DNA.



B. Analysis of proteins

The kinds and amounts of proteins in cells do not always directly correspond to the amounts of mRNA present. Some mRNAs 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 antibodies are used to detect and quantify specific proteins and to determine posttranslational modifications.

1. **Enzyme-linked immunosorbent assays (ELISAs):** These assays are performed in the wells of a plastic microtiter dish. The antigen (protein) is bound to the plastic of the dish. The probe used consists of an antibody specific for the particular protein to be measured. The antibody 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 antibody present and, indirectly, to the amount of protein in a test sample.

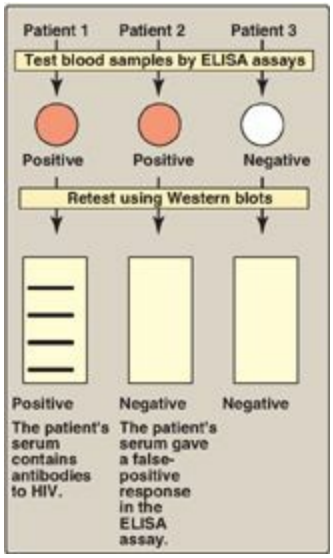
2. **Western blots:** Western blots (also called immunoblots) are similar to Southern blots, except that protein molecules in the sample are separated by electrophoresis and blotted (transferred) to a membrane. The probe is a labeled antibody, which produces a band at the location of its antigen.

3. **Detecting exposure to human immunodeficiency virus (HIV):** ELISA and Western blots are commonly used to detect exposure to HIV by measuring the amount of anti-HIV antibodies 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 (Figure 33.23). [Note: ELISA and Western blots can only detect HIV exposure after anti-HIV antibodies appear in the bloodstream. PCR-based testing for HIV is more useful in the first few months after exposure.]

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 33.24 compares some of the analytic techniques discussed in this chapter.

Figure 33.23 Testing for HIV exposure by enzymelinked immunosorbent assays (ELISAs) and Western blots.



IX. GENE THERAPY

The goal of gene therapy is to treat disease through insertion of the normal, cloned DNA for a 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, it is the germ cells that are modified, and so the change is passed on. A long-standing moratorium on germline gene therapy is in effect world-wide.] 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 vector (viral or nonviral) to deliver the DNA into the target cell. 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 (1990) involved two patients with severe combined immunodeficiency disease (SCID) caused by mutations to the gene for adenosine deaminase (see p. 301). It utilized mature T lymphocytes transduced ex vivo with a viral vector (Figure 33.25). 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.

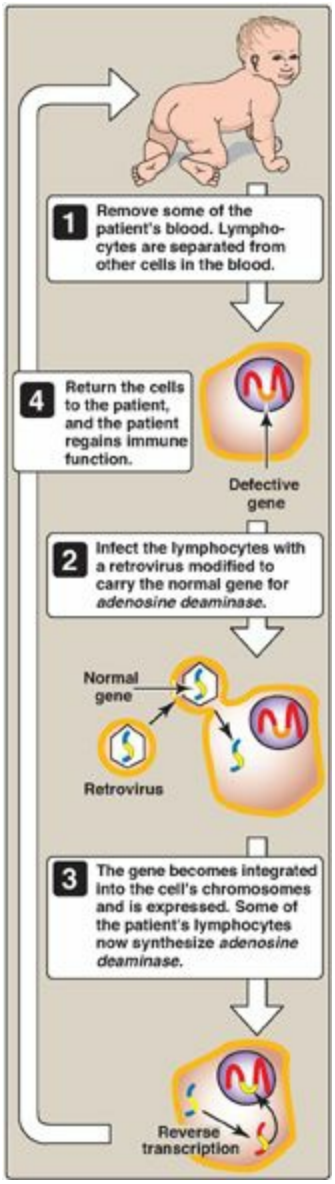
Figure 33.24 Techniques used to analyze DNA, RNA, and proteins. ASO = allele-specific oligonucleotides. ELISA = enzymelinked immunosorbent assay; cDNA = complementary DNA; mRNA = messenger RNA.

TECHNIQUE	SAMPLE ANALYZED	GEL USED	PURPOSE
Southern blot	DNA	Yes	Detects large changes in DNA; detects point mutations that create or destroy restriction sites
Northern blot	RNA	Yes	Measures mRNA amounts and sizes
Western blot	Protein	Yes	Measures protein amounts
ASO	DNA	No	Detects small changes in DNA
Microarray	cDNA or genomic DNA	No	Measures many mRNA levels at once; detects genomic changes
	Protein	No	Measures protein expression levels and protein–protein interactions
ELISA	Proteins	No	Detects proteins (antigens) or antibodies

X. TRANSGENIC ANIMALS

Transgenic animals can be produced by injecting a cloned gene into the fertilized egg. If the gene becomes successfully integrated into a chromosome, it will be present in the germline of the resulting animal and can be passed along 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. [Note: Transgenic animals have been designed that produce human proteins in their milk. Antithrombin, an anticlotting protein, was produced by transgenic goats and approved for clinical use in 2009 (see online Chapter 34).] Sometimes, rather than introducing a functional gene into a mouse, a nonfunctional version is inserted. Such genetically engineered animals can be used to produce a colony of "knockout mice" that lack the product of the affected gene. Such animals can then serve as models for the study of a corresponding human disease. [Note: Knock-in mice result if the inserted gene expresses a mutated product or under- or overexpresses a product.]

Figure 33.25 Gene therapy for SCID caused by adenosine deaminase deficiency. [Note: Bone marrow stem cells and a modified retroviral vector are now used.]



XI. CHAPTER SUMMARY

Restriction endonucleases are bacterial enzymes that cleave double-stranded DNA (dsDNA) into smaller fragments. Each enzyme cleaves at a specific four to eight–base pair sequence (a restriction site), producing DNA segments called **restriction fragments**. The sequences that are recognized are **palindromic**. Restriction enzymes form either **staggered cuts** (**sticky ends**) or **blunt-end cuts** on the DNA. **Bacterial DNA ligases** can join two DNA fragments from different sources if they have been cut by the same restriction endonuclease. This hybrid combination of two fragments is called a **recombinant DNA molecule**. Introduction of a foreign DNA molecule into a replicating cell permits the **amplification** (production of many copies) of the DNA, a process called **cloning**. A **vector** is a molecule of DNA to which the fragment of DNA to be cloned is joined. Vectors must be capable of **autonomous replication** within the host cell, must contain at least one specific nucleotide sequence recognized by a restriction endonuclease, and must carry at least one gene that confers the ability to select for the vector such as an **antibiotic resistance gene**. Prokaryotic organisms normally contain small, circular, extrachromosomal DNA molecules called **plasmids** that can serve as **vectors**. They can be readily isolated from the bacterium (or artificially constructed); joined with the DNA of interest; and reintroduced into the bacterium, which will replicate, thus making multiple copies of the **hybrid plasmid**. A **DNA library** is a collection of cloned restriction fragments of the DNA of an organism. **A genomic library** is a collection of fragments of dsDNA obtained by digestion of the total DNA of the organism with a restriction endonuclease and subsequent ligation to an appropriate vector. It ideally contains a copy of every DNA nucleotide sequence in the genome. In contrast, **complementary DNA** (**cDNA**) **libraries** contain only those DNA sequences that are complementary to messenger RNA (mRNA) molecules present in a cell and differ from one cell type to another. Because cDNA has no intervening sequences, it can be cloned into an **expression vector** for the synthesis of human proteins by bacteria or eukaryotes. Cloned, then purified, fragments of DNA can be sequenced, for example, using the **Sanger dideoxy method**. A **probe** is a small piece of RNA or single-stranded DNA (usually labeled with a radioisotope, such as ^{32}P, or another recognizable compound, such as biotin or a fluorescent dye) that has a nucleotide sequence complementary to the DNA molecule of interest (**target DNA**). Probes can be used to identify which clone of a library or which band on a gel contains the target DNA. **Southern blotting** is a technique that can be used to detect specific sequences present in DNA. The DNA is cleaved using a **restriction endonuclease**, and the pieces are separated by **gel electrophoresis** and are denatured and transferred (blotted) to a **nitrocellulose membrane** for analysis. The fragment of interest is detected using a **probe**. The human genome contains many thousands of polymorphisms (DNA sequence variations at a given locus). Polymorphisms can arise from single-base changes and from tandem repeats. A polymorphism can serve as a genetic marker that can be followed through

families. A **restriction fragment length polymorphism** (**RFLP**) is a genetic variant that can be observed by cleaving the DNA into restriction fragments using a restriction enzyme. A base substitution in one or more nucleotides at a restriction site can render the site unrecognizable by a particular restriction endonuclease. A new restriction site also can be created by the same mechanism. In either case, cleavage with the endonuclease results in fragments of lengths differing from the normal that can be detected by DNA hybridization. This technique can be used to diagnose genetic diseases early in the gestation of a fetus. The **polymerase chain reaction** (**PCR**), another method for **amplifying** a selected DNA sequence, does not rely on the biologic cloning method. 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. **Applications of the PCR technique** include: 1) efficient comparison of a normal gene with a mutant form of the gene, 2) detection of low-abundance nucleic acid sequences, 3) forensic analysis of DNA samples, and 4) prenatal diagnosis and carrier detection (for example, of cystic fibrosis). The **products of gene expression** (mRNA and proteins) can be measured by techniques such as the following: **Northern blots** are very similar to Southern blots except that the original sample contains a mixture of **mRNA** molecules that are separated by electrophoresis, then hybridized to a radiolabeled probe; **microarrays** are used to determine the differing patterns of gene expression in two different types of cells (for example, normal and cancer cells); **enzyme-linked immunosorbent assays** and **Western blots** (**immunoblots**) are used to detect specific proteins. **Proteomics** is the study of all the proteins expressed by a genome. The goal of **gene therapy** is the insertion of a normal cloned gene to replace a defective gene in a **somatic cell**. Insertion of a foreign gene into the germline of an animal creates a **transgenic animal** that can produce therapeutic proteins or serve as a model for human diseases.

Study Questions

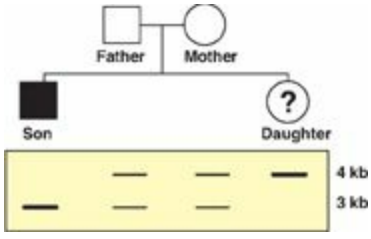
Choose the ONE best answer.

33.1 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′.

33.2 An Ashkenazi Jewish couple brings their 6-month-old son to you for evaluation of listlessness, poor head control, and a fixed gaze. You determine that he has Tay-Sachs disease, an autosomal recessive disorder. The couple also has a daughter. The family's pedigree is shown to the right, 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. 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

biological 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.

33.3 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 DNA expression.

33.4 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 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.

33.5 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 messenger RNA production. The lack of enzymic activity means that some aspect of protein synthesis is affected.

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.

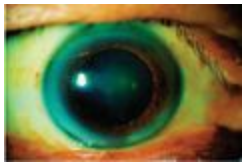
CASE 1: CHEST PAIN

Patient Presentation: A 35-year-old man with severe substernal chest pain of about 2 hoursl duration is brought by ambulance to his local hospital at 5 a.m. 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 (2–3 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 age 45 years and 39 years, respectively. His mother and younger (age 31 years) brother are said to be in good health.

Physical Examination (Pertinent Findings): The patient 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 a myocardial infarction (MI). Angiography reveals areas of severe stenosis (narrowing) of several coronary arteries. Initial results from the clinical laboratory include:

	Patient	Reference Range
Troponin	+	0
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 about 8 hours prior to the blood draw.]

Diagnosis: 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: The patient is given O_2, a vasodilator, pain medication, and

drugs to dissolve blood clots (thrombolytics) and reduce clotting (antithrombotics).

Long-term Treatment: Lipid-lowering drugs (for example, high-potency statins, bile acid [BA] sequestrants, and niacin), daily aspirin; β-blockers; and counseling on nutrition, exercise, and smoking cessation would be part of the long-term treatment plan.

Prognosis: Patients with heterozygous FH have ~50% of the normal number of functional LDL receptors and a hypercholesterolemia (two to three times normal) that puts them at high risk (>50% risk) for premature coronary heart disease (CHD). However, less than 5% of patients with hypercholesterolemia 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 (10–20 g/day) and plant sterols (2 g/day) for their hypocholesterolemic effects. Fiber increases BA excretion. This results in increased hepatic uptake of cholesterol-rich LDLs 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 the patient would identify affected individuals to allow their 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 by inhibiting hydroxymethylglutaryl coenzyme A (HMG CoA) reductase.

B. decrease expression of the gene for the LDL receptor by preventing the movement of the sterol regulatory element–binding protein-2 (SREBP-2) in complex with SREBP cleavage–activating protein (SCAP) from the membrane of the endoplasmic reticulum to the membrane of the Golgi.

C. increase the oxidation of cholesterol to $CO_2 + H_2O$.

D. interfere with the absorption of bile salts in the enterohepatic circulation, thereby causing the liver to take up cholesterol from the blood for use in bile acid synthesis.

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 competitive inhibitors is correct?

A. Competitive inhibitors are examples of irreversible inhibitors.

B. Competitive inhibitors increase both the apparent Michaelis constant (K_m) and the apparent maximal velocity (V_{max}).

C. Competitive inhibitors increase the apparent K_m and have no effect on the V_{max}.

D. Competitive inhibitors decrease both the apparent K_m and the apparent V_{max}.

E. Competitive inhibitors have no effect on the K_m and decrease the apparent V_{max}.

RQ4. In a myocardial infarction, a blood clot forms as a result of injury to a blood vessel that leads to production of a platelet plug and a meshwork of thrombin. The clot occludes the blood vessel, preventing blood flow and, therefore, delivery of O_2. Destruction of the clot (thrombolysis) restores blood flow. Which one of the following is an example of a thrombolytic agent?

A. Activated protein C complex

B. Antithrombin III

C. Aspirin

D. Factor XIII
E. Heparin
F. Tissue plasminogen activator
G. Vitamin K
H. Warfarin

RQ5. 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 adenosine triphosphate synthesis.
B. ratio of the oxidized form of nicotinamide adenine dinucleotide (NAD^+) to the reduced form (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 to provide the reducing equivalents needed for oxidative phosphorylation to occur.

RQ6. Genetic screening of the patient's first-degree relatives would be accomplished by mutation analysis via polymerase chain reaction–based amplification followed by automated sequencing of the promoter region and the 18 exons of the LDL receptor gene. This process would involve the:

A. generation and use of complementary DNA (cDNA).
B. initiation of DNA synthesis with dideoxynucleotides.
C. isolation of genomic DNA from germ cells.
D. use of fluorescently labeled nucleotides.

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 E-2, the isoform that only poorly binds its receptor?

TQ2. Why was aspirin prescribed? **Hint**: What pathway of lipid metabolism is affected 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 in the patient is that these changes raise the level of HDLs, 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: Jason S. is a 4-month-old boy whose mother is concerned about the "twitching" movements he makes just before feedings. She tells the pediatrician that the movements started about 1 week ago, are most apparent in the morning, and disappear shortly after eating.

Focused History: Jason is the product of 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. Jason last ate a few hours ago.

Physical Examination (Pertinent Findings): Jason appears sleepy and feels clammy to the touch. His respiratory rate is elevated. His temperature is normal. Jason has a protuberant, firm abdomen that appears to be nontender. His liver is palpable 4 cm below the right costal margin and is smooth. His kidneys are enlarged and symmetrical.

Pertinent Test Results:

	Patient	Pediatric Reference Range
Glucose	50 mg/dl (**L**)	60–105
Lactate	3.4 mmol/l (**H**)	0.6–3.2
Urate	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

Jason is sent to the regional children's hospital for further evaluation. Ultrasound studies confirm hepatomegaly and renomegaly and show no evidence of tumors. A liver biopsy is performed. The hepatocytes are distended. 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 less than 10% of the normal activity of glucose 6-phosphatase, an enzyme of the endoplasmic reticulum (ER) membrane in the liver and the kidneys.

