Chapter 5— Biological Membranes: Structure and Membrane Transport

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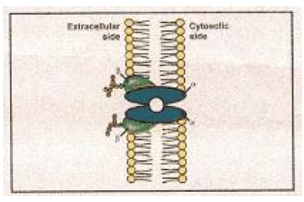

5.1— Overview

Biological membranes from either eukaryotic or prokaryotic cells have the same classes of chemical components, a similarity in structural organization, and many properties in common. There are major differences in specific lipid, protein, and carbohydrate components but not in physicochemical interaction of these molecules. Membranes have a trilaminar appearance when viewed by electron microscopy (Figure 5.1), with two dark bands on each side of a light band. The overall width of most mammalian membranes is 7–10 nm but some have significantly smaller widths. Intracellular membranes are usually thinner than plasma membranes. Many do not appear symmetrical, with an inner dense layer often thicker than an outer dense layer; there is a chemical asymmetry of membranes. With development of sophisticated techniques for preparation of tissue samples and staining, including negative staining and freeze fracturing, surfaces of membranes have been viewed; at the molecular level surfaces are not smooth but dotted with protruding globular-shaped masses.

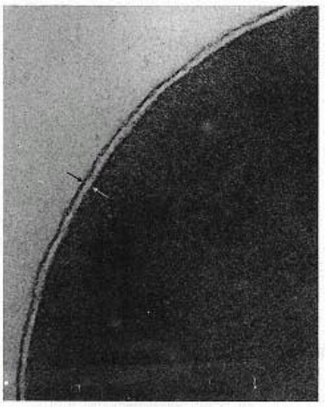

Figure 5.1
Electron micrograph of the erythrocyte plasma membrane showing the trilaminar appearance.
A clear space separates the two electrondense lines. Electron microscopy has demonstrated that the inner dense line is frequently thicker than the outer line. Magnification about ×150,000.
Courtesy of Dr J. D. Robertson, Duke University, Durham, North Carolina.

Membranes are very dynamic structures with a movement that permits cells as well as subcellular structures to adjust their shapes and to change position. Chemical components of membranes, that is, lipids and protein, are ideally suited for their dynamic role. Membranes are an organized sea of lipid in a fluid state, a nonaqueous compartment of cells, in which various components are able to move and interact.

Cellular membranes control the composition of space that they enclose by excluding a variety of molecules and by selective transport systems allowing movement of specific molecules from one side to the other. These transporters are proteins. By controlling translocation of substrates, cofactors, ions, and so on, membranes modulate the concentration of substances in cellular compartments, thereby exerting an influence on metabolic pathways. Hormones, and growth and metabolic regulators bind to specific protein receptors on plasma membranes (Chapter 20) and the information to be imparted to the cell is transmitted by the membrane component to the appropriate metabolic pathway by a series of intracellular intermediates, termed second messengers. Plasma membranes of eukaryotic cells also have a role in cell-cell recognition, maintenance of the shape of cells, and cell locomotion.

The discussion that follows is directed to the chemistry and transport functions of membranes primarily of mammalian cells but the observations and activities described are applicable to all biological membranes.

5.2— Chemical Composition of Membranes

Lipids and proteins are the two major components of all membranes. The amount of each varies greatly between different membranes (Figure 5.2). Protein ranges from about 20% in the myelin sheath to over 70% in the inner membrane of the mitochondria. Intracellular membranes have a high percentage of protein because of the large number of enzymic activities of these membranes. Membranes also contain a small amount of various polysaccharides in the form of glycoprotein and glycolipid; there is no free carbohydrate in membranes.

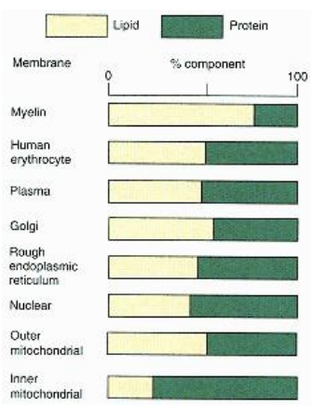

Figure 5.2
Representative values for the percentage of lipid and protein in various cellular membranes.
Values are for rat liver, except for the myelin and human erythrocyte plasma membrane. Values for liver from other species, including human, indicate a similar pattern.

Lipids Are a Major Component of Membranes

The three major lipid components of eukaryotic cell membranes are glycerophospholipids, sphingolipids, and cholesterol. Glycerophospholipids and sphingomyelin, a sphingolipid containing phosphate, are classified as **phospholipids.** Bacteria and blue-green algae contain glycerolipids where a carbohydrate is attached directly to the glycerol. Individual cellular membranes also contain small quantities of other lipids, such as triacylglycerol and diol derivatives (see the Appendix).

Glycerophospholipids Are the Most Abundant Lipids of Membranes

Glycerophospholipids (phosphoglycerides) have a glycerol molecule as the basic component to which phosphoric acid is esterified at the α carbon (Figure 5.3) and two long-chain fatty acids are esterified at the remaining carbon atoms (Figure 5.4). Glycerol does not contain an asymmetric carbon, but the α-carbon atoms are not stereochemically identical. Esterification of a phosphate to an α carbon makes the molecule asymmetric. The naturally occurring glycerophospholipids are designated by the stereospecific numbering system (*sn*)(Figure 5.3) discussed on p. 397.

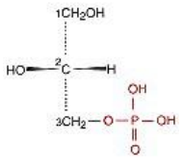

Figure 5.3
Stereochemical con figuration of L-glycerol 3-phosphate (*sn*-glycerol 3-phosphate).
The H and OH attached to C-2 are above and C-1 and C-3 are below the plane of the page.

1,2-Diacylglycerol 3-phosphate, **phosphatidic acid,** is the parent compound of a series of glycerophospholipids, where different hydroxyl-containing compounds are esterified to the phosphate. The major compounds attached by a phosphodiester bridge to glycerol are choline, ethanolamine, serine, glycerol, and inositol. These structures are presented in Figure 5.5. **Phosphatidylethanolamine** (ethanolamine glycerophospholipids or the trivial name cephalin) and **phosphatidylcholine** (choline glycerophospholipid or lecithin) are the most common glycerophospholipids in membranes (Figure 5.6). **Phosphatidylglycerol phosphoglyceride** (Figure 5.7) (diphosphatidylglycerol or cardiolipin) contains two phosphatidic acids linked by a glycerol and is found nearly exclusively in mitochondrial inner membranes and bacterial membranes.

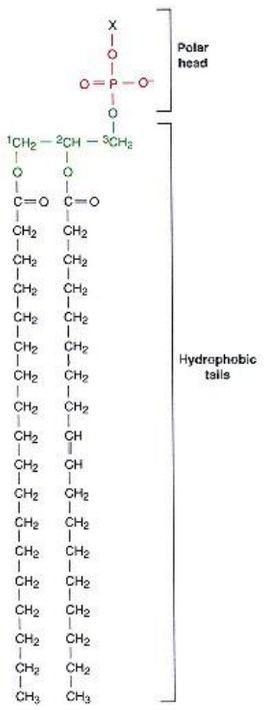

Figure 5.4
Structure of glycerophospholipid.
Long-chain fatty acids are esterified at C-1 and C-2 of the L-glycerol 3-phosphate. X can be a H (phosphatidic acid) or one of several alcohols presented in Figure 5.5.

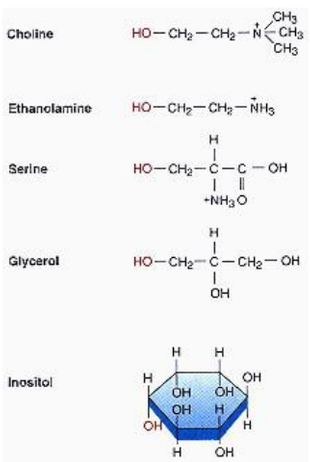

Figure 5.5
Structures of the major alcohols esterified to phosphatidic acid to form the glycerophospholipid.

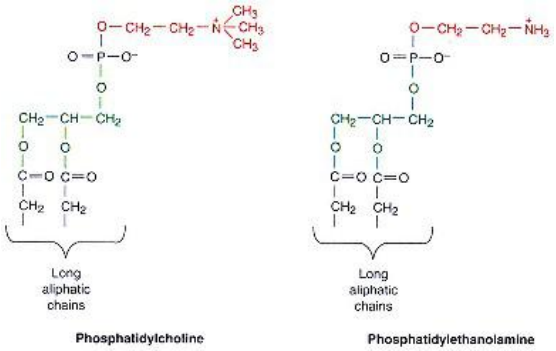

Figure 5.6
Structures of the two most common glycerophospholipids—phosphatidylcholine and phosphatidylethanolamine.

Inositol, a hexahydroxy alcohol, is esterified to phosphate in phosphatidylinositol (Figure 5.8). 4-Phospho- and **4,5-bisphosphoinositol glycerophospholipids** (Figure 5.8) are present in plasma membranes; the latter is the source of **inositol trisphosphate** and diacylglycerol that are involved in hormone action (see p. 865).

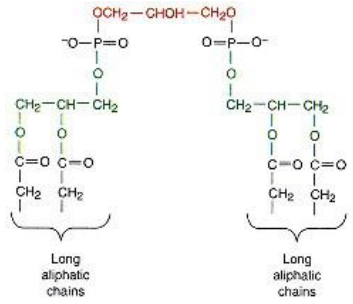

Figure 5.7
Phosphatidylglycerol phosphoglyceride (cardiolipin).

Glycerophospholipids contain two fatty acyl groups esterified to carbon atoms 1 and 2 of glycerol; some of the major fatty acids found in glycerophospholipids are presented in Table 5.1. A saturated fatty acid is usually found on C-1 of the glycerol and an unsaturated fatty acid on C-2. Designation of different glycerophospholipids does not specify which fatty acids are present. Phosphatidylcholine usually contains palmitic or stearic in the *sn*-1 position and a C18 unsaturated fatty acid, oleic, linoleic, or linolenic, on the *sn*-2 carbon. Phosphatidylethanolamine contains palmitic or oleic on *sn*-1 but a long-chain polyunsaturated fatty acid, such as arachidonic, on the *sn*-2 position.

A saturated fatty acid is a straight chain, as is a fatty acid with an unsaturation in the *trans* position. A *cis* double bond, however, creates a kink in the hydrocar-

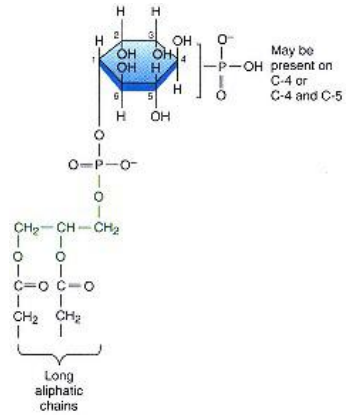

Figure 5.8
Phosphatidylinositol.
Phosphate groups are also found on C-4 or C-4 and C-5 of the inositol. The additional phosphate groups increase the charge on the polar head of this glycerophospholipid.

TABLE 5.1 Major Fatty Acids in Glycerophospholipids

Common Name	*Systematic Name*	*Structural Formula*
Myristic acid	*n*-Tetradecanoic	$CH_3–(CH_2)_{12}–COOH$
Palmitic acid	*n*-Hexadecanoic	$CH_3–(CH_2)_{14}–COOH$
Palmitoleic acid	*cis*-9-Hexadecenoic	$CH_3–(CH_2)_5–CH=CH–(CH_2)_7–COOH$
Stearic acid	*n*-Octadecanoic	$CH_3–(CH_2)_{16}–COOH$
Oleic acid	*cis*-9-Octadecenoic acid	$CH_3–(CH_2)_7–CH=CH–(CH_2)_7–COOH$
Linoleic acid	*cis,cis*-9,12-Octadecadienoic	$CH_3–(CH_2)_3–(CH_2–CH=CH)_2–(CH_2)_7–COOH$
Linolenic acid	*cis,cis,cis*-9,12,15-Octadecatrienoic	$CH_3–(CH_2–CH=CH)_3–(CH_2)_7–COOH$
Arachidonic acid	*cis,cis,cis,cis*-5,8,11,14-Icosatetraenoic	$CH_3–(CH_2)_3–(CH_2–CH=CH)_4–(CH_2)_3–COOH$

bon chain (Figure 5.9). A straight chain diagram, as shown in Figures 5.4 and 5.9, does not adequately represent the chemical configuration of a long-chain fatty acid. Actually, there is a high degree of coiling of the hydrocarbon chain in a glycerophospholipid that is disrupted by a double bond. The presence of unsaturated fatty acids has a marked effect on the physicochemical state of the membrane (see p. 195).

Glycerol ether phospholipids contain a long aliphatic chain in ether linkage to the glycerol at the *sn*-1 position (Figure 5.10). Ether phospholipids contain an alkyl group (alkyl acylglycerophospholipid) or an α, β-unsaturated ether, termed a **plasmalogen.** The latter groups are more prevalent in membranes. Plasmalogens containing ethanolamine (ethanolamine plasmalogen) and choline (choline plasmalogen) esterified to the phosphate are abundant in nervous tissue and heart but not in liver. In human hearts more than 50% of the ethanolamine glycerophospholipids are plasmalogens.

Glycerophospholipids are **amphipathic,** containing both a polar end, or head group, due to the charged phosphate and substitutions on the phosphate, and a nonpolar tail due to hydrophobic hydrocarbon chains of the fatty acyl

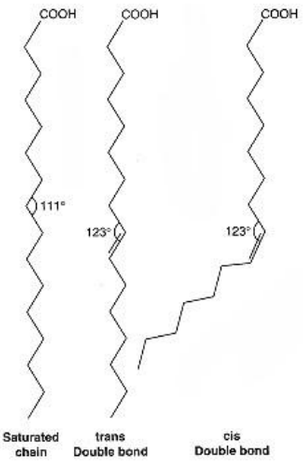

Figure 5.9
Conformation of fatty acyl groups in phospholipids.
The saturated and unsaturated fatty acids with trans double bonds are straight chains in their minimum energy conformation, whereas a chain with a cis double bond has a bend. The trans double bond is rare in naturally occurring fatty acids.

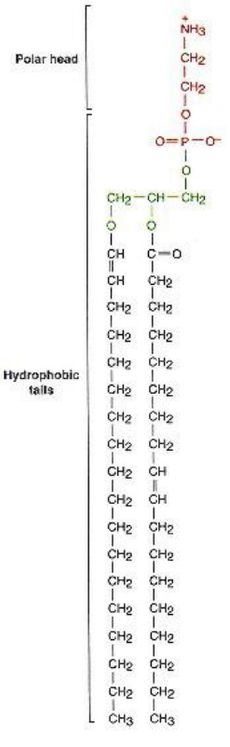

Figure 5.10
Ethanolamine plasmalogen.
Note the ether linkage of the aliphatic chain on C-1 of glycerol.

TABLE 5.2 Predominant Charge on Glycerophospholipids and Sphingomyelin at pH 7.0

Lipid	*Phosphate Group*	*Base*	*Net Charge*
Phosphatidylcholine	–1	+1	0
Phosphatidylethanolamine	–1	+1	0
Phosphatidylserine	–1	+1, –1	–1
Phosphatidylglycerol	–1	0	–1
Diphosphatidylglycerol (cardiolipin)	–2	0	–2
Phosphatidylinositol	–1	0	–1
Sphingomyelin	–1	+1	0

groups. The polar groups are charged at pH 7.0 with a negative charge due to ionization of the phosphate group (p*K* 2) and charges from groups esterified to phosphate (Table 5.2). Choline and ethanolamine glycerophospholipids are zwitterions at pH 7.0, with both a negative charge from phosphate and a positive charge on nitrogen. Phosphatidylserine has two negative charges, one on phosphate and one on the carboxyl group of serine, and a positive charge on the α-amino group of serine, with a net charge of –1 at pH 7.0. In contrast, glycerophospholipids containing inositol and glycerol have only a single negative charge on phosphate; 4-phospho- and 4,5-bisphosphoinositol derivatives are very polar compounds with additional negative charges on the phosphate groups.

Every tissue and cellular membrane has a distinctive composition of glycerophospholipids and a definite pattern in fatty acid composition. There is a greater variability in the fatty acyl groups of different tissues in a single species than in the fatty acyl groups of the same tissue in a variety of species. In addition, the fatty acid content of the glycerophospholipids can vary, depending on the physiological or pathophysiological state of the tissue.

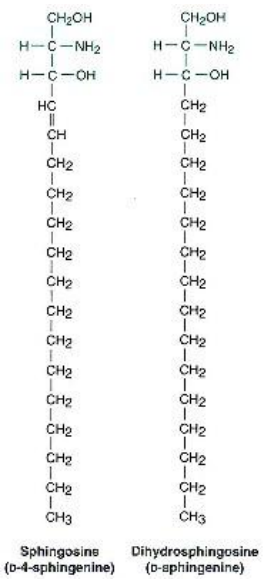

Figure 5.11
Structures of sphingosine and dihydrosphingosine.

Sphingolipids Are Also Present in Membranes

The amino alcohols **sphingosine** (D-4-sphingenine) and **dihydrosphingosine** (Figure 5.11) are the basis for another series of membrane lipids, the **sphingolipids.** A **ceramide** is sphingosine with a saturated or unsaturated long-chain fatty acyl group in amide linkage on the amino group (Figure 5.12). With two nonpolar tails a ceramide is similar in structure to diacylglycerol. Various substitutions are found on the hydroxyl group at position 1. The sphingomyelin series has phosphorylcholine esterified to the 1-OH (Figure 5.13) and is the most abundant sphingolipid in mammalian tissues. The similarity of this structure to choline glycerophospholipids is apparent, and they have many properties in common; note that the sphingomyelins are amphipathic compounds with a charged head group. Sphingomyelins and glycerophospholipids are classified as phospholipids. The sphingomyelin of myelin contains predominantly the longer chain fatty acids, with carbon lengths of 24; as with glycerophospholipids, there is a specific fatty acid composition of the sphingomyelin, depending on the tissue.

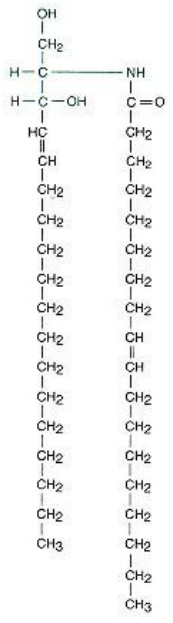

Figure 5.12
Structure of a ceramide.

Glycosphingolipids do not contain phosphate and have a sugar attached by a β-glycosidic linkage to the 1-OH group of the sphingosine in a ceramide. One subgroup is the **cerebrosides,** which contain either a glucose (**glucocerebrosides**) or galactose (**galactocerebrosides**) attached to a ceramide (Figure 5.14). Cerebrosides are neutral compounds. Galactocerebrosides are found predominantly in brain and nervous tissue, whereas the small quantities of cerebrosides in nonneural tissues usually contain glucose. **Phrenosin,** a specific galac-

tocerebroside, contains a 2-OH C_{24} fatty acid. Galactocerebrosides may contain a sulfate group esterified on the 3 position of the sugar. They are called **sulfatides** (Figure 5.15). Cerebrosides and sulfatides usually contain fatty acids with 22–26 carbon atoms.

In place of monosaccharides, neutral glycosphingolipids often have 2 (dihexosides), 3 (trihexosides), or 4 (tetrahexosides) sugar residues attached to the 1-OH group of sphingosine. Diglucose, digalactose, *N*-acetylglucosamine, and *N*-acetyldigalactosamine are the usual sugars.

The most complex group of glycosphingolipids, the **gangliosides,** contain oligosaccharide head groups with one or more residues of sialic acid; these are amphipathic compounds with a negative charge at pH 7.0. The gangliosides represent 5–8% of the total lipids in brain, and some 20 different types have been identified differing in the number and relative position of the hexose and sialic acid residues. This is the basis of their classification; a detailed description of the nomenclature and structures of gangliosides is presented on p. 426.

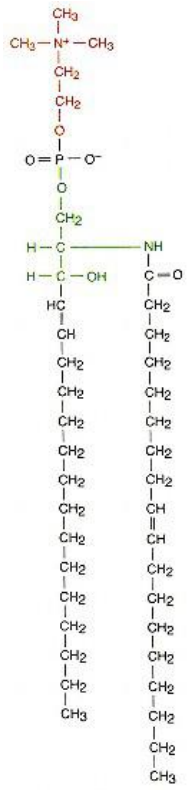

Figure 5.13
Structure of a choline containing sphingomyelin.

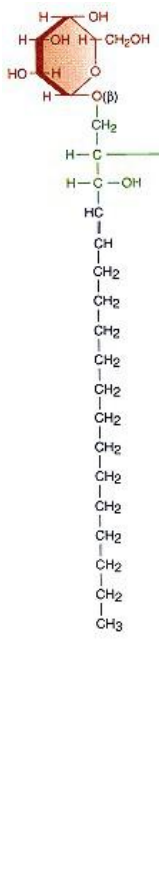

Figure 5.14
Structure of a galactocere-broside containing a C24 fatty acid.

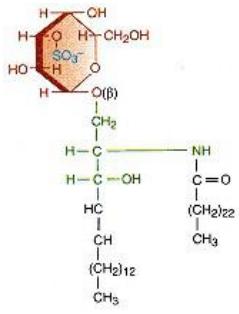

Figure 5.15
Structure of a sulfatide.

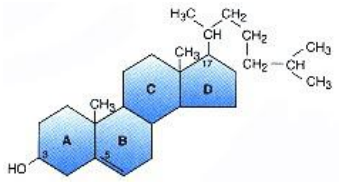

Figure 5.16
Structure of cholesterol.

Most Membranes Contain Cholesterol

Cholesterol is the third major lipid in membranes. With four fused rings and an eight-member branched hydrocarbon chain attached to the D ring at position 17, cholesterol is a compact, rigid, hydrophobic molecule (Figure 5.16). It also has a polar hydroxyl group at C-3.