Diagnosis: Glucose 6-phosphatase deficiency (glycogen storage disease [GSD] type Ia, von Gierke disease)

Treatment (Immediate): Jason 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. Jason's blood glucose levels were able to be maintained 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 glomerular function is impaired and can result in kidney failure. Patients are at increased risk for developing gout, but this rarely occurs before puberty.

Nutrition Nugget: Long-term medical nutrition therapy for Jason is designed to maintain his blood glucose levels in the normal range. Frequent (every 2–3 hours) daytime feedings rich in carbohydrate (provided by uncooked cornstarch that is slowly hydrolyzed) and nighttime nasogastric infusion (pump assisted) of glucose are advised. Avoidance of fructose and galactose is recommended because they are metabolized to glycolytic intermediates and lactate and can exacerbate the metabolic problems. Calcium and vitamin D supplements are prescribed.

Genetics Gem: GSD Ia is an autosomal-recessive disorder caused by more than 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 approximately 25% of all cases of GSDs 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 out of the cytosol and into the ER is the cause of GSD Ib.]

REVIEW QUESTIONS: Choose the ONE best answer.

RQ1. Jason is hypoglycemic because:

A. free (nonphosphorylated) glucose cannot be produced from either glycogenolysis or gluconeogenesis as a result of the deficiency in glucose 6-phosphatase.

B. glycogen phosphorylase is dephosphorylated and inactive, and glycogen cannot be degraded.

C. hormone-sensitive lipase is dephosphorylated and inactive, and fatty acid substrates for gluconeogenesis cannot be generated.

D. the decrease in the insulin/glucagon ratio upregulates glucose transporters in the liver and kidneys, resulting in increased uptake of blood glucose.

RQ2. Jason was prescribed calcium supplements because chronic acidosis can cause bone demineralization, resulting in osteopenia. Vitamin D was also prescribed because vitamin D:

A. binds G_q protein–coupled membrane receptors and causes a rise in inositol trisphosphate with release of calcium from intracellular stores.

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. is the coenzyme-prosthetic group for calbindin, a calcium transporter in the intestine.

RQ3. The hepatomegaly and renomegaly seen in Jason 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 membrane of the endoplasmic reticulum. Which of the following statements about such proteins is correct?

A. If glycosylated, the carbohydrate is on the 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 Jason's twitching movements?

TQ2. Why was the liver homogenate treated with detergent? **Hint**: Think about where the enzyme is located.

TQ3. Why is Jason'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. A. Why are triacylglycerols and cholesterol elevated? **Hint**: Glucose is the primary carbon source for their synthesis.

B. Why are ketone bodies not elevated?

CASE 3: HYPERGLYCEMIA AND HYPERKETONEMIA

Patient Presentation: A 40-year-old woman was brought to the hospital in a disoriented, confused state by her husband.

Focused History: As noted on her medical alert bracelet, the patient has had type 1 diabetes (T1D) for the last 24 years. Her husband reports that this is her first medical emergency in 2 years.

Physical Examination (Pertinent Findings): The patient displayed signs of dehydration (such as dry mucous membranes and skin, poor skin turgor, and low blood pressure) and acidosis (such as deep and rapid breathing [Kussmaul respiration]). Her breath had a faintly fruity odor. Her temperature was normal.

Pertinent Test Results: Rapid, bedside tests were strongly positive for glucose and acetoacetate and negative for protein. Results on 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 a urinary tract infection (UTI).

Diagnosis: The patient is in diabetic ketoacidosis that was precipitated by a UTI. [Note: Diabetes increases the risk for infections such as UTIs.]

Immediate Treatment: The patient was rehydrated with normal saline given intravenously (IV). She also was given insulin IV. Blood glucose, ketone bodies, and electrolytes were measured periodically. The patient was started on an antibiotic for her UTI.

Long-term Treatment: Diabetes increases the risk for macrovascular complications (such as 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 sixth 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 carbohydrate is primary in blood glucose control. Carbohydrate should come from whole grains, vegetables, legumes, and fruits. Low-fat dairy products and nuts

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

and fish rich in ω-3 fatty acids are encouraged. Intake of saturated and trans fats should be minimized.	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 glycosylated hemoglobin (HbA_{1C}) in the blood.

B. During periods of physiologic stress, the urine of an individual with T1D would likely test negative for reducing sugars.

C. T1D is associated with obesity and a sedentary lifestyle.

D. The characteristic metabolic abnormalities seen in T1D result from insensitivity to both insulin and glucagon.

E. Treatment with exogenous insulin allows normalization of blood glucose (euglycemia).

RQ2. Diabetic ketoacidosis occurs when the rate of ketone body production is greater than the rate of utilization. Which of the following statements concerning ketone body metabolism is correct? Ketone bodies:

A. are made in mitochondria from acetyl coenzyme A (CoA) 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. require 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 products is required for the generation of ketone bodies. Which of the following statements concerning the generation and use of fatty acids is correct?

A. Mitochondrial β-oxidation of fatty acids is inhibited by malonyl CoA.

B. Production of fatty acids from adipose lipolysis is upregulated by insulin.

C. The acetyl CoA product of fatty acid β-oxidation favors the use of pyruvate for gluconeogenesis by activating the pyruvate dehydrogenase complex.

D. The β-oxidation of fatty acids utilizes reducing equivalents generated by gluconeogenesis.

E. The fatty acids produced by lipolysis are taken up by the brain and oxidized for energy.

THOUGHT QUESTIONS

TQI. At admission, the patient was hypoinsulinemic, and she was given insulin. Why did the patient's hypoinsulinemia result in hyperglycemia? **Hint**: What is the role of insulin in glucose metabolism?

TQ2. Why is there glucose in the patient's urine (glucosuria)? How is the glucosuria related to her dehydrated state?

TQ3. Why is the majority of the acetyl CoA from fatty acid β-oxidation being used for ketogenesis rather than being oxidized in the tricarboxylic acid cycle?

TQ4. Was the patient in positive or negative nitrogen balance when she was brought to the hospital?

TQ5. What response to the ketoacidosis is apparent in the 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 fatty acid oxidation?

CASE 4: HYPOGLYCEMIA, HYPERKETONEMIA, AND LIVER DYSFUNCTION

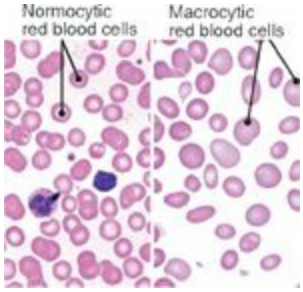
Patient Presentation: AK, a 59-year-old male with slurred speech, ataxia (loss of skeletal muscle coordination), and abdominal pain, was dropped off at the Emergency Department (ED).

Focused History: AK is known to the ED staff from previous visits. He has a 6-year history of chronic, heavy alcohol consumption. He is not known to take illicit drugs. At this ED visit, AK reports that he has been drinking heavily in the past day or so. He cannot recall having eaten anything in that time. There is evidence of recent vomiting, but no blood is apparent.

Physical Examination (Pertinent Findings): The physical examination was remarkable for the emaciated appearance of the patient. (His body mass index was later determined to be 17.5, which put him in the underweight category.) The cheeks of his face 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) was 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 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 sec (**H**)	11.0–13.2
	H = High; **L** = Low	*driving under the influence



Additional Tests: Complete blood count (CBC) and blood smear revealed a macrocytic anemia (right Image). Folate and B_{12} levels were ordered.

Diagnosis: Alcoholism

Treatment (Immediate): Thiamine and glucose were given intravenously.

Prognosis: Alcoholism (alcohol dependence) is the third most common cause of preventable death in the United States. People with alcoholism are at increased risk for

liver cirrhosis, pancreatitis, gastrointestinal bleeding, and some cancers.

Nutrition Nugget: Those with alcoholism 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). The majority of individuals of East Asian (but not European or African) heritage 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 the 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 mitochondrion. 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 reticulum 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 of the nicotinamide adenine dinucleotide phosphate (NADPH) that provides the reducing equivalents needed for activity of CYP proteins and the regeneration of functional glutathione.

RQ3. Alcohol is known to modulate the levels of serotonin in the central nervous system, where it 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, which also degrades the catecholamines.

C. released by activated platelets.

D. synthesized from tyrosine in a two-step process that utilizes a tetrahydrobiopterin-requiring hydroxylase and a pyridoxal phosphate-requiring carboxylase.

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, with the characteristic structural changes that result in decreased pancreatic function, 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 (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?

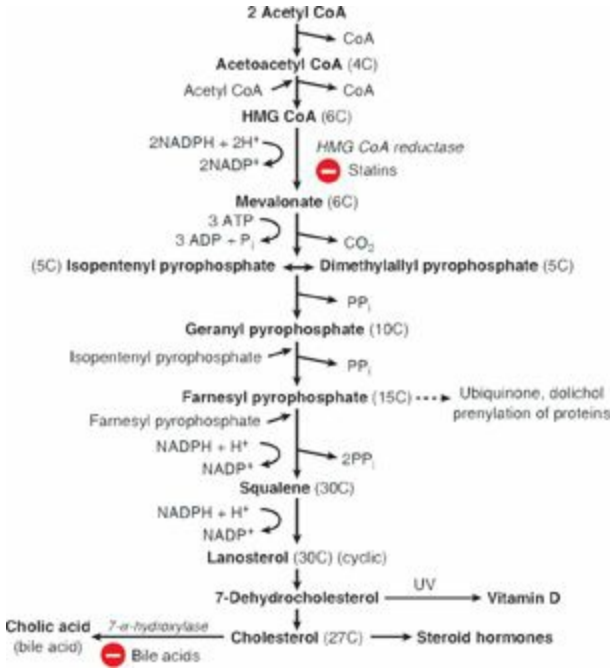
TQ4. Folate and vitamin B_{12} deficiencies cause a macrocytic anemia that may be seen in those with alcoholism. Why is it advisable to measure vitamin B_{12} levels before supplementing with folate in an individual with macrocytic anemia?

II. INTEGRATIVE CASE ANSWERS

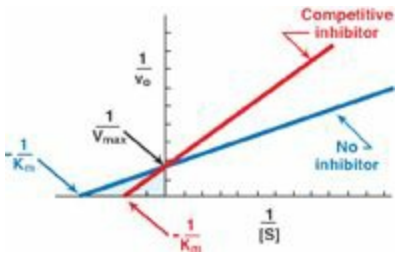
CASE 1: Answers to Review Questions

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 are derived from the 20-carbon polyunsaturated fatty acid arachadonic 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 reduction of HMG CoA to mevalonate and decreasing cholesterol biosynthesis (see Figure). 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, where 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 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 bile acids, thereby reducing blood cholesterol levels. Steroid hormones are synthesized from cholesterol, and vitamin D is synthesized 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. Once bound, they prevent the substrate from binding. This results in an increase in the apparent K_m, that substrate 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). It is noncompetitive inhibitors that decrease the apparent V_{max} and have no effect on K_m.



RQ4. Answer = F. Tissue plasminogen activator converts plasminogen to plasmin that degrades fibrin (fibrinolysis), thereby degrading the clot (thrombolysis). Aspirin, an inhibitor of cyclooxygenase, is an antiplatelet drug. Antithrombin III (ATIII) removes thrombin from the blood, and its action is potentiated by heparin. Activated protein C (APC) complex cleaves the accessory proteins factor (F)Va and FVIIIa. ATIII and APC are involved in anticoagulation. FXIII is a transglutaminase that cross-links the fibrin mesh. Vitamin K is a fat-soluble vitamin required for the γ-carboxylation of FII, FVII, FIX, and FX. Warfarin prevents regeneration of the functional, reduced form of vitamin K.

RQ5. Answer = D. In hypoxia, substrate level phosphorylation in glycolysis provides adenosine triphosphate (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.

RQ6. Answer = D. Fluorescently labeled nucleotides allow the base sequence of the DNA of interest to be detected. Complementary DNA (cDNA) is generated from processed mRNA and would not contain the promoter. Dideoxynucleotides lack the 3-OH needed to form the 3′→5′ phosphodiester bond that joins the nucleotides and, thus, will terminate DNA synthesis. Genomic DNA obtained from white cells isolated from a blood sample would be the source of the DNA.

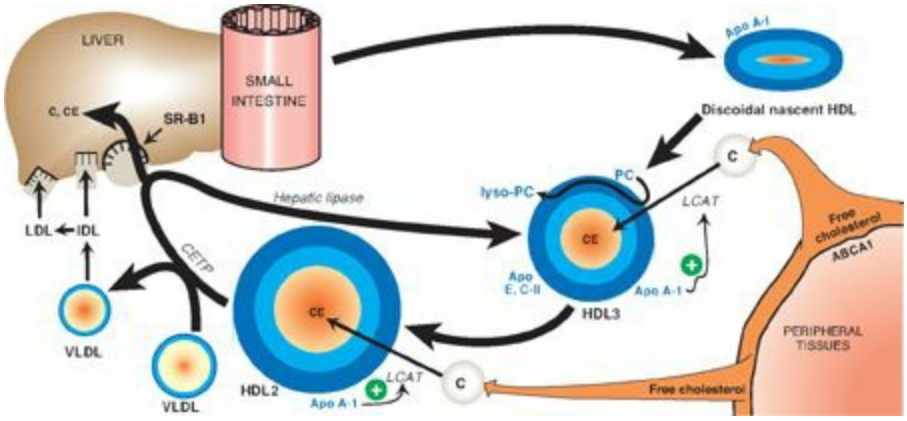
CASE 1: Answers to Thought Questions

TQ1. The phenotype would be the same. In familial defective apolipoprotein 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 apolipoprotein E-2 isoform, chylomicron remnants and intermediate-density lipoproteins would accumulate in blood.

TQ2. Aspirin irreversibly inhibits cyclooxygenase (COX) and, therefore, the synthesis of prostaglandins (PGs), such as PGI_2 in vascular endothelial cells, and thromboxanes (TXs), 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 can't 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.

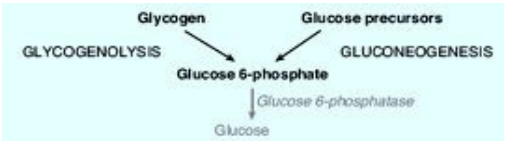
TQ3. The decrease in ATP (as the result of a decrease in O_2 and, thus, a decrease in oxidative phosphorylation) causes an increase in adenosine monophosphate (AMP). AMP allosterically activates phosphofructokinase-1, the key regulated enzyme of glycolysis. The rise in glycolysis increases the production of ATP by substrate-level phosphorylation. It also increases the ratio of the reduced to oxidized forms of nicotinamide adenine dinucleotide (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.

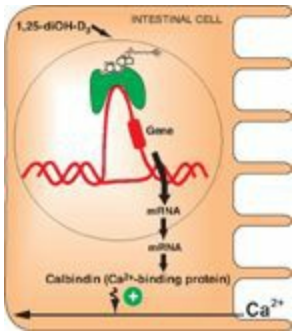
TQ4. High-density lipoprotein (HDL) functions in "reverse" cholesterol transport. It takes cholesterol from nonhepatic (peripheral) tissues (for example, the endothelial layer of arteries) and brings it to the liver (see Figure below). The ABCA1 transporter mediates the efflux of cholesterol to HDL. The cholesterol is esterified by lecithin-cholesterol acyl transferase (LCAT) that requires apo A-1 as a coenzyme. Some cholesteryl ester is transferred to very-low-density lipoproteins (VLDLs) by cholesteryl-ester transfer protein (CETP) in exchange for triacyl-glycerol. 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 from the liver to peripheral tissues.]