Lipid Composition Varies in Different Membranes

There are quantitative differences between the classes of lipids and individual lipids in various cell membranes (Figure 5.17). The lipid composition is very similar in the same intracellular membrane of a specific tissue in different species. The plasma membrane exhibits the greatest variation in percentage composition because the amount of cholesterol is affected by the nutritional state of the animal. Plasma membranes have the highest concentration of neutral lipids and sphingolipids; myelin membranes of axons of neural tissue are rich in sphingolipids, with a high proportion of glycosphingolipids. Intracellular membranes primarily contain glycerophospholipids with little sphingolipids or cholesterol. The membrane lipid composition of mitochondria, nuclei, and rough endoplasmic reticulum are similar, with Golgi membrane being somewhere between other intracellular membranes and the plasma membrane. As indicated previously, cardiolipin is found nearly exclusively in the inner mitochondrial membrane. Choline containing lipids, phosphatidylcholine, and sphingomyelin, are predominant, with ethanolamine glycerophospholipid second. The constancy of composition of various membranes indicates the relationship between lipids and the specific functions of individual membranes.

Membrane Proteins Are Classified Based on Their Ease of Removal

Membrane proteins are classified on the basis of ease of removal from isolated membrane fractions. **Peripheral (or extrinsic) proteins** are released from a

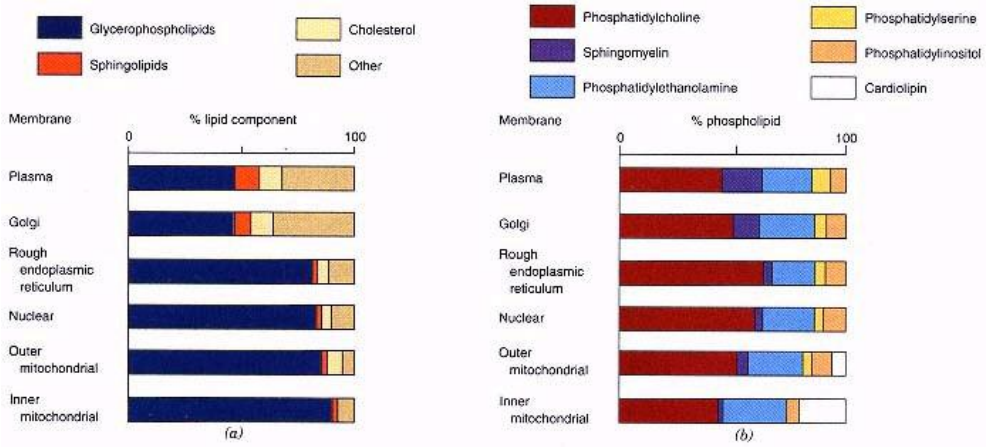

Figure 5.17
Lipid composition of cellular membranes isolated from rat liver.
(a) Amount of major lipid components
as percentage of total lipid. The area labeled "Other" includes mono-, di-, and triacylglycerol, fatty acids, and cholesterol esters.
(b) Phospholipid composition as a percentage of total phospholipid.
Values from R. Harrison and G. G. Lunt, *Biological Membranes*. New York Wiley, 1975.

membrane by treatment with salt solutions of different ionic strength or extremes of pH, and named to imply a physical location on the surface of the membrane. Peripheral proteins, many of which are enzymes, are usually soluble in water and free of lipids. **Integral (or intrinsic) proteins** require rather drastic treatment, such as use of detergents or organic solvents, to be separated from a membrane They usually contain tightly bound lipid, which if removed leads to denaturation of the protein and loss of biological function. Integral proteins have sequences of hydrophobic amino acids, which create hydrophobic domains in the tertiary structure. These hydrophobic regions interact with the hydrophobic hydrocarbons of the lipids stabilizing the protein–lipid complex. Removal of integral proteins leads to disruption of the membrane, whereas peripheral proteins can be removed with little or no change in the integrity of the membrane.

Proteolipids are hydrophobic **lipoproteins** soluble in chloroform and methanol but insoluble in water. They are present in many membranes but particularly in myelin, where they represent about 50% of the protein component. An example is lipophilin, a major lipoprotein of brain myelin that contains over 65% hydrophobic amino acids and covalently bound fatty acids.

Another class of integral membrane proteins is the **glycoproteins;** plasma membranes of cells contain a number of different glycoproteins, each with its own unique carbohydrate content.

The complexity, variety, and interaction of membrane proteins with lipids are just being resolved. Many of the proteins are enzymes located within or on the cellular membranes. Membrane proteins have a role in transmembrane movement of molecules and as receptors for the binding of hormones and growth factors. In many cells, such as neurons and erythrocytes, membrane proteins have a structural role to maintain the shape of the cell. Thus individual membrane proteins can have a *catalytic, transport, receptor, structural*, or *recognition role*. It is not surprising to find a high protein content in a membrane being correlated with the complexity and variety of functions of a membrane.

Carbohydrates of Membranes Are Present As Glycoproteins or Glycolipids

Carbohydrates present in membranes are **oligosaccharides** covalently attached to proteins to form glycoproteins and to a lesser amount to lipids to form glycolipids. The sugars found in glycoproteins and glycolipids include glucose, galactose, mannose, fucose, *N*-acetylgalactosamine, *N*-acetylglucosamine, and sialic acid (see Figure 5.18 and the Appendix for structures). Structures of glycoproteins and glycolipids are presented on pages 348 and 422, respectively. The carbohydrate is on the exterior side of the plasma membrane or the luminal side of the endoplasmic reticulum. Roles for membrane carbohydrates include cell-cell recognition, adhesion, and receptor action.

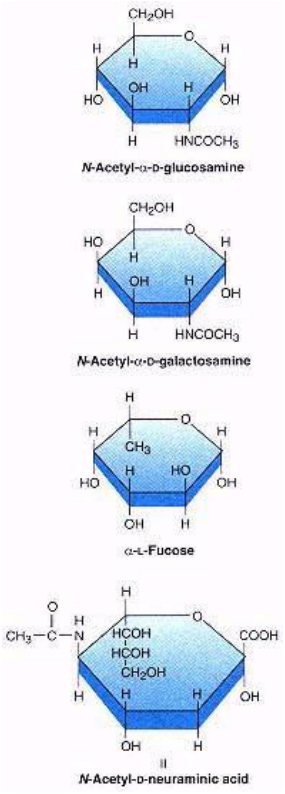

Figure 5.18
Structures of some membrane carbohydrates.

5.3— Micelles and Liposomes

Lipids Form Vesicular Structures

The basic structural characteristic of membranes is derived from the physicochemical properties of the major lipid components, the glycerophospholipids and sphingolipids. These amphipathic compounds, with a hydrophilic head and a hydrophobic tail (Figure 5.19*a*), will at appropriate concentrations interact in an aqueous system to form spheres, termed **micelles** (Figure 5.19*b*). The hydrophobic tails interact to exclude water and charged polar head groups will be on the outside of the sphere. The specific concentration of lipid required for micelle formation is referred to as the **critical micelle concentration.** Micelles with a single lipid or a mixture of lipids can be made. Formation of

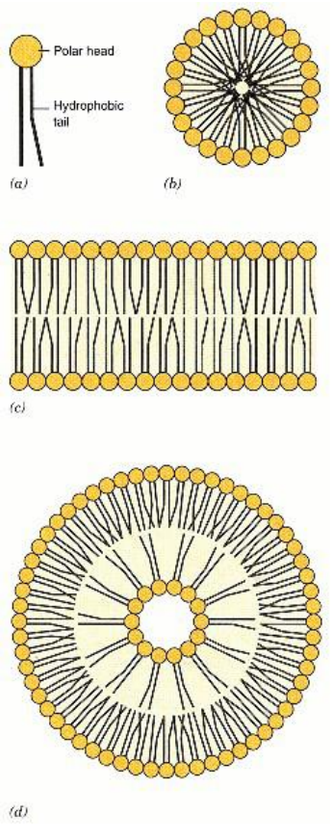

Figure 5.19
Representations of the interactions of phospholipids in an aqueous medium.
(a) Representation of an amphipathic lipid. (b) Cross-sectional view of the structure of a micelle. (c) Cross-sectional view of the structure of lipid bilayer. (d) Cross section of a liposome. Each structure has an inherent stability due to the hydrocarbon chains and the attraction of the polar head groups to water.

micelles depends also on the temperature of the system and, if a mixture of lipids are used, on the ratio of concentrations of the different lipids in the mixture (see p. 1079). The micelle structure is very stable because of hydrophobic interaction of hydrocarbon chains and attraction of polar groups to water. Micelles are important in the digestion of lipids (see p. 1081).

Liposomes Have a Membrane Structure Similar to Biological Membranes

Depending on conditions, amphipathic lipids such as glycerophospholipids will form a bimolecular leaf structure with two layers of lipid. The polar head groups will be at the interface between the aqueous medium and the lipid, and the hydrophobic tails will interact to form an environment that excludes water (Figure 5.19*c*). This bilayer conformation is the basic lipid structure of all biological membranes.

Lipid bilayers are extremely stable structures held together by noncovalent interactions of the hydrocarbon chains and ionic interactions of charged head groups with water. Hydrophobic interactions of the hydrocarbon chains lead to the smallest possible area for water to be in contact with the chains, and water is essentially excluded from the interior of the bilayer. If disrupted, bilayers will self-seal because hydrophobic groups will seek to establish a structure in which there is minimal contact of the hydrocarbon chains with water, a condition that is most favorable thermodynamically. A lipid bilayer will close in on itself, forming a spherical vesicle separating the external environment from an internal compartment. Such vesicles are termed **liposomes.** Because individual lipid–lipid interactions have low energies of activation, lipids in a bilayer have a circumscribed mobility, breaking and forming interactions with surrounding molecules but not readily escaping from the lipid bilayer (Figure 5.19*d*). Self-assembly of amphipathic lipids into bilayers is an important characteristic and is involved in formation of cell membranes.

Individual phospholipid molecules exchange places with neighboring molecules in a bilayer, leading to rapid lateral diffusion in the plane of the membrane (Figure 5.20). There is rotation around the carbon–carbon bonds in fatty acyl chains; in fact, there is a greater degree of rotation nearer the methyl end,

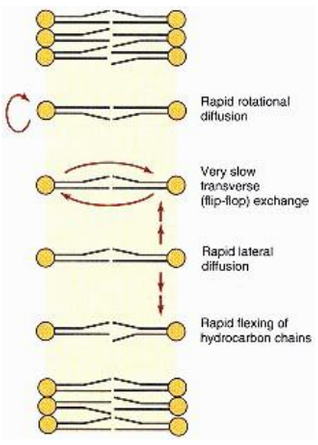

Figure 5.20
Mobility of lipid components in membranes.

leading to greater motion at the center than the peripheral region of the lipid bilayer. Individual lipid molecules, however, do not migrate readily from one monolayer to the other, a transverse movement, termed flip-flop, because of the thermodynamic constraints on movement of a charged head group through the lipophilic core. Thus lipid bilayers have an inherent stability and a fluidity in which individual molecules move rapidly in their own monolayer but do not readily exchange with an adjoining monolayer. In artificial bilayer membranes composed of different lipids, the components will be distributed randomly.

Artificial membrane systems have been studied extensively in order to understand the properties of biological membranes. A variety of techniques are available to prepare liposomes, using synthetic phospholipids and lipids extracted from natural membranes. Depending on the procedure, unilamellar and multilamellar (vesicles within vesicles) vesicles of various sizes (20 nm to 1 μm diameter) can be prepared. Figure 5.19*d* contains a representation of a liposome structure. The interior of the vesicle is an aqueous environment, and it is possible to prepare liposomes with different substances entrapped. Both the external and internal environments of liposomes can be manipulated and properties—including ability to exclude molecules, interaction with various substances, and stability under different conditions—of these synthetic membranes have been studied. Na^+, K^+, Cl^-, and most polar molecules do not readily diffuse across lipid bilayers of liposomes, whereas the bilayer presents no barrier to water. Lipid-soluble nonpolar substances such as triacylglycerol and undissociated organic acids readily diffuse into the membrane remaining in the hydrophobic environment of the hydrocarbon chains. Proteins have been incorporated into liposomes to mimic a natural membrane. Membrane-bound enzymes and proteins involved in translocating ions have been isolated from various tissues and incorporated into the membrane of liposomes for evaluation of the protein's function. With liposomes it is easier to manipulate the various parameters of membrane systems and thus study various activities free of interfering reactions present in cell membranes. Liposomes are used in delivery of drugs in humans (see Clin. Corr. 5.1).

CLINICAL CORRELATION 5.1

Liposomes As Carriers of Drugs and Enzymes

A major obstacle in the use of many drugs is lack of tissue specificity in the action of the drug. Administration of drugs orally or intravenously leads to a drug acting on many tissues and not exclusively on a target organ, resulting in toxic side effects. An example is the commonly observed suppression of bone marrow cells by anticancer drugs. Some drugs are metabolized rapidly and their period of effectiveness is relatively short. Liposomes have been prepared with drugs, enzymes, and DNA encapsulated inside and used as carriers for these substances to target organs. Liposomes prepared from purified phospholipids and cholesterol are nontoxic and biodegradable. Alteration of surface charge enhances drug incorporation and release. Attempts have been made to prepare liposomes for interaction at a specific target organ. Antibiotic, antineoplastic, antimalarial, antiviral, antifungal, and anti-inflammatory agents have been found to be effective when administered in liposomes. Some drugs have a longer period of effectiveness when administered encapsulated in liposomes. It may be possible to prepare liposomes with a high degree of tissue specificity so that drugs and perhaps even enzyme replacement can be carried out with this technique.

Ranade, V. V. Drug delivery systems. 1. Site-specific drug delivery using liposomes as carriers. *J. Clin. Pharmacol. 29*:685, 1989; Caplen, N. J., Gao, X., Hayes, P., et al. Gene therapy for cystic fibrosis in humans by liposome-mediated DNA transfer: the production of resources and the regulatory process. *Gene Ther.* 1:139, 1994; and Gregoriadis, G. Engineering liposomes for drug delivery: progress and problems. *Trends Biotechnol.* 13:527, 1995.

5.4— Structure of Biological Membranes

Fluid Mosaic Model of Biological Membranes

Based on evidence from physicochemical, biochemical, and electron microscopic investigations, knowledge of membrane structure has evolved. All biological membranes have a **bimolecular leaf** arrangement of lipids, as in liposomes. The amphipathic lipids and cholesterol are oriented so that hydrophobic portions of the molecules interact, minimizing their contact with water or other polar groups, and polar head groups of lipids are at the interface with the aqueous environment. J. D. Davson and J. Danielli in 1935 proposed this model for a membrane; their proposal was later refined by J. D. Robertson. A major question with the earlier models was how to explain the interaction of membrane proteins with the lipid bilayer. In the early 1970s, S. J. Singer and G. L. Nicolson proposed the mosaic model for membranes in which some proteins (intrinsic) are actually immersed in the lipid bilayer while others (extrinsic) are loosely attached to the surface of the membrane. It was suggested that some proteins spanned the lipid bilayer being in contact with the aqueous environment on both sides. Figure 5.21 is a current representation of a biological membrane and is referred to as the **fluid mosaic model** to indicate the movement of both lipids and proteins in the membrane. The characteristics of the lipid bilayer explain many of the observed cellular membrane properties, including fluidity, flexibility that permits changes of shape and form, ability to self-seal, and impermeability. The model continues to undergo modification and refinement;

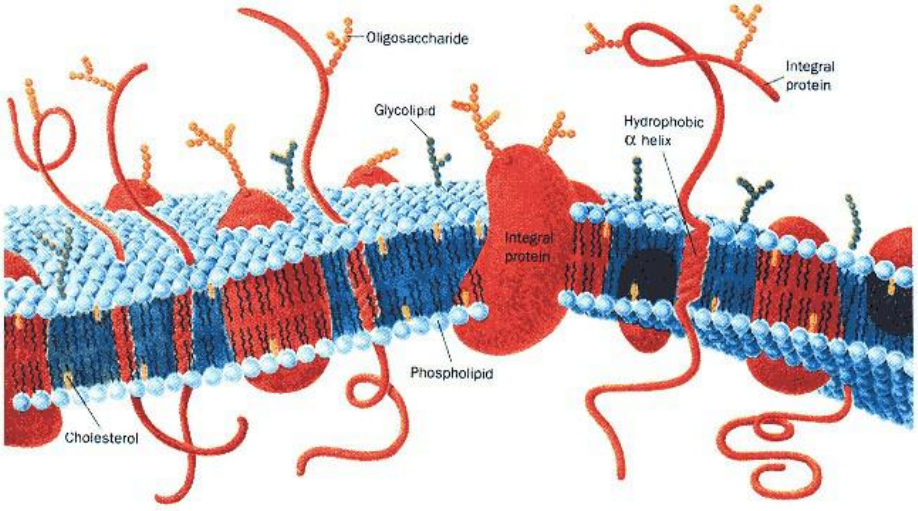

Figure 5.21
Fluid mosaic model of biological membranes.
Figure reproduced with permission from D. Voet and J. Voet, *Biochemistry*, 2nd ed. New York: Wiley, 1995.

as an example, under some conditions membrane lipids can assume structural variations other than the bimolecular leaf arrangement.

Integral Membrane Proteins Are Immersed in Lipid Bilayer

The development of techniques for isolation of integral membrane proteins, for determination of their primary structure, and for identification of specific functional domains in the protein has led to an understanding of the structural relationship between the hydrophobic lipid bilayer and membrane proteins. Figure 5.22 illustrates the various ways of attachment of proteins to a biological membrane. Some integral membrane proteins (see p. 187) span the membrane, whereas others may only be immersed partially in the lipid. Based on measurements of the hydrophobicity of the amino acid residues and partial proteolytic digestion of proteins, sequences of amino acids embedded in the membrane have been determined. Some proteins contain an α-helical structure consisting primarily of hydrophobic amino acids (such as leucine, isoleucine, valine, and phenylalanine), which is the transmembrane sequence. This is illustrated in Figure 5.22*a*. An example is **glycophorin** present in the plasma membrane of human erythrocytes; amino acid residues 73–91, of the 131 total amino acids, are the transmembrane sequence and are predominantly hydrophobic. Glycophorin has three domains: a sequence exterior to the cell containing the amino terminal end, the transmembrane sequence, and a sequence extending into the cell with the carboxyl-terminal end. In other transmembrane proteins the amino acid chain loops back and forth across the membrane (Figure 5.22*b*). In some cases there are 12 loops snaking across the lipid bilayer. Often these multiple α helices spanning the membrane are organized to form a tubular structure. The **anion channel** of human erythrocytes, which has 926 amino acids and is responsible for the exchange of Cl^- and HCO_{3-} across the membrane, is an example (see p. 204). Secondary and tertiary structures of proteins are critical

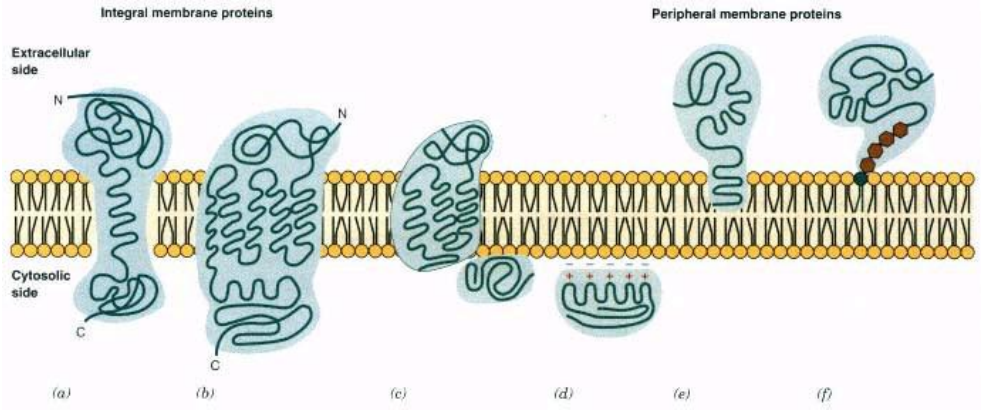

Figure 5.22
Interactions of membrane proteins with the lipid bilayer.
Diagram illustrates the multiple types of binding of proteins in or to the lipid bilayer:
(a) a single transmembrane segment;
(b) multiple transmembrane segments;
(c) bound to an integral protein;
(d) bound electrostatically to the lipid bilayer;
(e) attached by a short terminal hydrophobic sequence of amino acids; and
(f) attached by covalently bound lipid.

in the topography of the protein in the membrane. Some proteins in membranes form a quaternary structure with multiple subunits.

Integral membrane proteins have specific domains, for ligand binding, catalytic activity, and attachment of carbohydrate or lipid. The anion channel of the erythrocyte has two major domains: a hydrophilic amino-terminal domain on the cytosolic side of the membrane with binding sites for ankyrin, a protein that anchors the cytoskeleton and other cytosolic proteins, and a domain with 509 amino acids that traverses the membrane and mediates the exchange of Cl^- and HCO_{3-}. Glycophorin contains 60% carbohydrate, all of which is attached to the protein domain on the extracellular side of the membrane. With such well-defined domains, integral membrane proteins have a defined orientation in the membrane rather than a random one. Specific structural orientation demonstrates another important aspect of membrane structure; biological membranes are asymmetric, with each surface having specific characteristics. The orientation of proteins is fixed during the synthesis of the membrane or replacement of the protein; the bulkiness of the proteins, as well as thermodynamic restrictions, prevents transverse (flip-flop) movement.

Many enzymes that are integral membrane proteins require the presence of the membrane lipid for activity. As an example, D-β-hydroxybutyrate dehydrogenase, located in the inner mitochondrial membrane, requires phosphatidylcholine for activity. Cholesterol has been implicated in the activity of various membrane ion pumps, including Na^+,K^+– and Ca^{2+}–ATPases (see p. 206), and acetylcholine receptors. Some of these modulating effects of lipids may be a reflection of a change in ordering and fluidity of the membrane but the lipid may also have a direct influence on the activity.

Peripheral Membrane Proteins Have Various Modes of Attachment

Peripheral membrane proteins are loosely attached to membranes and if removed do not disrupt lipid bilayers. Some apparently bind to integral membrane proteins, such as **ankyrin** binding to the anion channel protein in erythro-

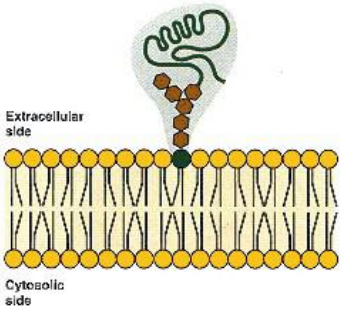

Figure 5.23
Attachment of a protein to a membrane by a glycosyl phosphatidylinositol anchor.

cytes (Figure 5.22*c*). Negatively charged phospholipids of membranes interact with positively charged regions of proteins allowing electrostatic binding (Figure 5.22*d*). Some peripheral proteins have sequences of hydrophobic amino acids at one end of the peptide chain that serve as an anchor in the membrane lipid (Figure 5.22*e*); cytochrome b_5 is attached to the endoplasmic reticulum by such an anchor.