LIVER
SMALL INTESTINE
Apo A-I
Discoidal nascent HDL
C, CE
SR-B1
PC
C
Hepatic lipase
lyso-PC
LCAT
Free cholesterol
ABCA1
LDL
IDL
CETP
CE
Apo E, C-II
Apo A-I
HDL3
PERIPHERAL TISSUES
VLDL
HDL2
LCAT
C
Free cholesterol
VLDL
Apo A-I

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: Jason's symptoms appeared only recently because, at age 4 months, he is going longer periods between feedings.] 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 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 transporter protein in the intestine (see Figure). 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 vitamin 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 kidneys. The increased availability of glucose 6-phosphate also drives glycolysis. The increase in glycolysis provides substrates for lipogenesis, thereby increasing synthesis of fatty acids and triacylglycerols (TAGs). 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

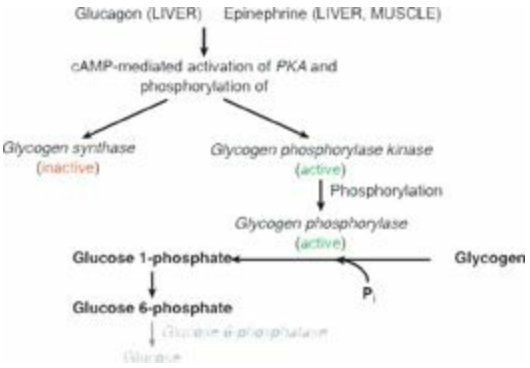
the most common posttranslational modification found in proteins. The glycosylated portion of membrane proteins is found on the outside face of the membrane. The membrane-spanning domain consists of approximately 22 hydrophobic amino acids. Proteins destined for secretion or for membrane, the ER lumen, 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 hyperglycemia persists.

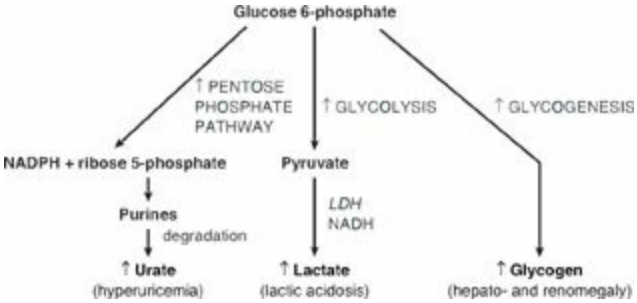
TQ2. Detergents are amphipathic molecules (that is they have both hydrophilic [polar] and hydrophobic [nonpolar] regions). Detergents solubilize membranes, thereby disrupting membrane structure. If the problem were the translocase needed to move the glucose 6-phosphate substrate into the ER, rather than the phosphatase, disruption of the ER membrane would allow the substrate access to the phosphatase.

TQ3. Glucagon, a peptide hormone released in hypoglycemia, binds its plasma membrane G protein–coupled receptor on hepatocytes. The a_s subunit of the 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 adenosine triphosphate (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). Consequently, administration of glucagon is unable to cause a rise in blood glucose. [Note: Epinephrine would be similarly ineffective.]

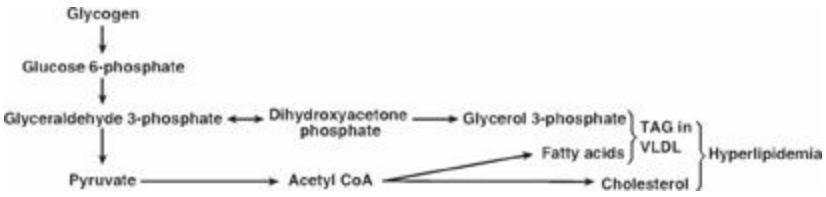


TQ4. The availability of inorganic phosphate (P_i) is decreased because it is trapped as phosphorylated glycolytic intermediates as a result of the upregulation of glycolysis by the rise in glucose 6-phosphate. Urate is elevated because the trapping of P_i decreases the ability to phosphorylate adenosine 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. Purines made beyond

need are degraded to urate (see Figure below). [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 (LDH) 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.



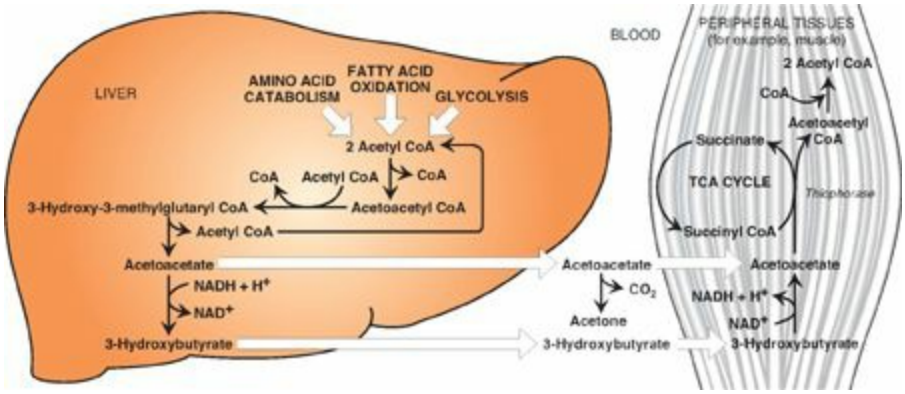
TQ5. Increased glycolysis results in increased availability of glycerol 3-phosphate for hepatic TAG synthesis. Additionally, some of the pyruvate generated in glycolysis will be oxidatively decarboxylated to acetyl coenzyme A (CoA). However, the tricarboxylic acid cycle is inhibited by the rise in NADH, and the acetyl CoA is transported to the cytosol as citrate. The rise of acetyl CoA in the cytosol results in increased fatty acid synthesis. Recall that citrate is an allosteric activator of acetyl CoA carboxylase (ACC). The malonyl product of ACC inhibits fatty acid oxidation at the carnitine palmitoyltransferase I step. Because mitochondrial fatty acid oxidation generates the acetyl CoA substrate for hepatic ketogenesis, ketone body levels do not rise. The fatty acids get esterified to the glycerol backbone, resulting in an increase in TAGs that get sent out of liver as components of very-low-density lipoproteins (VLDLs). [Note: The hypoglycemia results in release of epinephrine and the activation of TAG lipolysis with release of free fatty acids into the blood. The fatty acids are used in hepatic TAG synthesis.] The acetyl CoA is also a substrate for cholesterol synthesis. Thus, the increase in glycolysis results in the hyperlipidemia seen in the patient (see Figure).



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 (for example, 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.

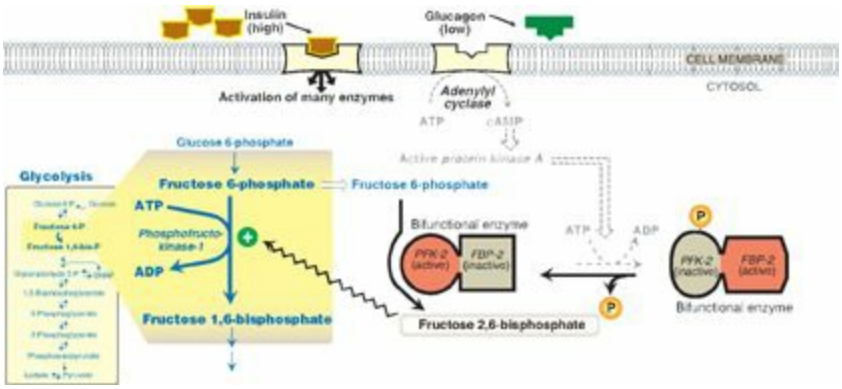
RQ2. Correct answer = E. 3-Hydroxybutyrate and acetoacetate are organic acids, and their ionization contributes to the proton load of the body. Ketone bodies are made in the mitochondria of liver cells using acetyl coenzyme A (CoA) generated primarily from the β-oxidation of fatty acids (see Figure below). Because they are water soluble, they do not require a transporter. 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 fatty acid synthesis, inhibits fatty acid β-oxidation through inhibition of carnitine palmitoyltransferase I. Lipolysis occurs when the insulin-to-counterregulatory hormone ratio decreases. Acetyl CoA, the product of fatty acid β-oxidation, inhibits pyruvate dehydrogenase (PDH) 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. The blood–brain barrier inhibits use of fatty acids 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-to-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 inhibits fructose 1,6-bisphosphatase, thereby inhibiting gluconeogenesis, and activates phosphofructokinase-1 (PFK-1) of glycolysis (see Figure). 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 fatty acid 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.

TQ4. The patient 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). [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 (UUN).]

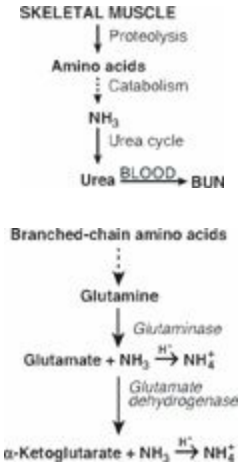
TQ5. The Kussmaul respiration seen in the patient is a respiratory response to the metabolic acidosis. Hyperventilation blows off carbon dioxide (CO_2) and water,

reducing the concentration of protons (H^+) and bicarbonate (HCO_3^-) as reflected in the following equation:

$$H^+ + HCO_3^- \leftrightarrow H_2CO_3 \text{ (carbonic acid)} \leftrightarrow CO_2 + H_2O.$$

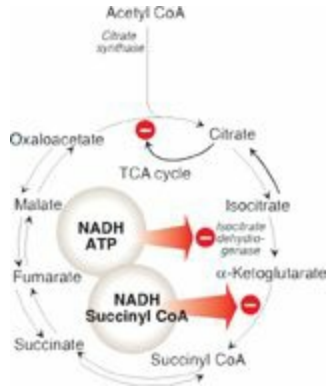
The renal response includes, in part, the excretion of H^+ as ammonium (NH_4^+). Degradation of branched-chain amino acids in skeletal muscle results in the release of large amounts of glutamine into the blood. The kidneys take up and catabolize the glutamine generating NH_3 in the process. The NH_3 is converted to NH_4^+ by secreted H^+ and is excreted (see Figure). [Note: When ketone bodies are plentiful, enterocytes shift to using them as a fuel instead of glutamine. This increases the amount of glutamine going to the kidney.]

TQ6. Because fatty acid 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 fatty acid 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 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). It also favors the reduction of oxaloacetate to malate (not malate to oxaloacetate), decreasing the availability of oxaloacetate for condensation with acetyl coenzyme A (CoA) in the TCA cycle and for gluconeogenesis. Fatty acid oxidation requires NAD^+ for the 3-hydroxyacyl CoA dehydrogenase step and, thus, is inhibited by the rise in NADH. The decrease in fatty acid oxidation decreases the production of adenosine triphosphate (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.

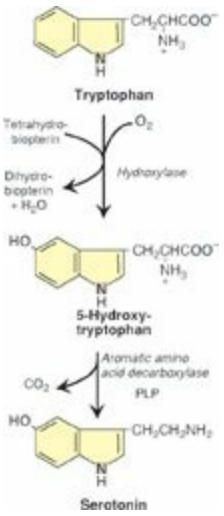


RQ2. Answer = E. The irreversible, oxidative portion of the pentose phosphate pathway provides the nicotinamide adenine dinucleotide phosphate (NADPH) that supplies the reducing equivalents needed for activity of cytochrome P450 (CYP) proteins and for the regeneration of functional (reduced) glutathione. It is also an important source of NADPH for reductive biosynthetic processes in the cytosol, such as fatty acid and cholesterol synthesis. 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 reticulum membrane that are involved in detoxification reactions. Those of the inner mitochondrial membrane are involved in the synthesis of steroid hormones, bile acids, and calcitriol. 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 serotonin that was made in the intestine and secreted into the blood.] Serotonin is associated with a feeling of well-being. It is degraded by monoamine oxidase (MAO) that catalyzes oxidative deamination. It is catechol-O-methyl transferase (COMT) that catalyzes the methylation step in the

degradation of the catecholamines. Serotonin is synthesized from tryptophan in a two-step process that utilizes tetrahydrobiopterin-requiring tryptophan hydroxylase and a pyridoxal phosphate (PLP)-requiring decarboxylase (see Figure).

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.

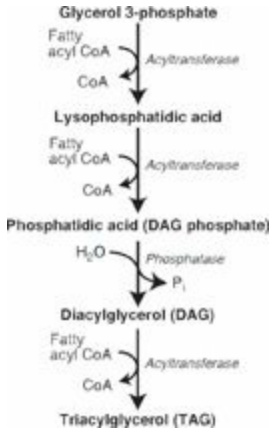


CASE 4: Answers to Thought Questions

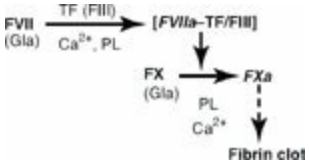
TQ1. A. The rise in cytosolic NADH seen with ethanol metabolism inhibits glycolysis. The glyceraldehyde 3-phosphate dehydrogenase step requires NAD^+, which gets reduced as glyceraldehyde 3-phosphate gets oxidized. With the rise in NADH, glyceraldehyde 3-phosphate accumulates.

B. The glyceraldehyde 3-phosphate from glycolysis is converted to glycerol 3-phosphate, the initial acceptor of fatty acids in triacylglycerol (TAG) synthesis (see Figure). Fatty acids 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 and decreased use in the TCA cycle), increased availability from lipolysis in adipose tissue, and decreased degradation. The TAGs 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-induced 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 the patient.]



TQ3. Prothrombin time (PT) measures the time it takes for plasma to clot after the addition of tissue factor (FIII), thereby allowing evaluation of the extrinsic (and common) pathways of coagulation (see Figure). In the extrinsic pathway, FIII activates FVII. FVII, like most of the proteins of clotting, is made by the liver. Alcohol-induced liver damage can decrease its synthesis. Additionally, FVII has a short half-life and, as a g-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 thromboplasin time, or aPPt.]



TQ4. Administration of folate can mask a deficiency in vitamin B_{12} by reversing the hemotologic manifestations 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: JS is a 24-year-old man who is being evaluated as a follow-up to a pre-placement medical evaluation he had prior to starting his new job.

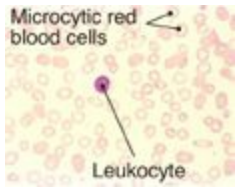
Focused History: JS has no significant medical issues. His family history is unremarkable, but he knows little of the health status of those family members who remain in Greece.

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:

	Patient	Reference Range
HbA*	90% (**L**)	96–98
HbA_2	6% (**H**)	<3
HbF	4% (**H**)	<2
*Includes HbA_{1c}	**H** = High; **L** = Low.	



Diagnosis: JS has β-thalassemia trait (β-thalassemia minor) that is causing a microcytic anemia (see Image). Ethnicity (such as being of Mediterranean origin) influences the risk for thalassemia.

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**l** end of eukaryotic mRNA requires a methyl donor.

E. Splicing of eukaryotic mRNA involves removal of exons and joining of introns by the proteasome.

Q2. Hemoglobin A (HbA), a tetramer of 2 α and 2 β 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 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: KL is a 34-year-old woman who presents with a red, nonitchy rash on her left thigh and flulike symptoms.

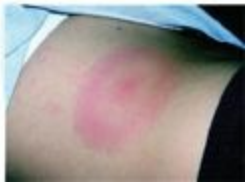
Focused History: KL reports that the rash first appeared a little over 2 weeks ago. It started out small but has gotten larger. She also thinks she is getting the flu because her muscles and joints ache (myalgia and arthralgia, respectively), and she has had a headache for the last few days. Upon questioning, KL tells you she and her husband took a camping trip through New England last month.