Phosphatidylinositol has a role in anchoring proteins to membranes (Figures 5.22*f* and 5.23). A **glycan,** consisting of ethanolamine, phosphate, mannose, mannose, mannose, and glycosamine is covalently bound to the carboxyl terminal of the protein. This glycan has been conserved throughout evolution because it is found in different species attached to carboxyl-terminal amino acid residues of various membrane-bound proteins. Additional carbohydrate can be attached to the last mannose. The glycosamine of the glycan is covalently bonded to phosphatidylinositol. The fatty acids of this glycerophospholipid are inserted into the lipid membrane, thus anchoring the protein. These molecules are now referred to as **glycosyl phosphatidylinositol** (GPI) **anchors.** Various proteins are attached in this manner including enzymes, antigens, and cell adhesion proteins; a partial list is presented in Table 5.3. Fatty acyl groups of phosphatidylinositol are apparently specific for different proteins. To date, proteins found to be attached by a GPI anchor are on the external surface of plasma membranes. The significance of this form of anchoring has yet to be determined but it may be important for localization of the protein on a membrane, control of function of the protein, and controlled release of the protein from the membrane. A specific phosphatidylinositol-specific phospholipase C catalyzes the hydrolysis of the phosphate-inositol bond leading to release of the protein.

Myristic and **palmitic acids** can also be covalently linked to proteins and serve to anchor proteins by insertion of the acyl chain into the lipid bilayer (Figure 5.22*f*). Myristic acid (C_{14}) is attached by an amide linkage to an amino-terminal glycine, and palmitic acid (C_{16}) is most often attached by a thioester linkage to cysteine or by a hydroxyester bond to serine or threonine.

Even though membrane models suggest that proteins are randomly distributed throughout and on the membrane, there is a high degree of functional organization with definite restrictions on the localization of some proteins. As an example, proteins participating in electron transport in the inner membrane of mitochondria function in consort and are organized into functional units both laterally and transversely. The location of specific proteins on the surface of plasma membranes is also controlled. Cells lining the lumen of kidney nephrons have specific plasma membrane enzymes on the luminal surface but not on the contraluminal surface of cells; enzymes restricted to a particular region of the membrane are located to meet specific functions of these cells. Thus there is a high degree of molecular organization of biological membranes that is not apparent from diagrammatic models.

Human Erythrocytes Are Ideal for Studying Membrane Structure

The structure of the plasma membrane of the human erythrocyte has been investigated extensively because of the ease with which the membrane can be purified from other cellular components. Figure 5.24 is a representation of the interaction of some of the many proteins in this membrane.

TABLE 5.3 Proteins with a Glycosyl Phosphatidylinositol Anchor

Alkaline phosphatase
5 -Nucleotidase
Acetylcholinesterase
Trehalase
Renal dipeptidase
Lipoprotein lipase
Carcinoembryonic antigen
Neural cell adhesion molecule
Scrapie prion protein
Oligodendrocyte-myelin protein

Source: M. G. Low, Glycosyl-phosphatidylinositol: a versatile anchor for cell surface proteins. *FASEB J.* 3:1600, 1989.

Lipids Are Distributed in an Asymmetric Manner in Membranes

There is an **asymmetric distribution of lipid** components across biological membranes in contrast to the random distribution of lipids between the outer and inner lipid monolayers of liposomes. Each layer of the bilayer has a different composition with respect to individual glycerophospholipids and sphingolipids. An example is the asymmetric distribution of lipids in the human erythrocyte

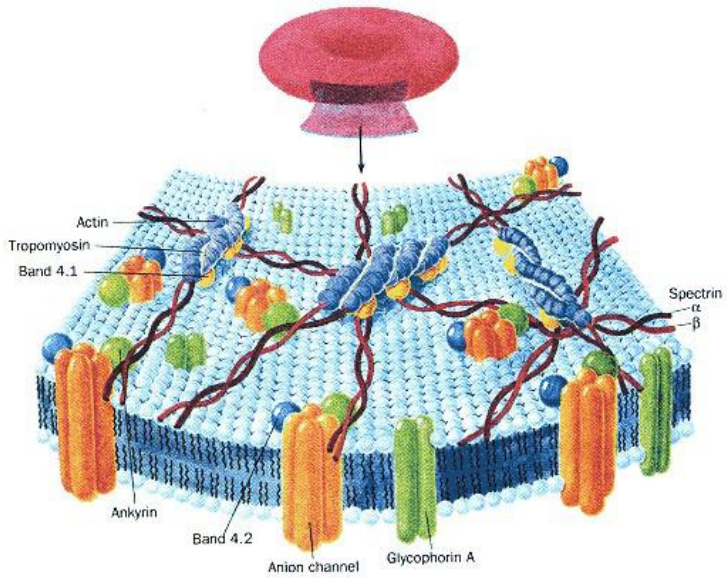

Figure 5.24
Schematic diagram of the erythrocyte membrane.
Diagram indicates the relationship of four membrane-associated proteins with the lipid bilayer. Glycophorin is a glycoprotein that contains 131 amino acids but whose function is unknown. Band 3, so designated because of its mobility in electrophoresis, contains over 900 amino acids and is involved in interacting with ankyrin and possibly in the facilitated diffusion of Cl^- and HCO_{3-} (see Section 5.1). Ankyrin and spectrin are part of the cytoskeleton and are peripheral membrane proteins. Ankyrin binds to band 3 and spectrin is anchored to the membrane by ankyrin.

Figure reproduced with permission from D. Voet and J. Voet, Biochemistry, 2nd ed., New York: Wiley, 1995.

membrane (Figure 5.25). Sphingomyelin is predominantly in the outer layer, whereas phosphatidylethanolamine is predominantly in the inner lipid layer. In contrast, cholesterol is equally distributed on both sides of the plasma membrane.

Asymmetry of lipids may be maintained by specific membrane proteins that promote the transverse movement of specific lipids from one side to the other. Metabolic energy may be involved in this process. Uncatalyzed transverse movement from one side to the other (i.e., flip-flop movement) of the glycerophospholipids and sphingolipids is slow. The asymmetry of lipids in erythrocyte membranes is an example of how slow is the transverse movement of membrane lipids. Mature erythrocytes have a lifetime of about 120 days, during which there is no new membrane synthesis or even significant repair. Even so, there appears to be little mixing of phospholipids between molecular layers. Individual lipids can exchange with lipids in the cell matrix, as well as with lipids of other membranes. Specific mechanisms to maintain both the composition and asymmetry of lipids in membranes apparently exist.

Proteins and Lipids Diffuse in Membranes

Interactions among different lipids and between lipids and proteins are very complex and dynamic. There is a **fluidity** in the lipid portion of

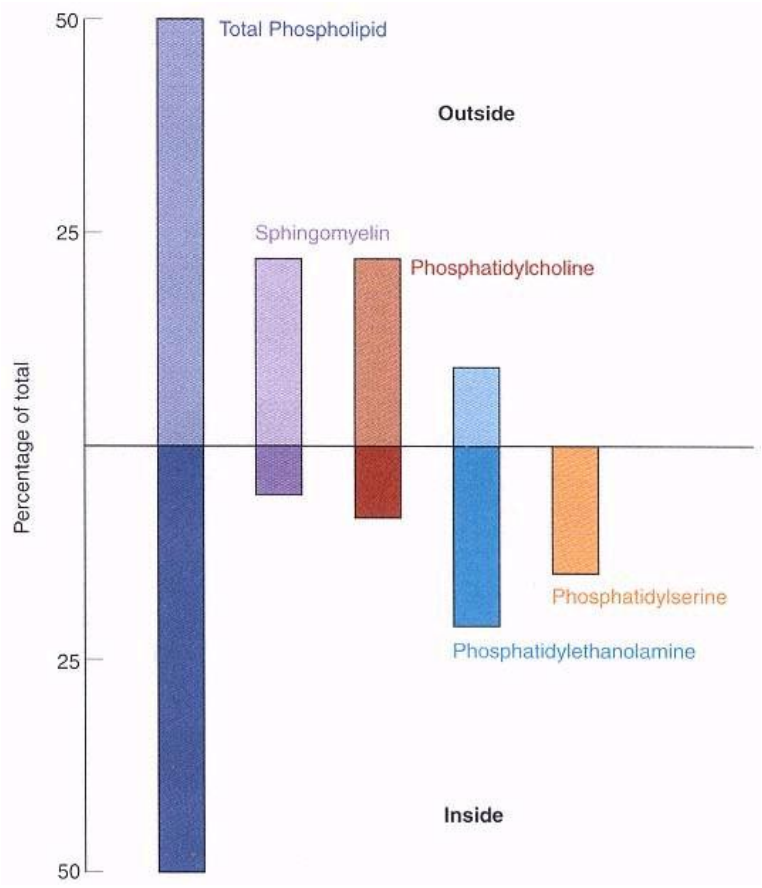

Figure 5.25
Distribution of phospholipids between inner and outer layers of the human erythrocyte membrane.
Values are percentage of each phospholipid in the membrane.
Redrawn from A. J. Verkeij, R. F. A. Zwaal, B. Roelofsen, P. Comfurius, D. Kastelijn, and L. L. M. Van Deenan. The asymmetric distribution of phospholipids in the human red cell membrane. Biochim. Biophys. Acta 323:178, 1973.

membranes in which both the lipids and proteins move. The degree of fluidity is dependent on the temperature and composition of the membrane. At low temperatures, lipids are in a gel–crystalline state, with lipids restricted in their mobility. As temperature is increased, there is a phase transition into a liquid–crystalline state, with an increase in fluidity (Figure 5.26). With liposomes prepared from a single pure phospholipid, the **phase transition temperature, T_m,** is rather precise; but with liposomes prepared from a mixture of lipids, T_m, becomes less precise because individual clusters of lipids may be in either the gel–crystalline or liquid–crystalline state. T_m is not precise for biological membranes because of their heterogeneous chemical composition. Interactions between lipids and proteins lead to variations in the gel–liquid state throughout the membrane and differences in fluidity in different areas of the membrane.

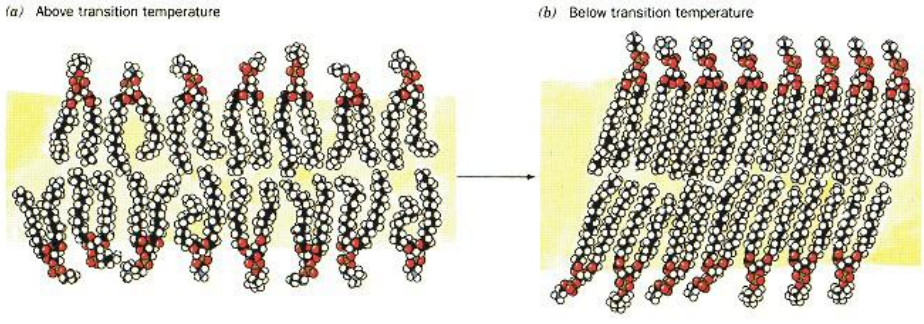

Figure 5.26
Structure of lipid bilayer above and below transition temperature.
Figure reproduced with permission from
D. Voet and J. Voet, *Biochemistry*, 2nd ed. New York: Wiley, 1995. (After Robertson, R. N., *The Lively Membranes*, Cambridge, MA: Cambridge University Press, 1983.)

The specific composition of individual biological membranes leads to differences in fluidity. Glycerophospholipids containing short-chain fatty acids will increase the fluidity as does an increase in unsaturation of the fatty acyl groups. Cis double bonds in unsaturated fatty acids of phospholipids lead to kinks in the hydrocarbon chain, preventing the tight packing of the chains and creating pockets in the hydrophobic areas. It is assumed that these spaces, which will also be mobile due to the mobility of the hydrocarbon chains, are filled with water molecules and small ions. **Cholesterol** with its flat stiff ring structure reduces the coiling of the fatty acid chain and decreases fluidity. Consideration has been given to the potential clinical significance of high blood cholesterol on the fluidity of cell membranes (see Clin. Corr. 5.2). **Ca^{2+}** ion decreases the fluidity of membranes because of its interaction with the negatively charged phospholipids, reducing repulsion between polar groups and increasing packing of lipid molecules. This ion causes aggregation of lipids into clusters, reducing membrane fluidity.

Fluidity at different levels within the membrane also varies. The hydrocarbon chains of the lipids have a motion, which produces a fluidity in the hydrophobic core. The central area of a bilayer is occupied by ends of the hydrocarbon chains and is more fluid than areas closer to the two surfaces, where there are more constraints due to stiffer portions of the hydrocarbon chains. Cholesterol makes membranes more rigid toward the periphery because it does not reach into the central core of membranes.

Individual lipids and proteins move rapidly in a lateral motion along the surface of membranes. Electrostatic interactions of polar head groups, hydrophobic interactions of cholesterol with selected phospholipids or glycolipids, and protein–lipid interactions, however, lead to constraints on movement. There are lipid domains in which lipids move together as a unit.

Movement of integral membrane proteins in the lipid environment has been demonstrated by fusion of human and rat cells. When antigenic membrane proteins on cells of each species were labeled with different antibody markers, the markers indicated the localization of the proteins on the membrane. Immediately following fusion of the cells, proteins on the membranes of the human and rat cells were segregated in different hemispheres of the new cell, but within 40 minutes the two groups of proteins were evenly distributed over the membrane of the new cell. Movement of protein is slower than that of lipids and may be restricted by other membrane proteins, matrix proteins, or cellular structural elements such as microtubules or microfilaments to which they may be attached.

CLINICAL CORRELATION 5.2

Abnormalities of Cell Membrane Fluidity in Disease States

Membrane fluidity can control the activity of membrane-bound enzymes, membrane functions such as phagocytosis, and cell growth. A major factor in controlling the fluidity of the plasma membrane in higher organisms and mammals is the presence of cholesterol. With increasing cholesterol content the lipid bilayers become less fluid on their outer surface but more fluid in the hydrophobic core. Erythrocyte membranes of individuals with spur cell anemia have an increased cholesterol content. This condition occurs in severe liver disease such as cirrhosis of the liver in alcoholics. Erythrocytes have a spiny shape and are destroyed prematurely in the spleen. The cholesterol content is increased 25–65%, and the fluidity of the membrane is decreased. The erythrocyte membrane requires a high degree of fluidity for its function and any decrease would have serious effects on the cell's ability to pass through the capillaries. The increased plasma membrane cholesterol in other cells leads to an increase in intracellular membrane cholesterol, which also affects their fluidity. The intoxicating effect of ethanol on the nervous system is probably due to modification of membrane fluidity and alteration of membrane receptors and ion channels. Individuals with abetalipoproteinemia have an increase in sphingomyelin content and a decrease in phosphatidylcholine, thus causing a decrease in fluidity. The ramifications of these changes in fluidity are not completely understood, but it is presumed that, as techniques for the measurement and evaluation of cellular membrane fluidity improve, some of the pathological manifestations in disease states will be explained on the basis of changes in membrane structure and function.

Cooper, R. A. Abnormalities of cell membrane fluidity in the pathogenesis of disease. *N. Engl. J. Med.* 297:371, 1977.

Evidence is accumulating that the fluidity of cellular membranes can change in response to changes in diet or physiological state. Fatty acid and cholesterol content of membranes is modified by a variety of factors. In addition, pharmacological agents may have a direct effect on membrane fluidity. Anesthetics that induce sleep and muscular relaxation may have their action because of their effect on membrane fluidity of specific cells. A number of structurally unrelated compounds induce anesthesia, but their common feature is lipid solubility. Anesthetics increase membrane fluidity *in vitro*.

Thus cellular membranes are in a constantly changing state, with not only movement of proteins and lipids laterally on the membrane but with molecules moving into and out of the membrane. The membrane creates a number of microenvironments, from the hydrophobic portion of the core of the membrane to the interface with the surrounding environments. It is difficult to express in words or pictures the very fluid and dynamic state, in that neither captures the time-dependent changes that occur in the structure of biological membranes. Figure 5.27 attempts to illustrate the structural and movement aspects of cellular membranes.

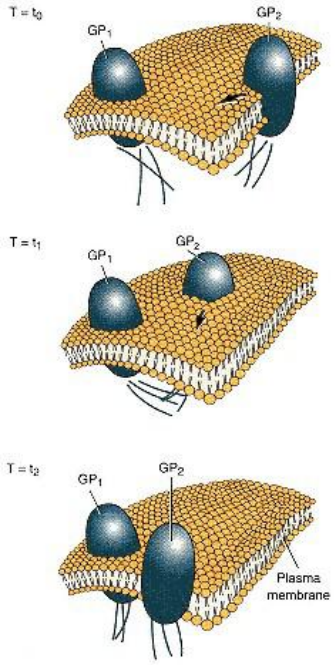

Figure 5.27
Modified version of the fluid mosaic model of biological membranes to indicate the mobility of membrane proteins.
t_0, t_1, and t_2 represent successive points in time. Some integral proteins (GP_2) are free to diffuse laterally in the plane of the membrane directed by the cytoskeletal components, whereas others (GP_1) may be restricted in their mobility.

5.5— Movement of Molecules through Membranes

The lipid nature of biological membranes severely restricts the type of molecules that diffuse readily from one side to another. Inorganic ions or charged organic molecules do not diffuse at a significant rate because of their attraction to water molecules and exclusion of charged species by the hydrophobic environment of lipid membranes. The diffusion rate of carbohydrates, amino acids, and inorganic ions, however, is not zero but may be too slow to accommodate a cell's requirements for the substance. Where there is a need to move a substance across a cell membrane, specific mechanisms are available for its translocation.

The basic mechanisms by which molecules cross cellular membranes is presented in the following sections with examples of the processes for illustrative purposes. Specific systems are described in the context of individual metabolic processes in later chapters.

Some Molecules Can Diffuse through Membranes

Diffusion of a substance through a membrane involves three major steps: (1) solute must leave the aqueous environment on one side and enter the membrane; (2) solute must traverse the membrane; and (3) solute must leave the membrane to enter a new environment on the opposite side (Figure 5.28). Each step involves an equilibrium of solute between two states. Thermodynamic and kinetic constraints control the concentration equilibrium of a substance on two sides of a membrane and the rate at which it can attain equilibrium. Diffusion of gases such as O_2, N_2, CO_2, and NO occur rapidly and depend entirely on the concentration gradient. **Water** diffuses readily through biological membranes; its movement occurs via gaps in the hydrophobic environment created by random movement of fatty acyl chains of lipids. Water and other small molecules move into these **transitory spaces** and equilibrate across the membrane from one gap to another. For diffusion of a solute with strong interaction with water molecules, the shell of water surrounding the solute must be stripped away before it enters the lipid milieu and then regained on leaving the membrane. Distribution of hydrophobic substances between the aqueous phase and lipid membrane will depend on the degree of lipid solubility of the substance; very lipid-soluble materials will dissolve in the membrane.

The **rate of diffusion** of a lipophilic substance is directly proportional to its lipid solubility and diffusion coefficient in lipids; the latter is a function of the size and shape of the substance. Uncharged lipophilic molecules, for exam-

ple, fatty acids and steroids, diffuse relatively rapidly but water-soluble substances, for example, sugars and inorganic ions, diffuse very slowly.

Direction of movement of solutes by diffusion is always from a higher to a lower concentration and the rate is described by **Fick's first law of diffusion:**

$$J = -D\left(\frac{\delta c}{\delta x}\right)$$

where J is the net amount of substance moved per time, D is the diffusion coefficient, and $\delta c/\delta x$ is the chemical gradient of substance. As the concentration of solute on one side of the membrane is increased, there will be an increasing *initial rate* of diffusion as illustrated in Figure 5.29. A *net movement* of molecules from one side to another will continue until the concentration in each is at chemical equilibrium. A continued exchange of solute molecules from one side to another occurs after equilibrium is attained but no net accumulation on one side can occur because this would recreate a concentration gradient if it occurred.

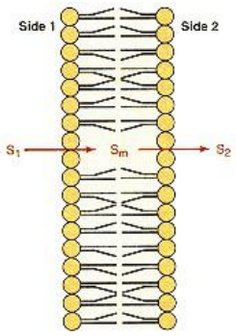

Figure 5.28
Diffusion of a solute molecule through a membrane.
S_1 and S_2 are solutes on each side of the membrane, and S_m is a solute in the membrane.

Movement of Molecules across Membranes Can be Facilitated

Mechanisms for membrane translocation of various substances including sugars, amino acids, metabolic intermediates, inorganic ions, and even H^+ have been determined. The plasma membrane of both prokaryotic and eukaryotic cells, as well as membranes of subcellular organelles, contain **transport systems** that have an important role in the uptake of nutrients, maintenance of ion concentrations, and control of metabolism. These systems involve intrinsic membrane proteins and are classified on the basis of their mechanism of translocation of substrate across the membrane and the energetics of the system. A classification of transport systems is presented in Table 5.4. Each will be discussed in more detail in subsequent sections but for now it is important to distinguish the three main types.

Membrane Channels

Membranes of most cells contain specific **channels,** in some cases referred to as **pores,** which permit the rapid movement of specific molecules or ions from one side of a membrane to the other. The tertiary and quaternary structures of these intrinsic membrane proteins create an aqueous hole in the membrane that permits diffusion of substances in both directions through the membrane. Like diffusion, the substances will move only in the direction of lower concentration, that is, down a concentration gradient. In contrast to transporters, the channel proteins do not bind the molecules or ions to be transported. The

TABLE 5.4 Classification of Membrane Translocation Systems

Type	*Class*	*Example*
Channel	1. Voltage regulated	Na^+ channel
	2. Chemically regulated	Acetylcholine receptor
	3. cAMP regulated	Cl^- channel
	4. Other	Pressure sensitive
Transporter	1. Passive mediated	Glucose transporter
	2. Active mediated	
	a. Primary-redox coupled	Respiratory chain linked
	Primary-ATPases	Na^+, K^+–ATPase
	b. Secondary	Na^+-dependent glycose transport
Group translocation		Amino acid translocation

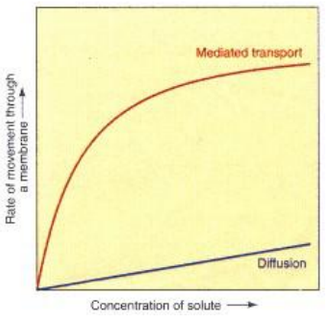

Figure 5.29
Kinetics of movement of a solute molecule through a membrane.
The initial rate of diffusion is directly proportional to the concentration of the solute. In mediated transport, the rate will reach a V_{max} when the carrier is saturated.

channels have some degree of specificity, however, based on the size and charge of the substance. Flow through the channel can be regulated by opening and shutting the passageway, like a gate to a garden.