Pertinent Findings: The physical examination is remarkable for the presence of a red, circular, flat lesion about 11 cm in size that resembled a bullseye (erythema migrans) (see image). KL also has a low-grade fever.

Diagnosis: KL 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 the Northeast region of the United States.

Treatment: The patient is prescribed doxycycline, an antibiotic in the tetracycline family. Monitoring of KL 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) 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. Peptidyl transferase 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 eukaryotic messenger RNA (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) follow 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 mRNAs.

B. Both techniques involve the use of antibodies.

C. ELISAs require the use of electrophoresis.

D. Western blots require use of the polymerase chain reaction.

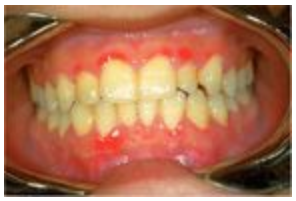
Q3. Why are eukaryotic cells unaffected by antibiotics in the tetracycline class?

CASE 3: BLOOD ON THE TOOTHBRUSH

Patient Presentation: LT is an 84-year-old man whose gums have been bleeding for several months.

Focused History: LT is a widower and lives alone in a suburban community on the East Coast. He no longer drives. His two children live on the West Coast and come east infrequently. 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). Several of the patient's 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 (petechia) around some of the hair follicles. Blood was drawn for testing.



Results of tests on the patient's blood:

	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 μm^3 (**L**)	80–100
Serum iron	40 µg/dl (**L**)	50–170
Platelets	250×10^9/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:

	Patient	Reference Range
Vitamin C (plasma)	0.16 mg/dl (**L**)	0.2–2

H = High; **L** = Low

Diagnosis: Vitamin C deficiency with a microcytic, hypochromic anemia secondary to the deficiency

Treatment: LT 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 excellent.

CASE-RELATED QUESTIONS: Choose the ONE best answer.

Q1. Which of the following statements about vitamin C is correct? Vitamin C is:

A. a competitor of iron absorption in the intestine.

B. a fat-soluble vitamin with a 3-month supply typically stored in adipose tissue.

C. a coenzyme in several enzymic reactions such as the hydroxylation of proline.

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 enzymes catalyzing 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: BE is a 45-year-old woman who presents with concerns about sudden (paroxysmal), intense, brief episodes of headache, sweating (diaphoresis), and a racing heart (palpitations).

Focused History: BE reports that the attacks started about 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 the drug store and had her blood pressure taken. She was told it was 165/110 mm Hg. BE notes that she has lost weight (about 8 lbs) in this period even though her appetite has been good.

Pertinent Findings: The physical examination was remarkable for the patient's thin, pale appearance. Blood pressure was elevated (150/100 mm Hg), as was the heart rate (110–120 beats/minute). Based on the patient's history, blood levels of normetanephrine and metanephrine were ordered. They were found to be elevated.

Diagnosis: Pheochromocytoma, a rare catecholamine-secreting tumor of the adrenal medulla

Treatment: Imaging studies of the abdomen were performed to locate the tumor. Surgical resection of the tumor was performed. The tumor was found to be nonmalignant. 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 over 95%.

CASE-RELATED QUESTIONS: Choose the ONE best answer.

Q1. Pheochromocytomas secrete norepinephrine 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-methytransferase 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 norepinephrine are correct?

A. Norepinephrine 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, which are a class of nuclear receptors.

D. They result in the activation of glycogen and triacylglycerol synthesis.

Q3. Norepinephrine bound to certain receptors causes vasoconstriction and an increase in blood pressure. Why might norepinephrine be used clinically in the treatment of septic shock?

CASE 5: SUN SENSITIVITY

Patient Presentation: AZ is a 6-year-old boy who is being evaluated for freckle-like areas of hyperpigmentation on his face, neck, forearms, and lower legs.

Focused History: AZ's father reports that the boy 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 face was biopsied, and two were later determined to be squamous cell carcinomas.

Diagnosis: 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. Which of the following statements about DNA repair mechanisms is correct? 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 ultraviolet 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. Which one of the following statements about DNA synthesis (replication) is correct? 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: JF is a 13-year-old girl who presents with fatigue and yellow sclerae.

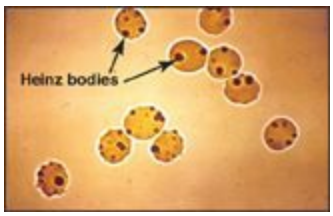
Focused History: JF began treatment about 4 days ago with a sulfonamide antibiotic and a urinary analgesic for a urinary tract infection. She had been told that her urine would change color (become reddish) with the analgesic, but she reports that it has gotten darker (more brownish) over the last 2 days. Last night her mother noticed that her eyes had a yellow tint. JF says she feels as though she has no energy.

Pertinent Findings: The physical examination was remarkable for the pale appearance of the patient, mild scleral icterus (jaundice), mild splenomegaly, and an increased heart rate (tachycardia). JF's urine tested positive for hemoglobin (hemoglobinuria). A peripheral blood smear reveals a lower-than-normal number of red blood cells (RBCs), with some containing precipitated hemoglobin (Heinz bodies; see Image), and a higher-than-normal number of reticulocytes (immature RBCs). Results of the complete blood count (CBC) and blood chemistry tests are pending.

Diagnosis: 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. JF will be switched to a different antibiotic. She will be advised that she is susceptible to certain drugs (for example, sulfa drugs), foods (fava or broad beans), and certain chemicals (for example, naphthalene), and must avoid exposure to them.

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. Glucose 6-phosphate dehydrogenase (G6PD) catalyzes the regulated step in the pentose phosphate pathway. Which of the following statements concerning G6PD and the pentose phosphate pathway is correct?

A. Deficiency of G6PD occurs only in red blood cells.

B. Deficiency of G6PD results in an inability to keep glutathione in its functional, reduced form.

C. The pentose phosphate pathway includes one reversible reductive reaction followed by a series of phosphorylated sugar interconversions.

D. The reduced nicotinamide adenine dinucleotide phosphate (NADPH) product of the pentose phosphate pathway is utilized in processes such as fatty acid oxidation.

Q2. When received, the results of the blood count 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 can cause deposition of bilirubin in the skin and sclerae resulting in jaundice.

B. The solubility of bilirubin is increased by conjugating it with 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: BJ is a 22-year-old male who presents for follow-up 10 days after having been treated in the Emergency Department (ED) for severe inflammation at the base of his thumb.

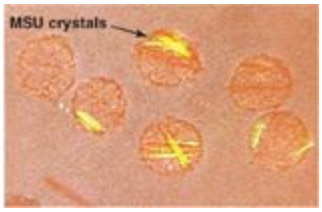
Focused History: This was BJ's first occurrence of severe joint pain. In the ED, he was given an anti-inflammatory medication. Fluid aspirated from the carpometacarpal joint of the thumb was negative for organisms but positive for needle-shaped monosodium urate (MSU) crystals (see Image). The inflammatory symptoms have since resolved. BJ 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 reveal that BJ is not an undersecretor of uric acid. 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: Gout (MSU crystal deposition disease), a type of inflammatory arthritis

Treatment: BJ was given prescriptions for allopurinol and colchicine. The treatment goals are to reduce his blood urate levels to <6.0 mg/dl and 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 Michaelis constant (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. Purines are one type of nitrogenous base found in nucleotides. Pyrimidines are the other. 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. BJ 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: MW is a 48-hour-old female who has not yet had a bowel movement.

Focused History: MW is the full-term product of a normal pregnancy and delivery. She appeared normal at birth. MW is the first child of parents of Northern European ethnicity. The parents are both in good health, and their family histories are unremarkable.

Pertinent Findings: MW 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 CF was subsequently confirmed with a chloride sweat test.

Treatment: The ileus was successfully treated nonsurgically. For management of the CF, 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.

CASE-RELATED QUESTIONS: Choose the ONE best answer.

Q1. CF is the result of mutations to the gene that codes for the cystic fibrosis transmembrane conductance regulator (CFTR) protein that functions as a chloride channel in the apical membrane of epithelial cells on a mucosal surface. 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 mucous on the epithelial surface to be excessively thick and sticky.

B. Excessive pancreatic secretion of insulin commonly results in hypoglycemia.

C. Genetic testing for CF may involve the use of a set of probes for the most common mutations, a technique known as restriction fragment length polymorphism analysis.

D. Some mutations result in premature degradation of the CFTR protein through tagging with ubiquinone followed by proteasome-mediated proteolysis.

E. The most common mutation, ΔF508, results in the loss of a codon for phenylalanine 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 (that is, 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: RL is a 40-hour-old male with signs of cerebral edema.

Focused History: RL is the full-term product of a normal pregnancy and delivery. He appeared normal at birth. At age 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 level, and decreased blood urea nitrogen level were found. An amino acid screen revealed that argininosuccinate was increased more than 60-fold over baseline, and citrulline was increased 4-fold. Glutamine was elevated, and arginine was decreased relative to normal.

Diagnosis: 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 arginine. Long-term treatment will include lifelong limitation of dietary protein; supplementation with essential amino acids; and administration of arginine, 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 arginine 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: CR is a 19-year-old female who is being evaluated for pain and swelling in her right calf.

Focused History: Ten days ago, CR 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. CR is no longer taking pain medication but has continued her oral contraceptives.

Pertinent Findings: CR's 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: CR has a deep venous thrombosis (DVT). Oral contraceptives are a risk factor for DVT, as are surgery and immobilization.

Treatment (Immediate): Heparin and warfarin are administered.

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. A deep venous thrombosis is a blood clot that occludes the lumen of a deep vein, most commonly in the leg. Which of the following statements about the clotting cascade is correct?

A. A deficiency in factor (F)IX of the intrinsic pathway results in hemophilia A.

B. FIII of the extrinsic pathway is a serine protease.

C. Formation of the fibrin meshwork is referred to as primary hemostasis.

D. Thrombin proteolytically activates components of the extrinsic, intrinsic, and common pathways.

E. Vitamin K is required for the activation of fibrinogen.

Q2. 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. Hypoprothrobinemia

E. von Willebrand disease

Q3. Compare and contrast the actions of heparin and warfarin.

IV. FOCUSED CASES: ANSWERS TO CASE-BASED QUESTIONS

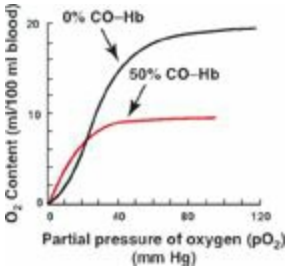
CASE 1: Anemia with β-Thalassemia Minor

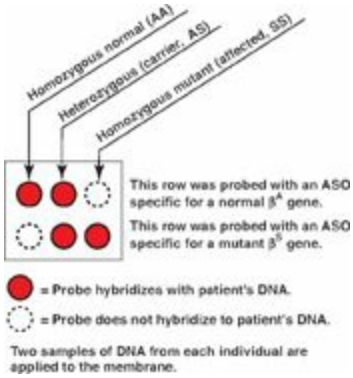
Q1. Answer = B. Transcription (synthesis of single-stranded RNA from the template strand of double-stranded DNA) requires the binding of proteins (trans-acting factors) to sequences on the DNA (cis-acting elements). Eukaryotic messenger RNA (mRNA) is monocistronic because it contains information from 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′ 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 (see Figure). 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 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.

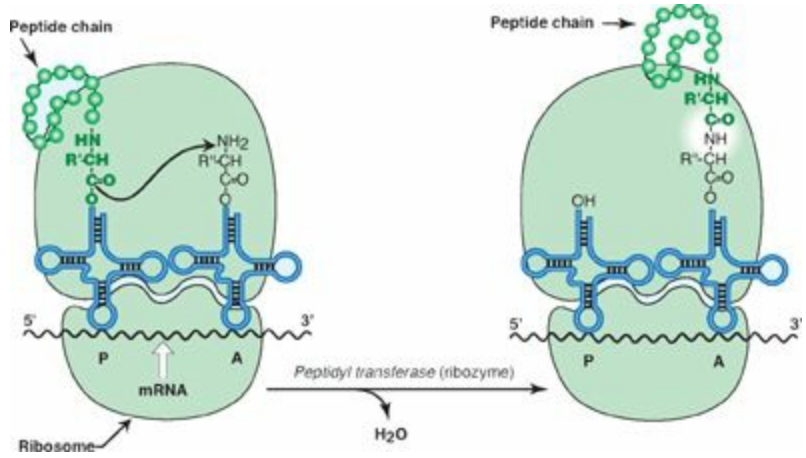
Q4. Sickle cell anemia is caused by a single point mutation (A→T) in the gene for β globin that results in the replacement of glutamate by valine at the sixth amino acid position in the protein. Mutational analysis using allele-specific oligonucleotide (ASO) probes for that mutation (β^S) and for the normal sequence (β^A) is used in diagnosis (see Figure). 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 (for example, those of Greek ancestry). However, less common mutations are often not included in the panel and can be detected only by DNA sequencing.




Homozygous normal (AA)
Heterozygous (carrier, AS)
Homozygous mutant (affected, SS)
This row was probed with an ASO specific for a normal β^A gene.
This row was probed with an ASO specific for a mutant β^S gene.
= Probe hybridizes with patient's DNA.
= Probe does not hybridize to patient's DNA.
Two samples of DNA from each individual are applied to the membrane.

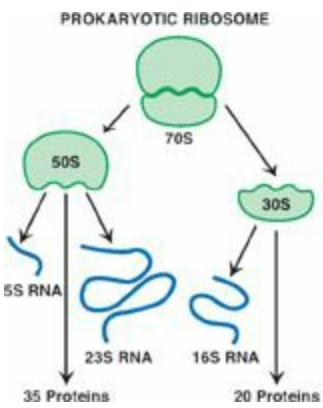
CASE 2: Skin Rash with Lyme Disease

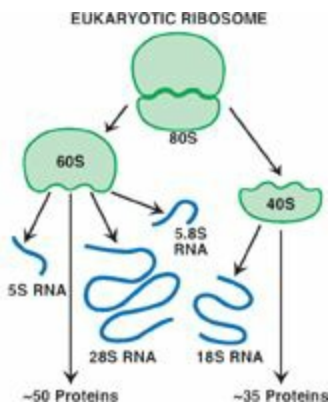
Q1. Answer = C. Peptide bond formation between the amino acid in the A site of the ribosome and the amino acid last added to the growing peptide in the P site is catalyzed by an RNA of the large ribosomal subunit. Any RNA with catalytic activity is referred to as a ribozyme (see Figure below). Formylated methionine is used to initiate prokaryotic translation. The charged initiating transfer RNA (tRNA) 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 (SD) 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 ribosomal subunit (30S) 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 (see Figure). Their small (40S) subunit contains 18S rRNA, which does not bind tetracycline.


PROKARYOTIC RIBOSOME
70S
50S
30S
5S RNA
23S RNA
16S RNA
35 Proteins
20 Proteins

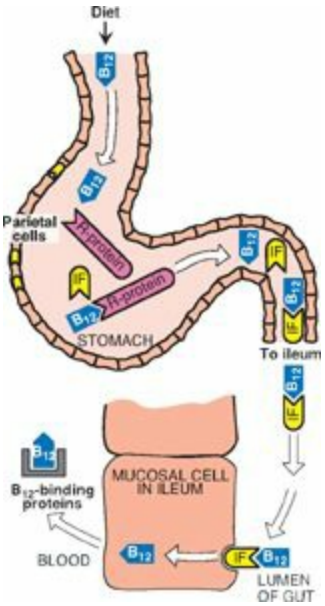

EUKARYOTIC RIBOSOME
80S
60S
40S
5.8S RNA
5S RNA
28S RNA
18S RNA
~50 Proteins
~35 Proteins

CASE 3: Blood on the Toothbrush with Vitamin C Deficiency

Q1. Answer = C. Vitamin C (ascorbic acid) functions as a coenzyme in the hydrolylation of proline and lysine in the synthesis of collagen, a fibrous protein of the extracellular matrix. Vitamin C reduces dietary iron from the ferric (Fe^{3+}) to the ferrous (Fe^{2+}) form that is required for absorption. 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). 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 required to reduce 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 (PLP) that is the coenzyme for most reactions involving amino acids. (Note that tetrahydrobioptrin is required by aromatic amino acid hydroxylases.)

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 and RBC number is decreased.

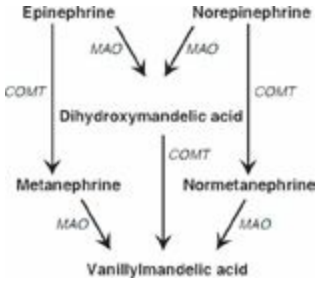


CASE 4: Rapid Heart Rate, Headache, and Sweating with a Pheochromocytoma

Q1. Answer = D. Degradation of both epinephrine and norepinephrine involves methylation by catechol-O-methytransferase (COMT) that produces normetanephrine from norepinephrine and metanephrine from epinephrine (see Figure). 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 that most carboxylases require biotin.) Norepinephrine is converted to epinephrine by methylation, and S-adenosylmethionine provides the methyl group.

Q2. Answer = A. Norepinephrine 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 (for example, glucose and triacylglycerols). These actions are mediated by the binding of norepinephrine 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.

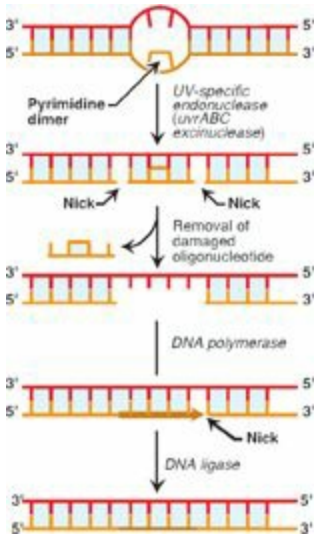
Q3. Septic shock is vasodilatory hypotension (low blood pressure caused by blood vessel dilation) resulting from the production of large amounts of nitric oxide by inducible nitric oxide synthase (iNOS) in response to infection. Norepinephrine bound to receptors on smooth muscle cells causes vasoconstriction and, thus, raises blood pressure.



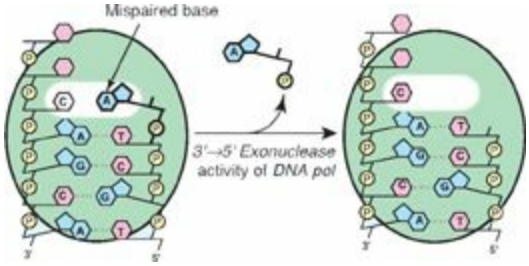
CASE 5: Sun Sensitivity with Xeroderma Pigmentosum

Q1. Answer = D. Pyrimidine dimers are the characteristic DNA lesion 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. (See Figure 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 method of double-strand break repair is much less prone to error than is the nonhomologous–end-joining method because any DNA that was lost is replaced. Mismatched-base repair 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, 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 (pols) cannot initiate DNA synthesis. The chromatin of eukaryotes gets decondensed (relaxed) for replication. Relaxation can be accomplished, for example, by acetylation via histone acetyltransferases (HATs). 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 (for example, DnaA in prokaryotes). Deoxynucleoside monophosphates (dNMPs) are joined by a phosphodiester bond that links the 3-hydroxyl group of the last dNMP added with the 5-phosphate group of the incoming nucleotide, thereby forming a 3′→5′ phosphodiester bond as pyrophosphate is released.

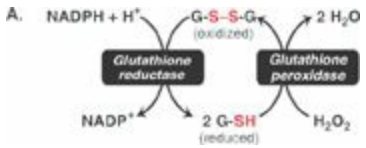


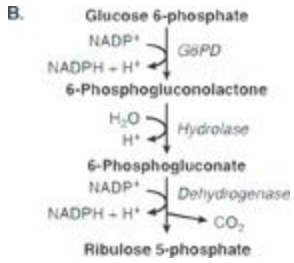
Q3. Proofreading occurs during replication in the S phase of the cell cycle and involves the 3′→5′ exonuclease activity possessed by some DNA pols (see Figure). Repair can occur independently of replication and, therefore, can be performed outside of the S phase.


Mispaired base
3'→5' Exonuclease
activity of DNA pol

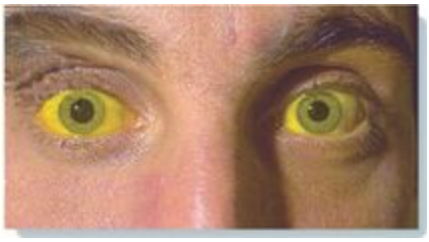
CASE 6: Dark Urine and Yellow Sclerae with Glucose 6-Phosphate Dehydrogenase Deficiency

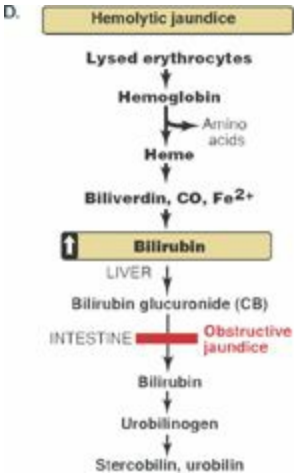
Q1. Answer = B. Glutathione in its reduced form (G-SH) is an important antioxidant. The enzyme glutathione peroxidase reduces hydrogen peroxide (a reactive oxygen species) to water as glutathionine is oxidized (G-S-S-G). Reduced nicotinamide adenine dinucleotide phosphate (NADPH)-requiring glutathionine reductase regenerates the reduced, functional form of glutathione (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.





Q2. Answer = A. Jaundice (icterus) refers to the yellow color of the skin, nail beds, and sclerae that results from the deposition of bilirubin 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 (UDP)-glucuronic acid in the liver, forming bilirubin diglucuronide or conjugated bilirubin (CB). In hemolytic conditions, such as G6PD deficiency, both conjugated 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 bilirubiin to isomeric forms that are more water soluble. Bilirubin is the product of heme degradation in cells of the reticuloendothelial system, particularly in the liver and the spleen. The porphyrias are pathologies of heme synthesis and, therefore, are not characterized by hyperbilirubinemia.

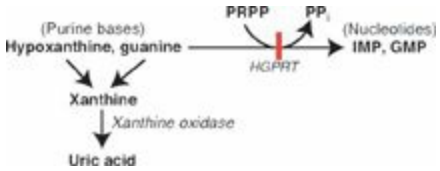
C. 

D. 

Q3. With hemolysis, more bilirubin is produced and conjugated. CB 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).

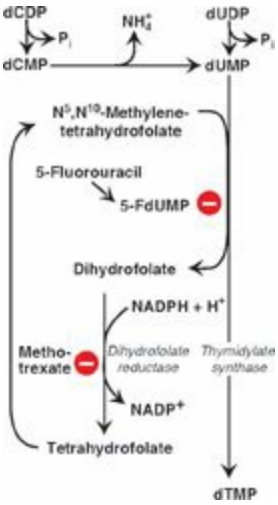
CASE 7: Joint Pain with Gout

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). 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 an 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); see Figure. 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.

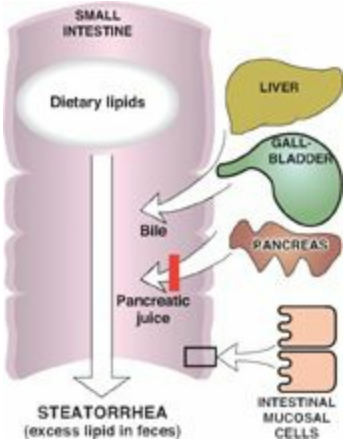

dCDP
P_i
NH_4^+
dUDP
P_i
dCMP
dUMP
N^5,N^{10}-Methylene-tetrahydrofolate
5-Fluorouracil
5-FdUMP
Dihydrofolate
NADPH + H^+
Metho-trexate
Dihydrofolate reductase
Thymidylate synthase
$NADP^+$
Tetrahydrofolate
dTMP

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. The genetic testing technique described, and one used in the diagnosis of CF, is the use of allele-specific oligonucleotides (ASOs). Some mutations do result in increased degradation of the cystic fibrosis 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. The ΔF509 mutation is caused by the loss of three nucleotides that code for phenylalanine at position 509 in the CFTR protein and, therefore, is not a frameshift mutation.

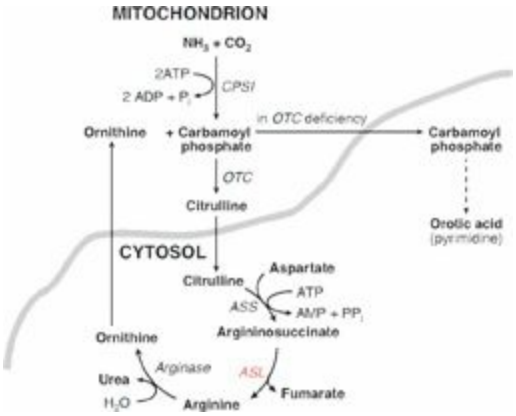
Q2. Answer = A. Targeting of proteins destined to function as components of the plasma membrane is an example of cotranslational targeting. It involves the initiation of translation on cytosolic ribosomes; the recognition of the amino (N)-terminal signal sequence in the protein; the movement of the protein-synthesizing complex to the outer face of the membrane of the endoplasmic reticulum (ER); and the continuation of protein synthesis such that the protein is threaded into the lumen of the ER, 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 posttranslationally 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, which is foul-smelling and bulky and may float (see Figure). Patients are at risk for malnutrition and deficiencies in fat-soluble vitamins. Oral supplementation of pancreatic enzymes is the treatment.



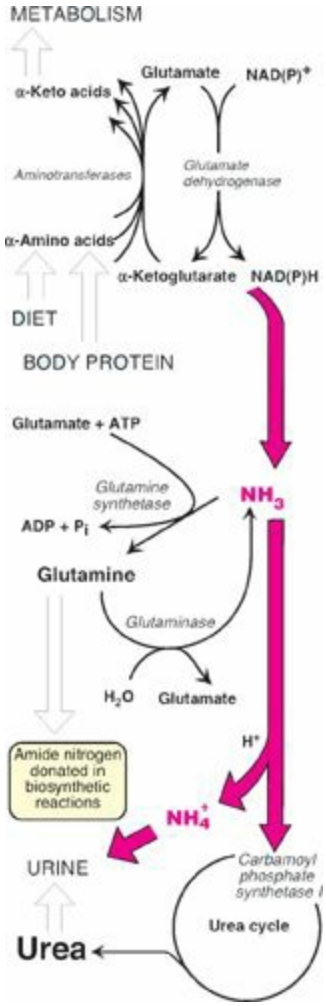
CASE 9: Hyperammonemia with a Urea Cycle Defect

Q1. Answer = B. Argininosuccinate lyase (ASL) cleaves argininosuccinate to arginine and fumarate. The increase in argininosuccinate and citrulline and the decrease in arginine seen in the patient indicate a deficiency in ASL (see Figure below). With arginase deficiency, arginine would be increased, not decreased. Additionally, with arginase deficiency, the hyperammonemia would be less severe. 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 arginine and citrulline. Deficiency of ornithine transcarbamoylase (OTC), the only X-linked enzyme of the urea cycle, would result in low levels of arginine 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. Arginine supplementation is helpful because the arginine will be hydrolyzed to urea plus ornithine by arginase. The ornithine will be combined with carbamoyl phosphate to form citrulline (see Figure above). With ASL (and ASS) deficiency, citruline accumulates and is excreted, thereby carrying waste nitrogen out of the body.

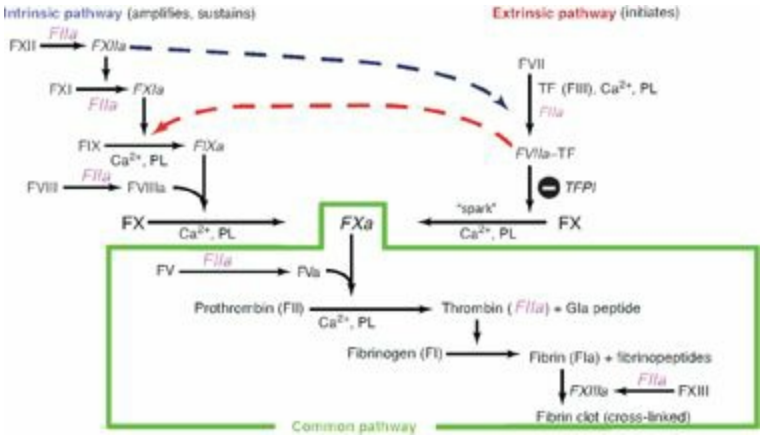
Q3. Answer = D. In individuals with milder (partial) deficiencies in the enzymes of the urea cycle, hyperammonemia may be triggered by physiologic stress (for example, an illness or prolonged fasting) that decreases the insulin-to-counterregulatory hormone ratio. [Note: The degree of the hyperammonemia 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 plus glutamate. The glutamate undergoes oxidative deamination to α-ketoglutarate and ammonia (NH_3) by glutamate dehydrogenase (GDH): see Figure. [Note: GDH is unusual in that it uses both nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) as coenzymes.]



The ammonia (toxic) can be transported to the liver as glutamine and alanine. The glutamine is generated by the amination of glutamate by adenosine triphosphate–requiring glutamine synthetase. In the liver, the enzyme glutaminase removes the ammonia, which can be converted to urea by the urea cycle (see Figure). Glutamine, then, is a nontoxic vehicle of ammonia transport in the blood. Alanine is generated in skeletal muscle from the catabolism of the branched-chain amino acids. In the liver, alanine is transaminated to pyruvate (used in gluconeogenesis) and glutamate. Thus, alanine carries nitrogen to the liver for conversion to urea. Therefore, defects in the urea cycle would result in an elevation in ammonia, glutamine, and alanine. The elevated ammonia 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 ammonia to glutamine (in the astrocytes of the brain) results in osmotic effects that cause the brain to swell. Additionally, the rise in glutamine decreases the availability of glutamate, an excitatory neurotransmitter.]

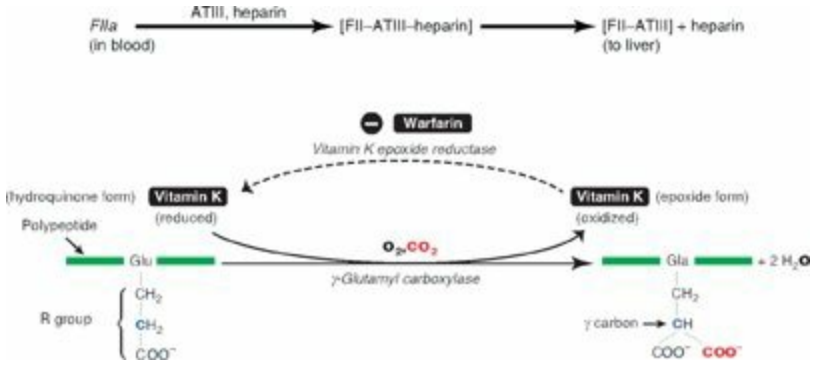
CASE 10: Swollen, Painful Calf with Deep Venous Thrombosis

Q1. Answer = D. Thrombin, a serine protease, is activated by the prothrombinase complex of factor (F)Xa + FVa. Once formed, activated thrombin proteolytically activates components of the extrinsic (FVII) and intrinsic (FXI, FVIII) pathways, generating FXa. Thrombin can also activate FV, FI, and FXIII of the common pathway (see Figure below). Hemophilia A is caused by a deficiency in FVIII. FIX deficiency results in hemophilia B. FIII, also known as tissue factor (TF), is a transmembrane glycoprotein of the vascular endothelium. It functions as an accessory protein and not a protease. Formation of the platelet plug is primary hemostasis, and formation of the fibrin meshwork is secondary hemostasis. Vitamin K is required for the activation (g-carboxylation) of FII, FVII, FIX, and FX but not for FI (fibrinogen).