Transporters

Transporters actually translocate the molecule or ion across the membrane by binding and physically moving the substance. The activity can be evaluated in the same kinetic terms as an enzyme-catalyzed reaction except no chemical reaction occurs. Transporters have specificity for the substance to be transported, frequently referred to as the substrate, have defined reaction kinetics, and can be inhibited by both competitive and noncompetitive inhibitors. Some transporters only move substrates down their concentration gradient (referred to as passive transport), while others can move the substrate against its concentration gradient (active transport) requiring the expenditure of some form of energy. With both channels and transporters the molecule is unchanged following translocation across the membrane.

A major difference between membrane channels and transporters is the rate of substrate translocation; for a channel, rates in the range of 10^7 ions s^{-1} are usual, whereas with a transporter the rate is in the range of 10^2–10^3 molecules s^{-1}. The activity of all translocation systems can be modulated, permitting cells and tissues to control the movement of substances across membranes. Drugs for specific channels and transporters have been developed to control these processes.

Group Translocation

Group translocation involves not only movement of the substance across the membrane but also chemical modification of the substance during the process. One mechanism of uptake of sugars by bacteria involves transport and then phosphorylation of the sugar before release into the cytosol of the cell. In some mammalian cells uptake of amino acids involves a group translocation mechanism.

Membrane Transport Systems Have Common Characteristics

Membranes of all cells contain highly specific transporters for the movement of inorganic anions and cations (e.g., Na^+, K^+, Ca^{2+}, HPO_{4}^{2+}, Cl^-, and HCO_3^-), and uncharged and charged organic compounds (e.g., amino acids and sugars). Different cellular membranes have different transport systems; as an example, the mitochondrial membrane has a specific mechanism to translocate ADP and ATP that is not present in other cellular membranes. Transport systems involve integral membrane proteins with a high degree of specificity for the substances transported. These proteins or protein complexes have been designated by a variety of names, including **transporter, translocase, translocator, permease,** and **pump,** or termed **transporter system, translocation mechanism,** and **mediated transport system.** The designations above are used interchangeably, but for convenience we will use transporter or translocase when referring to the proteins involved in translocation.

Membrane transporters have a number of characteristics in common. Each facilitates the movement of a molecule or molecules through the lipid bilayer at a rate that is significantly faster than can be accounted for by simple diffusion. If S_1 is the solute on side 1 and S_2 on side 2, then the transporter promotes establishment of an equilibrium as follows:

$$[S_1] \rightleftharpoons [S_2]$$

where the brackets represent the concentration of solute. If the transporter (T) is included in the equilibrium the rection is

$$[S_1] + T \rightleftharpoons [S - T] \rightleftharpoons [S_2] + T$$

TABLE 5.5 Characteristics of Membrane Transporters

Passive Mediated	*Active Mediated*
1. Saturation kinetics	1. Saturation kinetics
2. Specificity for solute transported	2. Specificity for solute transported
3. Can be inhibited	3. Can be inhibited
4. Solute moves down concentration gradient	4. Solute can move against concentration gradient
5. No expenditure of energy	5. Requires coupled input of energy

If no energy is put into the system, the concentration on both sides of the membrane will be equal at equilibrium; but if there is an expenditure of energy, a concentration gradient can be established. Note the similarity of the role of a transporter to that of an enzyme; in both cases the protein increases the rate but does not determine the final equilibrium.

Table 5.5 lists major characteristics of membrane transport systems. As presented in Figure 5.29, they demonstrate **saturation kinetics;** as the concentration of the substance to be translocated increases, the initial rate of transport increases but reaches a maximum when the substance saturates the protein transporter. Simple diffusion does not have saturation kinetics. Constants such as V_{max} and K_m can be calculated for transporters. As with enzymes, transporters can catalyze movement of a solute in both directions across the membrane depending on the ΔG for the reaction.

Most transporters have a high degree of structural and stereo specificity for the substance transported. An example is mediated transport of D-glucose in erythrocytes, where the K_m for D-galactose is 10 times larger and for L-glucose 1000 times larger than for D-glucose. The transporter has essentially no activity with D-fructose or disaccharides. Competitive and noncompetitive inhibitors have been found for many transporters. Structural analogs of the substrate inhibit competitively and reagents that react with specific groups on proteins are noncompetitive inhibitors.

There Are Four Common Steps in the Transport of Solute Molecules

We need to expand the equation above and consider four aspects of mediated transport (Figure 5.30). These are (1) *recognition by transporter of appropriate solute from a variety of solutes in the aqueous environment,* (2) *translocation of solute across membrane,* (3) *release of solute by transporter,* and (4) *recovery of transporter to its original condition to accept another solute molecule*.

The first step, **recognition** of a specific substrate by the transporter, is explained on the same basis as that described for recognition of a substrate by an enzyme. The presence of very specific binding sites on the protein permits the transporter to recognize the correct structure of the solute to be translocated.

The second step, **translocation,** is not completely understood. A reasonable mechanism (Figure 5.31) is one in which the protein transporter creates a channel between the environments on each side of the membrane with access through the channel being controlled by a gating mechanism in order to control which solutes can move into the channel. Transporters have receptor sites to which the solute attaches. After binding of solute and transporter, a conformational change of the protein moves the solute molecule a short distance, perhaps only 2 or 3 Å, but into the environment of the opposite side of the membrane. In this manner, it is not necessary for the transporter to move the molecule the entire distance across the membrane. Earlier suggestions for the translocation step included the possibility of a diffusible or rotating carrier, but both are improbable considering that transporters are large integral membrane proteins that do not diffuse transversely.

Figure 5.30
Reactions involved in mediated transport across a biological membrane.
S_1 and S_2 are the solutes on sides 1 and 2 of the membrane, respectively; T_1 and T_2 are the binding sites on the transporter on sides 1 and 2, respectively.

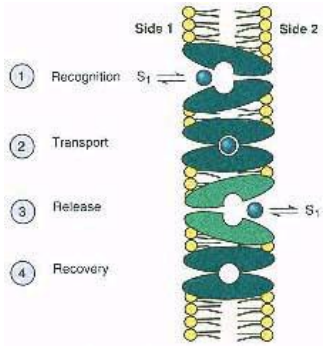

Figure 5.31
Model for a mediated transport system in a biological membrane.
Model is based on the concept of specific sites for binding of substrate and a conformational change in the transporter to move the bound solute a short distance but into the environment of the other side of the membrane. Once moved, the solute is released from the transporter.

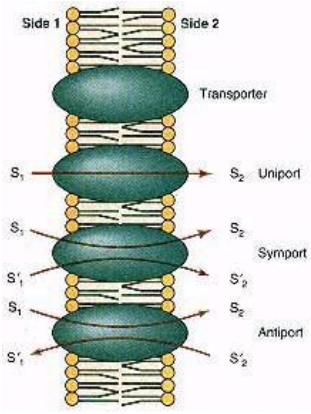

Figure 5.32
Uniport, symport, and antiport mechanisms for translocation of substances.
S and S 1represent different molecules.

Release, step 3, of the solute can occur readily if the concentration of solute is lower in the new compartment than on the initial side of binding. Without a change in the affinity (K_{eq}), there would be a shift in the equilibrium and release of a portion of the solute. For those transporters that move a solute against a concentration gradient, release of the solute at the higher concentration requires a decrease in the affinity for the solute by the transporter. A change in the conformation of the transporter decreases the affinity. In group translocation (p. 210) the solute is chemically altered while attached to the transporter and the modified molecule has a lower affinity for the transporter.

Finally, in **recovery,** step 4, the transporter must return to its original state. If a conformational change has occurred, the transporter reverts to the original conformation.

The discussion above has centered on the movement of a single solute molecule by the transporter. There are systems that move two molecules simultaneously in one direction **(symport mechanisms),** two molecules in opposite directions **(antiport mechanism),** as well as a single molecule in one direction **(uniport mechanism)** (Figure 5.32). When a charged substance, such as K^+, is translocated and no ion of the opposite charge is moved, a charge separation occurs across the membrane. This mechanism is termed electrogenic and leads to development of a membrane potential. If an oppositely charged ion is moved to balance the charge, the mechanism is called neutral or electrically silent.

Energetics of Membrane Transport Systems

The change in **free energy** when an uncharged molecule moves from a concentration of C_1 to a concentration of C_2 on the other side of a membrane is given by Eq. 5.1:

$$\Delta G' = 2.3RT\log\left(\frac{C_2}{C_1}\right) \qquad \textbf{(5.1)}$$

When ΔG is negative—that is, there is release of free energy—movement of solute will occur without the need for a driving force. When ΔG is positive, as would be the case if C_2 is larger than C_1, then there needs to be an input of energy to drive the transport. For a charged molecule (e.g., Na^+) both the electrical potential and concentrations of solute are involved in calculating the change in free energy as indicated in Eq. 5.2:

$$\Delta G' = 2.3RT\log\left(\frac{C_2}{C_1}\right) + Z\mathscr{F}\Psi \qquad \textbf{(5.2)}$$

where Z is the charge of the species moving, $\mathscr{F}$ is the Faraday constant (23.062 kcal V^{-1} mol^{-1}), and Ψ is the difference in electrical potential in volts across the membrane. The electrical component is the membrane potential and ΔG is the electrochemical potential.

A **passive transport** system is one in which ΔG is negative and the movement of solute occurs spontaneously. When ΔG is positive, coupled input of energy from some source is required for movement of the solute and the process is called active transport. **Active transport** is driven by either hydrolysis of ATP to ADP or utilization of an electrochemical gradient of Na^+ or H^+ across the membrane. In the first the chemical energy released on hydrolysis of a pyrophosphate bond drives the reaction, whereas in the latter an electrochemical gradient is dissipated to transport the solute.

Transport systems that can maintain very large concentration gradients are present in various membranes. An example is the plasma membrane transport system that maintains the Na^+ and K^+ gradients. One of the most striking examples of an active transport system is that present in the parietal cells of gastric glands, which are responsible for secretion of HCl into the lumen of the stomach (see p. 1069). The pH of plasma is about 7.4 (4×10^{-8} M H^+), and

the luminal pH of the stomach can reach 0.8 (0.15 M H^+). The cells transport H^+ against a concentration gradient of $1 \times 10^{6.6}$. Assuming there is no electrical component, the energy for H^+ secretion under these conditions can be calculated from Eq. 5.1 and is 9.1 kcal mol^{-1} of HCl.

5.6— Channels and Pores

Channels and Pores in Membranes Function Differently

Membrane channels are differentiated from membrane pores on the basis of their degree of specificity for molecules crossing the membrane. **Channels** are selective for specific inorganic cations and anions, whereas pores are not selective, permitting inorganic and organic molecules to pass through the membrane. The Na^+ channel of plasma membranes of eukaryotic cells, for example, permits movement of Na^+ at a rate more than ten times greater than that for K^+. This difference between channels and pores is due to differences in size of the aqueous area created in the protein structure as well as amino acid residues lining the channel area. Channels and pores are intrinsic membrane proteins and amino acid sequences in the proteins of many channels suggest existence of structurally related superfamilies of proteins in which similar amino acid sequences occur. A common motif is a structure formed by amphipathic α-helices of associated protein subunits or from domains within a single polypeptide chain creating a central aqueous space as pictured in Figure 5.33. Exceptions to the α-helical structure are the porins (see below) of Gram-negative bacteria, which have α,β-sheet structure lining the central pore. The opening and closing of membrane channels involve a conformational change in the channel protein.

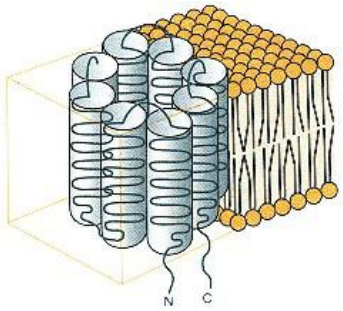

Figure 5.33
Arrangement of protein subunits
or domains to form a membrane channel.

Opening and Closing of Channels Are Controlled

As indicated in Table 5.4, the opening and closing of some channels can be controlled by changes in the **transmembrane potential.** These are referred to as **voltage-gated channels.** In the case of the Na^+ channel, depolarization of the membrane leads to an opening of the channel. Voltage-gated channels for Na^+, K^+, and Ca^{2+} are present in the plasma membrane of most cells. Clinical Correlation 22.8 (p. 956) describes changes in voltage-gated channels in myotonic muscle disorders. Mitochondria have a voltage-dependent channel for anions. Binding of a specific agent, termed an **agonist,** can also control the opening of a channel. A channel opens in the **nicotinic–acetylcholine receptor** on binding of acetylcholine allowing the flow of Na^+ into the cell. This mechanism is important to neuronal electrical signal transmission (see p. 928). In addition, some channels are controlled by cAMP (see p. 862); Clin. Corr. 5.3 describes the modification of the Cl^- channel in cystic fibrosis. These forms of control for opening channels are very fast, permitting bursts of ion flow through the membrane at rates of over 10^7 ions s^{-1}, which is near the diffusion rate of these ions in water. This rate is necessary because these channels are involved in nerve conduction and muscle contraction. A number of pharmacological agents that modulate these channels are used therapeutically.

Sodium Channel

Voltage-sensitive **sodium channels** mediate rapid increase in intracellular Na^+ following depolarization of the plasma membrane in nerve and muscle cells. The channel consists of a single large glycopolypeptide and several smaller glycoproteins. The genes for some of the Na^+ channels have been cloned and the amino acid sequences have been determined. There are four repeat homology units, each with six transmembrane α-helices. A model for this trans-

CLINICAL CORRELATION 5.3

Cystic Fibrosis and the Cl^- Channel

Cystic fibrosis (CF), an autosomal recessive disease, is the commonest, fatal, inherited disease of caucasians, occurring with a frequency of 1 in 2000 live births. It is a multi-organ disease, with a principal manifestation being pulmonary obstruction; thick mucous secretions obstruct the small airways allowing recurrent bacterial infections. Exocrine pancreatic dysfunction occurs early and leads to steatorrhea (fatty stool) in CF patients; see page 1059 for a discussion of the role of the pancreas in fat digestion and absorption. CF patients have reduced Cl^- permeability, which impairs fluid and electrolyte secretion, leading to luminal dehydration. Diagnosis of CF is confirmed by a significant increase of Cl^- in sweat of affected in comparison to normal individuals.

The gene responsible for CF was identified in 1989 and over 400 mutations leading to CF have been found. The most common mutation (about 70%) leads to a deletion of a single phenylalanine at position 508 on the protein, but missense, nonsense, frameshift, and splice-junction mutations (see p. 628) have also been reported. The CF gene product is the cystic fibrosis transmembrane conductance regulator (CFTR), which is a cAMP-dependent Cl^- channel. CFTR is composed of 1480 amino acids with structural homology to a family of transport proteins termed the transport ATPases. The gene has been cloned (see p. 765) and a major effort is under way to treat the disease by gene therapy, using both viral and nonviral vectors including liposomes (see Clin. Corr. 5.1).

Alton, E. W., and Geddes, D. M. Gene therapy for cystic fibrosis; a clinical perspective. *Gene Ther*. 2:88, 1995; Frizzell, R. A. Functions of the cystic fibrosis transmembrane conductance regulator protein. *Am. J. Respir. Crit. Care Med.* 151:54, 1995; and Wagner, J. A., Chao, A. C., and Gardner, P. Molecular strategies for therapy of cystic fibrosis. *Annu. Rev. Pharmacol. Toxicol.* 35:257, 1995.

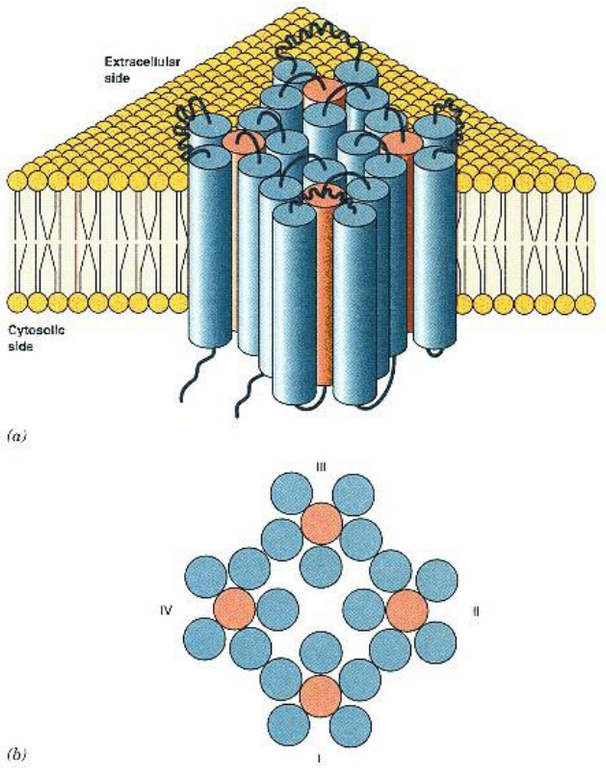

Figure 5.34
Possible model of the Na^+ channel.
(a) The single peptide consists of four repeating units with each unit folding into six transmembrane helices.
(b) Proposed arrangement of the transmembrane sequences as viewed down on the membrane.
Redrawn from M. Noda et al., *Nature* 320:188, 1986.

porter is presented in Figure 5.34*a* and a possible arrangement of the helices in the membrane as viewed down on a membrane is presented in Figure 5.34*b*. One transmembrane segment, labeled S4, has a positively charged amino acid at every third position and may serve as a voltage sensor. A mechanical shift of this region due to a change in the membrane potential may lead to a conformational change in the protein, resulting in the opening of the channel. The channel size created by the protein, however, cannot totally explain the specificity for Na^+.

Nicotinic–Acetylcholine Channel (nAChR)

The **nicotinic–acetylcholine channel,** also referred to as the **acetylcholine receptor,** is an example of a chemically regulated channel, where the binding of acetylcholine (Figure 5.35) opens the channel. The dual name is used to differentiate this receptor from other acetylcholine receptors, which function in a different manner. **Acetylcholine,** a neurotransmitter, is released at the

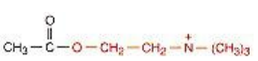

Figure 5.35
Structure of acetylcholine.

neuromuscular junction by a neuron when electrically excited. The acetylcholine diffuses to the skeletal muscle membrane where it interacts with the acetylcholine receptor, opening the channel and allowing selective cations to move across the membrane (see p. 928). The change in transmembrane potential leads to a series of events culminating in muscle contraction. The nicotinic–acetylcholine receptor consists of five polypeptide subunits, with two α subunits and one each of β, γ, and δ; each α subunit is phosphorylated and glycosylated and two others contain covalently bound lipid. The channel opens when two acetylcholine molecules bind to subunits and cause a change in protein conformation; reclosure of the channel occurs within a millisecond due to hydrolysis of acetylcholine to acetate and choline and release of bound ligand. A desensitized state of the receptor has been reported that does not open when acetylcholine binds. In the open conformation, cations and small nonelectrolytes can flow through the channel but not anions; negatively charged amino acid residues in the channel are sufficient to repel negatively charged ions from passing.

The nicotinic–acetylcholine receptor is inhibited by a number of deadly neurotoxins including ***d*-tubocurarine,** the active ingredient of curare, and several toxins from snakes including ***α*-bungarotoxin, erabutoxin,** and **co-bratoxin,** the latter from the cobra. **Succinylcholine,** a muscle relaxant, activates the channel leading to depolarization of the membrane; succinyl choline is used in surgical procedures because its activity is reversible due to the rapid hydrolysis of the compound after cessation of administration.

Examples of Pores Are Gap Junctions and Nuclear Pores

Plasma membrane gap junctions and **nuclear membrane pores** are relatively large aqueous openings in the membrane created by specific proteins. **Gap junctions** are clusters of membrane channels lined by proteins spanning two plasma membranes that create aqueous connections between two cells. They permit the exchange between cells of ions and metabolites but not large molecular weight compounds such as proteins. The diameter of the opening ranges from 12 to 20 Å. Oligomers of the gap junction polypeptide (32 kDa), referred to as **connexin,** form the channel. Twelve subunits, six from each cell, form a hexameric structure in each membrane as shown in Figure 5.36. The channels are normally open but increases in cytosolic Ca^{2+}, a change in metabolism, a drop in transmembrane potential, or acidification of the cytosol cause closure. When the channel is open subunits appear to be slightly tilted

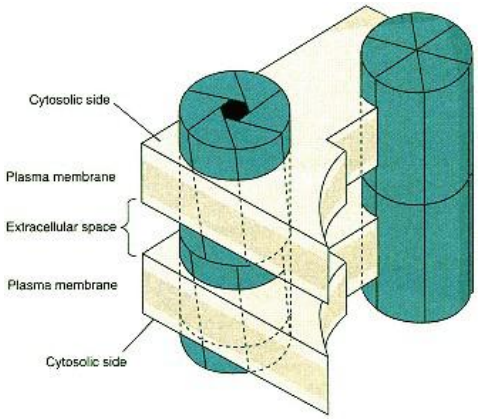

Figure 5.36
Model for a channel in the gap junction.

but when closed they appear to be more nearly parallel to a perpendicular to the membrane, suggesting that subunits slide over each other. The detailed mechanism of opening and closing, however, is unknown.

Like gap junctions **nuclear pores** cover two membranes, creating aqueous channels in the nuclear envelope. Pores are about 90 Å in diameter and permit the movement of large macromolecules. They are presumably lined with protein. The plasma membranes of Gram-negative bacteria also contain protein pores, termed **porins.** Over 40 different porins have been isolated and they range in size from 28 to 48 kDa. In contrast to most mammalian channels, these transmembrane segments are β-sheets not α-helices and exist in the membrane as trimers. Porins are water-filled transmembrane channels and range in diameter from 6 to 23 Å with some degree of selectivity for inorganic ions; some, however, permit the uptake of sugars.

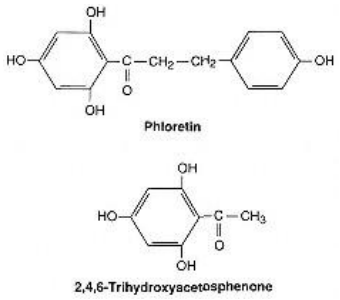

Figure 5.37
Inhibitors of passive mediated transport of D-glucose in erythrocytes.

5.7— Passive Mediated Transport Systems

Passive mediated transport, also referred to as **facilitated diffusion,** leads to translocation of solutes through cell membranes without expenditure of metabolic energy (see Table 5.5, p. 199). As with nonmediated diffusion the direction of flow is always from a higher to a lower concentration. The distinguishing differences between measurements of simple diffusion and passive mediated transport are the demonstration of saturation kinetics, a structural specificity for the class of molecule moving across the membrane, and specific inhibition of solute movement.

Glucose Transport Is Facilitated

A family of passive mediated transporters for D-glucose, frequently referred to as **glucose permeases,** has been identified in the plasma membrane of mammalian cells. Six members have been described and are termed GLUT-1, GLUT-2, and so on. All have 12 hydrophobic segments considered to be the transmembrane regions. The physiological direction of movement is into the cell because the extracellular level of glucose is about 5 mM and most cells metabolize glucose rapidly, thus maintaining low intracellular concentrations. The transporter catalyzes a uniport mechanism and is most active with D-glucose. D-Galactose, D-mannose, D-arabinose, and several other D-sugars as well as glycerol are translocated by the same transporter. L-Isomers are not transported. It has been proposed that the β-D-glucopyranose is transported with carrier interaction at the hydrogen atoms on at least C-1, C-3, and C-6 of the sugar. The affinity of erythrocyte translocase for D-glucose is highest with a K_m of ~6.2 mM, whereas for other sugars K_m values are much higher. The transporter has a very low affinity for D-fructose, precluding a role in cellular uptake of fructose; a separate carrier for fructose has been proposed. With isolated erythrocytes, glucose will move either into or out of the erythrocyte, depending on the direction of the experimentally established concentration gradient, demonstrating the reversibility of the system. Several sugar analogs as well as phoretin and 2,4,6-trihydroxyacetophenone (Figure 5.37) are competitive inhibitors. Some physiological aspects of the glucose translocase are presented on p. 881.

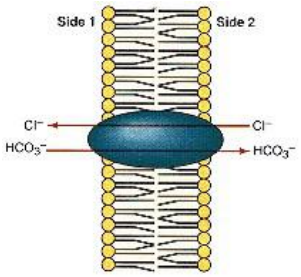

Figure 5.38
Passive anion antiport mechanism for movement of Cl^- and HCO_{3-} across the erythrocyte plasma membrane.

Cl^- and HCO_{3-} Are Transported by an Antiport Mechanism

An anion transporter in erythrocytes involves the antiport movement of Cl^- and HCO_{3-} (Figure 5.38). The transporter is referred to as the **Cl^-—HCO_{3-} exchanger, anion exchange protein,** or **band 3,** the latter because of its position in SDS polyacrylamide gel electrophoresis of erythrocyte membrane proteins. The direction of ion flow is reversible and depends on the concentra-

tion gradients of the ions across the membrane. The transporter has an important role in adjusting the erythrocyte HCO_3^- concentration in arterial and venous blood (see p. 1035).

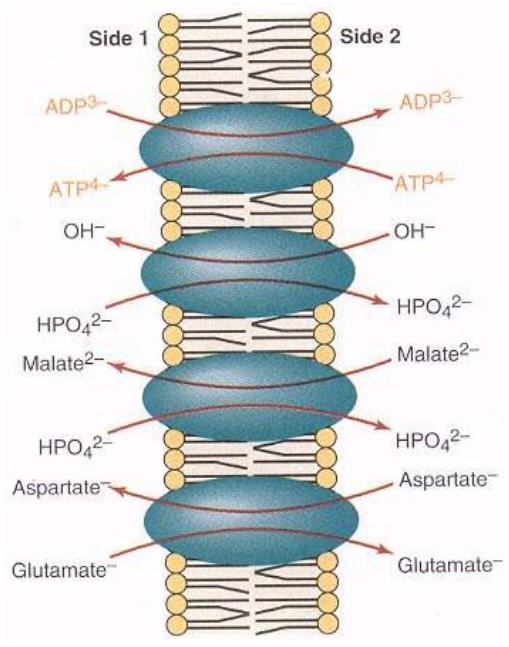

Figure 5.39
Representative anion transport systems in liver mitochondria.
Note that each is an antiport mechanism. Several other transport systems are known and are discussed in Chapter 6.

Mitochondria Contain a Number of Transport Systems

The inner mitochondrial membrane contains several antiport systems for the exchange of anions between the cytosol and mitochondrial matrix. These include (1) a transporter for exchange of ADP and ATP, (2) a transporter for exchange of phosphate and OH^-, (3) a dicarboxylate carrier that catalyzes an exchange of malate for phosphate, and (4) a translocator for exchange of aspartate and glutamate (Figure 5.39). The relationship of these translocases and energy coupling are discussed on page 243. In the absence of an input of energy these transporters will catalyze a passive exchange of metabolites down their concentration gradient to achieve a thermodynamic equilibrium of all intermediates. As an antiport mechanism, a concentration gradient of one compound can drive the movement of the other solute. In several cases, the transporter catalyzes the antiport movement of an equal number of charges on the substrate; in such movement the mitochondrial membrane potential influences the equilibrium and the anions can be moved against their concentration gradients. ADP–ATP and the phosphate transporters, as well as an uncoupling protein that translocates H^+, have significant amino acid homology and are presumably derived from a common ancestor. It has been suggested that each subunit has six transmembrane -helices. The **uncoupling protein,** found in mitochondria of brown adipose tissue, has been proposed to be involved in generation of heat.

The **ATP–ADP translocase** is very specific for ATP and ADP and deoxyribose derivatives, dATP and dADP, but does not transport AMP or other nucleotides. It is a dimer containing two subunits of 33 kDa each and represents about 12% of the total protein in heart mitochondria. It is very hydrophobic and can exist in two conformations. Atractyloside and bongkrekic acid (Figure 5.40) are

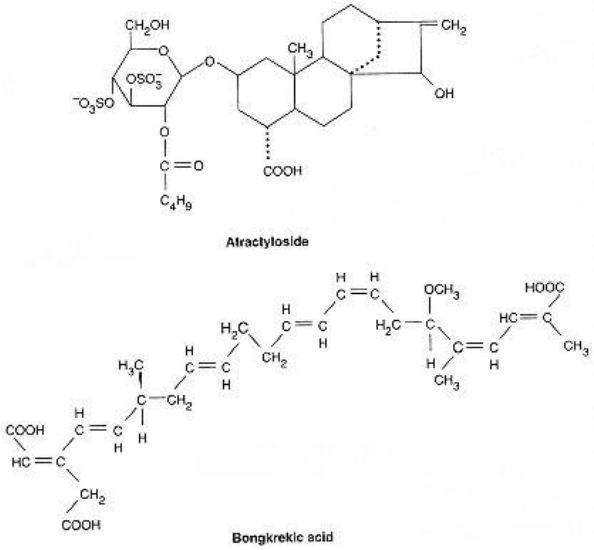

Figure 5.40
Structure of two inhibitors of the ATP–ADP transport system of liver mitochondria.

specific inhibitors, each apparently reacting with a different conformation of the protein. The mitochondrial membrane potential can drive the movement of the nucleotides by this translocator, but in the absence of the membrane potential it functions as a passive mediated transporter.

It is sometimes difficult to differentiate passive mediated transport from simple diffusion, but inhibition of the process is good evidence of a carrier.

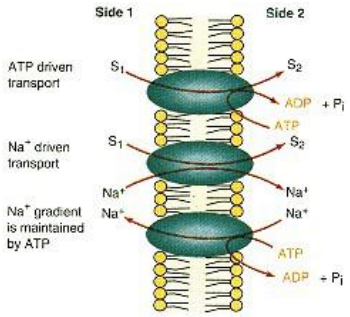

Figure 5.41
Involvement of metabolic energy (ATP) in active mediated transport systems. The chemical energy released on the hydrolysis of ATP to ADP and inorganic phosphate is used to drive the active transport of various substances, including Na^+. The transmembrane concentration gradient of Na^+ is also used for the active transport of substances.

5.8— Active Mediated Transport Systems

Active mediated transporters have the same three characteristics as passive transporters, that is, saturation kinetics, substrate specificity, and inhibitability (see Table 5.5, p. 199). They also require the utilization of energy to translocate solutes and if the energy source is removed or inhibited, the transport system will not function. These active transporters can be classified as either **primary active transporters,** if they utilize ATP directly, or **secondary active transporters** if a transmembrane chemical gradient of Na^+ or H^+ is utilized (Figure 5.41). The transporters that utilize ATP are also referred to as an **ATPase** because during the translocation ATP is hydrolyzed to ADP and phosphate. They are classified as either **P, V, or F type transporters or ATPases.** P type translocases are phosphorylated and dephosphorylated during the transport activity; the Na^+, K^+-translocase is an example of a P type. V, for vacuole, type are present in membranes of lysosomes, endosomes, Golgi vesicles, and secretory vesicles and are responsible for acidification of the interior of these vesicles. F type translocases, present in mitochondria and chloroplasts, are involved in ATP synthesis (see p. 263). A special case of active transport is the translocation of protons across the inner mitochondrial membrane during electron transport; this mechanism is discussed in detail on page 262 and will not be reviewed here. Active mediated transporters, which use the transmembrane Na^+ or H^+ gradient, require maintenance of the gradient; for Na^+ this is achieved by expenditure of ATP (Figure 5.41). Inhibition of ATP synthesis leads to a dissipation of the Na^+ gradient, which in turn causes a cessation of transport activity.

Translocation of Na^+ and K^+ Is a Primary Active Transport System

All mammalian cells contain a Na^+,K^+ antiporter, type P, which utilizes ATP to drive the movement of the ions. Knowledge of this transporter has developed along two paths: (1) from studies of a membrane enzyme, the **Na^+,K^+–ATPase,** that catalyzes ATP hydrolysis and has a requirement for Na^+ and K^+ ions, and (2) from measurements of Na^+ and K^+ movements across intact plasma membranes by a protein referred to as the **Na^+,K^+ pump.** The two activities are catalyzed by the same protein.

All Plasma Membranes Contain a Na^+,K^+-Activated ATPase

All mammalian plasma membranes catalyze the reaction

$$ATP \xrightarrow[Mg^{2+}]{Na^+ + K^+} ADP + P_i$$

The enzyme, officially termed the **Na^+,K^+-exchanging ATPase,** has a requirement for both Na^+ and K^+ ions, as well as Mg^{2+}, which is a cofactor for ATP-requiring reactions. The level of the ATPase in plasma membranes correlates with the Na^+,K^+ transport activity. Excitable tissues, such as muscle and nerve, and cells actively involved in the movement of Na^+ ion, such as those in the salivary gland and kidney cortex, have high activities of both Na^+,K^+-ATPase and Na^+,K^+ transport system. The protein responsible for the Na^+,K^+–ATPase activity is an oligomer containing two α subunits of about 110 kDa each and

two β subunits of about 55 kDa each. The smallest subunits are glycoproteins, and the complex has the characteristics of an integral membrane protein. Figure 5.42 is a schematic diagram of the Na^+,K^+-exchanging ATPase. The ATPase activity has a requirement for phospholipids indicating its close relationship to the membrane. During transport the larger subunit is *cyclically phosphorylated* and *dephosphorylated* on a specific aspartic acid residue forming a β-aspartyl phosphate. Phosphorylation of the protein requires Na^+ and Mg^{2+} but not K^+, whereas dephosphorylation of the protein requires K^+ but not Na^+ or Mg^{2+}. The isolated enzyme has an absolute requirement for Na^+, but K^+ can be replaced with NH_{4+} or Rb_+. Two distinguishable conformations of the protein complex have been observed and thus it is classified as an **E_1–E_2 type transporter.** A possible sequence of reactions for the enzyme is presented in Figure 5.43.

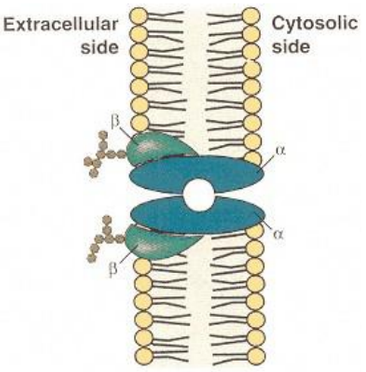

Figure 5.42
Schematic drawing of the Na^+,K^+-transporting ATPase of plasma membranes.

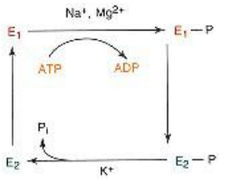

Figure 5.43
Proposed sequence of reactions and intermediates in hydrolysis of ATP by the Na^+,K^+–ATPase.
E_1 and E_2 are different conformations of the enzyme. Phosphorylation of he enzyme requires Na^+ and Mg^{2+} and dephosphorylation involves K^+.

Of significance to its physiological role as a transporter, the enzyme is inhibited by a series of **cardiotonic steroids.** These pharmacological agents, which include **digitalis,** increase the force of contraction of heart muscle by altering the excitability of the tissue, which is a function of the Na^+–K^+ concentrations across the membrane. **Ouabain** (Figure 5.44) is one of the most active Na^+,K^+–ATPase inhibitors of the series; its site of binding is on the smaller subunit of the enzyme complex and at some distance from the ATP-binding site on the larger monomer. An inhibitor in human serum of the transporter competes with ouabain binding and may be involved in the control of Na^+,K^+ transport.

Erythrocyte Ghosts Are Used to Study Na^+,K^+ Translocation

Studies of the **Na^+,K^+ transporter** activity have been facilitated by use of erythrocyte preparations free of hemoglobin, referred to as **erythrocyte ghosts.** By carefully adjusting the tonicity of the medium, erythrocytes will swell with breaks in the phospholipid bilayer, permitting leaking from cells of cytosolic material, including hemoglobin. The cytosol can be replaced with a defined medium by readjusting the tonicity so that the membrane reseals, trapping isolation medium inside. In this manner intracellular ionic and substrate composition and even protein content can be altered. With erythrocyte ghosts intra- and extracellular Na^+ and K^+ can be manipulated as well as ATP or inhibitor content. Movement of Na^+ and K^+ is an antiport vectorial process, with Na^+ moving out and K^+ moving into the cell. This transporter is responsible for maintaining the high K^+ and low Na^+ concentrations in a mammalian cell (see p. 14). ATP-binding site on the protein is on the inner surface of the membrane in that hydrolysis occurs only if ATP, Na^+, and Mg^{2+} are inside the cell. K^+ ion is required externally for internal dephosphorylation of protein. Ouabain inhibits translocation of Na^+ and K^+ but only if present externally. There are between 100 and 200 transporter molecules per erythrocyte, but the number is significantly larger for other tissues.

ATP hydrolysis by the translocase occurs only if Na^+ and K^+ are translocated, demonstrating that the enzyme is not involved in dissipation of energy in a useless activity. For each ATP hydrolyzed, three ions of Na^+ are moved out of the cell but only two ions of K^+ in, which leads to an increase in external positive charges. This electrogenic movement of Na^+ and K^+ is part of the mechanism for the maintenance of the transmembrane potential in tissues. Even though the energetics of the system dictate that it functions in only one direction, the translocator can be reversed *in vitro* by adjusting the Na^+ and K^+ levels; a small net synthesis of ATP has been observed when transport is forced to run in the reverse direction.

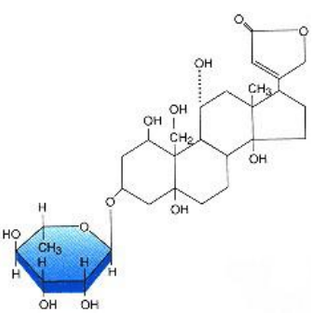

Figure 5.44
Structure of ouabain, a cardiotonic steroid, which is a potent inhibitor of the Na^+,K^+–ATPase and of active Na^+ and K^+ transport.

A hypothetical model for movement of Na^+ and K^+ is presented in Figure 5.45. The protein goes through conformational changes during which the Na^+ and K^+ are moved short distances. During the transition a change in the affinity

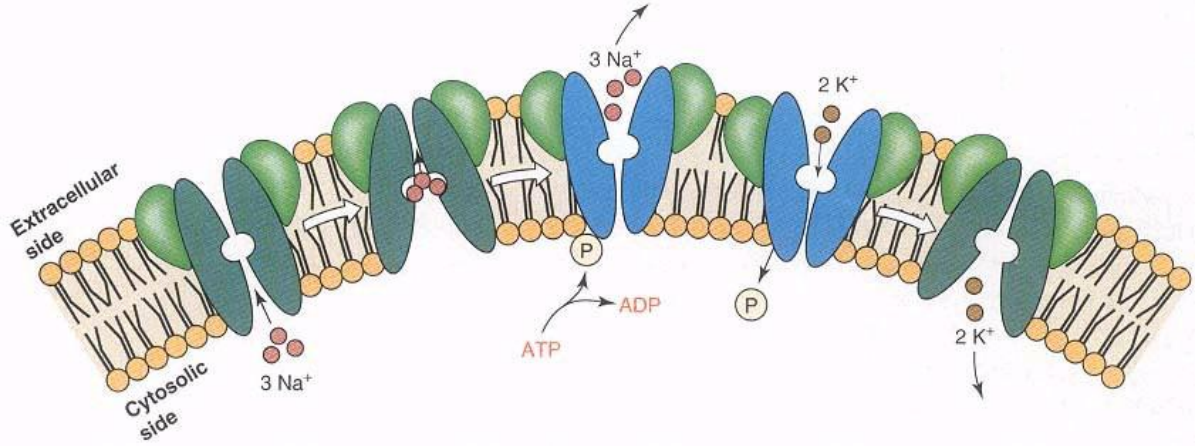

Figure 5.45
Hypothetical model for the translocation of Na^+ and K^+ across the plasma membrane by the Na^+,K^+–ATPase.
(1) Transporter in conformation 1 picks up Na^+.
(2) Transporter in conformation 2 translocates and releases Na^+.
(3) Transporter in conformation 2 picks up K^+.
(4) Transporter in conformation 1 translocates and releases K^+.

of the binding protein for the cations can occur such that there is a decrease in affinity constants, resulting in the release of the cation into a milieu where the concentration is higher than that from which it was transported.

As an indication of the importance of this enzyme, it has been estimated that Na^+,K^+–ATPase uses about 60–70% of the ATP synthesized by cells in nerve and muscle, and may utilize about 35% of ATP generated in a resting individual.

Ca^{2+} Translocation Is Another Example of a Primary Active Transport System

Ca^{2+} is an important **intracellular messenger** regulating cellular processes as varied as muscle contraction and carbohydrate metabolism. The signal initiated by some hormones, the primary messenger to direct cells to alter their function, is transmitted by changes in cytosolic Ca^{2+}; for this reason Ca^{2+} is referred to as a **second messenger.** Cytosolic Ca^{2+} is in the range of 0.10 μM, over 10,000 times lower than extracellular Ca^{2+}. Intracellular Ca^{2+} concentrations can be increased rapidly by (1) transient opening of Ca^{2+} channels in the plasma membrane, permitting flow of Ca^{2+} down the large concentration gradient, or (2) by release from stores of Ca^{2+} in endoplasmic or sarcoplasmic reticulum. In order to reestablish low cytosolic levels, Ca^{2+} is actively transported out of cells across the plasma membrane or into the endoplasmic or sarcoplasmic reticulum. With both membrane systems, a **Ca^{2+} transporter** of the E_1–E_2 type is involved in which ATP is hydrolyzed during translocation. The transporter catalyzes a Ca^{2+}-stimulated ATPase activity.

Ca^{2+}–ATPase of sarcoplasmic reticulum of muscle, which is involved in the contraction–relaxation cycles of muscle, represents 80% of the integral membrane protein of the sarcoplasmic reticulum and occupies one-third of the surface area (see p. 954); it has many properties similar to Na^+,K^+–ATPase. The protein has ten membrane-spanning helices and is phosphorylated on an aspartyl residue during the Ca^{2+} translocation reaction. Two Ca^{2+} ions are translocated for each ATP hydrolyzed and it can move Ca^{2+} against a very large concentration gradient.

The Ca^{2+} transporter of plasma membranes has properties similar to the enzyme of sarcoplasmic reticulum. In eukaryotic cells, the transporter is regulated by cytosolic Ca^{2+} levels through a calcium-binding protein termed **calmodulin.** As cellular Ca^{2+} levels rise, Ca^{2+} is bound to calmodulin, which has a dissociation constant of ~1 μM. The Ca^{2+}–calmodulin complex binds to the

Ca^{2+} transporter, leading to an increased rate in Ca^{2+} transport. The rate is increased by lowering the K_m for Ca^{2+} of the transporter from about 20 to 0.5 μM. Increased activity reduces cytosolic Ca^{2+} to its normal resting level (~0.10 μM) at which concentration the Ca^{2+}–calmodulin complex dissociates and the rate of the Ca^{2+} transporter returns to a lower value. Thus the Ca^{2+}–calmodulin complex exerts fine control on the Ca^{2+} transporter. Calmodulin is one of several Ca^{2+}-binding proteins, including **parvalbumin** and **troponin C,** all of which have very similar structures. The Ca^{2+}–calmodulin complex is also involved in control of other cellular processes, which are affected by Ca^{2+}. The protein (17 kDa) has the shape of a dumbbell with two globular ends connected by a seven-turn α-helix; there are four Ca^{2+}-binding sites, two high affinity on one lobe and two low affinity on the other. It is believed that the binding of Ca^{2+} to the lower affinity binding sites causes a conformational change in the protein, revealing a hydrophobic area that can interact with a protein that it controls. Each Ca^{2+}-binding site consists of a helix–loop–helix structural motif (Figure 5.46) and Ca^{2+} is bound in the loop connecting the helices. A similar structure is found in other Ca^{2+}-binding proteins. The motif is referred to as the **EF hand,** based on studies with parvalbumin where the Ca^{2+} is bound between helices E and F of the protein.