Q2. Answer = C. FV Leiden is a mutant form of FV that is resistant to proteolysis by the activated protein C complex. Decreased ability to degrade FV allows continued production of activated thrombin and leads to an increased risk of clot formation or thrombophilia. Antithrombin III (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 on FVIII and platelets.

Q3. Heparin and warfarin are anticoagulants. Heparin, a glycosaminoglycan, increases the affinity of ATIII for thrombin. Binding of ATIII removes thrombin from the blood and prevents it from converting fibrinogen to fibrin. Warfarin, a synthetic analog of vitamin K, inhibits vitamin K epoxide reductase and prevents the regeneration of the functional hydroquinone form of the vitamin that is required for the γ-carboxylation of glutamate residues to γ-carboxyglutamate (Gla) residues in FII, FVII, FIX, and FX (see Figures).


FIIa
(in blood)
ATIII, heparin
[FII–ATIII–heparin]
[FII–ATIII] + heparin
(to liver)
Warfarin
Vitamin K epoxide reductase
(hydroquinone form)
Vitamin K
(reduced)
Vitamin K
(oxidized)
(epoxide form)
Polypeptide
Glu
Gla
+ 2 H2O
γ-Glutamyl carboxylase
R group
γ carbon

Index

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

Z

Figure Sources

Figure 2.12: Modified from Garrett, R. H. and Grisham, C. M. Biochemistry. Saunders College Publishing, 1995. Figure 6.36, p. 193.

Figure 2.13 (panel C, top): Abdulla, S. Basic mechanisms of protein folding disease. Nature Publishing Group.

Figure 3.1A: Illustration: Irving Geis. Rights owned by Howard Hughes Medical Institute. Not to be used without permission

Figure 3.20: Photo curtesy of Photodyne Incorporated, Hartland, WI.

Figure 3.21: Corbis

Figure 4.3: Electron micrograph of collagen: Natural Toxin Research Center. Texas A&M University-Kingsville. Collagen molecule modified from Mathews, C. K., van Holde, K. E. and Ahern, K. G. Biochemistry, 3rd Ed., Addison Wesley Longman, Inc. 2000. Figure 6.13, p.175.

Figure 4.4: modified from Yurchenco, P. D., Birk, D. E., and Mecham, R. P., eds. (1994) Extracellular Matrix Assembly and Structure, Academic Press, San Diego, California

Figure 4.8: Kronauer and Buhler, Images in Clinical Medicine, N Engl J Med, June 15, 1995, Vol. 332, No. 24, p.1611.

Figure 4.10: Photo from Web site Derma.de

Figure 4.11: Jorde, L. B., Carey, J. C., Bamshad, M. J. and White, R. L. Medical Genetics, 2nd Ed. http://medgen.genetics.utah.edu/index.htm

Question 4.3: from Berge LN, Marton V, Tranebjaerg L, Kearney MS, Kiserud T, Oian P. Prenatal diagnosis of osteogenesis imperfecta. Acta Obstetricia et Gynecologica Scandinavica. 74(4):321-3, 1995 Apr.

Figure 13.11: The Crookston Collection, University of Toronto.

Figure 17.13: Urbana Atlas of Pathology, University of Illinois College of Medicine at Urbana-Champaign. Image number 26.

Figure 17.19: Interactive Case Study Companion to Robbins Pathologic Basis of Disease.

Figure 18.12: Custom Medical Stock Photo.

Figure 20.20: Success in MRCO phth. http://www.mrcophth.com/iriscases/albinism.html

Figure 20.22 (top): Rubin, E. and Farber, J. L. Pathology, 2nd Ed. J. B. Lippinoctt. 194. Figure 6-30, p. 244.

Figure 20.22 (bottom): Gilbert-Barness, E. and Barness L. Metabolic Disease. Eaton Publishing, 2000. Figure 15, p.42.

Figure 21.6: Rich, M. W. Porphyria cutanea tarda, Postgraduate Medicine, 105: 208-214 (1999).

Figure 21.7: Department of Dermatlogy, University of Pittsburgh. http://www.upmc.edu/dermatology/MedStudentInfo/introLecture/enlarged/vespct.htm.

Figure 21.11: Custom Medical Stock Photo, Inc.

Figure 21.14: Phototake.

Figure 22.16: Wuthrich, D. A., and Lebowitz. Tophaceous gout. Images in clinical Medicine. N Engl J Med, 332:646, 1995.

Figure 22.18: WebMD Inc. http://www.samed.com/sam/forms/index.htm

Figure 23.2: Childs, G. http://www.cytochemistry.net/.

Figure 23.13: Modified from Cryer, P. E., Fisher, J. N. and Shamoon, H. Hypoglycemia. Diabetes Care 17:734-753, 1994.

Figure 24.5: Phototake.

Figure 26.5: Corbis.

Figure 26.6: Gibson W, et al. The Journal of Clinical Endocrinology & Metabolism 89 (10):4821, 2004.

Figure 27.22 A + B: Centers for Disease Control and Prevention Public Health Image Library, Atlanta, Georgia, USA.

Figure 28.4: Matthews, J. H. Queen's University Department of Medicine, Division of Hematology/Oncology, Kingston, Canada.

Figure 29.7: Nolan, J., Department of Biochemistry, Tulane University, New Orleans, LA.

Figure 34.10: Foerster J, Lee G, Lukens J, et al. Wintrobe's Clinical Hematology, 10th Ed. Philadelphia. Lippincott Williams & Wilkins, 1998.

Figure 34.20: Cohen BJ, Taylor JJ. Memmler's The Human Body in Health and Disease, 10th Ed. Baltimore. Lippincott Williams & Wilkins, 2005.

Appendix p. 489: Gold DH, MD, and Weingeist TA, MD, PhD. Color Atlas of the Eye in Systemic Disease. Baltimore. Lippincott Williams & Wilkins, 2001.

Appendix p. 506: Goodheart HP, MD. Goodheart's Photographs of Common Skin Disorders, 2nd Ed. Philadelphia. Lippincott Williams & Wilkins, 2003.

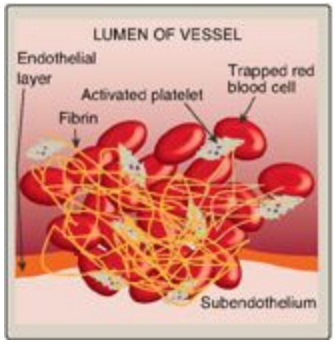
Appendix p. 510: Thomas H. McConnell. The Nature of Disease Pathology for the Health Professions. Philadelphia. Lippincott Williams & Wilkins, 2007.

Blood clotting 34

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 and a meshwork of the protein fibrin that stabilizes the platelet plug. Clotting occurs in association with membranes on the surface of platelets and damaged blood vessels (Figure 34.1). [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.]

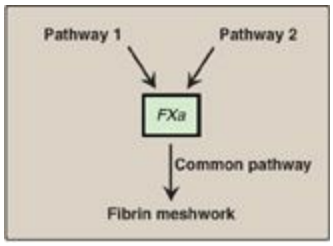
Figure 34.1 A blood clot formed by a plug of activated platelets and a meshwork of fibrin at the site of vessel injury.



II. FIBRIN MESHWORK FORMATION

The formation of the fibrin meshwork involves two unique pathways that converge to form a common pathway (Figure 34.2). In each pathway, the major components are proteins (called factors) designated by Roman numerals. The factors are glycoproteins that are synthesized and secreted by the liver, primarily. [Note: Several factors are also denoted by alternative names. For example, active factor X (FXa), the point of pathway convergence, is also known as Stuart factor.]

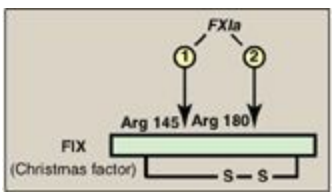
Figure 34.2 Three pathways involved in formation of the fibrin meshwork.



A. Protolytic cascade

Within the pathways, a cascade is set up in which proteins are converted from an inactive form, or zymogen, to an active form by proteolytic cleavage in which the protein product of one activation reaction initiates another. The active form of a factor is denoted by a lower case "a" after the numeral. The active proteins FIIa, FVIIa, FIXa, FXa, FXIa, and FXIIa are enzymes that function as serine proteases with trypsin-like specificity and, therefore, cleave a peptide bond on the carboxyl side of an arginine or lysine residue in a polypeptide. For example, FIX (Christmas factor) is activated through cleavage at arginine 145 and arginine 180 by FXIa (Figure 34.3). 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. [Note: Nonproteolytic proteins play a role as accessory proteins (cofactors) in the pathways. FIII, FV, and FVIII are the accessory proteins.]

Figure 34.3 Activation of FIX (Christmas factor) via proteolysis by the serine protease FXIa. [Note: Activation can occur by conformational change for some of the factors.] a = active; Arg = arginine.



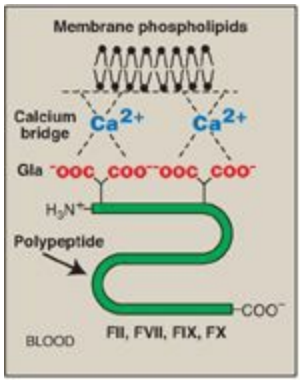
B. Role of phosphatidylserine and calcium

The presence of the negatively charged phospholipid phosphatidylserine (PS) and positively charged calcium ions (Ca^{2+}) accelerates the rate of some steps in the cascade.

1. **Negatively charged 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 certain clotting serine proteases (FII, FVII, FIX, and FX), facilitating the binding of these proteins to exposed phospholipids (Figure 34.4). The Gla residues are good chelators of Ca^{2+} because of their two adjacent negatively charged carboxylate groups (Figure 34.5). [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 34.4 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 9–12 glutamate residues (at the amino or N terminus of the target protein) get carboxylated at the γ carbon, thereby forming γ-carboxyglutamate (Gla) residues. The process occurs in the rough endoplasmic reticulum (RER) of the liver.

1. **γ-Carboxylation:** This carboxylation reaction requires a protein substrate, O_2, CO_2, γ-glutamyl carboxylase, and the hydroquinone form of vitamin K as a coenzyme (Figure 34.6). In the reaction, the hydroquinone form of vitamin K gets oxidized to its epoxide form as O_2 is reduced to water. [Note: Vitamin K, a fat-soluble vitamin (see p. 389), is reduced from the quinone form to the hydroquinone coenzyme form by vitamin K reductase (Figure 34.7).]

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 complex 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 is an anticoagulant that inhibits clotting by functioning as a vitamin K antagonist. Warfarin salts are used therapeutically to limit clot formation. [Note: Warfarin is used commercially as a pest-control agent such as in rat poison. It was developed by the Wisconsin Alumni Research Foundation, hence the name.]

Figure 34.5 Gla residue.

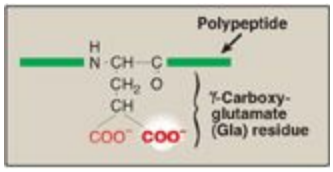
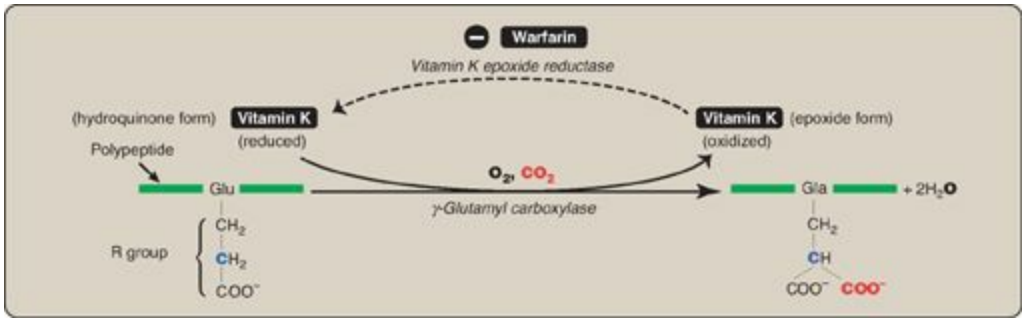


Figure 34.6 γ-Carboxylation of a glutamate (Glu) residue to γ-carboxyglutamate (Gla) by vitamin K-requiring γ-glutamyl carboxylase. The γ carbon is shown in blue.



Genetic differences (genotypes) in the gene for subunit 1 of the VKOR complex (VKORC1) influence patient response to warfarin. For example, a polymorphism 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 (package insert). The influence of genetics on an individual's response to drugs is known as pharmacogenetics.

D. Pathways

Three distinct pathways are involved in formation of the fibrin meshwork: the extrinsic pathway, the intrinsic pathway, and the common pathway. Production of FXa by the extrinsic and intrinsic pathways initiates the common pathway (see Figure 34.2).

1. **Extrinsic pathway:** This pathway involves a protein, tissue factor (TF), that is not

in the blood but becomes exposed when blood vessels get injured. TF (FIII) is a transmembrane glycoprotein abundant in vascular subendothelium. It is an extravascular accessory protein and not a protease. Any injury that exposes FIII 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 can also be activated proteolytically by thrombin (see Section 3. below).] Binding of FVII to TF requires the presence of Ca^{2+} and phospholipids. The TF–FVIIa complex then binds and activates FX by proteolysis (Figure 34.8). Therefore, activation of FX by the extrinsic pathway occurs in association with the membrane. The extrinsic pathway is quickly inactivated by tissue factor pathway inhibitor (TFPI) that, in a FXa-dependent process, binds to the TF–FVIIa complex and prevents further production of FXa. [Note: TF and FVII are unique to the extrinsic pathway.]

Figure 34.7 The vitamin K cycle. VKOR = vitamin K epoxide reductase.

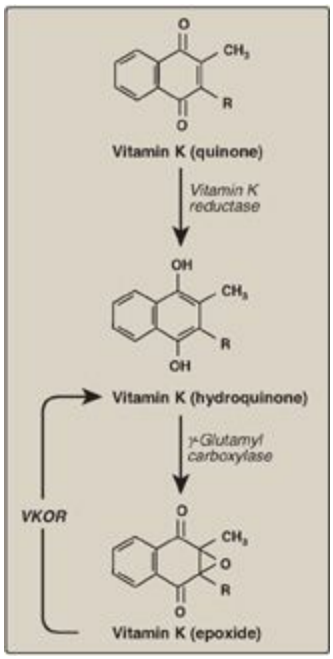
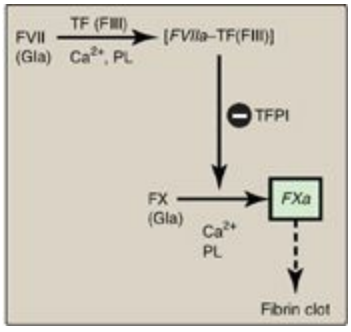


Figure 34.8 The extrinsic or tissue factor (TF) pathway. Binding of FVII to exposed TF (FIII) activates FVII. [Note: The pathway is quickly inhibited by tissue factor pathway inhibitor (TFPI).] F = factor; Gla = γ-carboxyglutamate; PL = phospholipid; a = active.



2. Intrinsic pathway: All of the protein factors involved in the intrinsic pathway are

present in the blood and are, therefore, intravascular. The intrinsic pathway involves two phases: the contact phase and the FX-activation phase, each with known deficiencies.