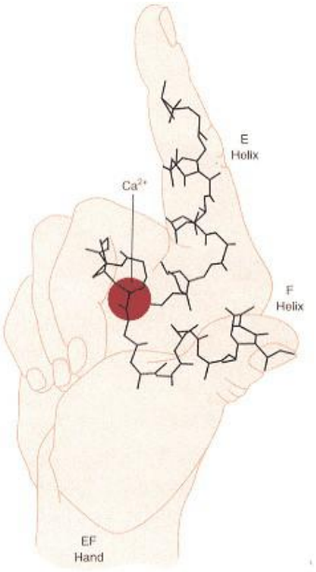

Figure 5.46
Binding site for Ca^{2+} in calmodulin.
Calmodulin contains four Ca^{2+}-binding sites, each with a helix–loop–helix motif. The Ca^{2+} ion is bound in the loop that connects two helices. This motif occurs in various Ca^{2+}-binding proteins and is referred to as the EF hand.

Na^+-Dependent Transport of Glucose and Amino Acids Are Secondary Active Transport Systems

The mechanisms described above for the active transport of cations involve the direct hydrolysis of ATP as the driving force. *Cells have another energy source, the gradient of Na^+ ion across the plasma membrane, which is utilized to move sugars, amino acids, and Ca^{2+} actively*. A symport translocation system involving simultaneous movement of both a Na^+ ion and glucose in the same direction is present in plasma membranes of cells of kidney tubule and intestinal epithelium. The general mechanism is presented in Figure 5.47. The diagram represents the **transport of D-glucose** driven by the movement of Na^+ ion down its concentration gradient. During transport of the sugar no hydrolysis of ATP occurs. There is an absolute requirement for Na^+, and in the process of translocation one Na^+ is moved with each glucose molecule. It can be considered that Na^+ is moving by passive facilitated transport down its chemical gradient and glucose carried along even against its concentration gradient. It is obligatory that the transporter translocates a glucose with the Na^+ ion. In the transport the chemical gradient of Na^+ ion is dissipated and unless the Na^+ ion gradient is continuously regenerated, transport of glucose will cease. The Na^+ gradient is maintained by the Na^+,K^+-exchanging ATPase described above and also represented in Figure 5.47. Thus metabolic energy in the form of ATP is indirectly involved in glucose transport because it is utilized to maintain the Na^+ ion gradient. Inhibition of ATP synthesis and a subsequent decrease in ATP will alter the Na^+ ion gradient and inhibit glucose uptake. Ouabain, the inhibitor of the Na^+,K^+ transporter, inhibits uptake of glucose by preventing the cell from maintaining the Na^+–K^+ gradient. Each glucose molecule requires only one-third of an ATP to be translocated because three Na^+ ions are translocated for the hydrolysis of each ATP in the Na^+,K^+-exchanging ATPase.

Amino acids are also translocated by the luminal epithelial cells of the intestines by Na^+-dependent pathways similar to the Na^+-dependent glucose transporter. At least seven different translocators have been identified for different classes of amino acids (see p. 1072 for details). The Na^+ gradient is also utilized to drive the transport of other ions, including a symport mechanism in the small intestines for the uptake of Cl^- with Na^+ and an antiport mechanism for the excretion of Ca^{2+} out of the cell.

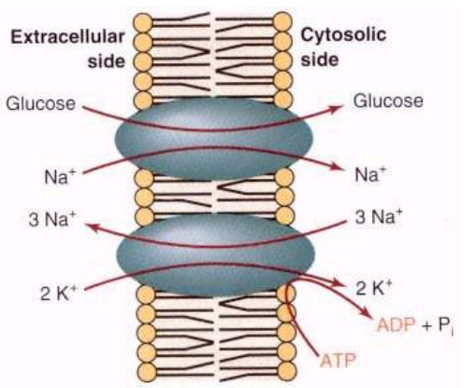

Figure 5.47
Na^+-dependent symport transport of glucose across the plasma membrane.

The chemical mechanism for the symport movement of molecules utilizing the Na^+ ion gradient involves a cooperative interaction of the Na^+ ion and the other molecule translocated on the protein. A conformational change of

the protein occurs following association of the two ligands, which moves them the necessary distance to bring them into contact with the cytosolic environment. Dissociation of Na^+ ion from the transporter because of the low Na^+ ion concentration inside the cell leads to a return of the protein to its original conformation, a decrease in affinity for the other ligand, and release of ligand into the cytosol.

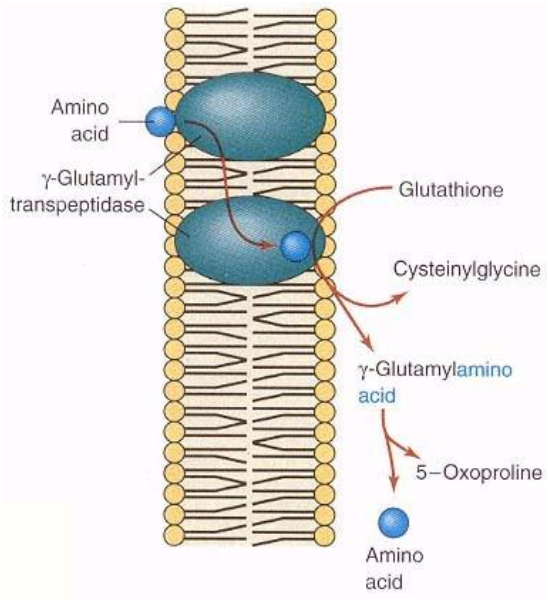

Figure 5.48
The γ-glutamyl cycle.
Represented are the key reactions involved in the group translocation of amino acids across liver cell plasma membranes. The continued uptake of amino acids requires the constant resynthesis of glutathione via a series of ATP-requiring reactions described in Chapter 11, page 485.

Group Translocation Involves Chemical Modification of the Substrate Transported

As discussed previously, a major hurdle for any active transport system is release of the transported molecule from the binding site after translocation. If affinity of the transporter for the translocated molecule does not change, there cannot be movement against a concentration gradient. In the active transport systems described above a change in affinity for the substance by the transporter occurs by a conformational change of the protein. An alternate mechanism for release of a substrate is chemical change of the molecule after translocation but before release from the transporter, leading to a new compound bound to the transporter with a lower affinity for the transporter. The process is termed **group translocation.** The ***γ*-glutamyl cycle** for the transport of amino acids across the plasma membrane of some tissues is an example where the substrate is altered during transport and released into the cell as a different molecule. Reactions of the transport mechanism are presented in Figure 5.48. The pathway involves the enzyme ***γ*-glutamyltranspeptidase,** a membrane-bound enzyme. This leads to formation of a dipeptide with the amino acid transported. The amino acid transported is the substrate to which the γ-glutamyl residue of **glutathione** (Figure 5.49; see p. 485) is transferred. The new dipeptide is not part of the chemical gradient across the membrane of the amino acid. The γ-glutamyl derivative is then hydrolyzed by a separate enzyme, not on the membrane, leaving the free amino acid and oxoproline.

All the amino acids except proline can be transported by group translocation. The energy for transport comes from the hydrolysis of a peptide bond in glutathione. For the system to continue, glutathione must be resynthesized, which requires the expenditure of three ATP molecules (see p. 485). Thus for each amino acid translocated, three ATPs are required. Recall that the expenditure of only one-third of an ATP is required for each amino acid transported in the Na^+-dependent translocase system. Group translocation is an expensive energetic mechanism for transport of amino acids. The pathway is present in many tissues but some doubt has been raised about its physiological significance in that individuals have been identified with a genetic absence of the γ-glutamyl-transpeptidase without any apparent difficulty in amino acid transport. Cells may have several alternate methods for the transport of amino acids and are not dependent on one mechanism.

A group translocation mechanism for uptake of sugars is found in bacteria. This pathway involves phosphorylation of the sugar, using phosphoenol-pyruvate as the phosphate donor. The mechanism is referred to as the **phospho-enolpyruvate-dependent phosphotransferase system** (PTS).

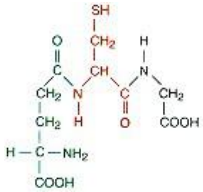

Figure 5.49
Glutathione
(-glutamylcysteinylglycine).

Summary of Transport Systems

The foregoing has presented the major mechanisms for movement of molecules across cellular membranes, particularly the plasma membrane. Cell organelles and membrane systems have a variety of transport systems. Mitochondria have transport mechanisms utilizing a proton gradient (see p. 243). Bacteria have transport systems analogous to those observed in mammalian cells. Table 5.6 summarizes characteristics of some major transport systems found in mammalian cells (see Clin. Corr. 5.4).

TABLE 5.6 Major Transport Systems in Mammalian Cells[a]

Substance Transported	*Mechanism of Transport*	*Tissues*
Sugars		
Glucose	Passive	Widespread
	Active symport with Na^+	Small intestines and renal tubular cells
Fructose	Passive	Intestines and liver
Amino acids		
Amino acid-specific transporters	Active symport with Na^+	Intestines, kidney, and liver
All amino acids except proline	Active group translocation	Liver
Specific amino acids	Passive	Small intestine
Other organic molecules		
ATP–ADP	Antiport transport of nucleotides; can be active transport	Mitochondria
Ascorbic acid	Active symport with Na^+	Widespread
Biotin	Active symport with Na^+	Liver
Cholic acid, deoxycholic acid, and taurocholic acid	Active symport with Na^+	Intestines
Dicarboxylic acids	Active symport with Na^+	Kidney
Folate	Active	Widespread
Lactate and monocarboxylic acids	Active symport with H^+	Widespread
Neurotransmitters (e.g., γ-amino butyric acid, norepinephrine, glutamate, dopamine)	Active symport with Na^+	Brain
Organic anions (e.g., malate, α-ketoglutarate, glutamate)	Antiport with counterorganic anion	Mitochondria
Peptides (2 to 4 amino acids)	Active symport with H^+	Intestines
Urea	Passive	Erythrocytes and kidney
Inorganic ions		
H^+	Active	Mitochondria
H^+	Active; vacuolar ATPase	Widespread; lysosomes, endosomes, and Golgi complex
Na^+	Passive	Distal renal tubular cells
Na^+, H^+	Active antiport	Proximal renal tubular cells and small intestines
Na^+, K^+	Active: ATP driven	Plasma membrane of all cells
Na^+, HPO_{42-}	Active cotransport	Kidney
Ca^{2+}	Active: ATP driven	Plasma membrane and endoplasmic (sarcoplasmic) reticulum
Ca^{2+}, Na^+	Active antiport	Widespread
H^+, K^+	Active antiport	Parietal cells of gastric mucosa secreting H^+
Cl^-/HCO_{3-}	Passive antiport	Erythrocytes and other cells

[a] The transport systems are only indicative of the variety of transporters known; others responsible for a variety of substances have been proposed. Most systems have been studied in only a few tissues and their localization may be more extensive than indicated.

5.9— Ionophores

An interesting class of antibiotics of bacterial origin facilitates the movement of monovalent and divalent inorganic ions across biological and synthetic lipid membranes. These molecules, called **ionophores,** are not large macromolecules such as proteins but are relatively small molecular weight compounds (up to several thousand daltons). Ionophores are divided into two major groups:

CLINICAL CORRELATION 5.4

Diseases Due to Loss of Membrane Transport Systems

A number of pathological conditions are due to an alteration in the transport systems for specific cellular components. Some of these are discussed in the appropriate sections describing the metabolism of the intermediates. Individuals have been observed with a decrease in glucose uptake from the intestinal tract due to a loss of the specific sodium-coupled glucose–galactose transporter. Fructose malabsorption syndromes have been observed, which are due to an alteration in the activity of the transport system for fructose. In Hartnup's disease there is a decrease in the transport of neutral amino acids in the epithelial cells of the intestine and renal tubules.

In cystinuria, renal reabsorption of cystine and the basic amino acids lysine and arginine is abnormal, resulting in formation of cystine kidney stones. In hypophosphatemic, vitamin D resistant rickets, renal absorption of phosphate is abnormal. Little is known concerning possible changes of transport activities in tissues such as muscle, liver, and brain but it has been suggested that there may be a number of pathological states due to the loss of specific transport mechanisms.

Evans, L., Grasset, E., Heyman, M., et. al. Congenital selective malabsorption of glucose and galactose. *J. Pediatr. Gastroenterol. Nutr.* 4:878, 1985.

TABLE 5.7 Major Ionophores

Compound	*Major Cations Transported*	*Action*
Valinomycin	K^+ or Rb^+	Uniport, electrogenic
Nonactin	NH_4^+, K^+	Uniport, electrogenic
A23187	Ca^{2+}/2 H^+	Antiport, electroneutral
Nigericin	K^+/H^+	Antiport, electroneutral
Monensin	Na^+/H^+	Antiport, electroneutral
Gramicidin	H^+, Na^+, K^+, Rb^+	Forms channels
Alamethicin	K^+, Rb^+	Forms channels

(1) **mobile carriers** are those ionophores that diffuse back and forth across the membrane carrying the ion from one side of the membrane to the other, and (2) ionophores that form a **channel** that transverses the membrane and through which ions can diffuse. With both types, ions are translocated by a passive mediated transport mechanism. The ionophores that diffuse back and forth across the membrane are more affected by changes in the fluidity of the membrane than those that form a channel. Some major ionophores are listed in Table 5.7.

Each ionophore has a definite ion specificity; **valinomycin,** whose structure is given in Figure 5.50, has an affinity for K^+ that is 1000 times greater than that for Na^+ and the antibiotic A23187 (Figure 5.51) translocates Ca^{2+} 10 times more actively than Mg^{2+}. Several of the diffusion type ionophores have a cyclic structure. The metal ion is coordinated to several oxygen atoms in the core of the ionophore; the periphery of the molecule consists of hydrophobic groups. The interaction of the ionophore leads to a chelation of the ion, stripping away its surrounding water shell and encompassing the ion by a hydrophobic shell. The ionophore-ion complex freely diffuses across the membrane. Since the interaction of ion and ionophore is an equilibrium reaction, a steady state develops in the concentration of ions on both sides of the membrane. The specificity of the ionophore is due in part to the size of the pore into which the ion fits and to the attraction of the ionophore for the ion in competition with water molecules.

Valinomycin transports K^+ by an electrogenic uniport mechanism and can create an electrochemical gradient across a membrane as it carries a positively charged K^+ across the membrane. **Nigericin** is an electrically neutral antiporter; its carboxyl group when dissociated binds a positive ion, such as K^+, leading to a neutral molecule that crosses a membrane. On diffusion back through the

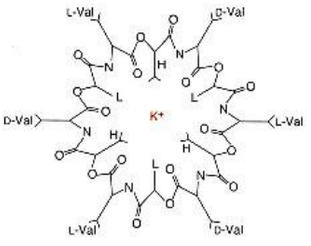

Figure 5.50
Structure of the valinomycin–K^+ complex.
Abbreviations: D-Val, D-valine; L-Val, L-valine; L, L-lactate; and H, D-hydroxyisovalerate.

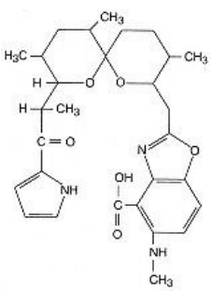

Figure 5.51
Structure of A23187, a Ca^{2+} ionophore.

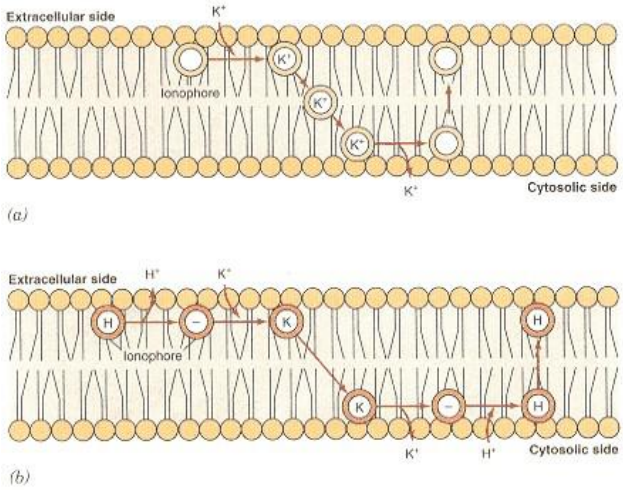

Figure 5.52
Proposed mechanism for the ionophoretic activities of valinomycin and nigericin.
(a) Transport by valinomycin.
(b) Transport by nigericin. The valinomycin–K^+ complex is positively charged and translocation of K^+ is electrogenic, leading to the creation of a charge separation across the membrane. Nigericin translocates K^+ in exchange for a H^+ across the membrane and the mechanism is electrically neutral.
Diagram adopted from B. C. Pressman, *Annu. Rev. Biochem.* 45:501, 1976.

membrane it transports a proton; overall K^+ exchanges for H^+. These mechanisms are presented in Figure 5.52.

Gramicidin A is an example of an ionophore that creates a pore in the membrane. This type of ionophore has a low ion selectivity because ions are diffusing through a hole in the membrane. Two molecules of gramicidin A form a channel and the dimer is in constant equilibrium with the free monomer form. By association and dissociation of the monomers in the membrane, channels can be formed and broken; the rate of interaction of two molecules of gramicidin A controls the rate of ion flux. The structure of the molecule suggests that polar peptide groups line the channel and hydrophobic groups are on the periphery of the channel interacting with the lipid membrane.

The antibiotic ionophores have been a valuable experimental tool in studies involving ion translocation in biological membranes and for the manipulation of the ionic compositions of cells. There have been reports that proteolipids, prostaglandins, and perhaps other lipids present in mammalian tissues may function as ionophores.

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Questions

C. N. Angstadt and J. Baggott

1. Cell membranes typically:

A. are about 90% phospholipid.

B. have both integral and peripheral proteins.

C. contain cholesteryl esters.

D. contain free carbohydrate such as glucose.

E. contain large amounts of triacylglycerols.

Refer to the following for Questions 2–5:

A. cerebrosides

B. gangliosides

C. phosphatidylcholines

D. phosphatidylinositols

E. sphingomyelins

2. Sphingolipids containing phosphorus.

3. Incorporate an oligosaccharide containing sialic acid.

4. Belong to the class of neutral glycosphingolipids.

5. Contain a hexahydroxy alcohol.

6. According to the fluid mosaic model of a membrane:

A. proteins are always completely embedded in the lipid bi-layer.

B. transverse movement (flip-flop) of a protein in the membrane is thermodynamically favorable.

C. the transmembrane domain has largely hydrophobic amino acids.

D. proteins are distributed symmetrically in the membrane.

E. peripheral proteins are attached to the membrane only by noncovalent forces.

7. Characteristics of a mediated transport system include:

A. nonspecific binding of solute to transporter.

B. release of the transporter from the membrane following transport.

C. a rate of transport directly proportional to the concentration of solute.

D. release of the solute only if the concentration on the new side is lower than that on the original side.

E. a mechanism for translocating the solute from one side of the membrane to the other.

8. Membrane channels:

A. have a large aqueous area in the protein structure so are not very selective.

B. commonly contain amphipathic α-helices.

C. are opened or closed only as a result of a change in the transmembrane potential.

D. are the same as gap junctions.

E. allow substrates to flow only from the outside to inside of the cell.

9. Which of the following require(s) a transporter that specifically binds a solute?

A. active mediated transport

B. gap junction

C. membrane channel

D. simple diffusion

E. all of the above

10. Which of the following can transport a solute against its concentration gradient?

A. active mediated transport

B. passive mediated transport

C. both of the above systems

D. neither of the above systems

11. The transport system that maintains the Na^+ and K^+ gradients across the plasma membrane of cells:

A. involves an enzyme that is an ATPase.

B. is a symport system.

C. moves Na^+ either into or out of the cell.

D. is an electrically neutral system.

E. in the membrane, hydrolyzes ATP independently of the movement of Na^+ and K^+.

12. A mediated transport system would be expected to:

A. show a continuously increasing initial rate of transport with increasing substrate concentration.

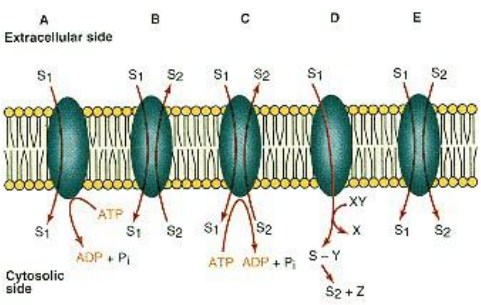
B. exhibit structural and/or stereospecificity for the substance transported.

C. be slower than that of a simple diffusion system.

D. establish a concentration gradient across the membrane.

E. exist only in plasma membranes.

The answers to Questions 13 and 14 are based on the following figure:



13. Represents a passive mediated antiport system.

14. Could represent the Na^+-driven uptake of glucose.

15. The translocation of Ca^{2+} across a membrane:

A. is a passive mediated transport.

B. is an example of a symport system.

C. involves the phosphorylation of a serine residue by ATP.

D. may be regulated by the binding of a Ca^{2+}-calmodulin complex to the transporter.

E. maintains $[Ca^{2+}]$ very much higher in the cell than in extracellular fluid.

16. The group translocation type of transport system:

A. does not require metabolic energy.

B. involves the transport of two different solute molecules simultaneously.

C. has been demonstrated for fatty acids.

D. results in the alteration of the substrate molecule during the transport process.

E. uses ATP to maintain a concentration gradient.

17. All of the following are correct about an ionophore EXCEPT it:

A. requires the input of metabolic energy for mediated transport of an ion.

B. may diffuse back and forth across a membrane.

C. may form a channel across a membrane through which an ion may diffuse.

D. may catalyze electrogenic mediated transport of an ion.

E. will have specificity for the ion it moves.

Answers

1. B (Figures 5.2 and 5.17). A: This is more than the total lipid. C: Cholesterol in membranes is unesterified. D: All carbohydrate in membranes is in the form of glycoproteins and glycolipids. E: This is a minor component, if present (pp. 180 and 187).

2. E A sphingomyelin is the only type of sphingolipid-containing phosphate (Figures 5.13).

3. B By definition, gangliosides contain sialic acid (p. 185).

4. A Cerebrosides are neutral; no phosphate; uncharged sugar. Gangliosides, by virtue of the presence of sialic acid, are acidic. Note: Sulfatides, which are acidic, are derived from cerebrosides but are not themselves classified as cerebrosides (pp. 184 and 185).

5. D The alcohol is inositol, which is often phosphorylated (p. 182).

6 C Hydrophobic domains will be in the interior; hydrophilic domains will be at either surface of the membrane (p. 189, Figure 5.19). A: Proteins may also be on the surface. B: Transverse motion of proteins is even less than that of lipids (p. 195). D: Both proteins and lipids are distributed asymmetrically. E: Glycans bind covalently to an amino acid as part of GPI anchors (p. 192).

7. E A: Specificity of binding is an integral part of the process. B: Recovery of the transporter to its original condition is one of the characteristics of mediated transport. C: Only at low concentrations of solute; transporters show saturation kinetics. D: Active transport, movement against a gradient, is also mediated transport (p. 198).

8. B A: This describes a pore; channels are quite specific. C: Voltage-gated channels, like that for Na^+, are controlled this way but others, like the nicotinic-acetylcholine channel, are chemically regulated. D: Clusters of membrane channels

work together to form a gap junction. E: Substances may move in either direction as dictated by the concentration gradient (p. 201).

9. A Specific binding by the transporter is a characteristic of mediated systems. B–D: These do not require a transporter (p. 196).

10. A Transportation against a gradient requires the input of energy (p. 204).

11. A The Na^+,K^+ transporter is the Na^+,K^+–ATPase. It is an antiport, vectorial (Na^+ out), electrogenic (3 Na^+, 2 K^+) system. ATP hydrolysis is not useless (p. 206).

12. B A and B: Mediated transport systems show saturation kinetics and substrate specificity (p. 199). C: The purpose of the transporter is to aid the transport of water-soluble substances across the lipid membrane (p. 197). D: This would be true only if the system were an active one. E: Mediated systems are also present in other membranes, for example, mitochondrial membrane (p. 199).

13. B The figure is a modified composite of Figures 5.32, 5.38, 5.39, and 5.48.

14. E All systems are mediated. A: An active uniport. B: A passive antiport; for example, Cl^--HCO_{3-}. C: An *active* antiport; for example, Na+,K+-ATPase. D: A group translocation representing a change in S_1 during transport. E: A symport system; in this case, S_1 could be glucose and S_2 could be Na^+.

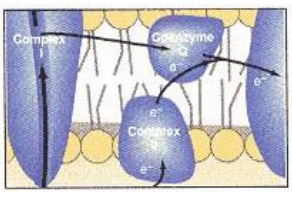
15. D This occurs with the eukaryotic plasma membrane (p. 208). A and B: Ca^{2+} translocation is an active uniport. C: Like Na^+,K^+–ATPase, phosphorylation occurs on an aspartyl residue. E: Extracellular is about 10,000 times higher.

16. D In eukaryotic cells, amino acids are transported by group translocation in which they are converted to a γ-glutamyl amino acid during transport (Figure 5.48). A and E: It is an active system with the ATP used to resynthesize the intermediate, glutathione. B and C: The system transports a single amino acid at a time (p. 210).

17. A Ionophores transport by passive mediated mechanisms (p. 212). B and C: These are the two major types of ionophores. D: Valinomycin transports K^+ by a uniport mechanism. There are also antiport systems that are electroneutral. E: For example, valinomycin has an affinity for K^+ 1000 times greater than for Na^+.

Chapter 6—
Bioenergetics and Oxidative Metabolism

Merle S. Olson



6.1— Energy-Producing and Energy-Utilizing Systems

Living cells are composed of a complex, intricately regulated system of energy-producing and energy-utilizing chemical reactions. Metabolic reactions involved in energy generation break down ingested or stored fuels such as carbohydrate, lipid, or protein in what are termed **catabolic** pathways. These reactions usually result in the conversion of large complex molecules to smaller molecules (ultimately CO_2 and H_2O), with production of storable or conservable energy, and often require the consumption of oxygen during this process. Such reactions are accelerated during periods of fuel deprivation or stress to an organism.

Energy-utilizing reactions perform various necessary, and in many instances tissue-specific, cellular functions, for example, nerve impulse conduction, muscle contraction, growth, and cell division. Metabolic pathways involved in the biosynthesis of large, complex molecules from smaller precursors are termed **anabolic** pathways and require the expenditure of energy. Such reactions are accelerated when energy is readily available, when precursor molecules are in abundance, or during periods of growth or regeneration of cellular material.

ATP Links Energy-Producing and Energy-Utilizing Systems

The relationship between energy-producing and energy-utilizing functions of the cell is illustrated in Figure 6.1. Energy may be derived from oxidation of metabolic fuels presented to the organism usually in the form of carbohydrate, lipid, and protein. The proportion of each fuel utilized as an energy source depends on the tissue and the dietary and hormonal state of the organism. For example, mature erythrocytes and adult brain in the fed state use only carbohydrate as a source of energy, whereas the liver of a diabetic or fasted individual metabolizes primarily lipid to meet the energy demands. Energy may be consumed during performance of various energy-linked (work) functions, some of which are indicated in Figure 6.1. Note that the liver and the pancreas are primarily involved in biosynthetic and secretory work functions, whereas the primary function of cardiac and skeletal muscle involves converting metabolic energy into mechanical energy during muscle contraction.

The essential link between energy-producing and energy-utilizing pathways is the nucleoside triphosphate, **adenosine 5′-triphosphate (ATP)** (Figure 6.2). The ATP molecule is a purine (adenine) nucleotide in which the adenine is attached in a glycosidic linkage to D-ribose. Three phosphoryl groups are esterified to the 5 position of the ribose moiety in **phosphoanhydride bonds.** The

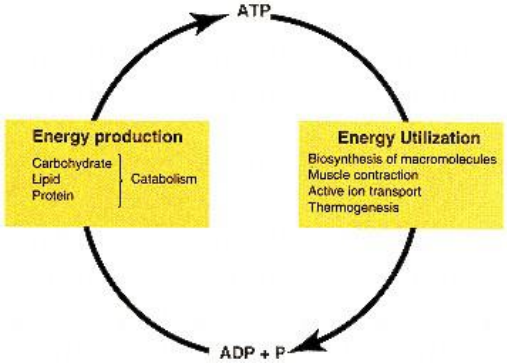


Figure 6.1
Relationship between energy production and energy utilization.

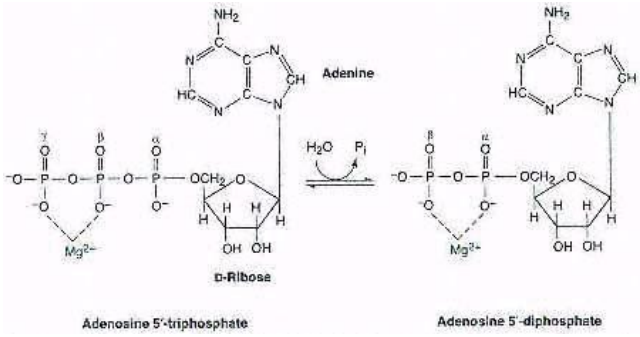


Figure 6.2
Structure of ATP and ADP complexed with Mg^{2+}.

two terminal phosphoryl groups (i.e., β and γ) are involved in the phosphoric acid anhydride bonding and are designated as energy-rich or **high-energy bonds.** Synthesizing ATP as a result of a catabolic process or consuming ATP in an energy-linked cellular function involves formation and either hydrolysis or transfer of the terminal phosphate group of ATP. The physiological form of this nucleotide is chelated with a divalent metal cation such as magnesium. Adenosine diphosphate also chelates magnesium, but the affinity of the metal cation for ADP is considerably less than for ATP. Although adenine nucleotides are mainly involved in energy generation or conservation, various nucleoside triphosphates, including ATP, are involved in transferring energy during biosynthetic processes. As indicated in Figure 6.3, the guanine nucleotide **GTP** serves as the source of energy in gluconeogenesis and protein synthesis, whereas

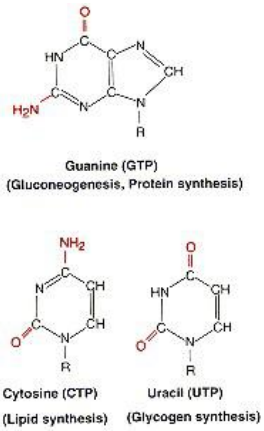


Figure 6.3
Structures of purine and pyrimidine bases involved in various biosynthetic pathways.

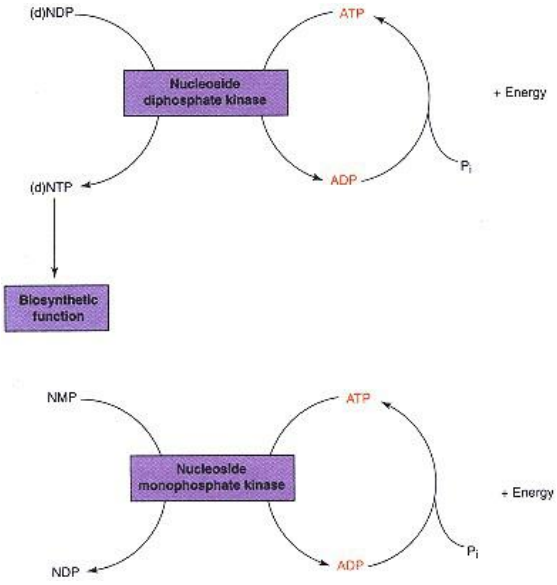


Figure 6.4
Nucleoside diphosphate kinase and nucleoside monophosphate kinase reactions.
N represents any purine or pyrimidine base; (d) indicates a deoxyribonucleotide.

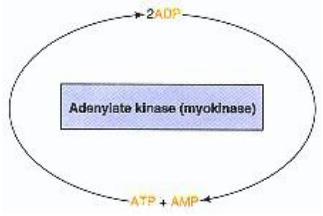


Figure 6.5
Adenylate kinase (myokinase) reaction.

UTP (uracil) and **CTP** (cytosine) are utilized in glycogen and lipid synthesis, respectively. The energy in the terminal phosphate bonds of ATP may be transferred to the other nucleotides, using either the **nucleoside diphosphate kinase** or the **nucleoside monophosphate kinase** reactions illustrated in Figure 6.4 (p. 219). Two nucleoside diphosphates can be converted to a nucleoside triphosphate and a nucleoside monophosphate in various nucleoside **monophosphate kinase** reactions, such as the adenylate kinase reaction (Figure 6.5). The utility of these types of enzymes is that the terminal energy-rich phosphate bonds of ATP may be transferred to the appropriate nucleotides and utilized in a variety of biosynthetic processes.

6.2— Thermodynamic Relationships and Energy-Rich Components

Because living cells interconvert different forms of energy and may exchange energy with their surroundings, it is necessary to review the principles of **hermodynamics,** which govern reactions of this type. Knowledge of these principles will facilitate a perception of how energy-producing and energy-utilizing metabolic reactions are permitted to occur within the same cell and how an organism is able to accomplish various work functions.

The **first law of thermodynamics** states that energy can neither be created nor destroyed. This law of energy conservation stipulates that, although energy may be converted from one form to another, the total energy in a system must remain constant. For example, chemical energy available in a metabolic fuel such as glucose can be converted in the process of glycolysis to another form of chemical energy, ATP. In skeletal muscle chemical energy involved in the energy-rich phosphate bonds of ATP may be converted to mechanical energy during the process of muscle contraction. The energy involved in an osmotic electropotential gradient of protons across the mitochondrial membrane may be converted to chemical energy using the proton gradient to drive ATP synthesis.

To discuss the **second law of thermodynamics** the term **entropy** must be defined. Entropy, designated by *S*, is a measure or indicator of the degree of disorder or randomness in a system. Entropy can be viewed as the energy in a system that is unavailable to perform useful work. All processes, whether chemical or biological, tend to progress toward a situation of maximum entropy. Equilibrium in a system will result when the randomness or disorder (entropy) is at a maximum. However, it is nearly impossible to quantitate entropy changes in biochemical systems and such systems are rarely at equilibrium. For simplicity and because of its inherent utility in these considerations, a quantity termed **free energy** is employed.

Free Energy Is the Energy Available for Useful Work

Free energy (denoted by *G*) of a system is that portion of the total energy in a system that is available for useful work. It can be further defined by

$$\Delta G = \Delta H - T\Delta S$$

In this expression for a system proceeding toward equilibrium at a constant temperature and pressure, G is the change in free energy, H is the change in enthalpy or the heat content, T is the absolute temperature, and S is the change in entropy of the system. It can be deduced from this relationship that at equilibrium $G = 0$. Furthermore, any process that exhibits a negative free-energy change proceeds to equilibrium, since energy is given off, and is called an **exergonic reaction.** A process that exhibits a positive free-energy change will not occur independently; energy from some other source must be applied to this process to allow it to proceed toward equilibrium, and this type of

process is termed an **endergonic reaction.** It should be noted that the change in free energy in a biochemical process is the same regardless of the pathway or mechanism employed to attain the final state. Whereas the rate of a given reaction depends on the free energy of activation, the magnitude of the G is not related to the rate of the reaction. The change in free energy for a chemical reaction is related to the equilibrium constant of that reaction. For example, an enzymatic reaction may be described as

$$A + B \rightleftharpoons C + D$$

And an expression for the equilibrium constant may be written as

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

The free-energy change (G) at a constant temperature and pressure is defined as

$$\Delta G = \Delta G^{\circ} + RT \ln \left(\frac{[C][D]}{[A][B]} \right)$$

where G is the free-energy change; G° is the standard free-energy change, which is a constant for each individual reaction; reactants and products in the reaction are present at concentrations of 1.0 M; R is the gas constant, which is 1.987 cal mol^{-1} K^{-1} or 8.134 J mol^{-1} K^{-1}, depending on whether the resultant free-energy change is expressed in calories (cal) or joules (J) per mole; and T is the absolute temperature in degrees Kelvin (K).

Because at equilibrium $G = 0$, the expression reduces to

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

or

$$\Delta G^{\circ} = -2.3RT \log K_{eq}$$

Hence, if the **equilibrium constant** for a reaction can be determined, the standard free-energy change (G°) for that reaction also can be calculated. The relationship between G° and K_{eq} is illustrated in Table 6.1. When the equilibrium constant of a reaction is less than unity, the reaction is endergonic, and G° is positive. When the equilibrium constant is greater than unity, the reaction is exergonic, and G° is negative.

In energy-producing and energy-utilizing metabolic pathways in cellular systems, free-energy changes characteristic of individual enzymatic reactions in an entire pathway are additive, for example,

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

$$\Delta G^{\circ}_{A \rightarrow D} = \Delta G^{\circ}_{A \rightarrow B} + \Delta G^{\circ}_{B \rightarrow C} + \Delta G^{\circ}_{C \rightarrow D}$$

Although any given enzymatic reaction in a sequence may have a characteristic positive free-energy change, as long as the sum of all the free-energy changes is negative, the pathway will proceed.

Another way of expressing this principle is that enzymatic reactions with positive free-energy changes may be coupled to or driven by reactions with negative free-energy changes associated with them. In a metabolic pathway such as glycolysis, various individual reactions either have positive G° values or G° values that are close to zero. On the other hand, there are other reactions that have large and negative G° values, which drive the entire pathway. The crucial consideration is that the sum of the G° values for the individual reactions in a pathway must be negative in order for such a metabolic sequence to be thermodynamically feasible. Also, as for all chemical reactions, individual enzymatic reactions in a metabolic pathway or the pathway as a whole would

TABLE 6.1 Tabulation of Values of K_{eq} and ΔG°

K_{eq}	ΔG° ($kcal\ mol^{-1}$)
10^{-4}	5.46
10^{-3}	4.09
10^{-2}	2.73
10^{-1}	1.36
1	0
10	−1.36
10^{2}	−2.73
10^{3}	−4.09
10^{4}	−5.46

TABLE 6.2 Free-Energy Changes and Caloric Values Associated with the Total Metabolism of Various Metabolic Fuels

Compound	*Molecular Weight*	$\Delta G°$ (*kcal mol*$^{-1}$)	*Caloric Value* (*kcal g*$^{-1}$)
Glucose	180	−686	3.81
Lactate	90	−326	3.62
Palmitate	256	−2380	9.30
Tripalmitin	809	−7510	9.30
Glycine	75	−234	3.12

be facilitated if the concentrations of the reactants (substrates) of the reaction exceed the concentrations of the products of the reaction.

The Caloric Value of Dietary Substances

During complete stepwise oxidation of glucose, a primary metabolic fuel in cells, a large quantity of energy is available. The free energy released during the oxidation of glucose in a functioning cell is illustrated in the following equation:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O \qquad \Delta G° = -686{,}000\ \text{cal mol}^{-1}$$

When this process occurs under aerobic conditions in most cells, it is possible to conserve less than one half of this "available" energy as 38 molecules of ATP. The $G°$ values for oxidation of other metabolic fuels are listed in Table 6.2. Carbohydrates and proteins (amino acids) have a caloric value of 3–4 kcal g^{-1}, while lipid (i.e., palmitate, a long-chain fatty acid, or a triacylglycerol) exhibits a caloric value nearly three times greater. The reason that more energy can be derived from lipid than from carbohydrate or protein relates to the average oxidation state of the carbon atoms in these substances. Carbon atoms in carbohydrates are considerably more oxidized (or less reduced) than those in lipids (Figure 6.6). Hence during sequential breakdown of lipid nearly three times as many **reducing equivalents** (a reducing equivalent is defined as a proton plus an electron, i.e., $H^+ + e^-$) can be extracted than from carbohydrate. Reducing equivalents may be utilized for ATP synthesis in the mitochondrial energy transduction sequence.

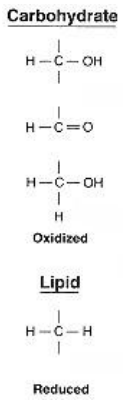


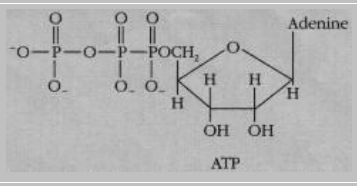
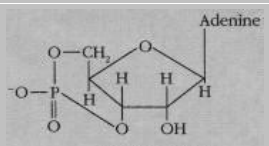
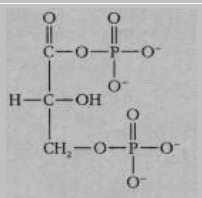
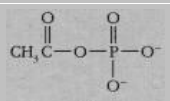
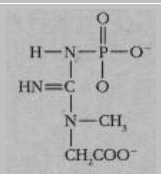
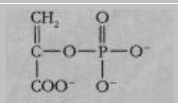
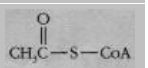
Figure 6.6
Oxidation states of typical carbon atoms in carbohydrates and lipids.

Compounds Are Classified on the Basis of Energy Released on Hydrolysis of Specific Groups

The two terminal phosphoryl groups of ATP contain energy-rich or high-energy bonds. What this description is intended to convey is that the free energy of hydrolysis of an energy-rich phosphoanhydride bond is much greater than would be obtained for a simple phosphate ester. High-energy is not synonymous with stability of the bonding arrangement in question, nor does it refer to the energy required to break such bonds. The concept of high-energy compounds implies that the products of the hydrolytic cleavage of the energy-rich bond are in more stable forms than the original compound. As a rule, simple phosphate esters (low-energy compounds) exhibit negative $G°$ values of hydrolysis in the range 1–3 kcal mol^{-1}, whereas high-energy bonds have negative $G°$ values in the range 5–15 kcal mol^{-1}. Phosphate esters such as glucose 6-phosphate and glycerol 3-phosphate are examples of low-energy compounds. Table 6.3 lists various types of energy-rich compounds with approximate values for their $G°$ values of hydrolysis.

There are various reasons why certain compounds or bonding arrangements

TABLE 6.3 Examples of Energy-Rich Compounds

Type of Bond	*$\Delta G°$ of Hydrolysis (kcal mol^{-1})*	*Example*
Phosphoric acid anhydrides	–7.3	 ATP
	–11.9	 3′5′ cyclic AMP
Phosphoric-carboxylic acid anhydrides	–10.1	 1,3-Bisphosphoglycerate
	–10.3	 Acetyl phosphate
Phosphoguanidines	–10.3	 Creatine phosphate
Enol phosphates	–14.8	 Phosphoenolpyruvate
Thiol esters	–7.7	 Acetyl CoA

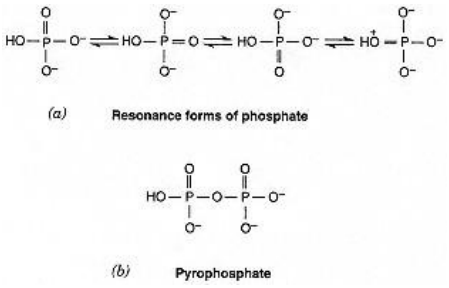


Figure 6.7
(a) Resonance forms of phosphate.
(b) Structure of pyrophosphate.

are energy rich. First, products of the hydrolysis of an energy-rich bond may exist in more **resonance forms** than the precursor molecule. The more possible resonance forms in which a molecule can exist stabilize that molecule. The resonance forms for inorganic phosphate (P_i) can be written as indicated in Figure 6.7.

Fewer resonance forms can be written for ATP or **pyrophosphate** (PP_i) (Figure 6.7) than for phosphate (P_i).

Second, many high-energy bonding arrangements have groups of similar electrostatic charges located in close proximity to each other in such compounds. Because like charges repel one another, hydrolysis of energy-rich bonds alleviates this situation and, again, lends stability to the products of hydrolysis. Third, hydrolysis of certain energy-rich bonds results in the formation of an unstable compound, which may isomerize spontaneously to form a more stable compound. Hydrolysis of phosphoenolpyruvate is an example of this type of compound (Figure 6.8). The G° for isomerization is considerable, and the final product, in this case pyruvate, is much more stable. Finally, if a product of the hydrolysis of a high-energy bond is an undissociated acid, dissociation of the proton and its subsequent buffering may contribute to the overall G° of the hydrolytic reaction. In general, any property or process that lends stability to products of hydrolysis tends to confer a high-energy character to that compound.

The high-energy character of **3′,5′-cyclic adenosine monophosphate (cAMP)** has been attributed to the fact that the phosphoanhydride bonding character in this compound is strained as it bridges the 3 and 5 positions on the ribose. The energy-rich character of thiol ester compounds such as acetyl CoA or succinyl CoA results from the relatively acidic character of the thiol group. Hence **acetyl CoA** is nearly equivalent to an anhydride rather than a simple thioester.