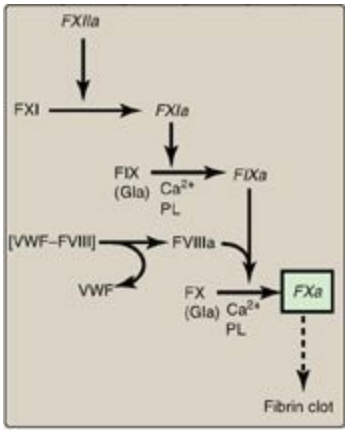
a. Contact phase: This phase results in the activation of FXII (Hageman factor) to FXIIa by conformational change through binding to a negative surface. Deficiencies in FXII (or in the other proteins of this phase, high-molecular-weight kininogen and prekallikrein) do not result in bleeding problems, calling into question the importance of this phase in coagulation. However, the contact phase does play a role in inflammation. [Note: FXII can be activated proteolytically by thrombin (see Section 3. below)].

b. Factor X–activation phase: The sequence of events leading to the activation of Factor X to FXa by the intrinsic pathway is initiated by FXIIa (Figure 34.9). FXIIa activates FXI, and FXIa activates FIX, a Gla-containing protein. FIXa combines with FVIIIa (a bloodborne accessory [nonenzymatic] protein), and the complex activates FX, a Gla-containing serine protease. [Note: The complex containing FIXa, FVIIIa, and FX forms on exposed negatively charged membrane regions, and FX gets activated to FXa. This complex is sometimes referred to as Xase. Binding of the complex to membrane phospholipids requires Ca^{2+}.]

c. Factor XII deficiency: A deficiency in FXII does not lead to a bleeding disorder. This is because FXI, the next protein in the cascade, can be activated proteolytically by thrombin (see Section 3. below).

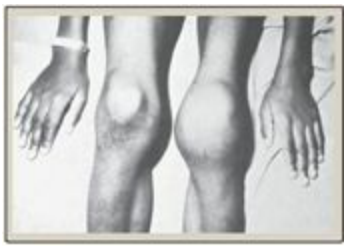
d. Hemophilia: Hemophilia is a coagulopathy, a defect in the ability to clot. Hemophilia A (the most common form of hemophilia) results from deficiency of FVIII, whereas deficiency of FIX results in hemophilia B. Each deficiency is characterized by decreased and delayed ability to clot and/or formation of abnormally friable (easily disrupted) clots. This can be manifested, for example, by bleeding into the joints (Figure 34.10). The extent of the factor deficiency determines the severity of the disease. Current treatment for hemophilia is factor replacement therapy using factors obtained from pooled human blood or from recombinant DNA technology. Gene replacement 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 34.9 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.



> 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.

Figure 34.10 Acute bleeding into joint spaces (hemarthrosis) in an individual with hemophilia.

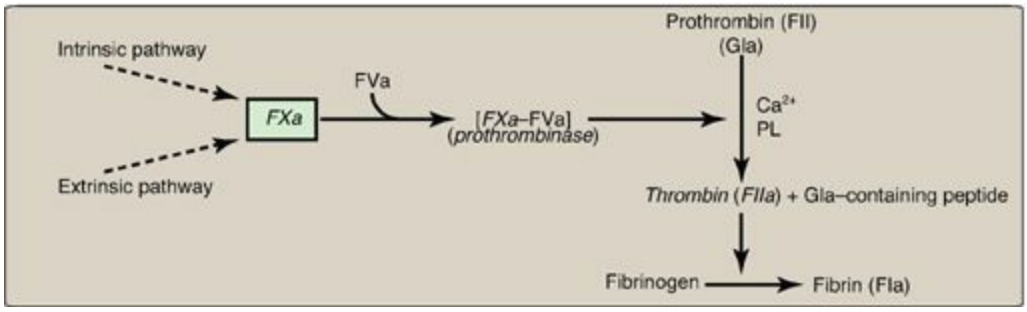


3. **Common pathway:** FXa produced by both the intrinsic and the extrinsic paths initiates the common pathway, a sequence of events that results in the generation of fibrin (FIa) (Figure 34.11) . FXa associates with FVa (a bloodborne accessory [nonenzymic] protein) and, in the presence of Ca^{2+} and 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 thrombin. Cleavage excises the Gla-containing region, releasing thrombin 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 limited clinical use as anticoagulants.

> 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

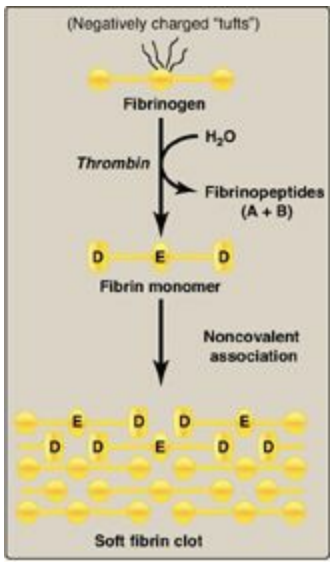
prothrombin leads to increased levels of prothrombin in the blood. This results in thrombophilia, a condition characterized by an increased tendency to clot.

Figure 34.11 Generation of fibrin by FXa and the common pathway. F = factor; Gla = γ-carboxyglutamate; PL = phospholipid; a = active.



a. Conversion of fibrinogen to fibrin by thrombin: Fibrinogen is a soluble glycoprotein made by the liver. It consists of dimers of three different polypeptide chains [$(A\alpha)_2(B\beta)_2(\gamma)_2$] held together at the N termini by disulfide bonds. [Note: Aα and Bβ each represent a single polypeptide.] The N termini of the Aα and Bβ chains form "tufts" on the central of three globular domains (Figure 34.12). The tufts are negatively charged and result in repulsion between fibrinogen molecules. Thrombin cleaves the charged tufts (releasing fibrinopeptides A and B), and fibrinogen becomes 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.

Figure 34.12 Conversion of fibrinogen to fibrin and formation of the soft fibrin clot. [Note: D and E refer to domains on fibrin.]



b. Cross-linking of fibrin: The associated fibrin molecules get covalently cross-linked. This converts 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 (Figure 34.13). [Note: FXIII is also activated by thrombin.]

c. **Importance of thrombin:** The activation of FX by the extrinsic path provides the "spark" of FXa that results in the initial activation of thrombin. Active thrombin then activates factors of the common (FV, FI, FXIII), intrinsic (FXI, FVIII), and extrinsic (FVII) pathways (Figure 34.14). It also activates FXII of the contact phase. 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, oral thrombin inhibitor. Recombinant hirudin has been approved for limited clinical use.] Additional crosstalk 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 34.15. The factors of the clotting cascade are shown organized by function in Figure 34.16.

Figure 34.13 Cross-linking of fibrin. FXIIIa forms a covalent isopeptide bond between a lysine residue and a glutamine residue. F = factor.

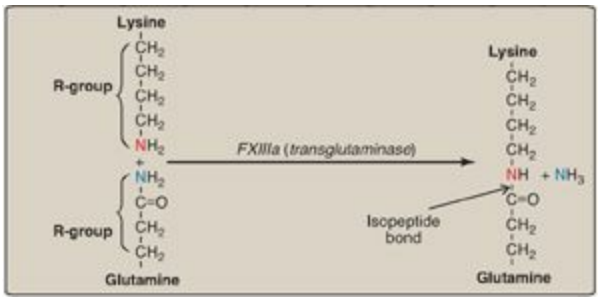


Figure 34.14 The importance of thrombin in formation of the fibrin clot. a = active; F = factor.

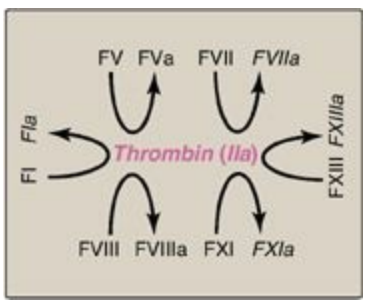
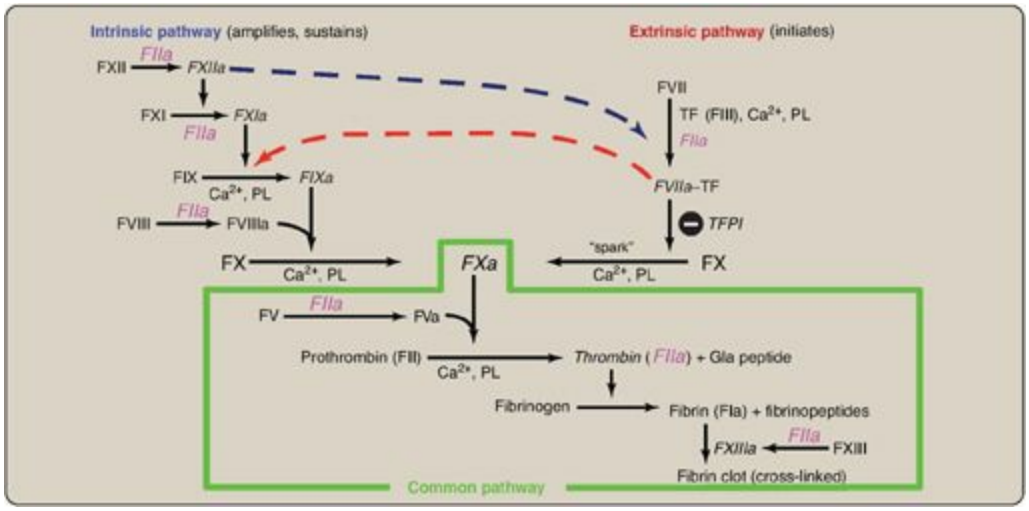


Figure 34.15 The complete picture of physiologic blood clotting via the formation of a cross-linked (hard) fibrin clot. a = active; F = factor; TF = tissue factor; TFPI = tissue factor pathway inhibitor; PL = phospholipid; Gla = γ-carboxyglutamate.



Clinical laboratory tests are available to evaluate 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 isn't needed to activate the intrinsic pathway.

Figure 34.16 Protein factors of the clotting cascade organized by function. The activated form would be denoted by an a after the numeral. [Note: Ca^{2+} is IV. There is no VI. I (fibrin) is neither a protease nor an accesory protein. XIII is a transglutaminase.] Gla = γ-carboxyglutamate.

Serine proteases	II, VII, IX, X, XI, XII
Gla-containing *proteases*	II, VII, IX, X
Accessory proteins	III, V, VIII

III. LIMITING CLOTTING

The ability to limit clotting to areas of damage (anticoagulation) and to 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 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.

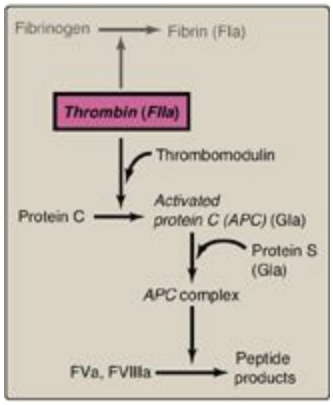
Figure 34.17 Inactivation of FIIa (thrombin) by binding of antithrombin III (ATIII) and transport to the liver. [Note: Heparin increases the affinity of ATIII for FIIa.] a = active; F = factor.

1. **Antithrombin:** Antithrombin III (ATIII), also referred to simply 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 (Figure 34.17). Thus, ATIII removes thrombin from the blood, 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. a_1-Antitrypsin (see p. 50) is also a serpin.] The affinity of ATIII for thrombin is greatly increased when ATIII is bound to heparin, an intracellular glycosaminoglycan 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, a long half-life, and is administered orally, heparin has a rapid onset, a short half-life, and requires intravenous administration. The two drugs are commonly used in an overlapping manner in the treatment of thrombosis.] ATIII also inactivates FXa and the other serine proteases of clotting, FIXa, FXIa, FXIIa, and the FVIIa–TF complex. [Note: AT 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.]

2. **Protein C–protein S complex:** Protein C, a circulating Gla-containing protein made in the liver, is activated by thrombin complexed with thrombomodulin.

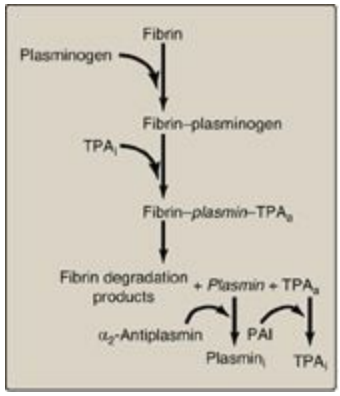
Thrombomodulin, an integral membrane glycoprotein of endothelial cells, binds thrombin, thereby decreasing thrombin's affinity for fibrinogen and increasing its affinity for protein C. Protein C in complex with protein S, also a Gla-containing protein, forms the activated protein C (APC) complex that cleaves the accessory proteins FVa and FVIIIa that are required for maximal activity of FXa (Figure 34.18). 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 (glutamine is substituted for arginine at position 506) that is resistant to APC. It is the most common inherited cause of thrombophilia in the United States, with highest frequency in the Caucasian population. Heterozygotes 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 34.18 Formation and action of the APC complex. Gla = γ-carboxyglutamate; a = active; F = factor.



Thrombophilia (hypercoagulability) can result from deficiencies of proteins C, S, and ATIII; from the presence of FV Leiden and antiphospholipid antibodies; and from excess production of prothrombin (G20210A mutation). [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.]

Figure 34.19 Fibrinolysis. TPA = tissue plasminogen activator; i = inactive; a = active; PAI = plasminogen activator inhibitor. [Note: Plasmin bound to fibrin is protected from its inhibitor.]

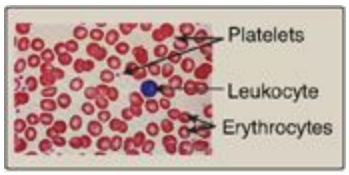


B. Fibrinolysis

Clots are temporary patches that must be removed once wound repair has begun. The fibrin clot is cleaved by the protein plasmin to fibrin-degradation products (Figure 34.19). [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 (see Figure 34.12).] 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 are protected from their inhibitors, α_2-antiplasmin (a serpin) and plasminogen activator inhibitors, respectively. Once the fibrin clot is dissolved, plasmin and TPA become available to their inhibitors. Therapeutic fibrinolysis in patients with an MI or an ischemic stroke can be achieved by treatment with commercially available TPA made by recombinant DNA techniques. [Note: Urokinase is a plasminogen activator (u-PA) made in a variety of tissues and originally isolated from urine. Streptokinase (from bacteria) also activates plasminogen and works on both free and fibrin-bound plasminogen.]

> Plasminogen contains structural motifs known as kringle domains that mediate protein–protein interactions. Lipoprotein(a) [Lp(a)] also contains kringle domains and, thus, competes with plasminogen for binding to fibrin. The potential to inhibit fibrinolysis may be the basis for the association of elevated Lp(a) with increased risk for cardiovascular disease (see p. 236).

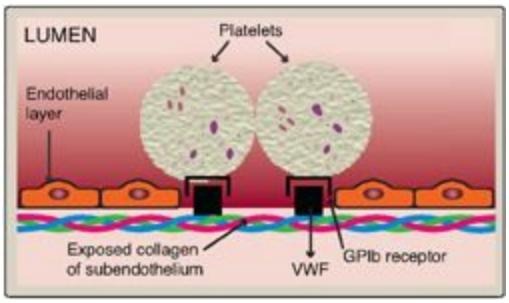
Figure 34.20 Size comparison of platelets, erythrocytes, and a leukocyte.



IV. 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 (Figure 34.20). Formation of the platelet plug is referred to as primary hemostasis. In a normal adult there are 150,000–450,000 platelets per µl of blood. They have a lifespan 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.

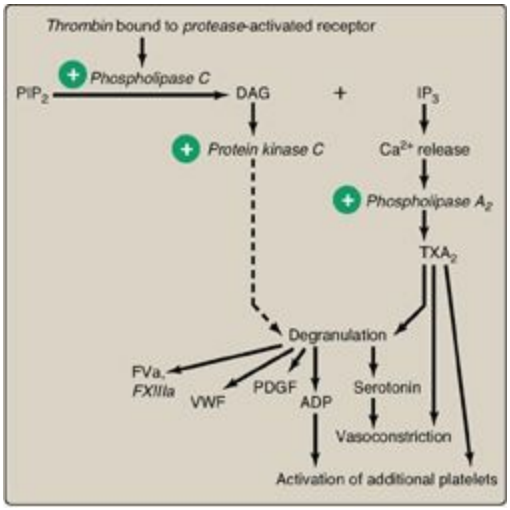
Figure 34.21 Binding of platelets via the receptor glycoprotein Ib (GPIb) to von Willebrand factor (VWF). VWF is bound to the exposed collagen at a site of injury.



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 (Figure 34.21). 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 function and number.] 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 defect in the ability to clot (coagulopathy). VWD coagulopathy 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 GPVI. Once adhered, platelets get activated. [Note: Damage to the endothelium also exposes FIII, initiating the extrinsic pathway of blood clotting and activation of FX (see Figure 34.8).]

Figure 34.22 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;TXA_2 = thromboxane A_2; ADP = adenosine diphosphate; PDGF = platelet-derived growth factor; VWF = von Willebrand factor; F = factor.



B. Activation

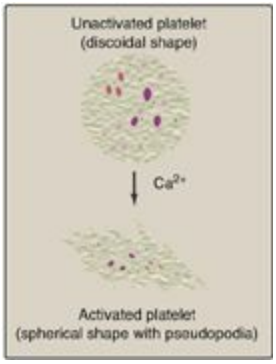
Once adhered to areas of injury, platelets get activated. Platelet activation involves morphologic (shape) changes and degranulation, the process by which platelets secrete the contents of their α and δ (or dense) storage granules. Activated platelets also expose PS on their surface. Thrombin is the 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 (Figure 34.22) . Thrombin is primarily associated with G_q proteins (see p. 205), 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 Figure 34.18).]

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. 214). TXA_2 causes vasoconstriction, augments degranulation, and binds to platelet GPCRs, 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 GPCRs 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. 202) synthesized by a variety of cell types including endothelial cells and platelets, binds PAF receptors (GPCRs) on the surface of platelets and activates them.]

2. **Shape change:** The change in shape of activated platelets from discoidal to spherical with pseudopod-like processes that facilitate platelet–platelet and platelet–

surface interactions (Figure 34.23) is initiated by the release of Ca^{2+} from dense granules. Ca^{2+} bound to calmodulin mediates the activation of myosin light-chain kinase that phosphorylates the myosin light chain (see p. 151), resulting in a major reorganization of the platelet cytoskeleton.

Figure 34.23 Activated platelets undergo Ca^{2+}-initiated shape change.

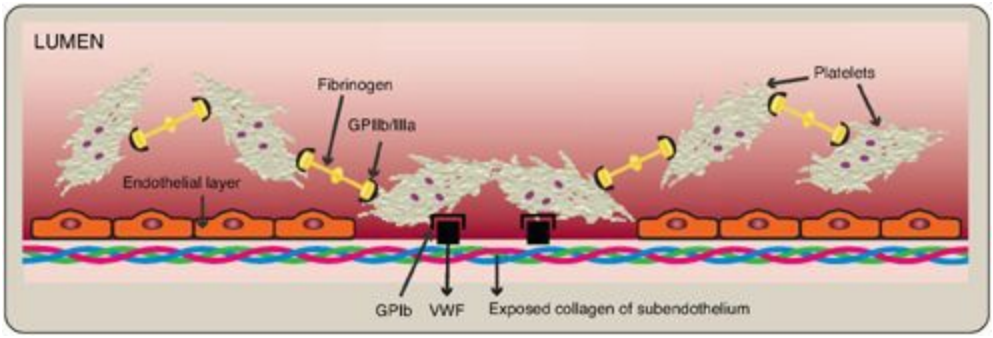


C. Aggregation

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 (Figure 34.24), with a single fibrinogen able to bind two platelets. The 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 thrombin.] Fibrin 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).]

> 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 AMP.

Figure 34.24 Linking of platelets by fibrinogen via the receptor glycoprotein (GP) IIb/IIIa. [Note: The shapes in the fibrinogen molecule represent the two D and one E domains.] GPIb = glycoprotein Ib receptor; VWF = von Willebrand factor.


LUMEN
Fibrinogen
GPIIb/IIIa
Platelets
Endothelial layer
GPIb
VWF
Exposed collagen of subendothelium

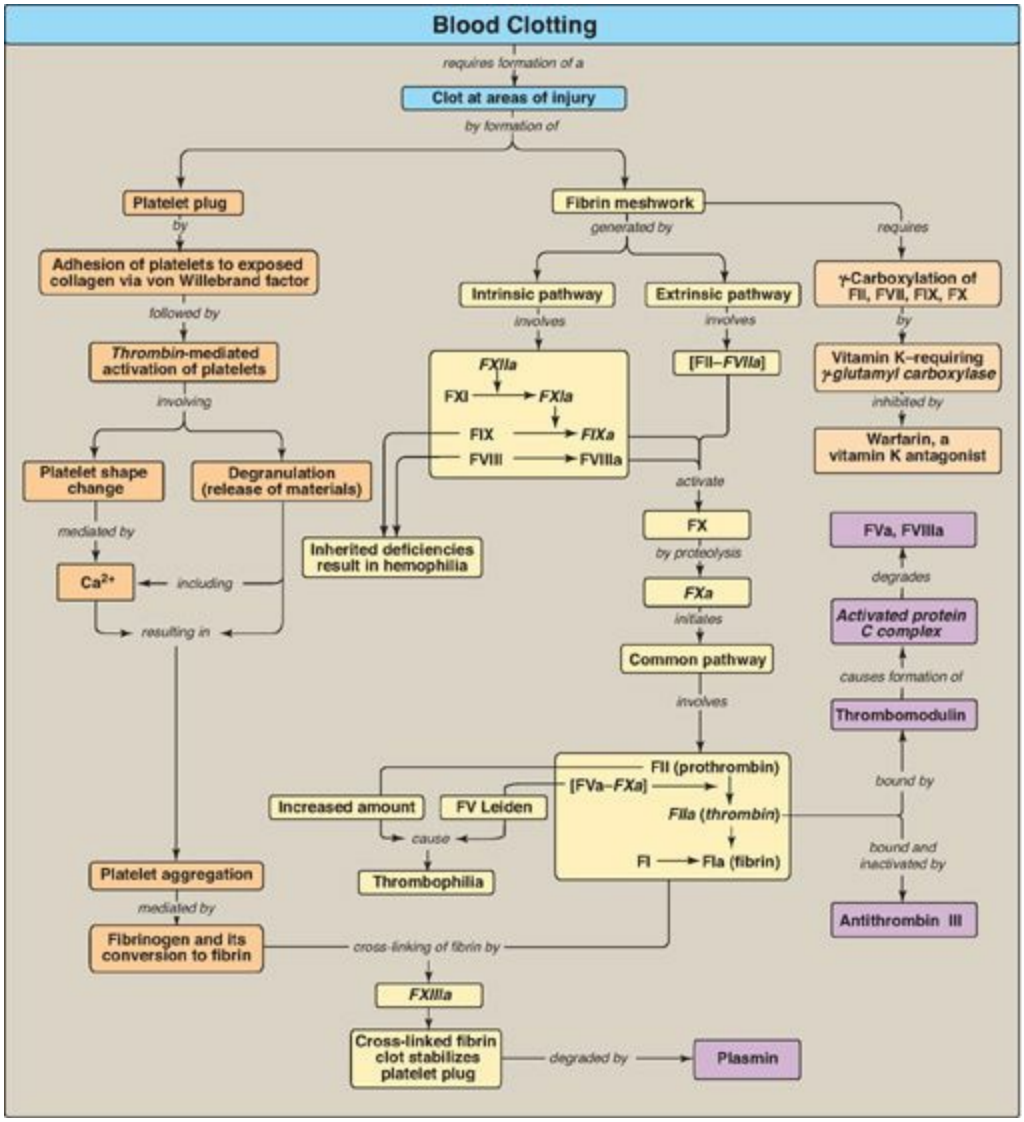
V. CHAPTER SUMMARY

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 formation of a **clot** (**thrombus**) consisting of a plug of **platelets** (**thrombocytes**) and a meshwork of the protein **fibrin** (Figure 34.25). Wound to a tissue damages blood vessels and exposes collagen. Platelets **adhere** to the **exposed collagen**, get **activated**, and **aggregate** to form a **platelet plug**. Adhesion is mediated by **von Willebrand Factor** (**VWF**). VWF binds collagen, and platelets bind VWF via GPIb within a receptor complex on the platelet surface. Deficiency of VWF results in **von Willebrand disease**, the most common inherited **coagulopathy**. Once adhered, platelets get activated. Platelet activation involves **changes in shape** (discoidal to spherical with pseudopodia) and **degranulation**, the process by which platelets release the contents of their storage granules. **Thrombin** is the most potent activator of platelets. Thrombin binds to **protease-activated G protein–coupled receptors** on the surface of platelets. Activated platelets release substances that cause **vasoconstriction** (**serotonin** and **thromboxane A2** [TXA_2]), **recruit** and **activate** other platelets (**adenosine diphosphate** and **TXA2**) and support the formation of a **fibrin clot** (**factor** [**F**] **V, FXIII,** and **fibrinogen**). Activation causes changes in platelets that lead to their **aggregation**. Structural changes in a surface receptor (GPIIb/IIIa) expose binding sites for **fibrinogen**. Fibrinogen molecules link activated platelets to one another. The fibrinogen is activated to **fibrin** by **thrombin** and then **cross-linked** by **FXIIIa**, a **transglutaminase** coming both from the blood and from platelets. The initial loose plug of platelets (**primary hemostasis**) is strengthened by the fibrin meshwork (**secondary hemostasis**).

The formation of the **fibrin meshwork** involves the **extrinsic** and **intrinsic** pathways (and their associated **protein factors**) that **converge** at **FXa** to form the **common pathway**. Many of the protein factors are **serine proteases** with trypsin-like specificity. Ca^{2+} binds the negatively charged **γ-carboxyglutamate** (**Gla**) residues present in certain of the clotting proteins (**FII**, **FVII**, **FIX**, and **FX**), facilitating the binding of these proteins to exposed **phosphatidylserine** at the site of injury and on the surface of platelets. **γ-Glutamyl carboxylase** and its coenzyme, the hydroquinone form of **vitamin K**, are required for formation of Gla residues. In the 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 **vitamin K epoxide reductase** that regenerates the functional reduced form. The **extrinsic pathway** is initiated by exposure of **FIII** (**tissue factor** [**TF**]), an accessory protein, in vascular **subendothelium**. Exposed TF binds a circulating Gla-containing protein, **FVII**, activating it through conformational change. The **TF–FVIIa** complex then binds and activates **FX** by proteolysis. The extrinsic pathway is rapidly inhibited by **tissue factor pathway**

inhibitor. The **intrinsic** pathway is initiated by **FXIIa**. FXIIa activates FXI, and **FXIa** activates FIX. **FIXa** combines with **FVIIIa** (an **accessory protein**), and the complex activates FX. FVIII deficiency results in **hemophilia A**, whereas FIX deficiency results in the less common **hemophilia B**. FXa associates with **FVa** (an **accessory protein**), forming **prothrombinase** that **cleaves prothrombin** (**FII**) to **thrombin** (**FIIa**). Thrombin then cleaves **fibrinogen** to **fibrin** (**FIa**). Fibrin monomers associate, forming a **soluble** (**soft**) **fibrin clot.** The fibrin molecules get cross-linked by **FXIIIa**, forming an **insoluble** (**hard**) **fibrin clot**. Proteins synthesized by the liver and by blood vessels themselves balance coagulation with **anticoagulation**. **Antithrombin III**, a serine protease inhibitor, or **serpin**, binds to and removes thrombin from the blood. Its affinity for thrombin is increased by **heparin**, which is used therapeutically to limit clot formation. **Protein C**, a Gla-containing protein, is activated by the **thrombin–thrombomodulin** complex. Thrombomodulin decreases thrombin's affinity for fibrinogen, converting it from a protein of coagulation to a protein of anticoagulation. Protein C in complex with **protein S** (a Gla-containing protein) forms the **activated protein C (APC**) complex that **cleaves** the accessory proteins **FVa** and **FVIIIa**. **Factor V Leiden** is resistant to APC. It is the most common inherited **thrombophilic** condition in the United States. The **fibrin clot** is **cleaved** (**fibrinolysis**) by the protein **plasmin,** a serine protease that is generated from **plasminogen** by **plasminogen activators** such as **tissue plasminogen activator** (**TPA, t-PA**). Recombinant TPA is used clinically. Disorders of platelets and coagulation proteins can result in deviations in the ability to clot. **Prothrombin time** and **activated partial thromboplastin time** are used to evalulate the clotting cascade.

Figure 34.25 Key concept map for blood clotting. a = active; F = factor.


Blood Clotting
requires formation of a
Clot at areas of injury
by formation of
Platelet plug
by
Adhesion of platelets to exposed collagen via von Willebrand factor
followed by
Thrombin-mediated activation of platelets
involving
Platelet shape change
Degranulation (release of materials)
mediated by
Ca2+
including
resulting in
Platelet aggregation
mediated by
Fibrinogen and its conversion to fibrin
Fibrin meshwork
generated by
Intrinsic pathway
Extrinsic pathway
involves
involves
FXIIa
FXI → FXIa
FIX → FIXa
FVIII → FVIIIa
[FII–FVIIa]
activate
Inherited deficiencies result in hemophilia
FX
by proteolysis
FXa
initiates
Common pathway
involves
FII (prothrombin)
[FVa–FXa]
FIIa (thrombin)
FI → FIa (fibrin)
Increased amount
FV Leiden
cause
Thrombophilia
cross-linking of fibrin by
FXIIIa
Cross-linked fibrin clot stabilizes platelet plug
degraded by
Plasmin
requires
γ-Carboxylation of FII, FVII, FIX, FX
by
Vitamin K–requiring γ-glutamyl carboxylase
inhibited by
Warfarin, a vitamin K antagonist
FVa, FVIIIa
degrades
Activated protein C complex
causes formation of
Thrombomodulin
bound by
bound and inactivated by
Antithrombin III

Study Questions

Choose the ONE best answer.

For Questions 31.1–31.5, match the most appropriate protein 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

34.1 This factor activates components of the intrinsic, extrinsic, and common pathways.

34.2 This factor converts the soluble clot to an insoluble clot.

34.3 This factor initiates the common pathway.

34.4 This factor is an accessory protein that potentiates the activity of factor Xa.

34.5 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. Factor (F)XIII, 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.

34.6 In which patient would prothrombin time be unaffected and activated partial thromboplastin time 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. Prothrombin time (PT) measures the activity of the extrinsic through the common pathways, and activated partial thromboplastin time (aPTT) measures the activity of the intrinsic through the common pathways. Patients with hemophilia are deficient in either factor (F)VIII (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.

34.7 Which one of the following can be ruled out in a patient with thrombophilia?

A. A deficiency of antithrombin III
B. A deficiency of factor IX
C. A deficiency of protein C
D. An excess of prothrombin
E. Expression of factor V 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.

34.8 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 III.
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. Tissue plasminogen activator (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.

34.9 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.

34.10 Several days after having had their home treated for an infestation of rats, the parents of a 3-year-old girl 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 factor (F)II, 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.