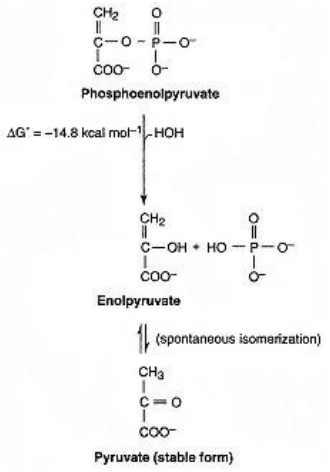


Figure 6.8
Hydrolysis of phosphoenolpyruvate.

Free-Energy Changes Can Be Determined in Coupled Enzyme Reactions

The G° value of hydrolysis of the terminal phosphate of ATP is difficult to determine by simply utilizing the K_{eq} of the hydrolytic reaction because of the position of the equilibrium.

$$ATP + HOH \rightleftharpoons ADP + P_i + H^+$$

However, the G° of hydrolysis of ATP can be determined indirectly because of the additive nature of free-energy changes. Hence free energy of hydrolysis of ATP can be determined by adding G° of an ATP-utilizing reaction such as hexokinase to G° of a reaction that cleaves the phosphate from the pro-

duct of the **hexokinase** reaction, **glucose 6-phosphate** (G6P), as indicated below:

$$\text{Glucose} + \text{ATP} \xrightleftharpoons{\text{hexokinase}} \text{G6P} + \text{ADP} + \text{H}^+ \qquad \Delta G^\circ = -4.0 \text{ kcal mol}^{-1}$$

$$\text{G6P} + \text{HOH} \xrightleftharpoons{\text{glucose 6-phosphatase}} \text{glucose} + \text{P}_i \qquad \Delta G^\circ = -3.3 \text{ kcal mol}^{-1}$$

$$\text{ATP} + \text{HOH} \rightleftharpoons \text{ADP} + \text{P}_i + \text{H}^+ \qquad \Delta G^\circ = -7.3 \text{ kcal mol}^{-1}$$

Free energies of hydrolysis for other energy-rich compounds can be determined in a similar fashion.

High-Energy Bond Energies of Various Groups Can Be Transferred from One Compound to Another

Energy-rich compounds can transfer various groups from the parent (donor) compound to an acceptor compound in a thermodynamically feasible fashion as long as an appropriate enzyme is present to facilitate the transfer. The energy-rich intermediates in the glycolytic pathway such as **1,3-bisphosphoglycerate** and phosphoenolpyruvate can transfer their high-energy phosphate moieties to ATP in the **phosphoglycerate kinase** and **pyruvate kinase** reactions, respectively (Figure 6.9*a*). The G° values of these two reactions are –4.5 and –7.5 kcal mol^{-1}, respectively, and hence transfer of "high-energy" phosphate is thermodynamically possible, and ATP synthesis is the result. ATP can transfer its terminal high-energy phosphoryl groups to form compounds of relatively similar high-energy character [i.e., **creatine phosphate** in the **creatine kinase** reaction (Figure 6.9*b*)] or compounds that are of considerably lower energy, such as glucose 6-phosphate formed in the hexokinase reaction (Figure 6.9*c*).

Thus phosphate or other transferable groups can be transferred from compounds that contain energy-rich bonding arrangements to compounds that have bonding characteristics of a lower energy in thermodynamically permissible enzymatic reactions. This principle is a major premise of the interaction between energy-producing and energy-utilizing metabolic pathways in living cells.

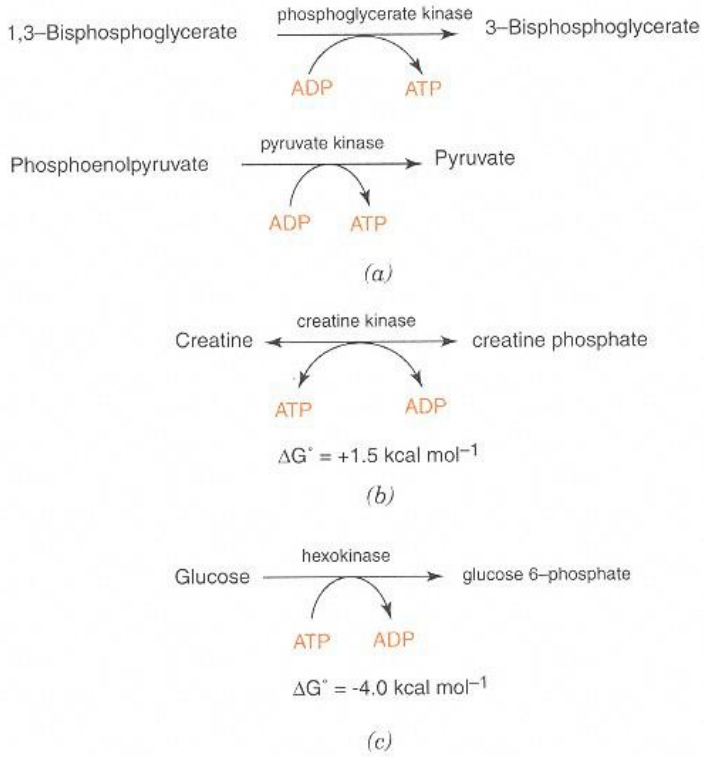


Figure 6.9
Examples of reactions involved in transfer of "high-energy" phosphate.

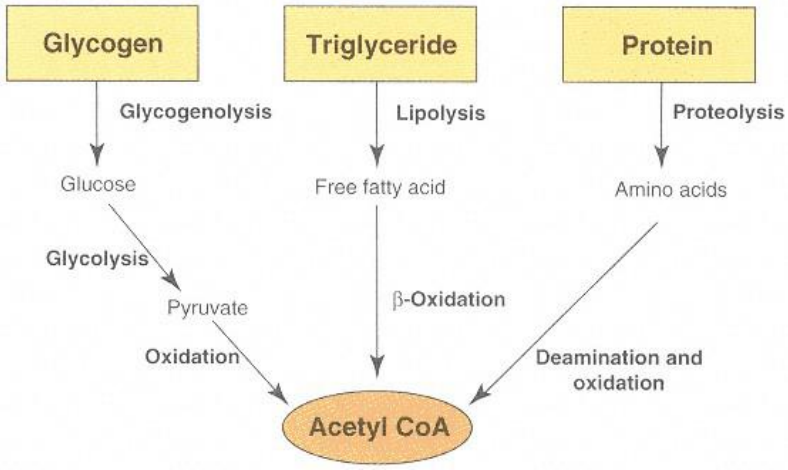


Figure 6.10
General precursors of acetyl CoA.

6.3—
Sources and Fates of Acetyl Coenzyme A

Most of the major energy-generating metabolic pathways of cells eventually result in production of the two-carbon unit **acetyl coenzyme A** (CoA). As illustrated in Figure 6.10, the catabolic breakdown of ingested or stored carbohydrate in the glycolytic pathway, of long-chain fatty acids in the ***β*-oxidation sequence,** or certain amino acids following **transamination** or **deamination** and subsequent oxidation provide precursors for the formation of acetyl CoA.

The structure of acetyl CoA is shown in Figure 6.11. This complex coenzyme, abbreviated either as CoA or CoASH, is composed of β-mercaptoethylamine, the vitamin **pantothenic acid,** and the adenine nucleotide, adenosine 3 -phosphate 5 -diphosphate. Coenzyme A exists as the reduced thiol (CoASH) and is involved in a variety of acyl group transfer reactions, where CoA alternately serves as the acceptor, then the donor, of the acyl group. Various metabolic pathways involve only acyl CoA derivatives, for example, β-oxidation of fatty acids and **branched-chain amino acid** degradation. Information on the

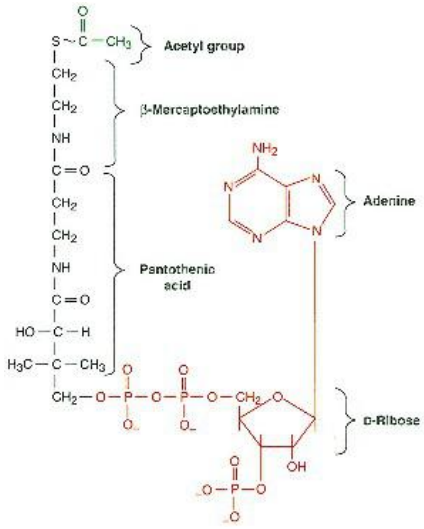


Figure 6.11
Structure of acetyl CoA.

nutritional aspects of the pantothenic acid will be described in Chapter 28. Like many other nucleotide species, CoA derivatives are not freely transported across cellular membranes. This property has necessitated the evolution of certain transport or shuttle mechanisms by which various intermediates or groups can be transferred across membranes. Such acyl transferase reactions for acetyl groups and long-chain acyl groups will be discussed in Chapter 9. Since the thiol ester linkage in acyl CoA derivatives is an energy-rich bond, these compounds can serve as effective donors of acyl groups in acyl transferase reactions. Also, to synthesize an acyl CoA derivative a high-energy bond of ATP must be expended, such as in the **acetate thiokinase** reaction,

$$\text{Pyruvate} + \text{NAD}^+ + \text{CoASH} \xrightarrow{\text{pyruvate dehydrogenase}} \text{acetyl CoA} + \text{CO}_2 + \text{NADH} + \text{H}^+ \qquad \Delta G^\circ = -8 \text{ kcal mol}^{-1}$$

The β-oxidation of fatty acids is a primary source of acetyl CoA in many tissues; a detailed description of the mobilization, transport, and oxidation of fatty acids is presented in Chapter 9. Note, however, that the products of the β-oxidation sequence are acetyl CoA and reducing equivalents (i.e., **NADH**). In certain tissues (e.g., cardiac muscle) and under somewhat special metabolic conditions in other tissues (e.g., in brain during prolonged starvation), acetyl CoA for energy generation may be derived from the **ketone bodies, acetoacetate** and **β-hydroxybutyrate.**

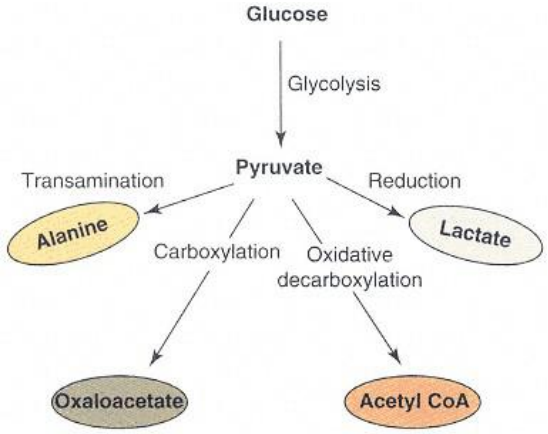


Figure 6.12
Metabolic fates of pyruvate.

Metabolic Sources and Fates of Pyruvate

During aerobic glycolysis (Chapter 7), glucose or other monosaccharides are converted to pyruvate, the end product of this cytosolic pathway. Also, degradation of amino acids such as alanine, serine, and cysteine results in the production of **pyruvate** (see p. 447). Pyruvate has a variety of metabolic fates, depending on the tissue and the metabolic state of that tissue. The major types of reactions in which pyruvate participates are indicated in Figure 6.12. The oxidative decarboxylation of pyruvate in the **pyruvate dehydrogenase** reaction is discussed next; the other reactions involving pyruvate are discussed in Chapter 7.

Pyruvate Dehydrogenase Is a Multienzyme Complex

Pyruvate is converted to acetyl CoA by the pyruvate dehydrogenase multienzyme complex.

$$\text{Pyruvate} + \text{NAD}^+ + \text{CoASH} \xrightarrow{\text{pyruvate dehydrogenase}} \text{acetyl CoA} + \text{CO}_2 + \text{NADH} + \text{H}^+ \qquad \Delta G^\circ = -8 \text{ kcal mol}^{-1}$$

This enzyme is located exclusively in the mitochondrial matrix and is present in high concentrations in tissues such as cardiac muscle and kidney. Because of the large negative G° of the pyruvate dehydrogenase reaction, under physiological conditions the reaction is irreversible. This fact is the primary reason that a net conversion of fatty acid carbon to carbohydrate cannot occur; for example, acetyl CoA from fatty acids cannot be converted to pyruvate. Molecular weights of the multienzyme complex derived from kidney, heart, or liver range from 7 to 8.5 × 10^6. The mammalian pyruvate dehydrogenase enzyme complex consists of three different types of catalytic subunits:

Number of Subunits/Complex	*Type*	*Molecular Weight*	*Subunit Structure*
20 or 30[a]	Pyruvate dehydrogenase	154,000	$\alpha_2\beta_2$ Tetramer
60	Dihydrolipoyl transacetylase	52,000	Identical
6	Dihydrolipoyl dehydrogenase	110,000	α_2 Dimer

[a] Depending on source.

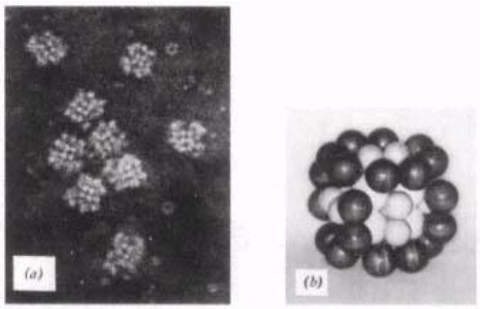


Figure 6.13
Pyruvate dehydrogenase complex from *E. coli*.
(a) Electron micrograph.
(b) Molecular model. The enzyme
complex wasnegatively stained with phosphotungstate (× 200,000).
Courtesy of Dr. Lester J. Reed, University of Texas, Austin.

The structure of the pyruvate dehydrogenase complex derived from *Escherichia coli* (particle weight, 4.6×10^6) is somewhat different from that of the mammalian enzyme. Electron micrographs of the bacterial enzyme complex (Figure 6.13) indicate that the transacetylase, which consists of 24 identical polypeptide chains (mol wt = 64,500), forms the cube-like core of the complex (white spheres in the model shown in Figure 6.11). Twelve pyruvate dehydrogenase dimers (black spheres; mol wt = 90,500) are distributed symmetrically on the 12 edges of the transacetylase cube. Six dihydrolipoyl dehydrogenase dimers (gray spheres; mol wt = 56,000) are distributed on the six faces of the cube. Five different coenzymes or prosthetic groups are involved in the pyruvate dehydrogenase reaction (Table 6.4 and Figure 6.14). The mechanism of the pyruvate dehydrogenase reaction occurs as illustrated in Figure 6.15.

Because of active participation of thiol groups in the catalytic mechanism, agents that either oxidize or complex with thiol groups are strong inhibitors of the enzyme complex. **Arsenite** is such an inhibitor.

Pyruvate Dehydrogenase Is Strictly Regulated

Two types of regulation of the pyruvate dehydrogenase complex have been characterized. First, two products of the pyruvate dehydrogenase reaction, acetyl

TABLE 6.4 Function of Coenzymes and Prosthetic Groups of the Pyruvate Dehydrogenase Reaction

Coenzyme or Prosthetic Group	*Location*	*Function*
Thiamine pyrophosphate	Bound to pyruvate dehydrogenase	Reacts with substrate, pyruvate
Lipoic acid	Covalently attached to a lysine residue on the dihydrolipoyl transacetylase	Accepts acetyl group from thiamine pyrophosphate
Coenzyme A	Free in solution	Accepts acetyl group from lipoamide group on the transacetylase
Flavin adenine dinucleotide (FAD)	Tightly bound to dihydrolipoyl dehydrogenase	Accepts reducing equivalents from reduced lipoamide group
Nicotinamide adenine dinucleotide	Free in solution	Terminal acceptor of reducing equivalents from the reduced flavoprotein

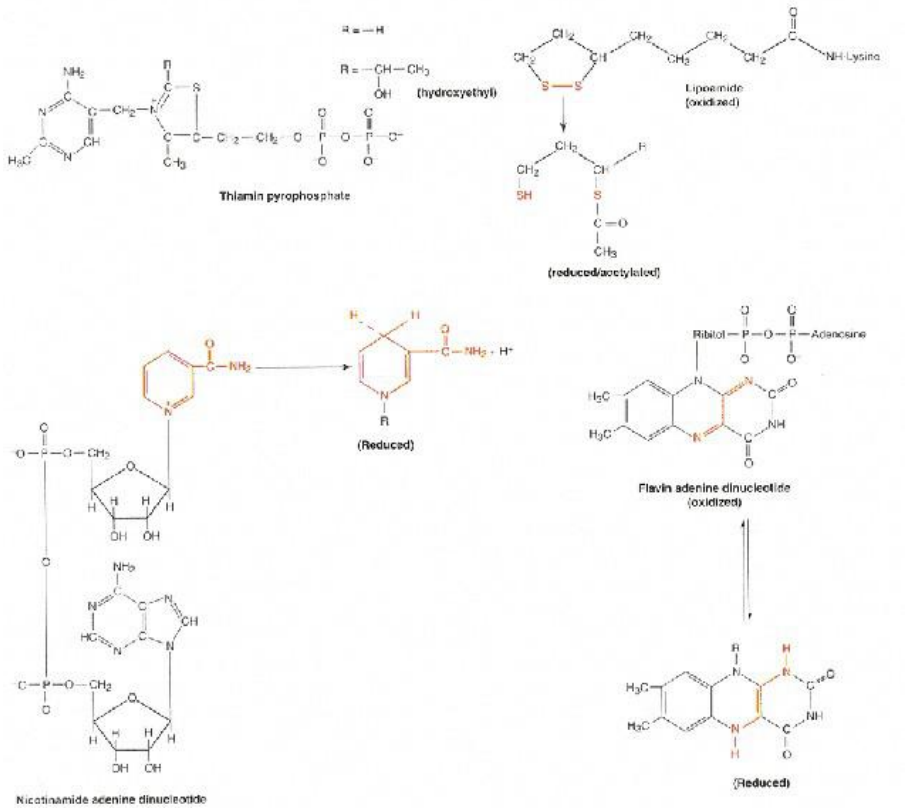


Figure 6.14
Structures of coenzymes involved in the pyruvate dehydrogenase reaction.
See Figure 6.11 for the structure of CoA.

CoA and NADH, inhibit the complex in a competitive fashion. Second, the pyruvate dehydrogenase complex exists in two forms: (1) an active, dephosphorylated complex and (2) an inactive, phosphorylated complex. Inactivation of the complex is accomplished by a Mg^{2+}–ATP-dependent **protein kinase,** which is tightly bound to the enzyme complex. Reactivation is accomplished by a **phosphoprotein phosphatase,** which dephosphorylates the complex in a Mg^{2+}- and Ca^{2+}-dependent reaction. Three separate serine residues on the α subunit of pyruvate dehydrogenase are phosphorylated by the protein kinase but the phosphorylation of only one serine is related to the activity of the complex. The differential regulation of the pyruvate dehydrogenase kinase and phosphatase is the key to the regulation of the pyruvate dehydrogenase complex. Essential features of this complex regulatory system are illustrated in Figure 6.16. Acetyl CoA and NADH, products of pyruvate dehydrogenase, inhibit the

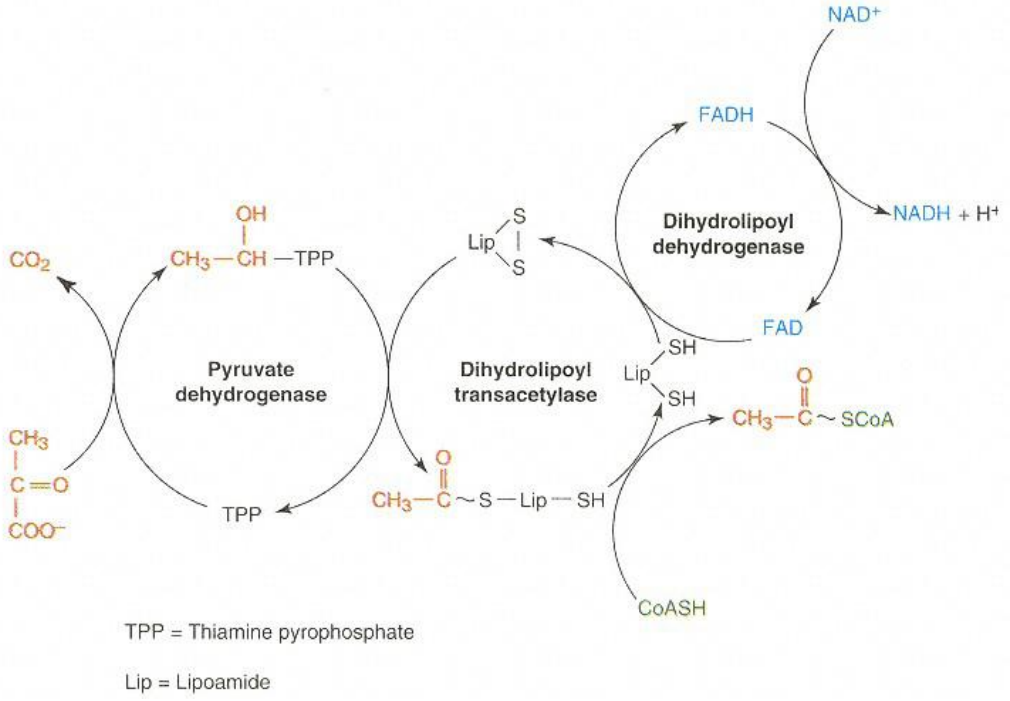


Figure 6.15
Mechanism of the pyruvate dehydrogenase reaction; the pyruvate dehydrogenase multienzyme complex.

dephospho (active) form of the enzyme, but these two compounds stimulate the protein kinase reaction, leading to an interconversion of the complex to its inactive form. In addition, free CoASH and NAD^+ inhibit the protein kinase. Hence, with any increase of the mitochondrial $NADH/NAD^+$ or acetyl CoA/CoASH ratio, such as during rapid β-oxidation of fatty acids, pyruvate dehydrogenase will be inactivated by the kinase reaction. In addition, pyruvate, the substrate of the enzyme, is a potent inhibitor of the protein kinase, and therefore in the presence of elevated tissue pyruvate levels the kinase will be inhibited and the complex maximally active. Finally, **insulin** administration activates

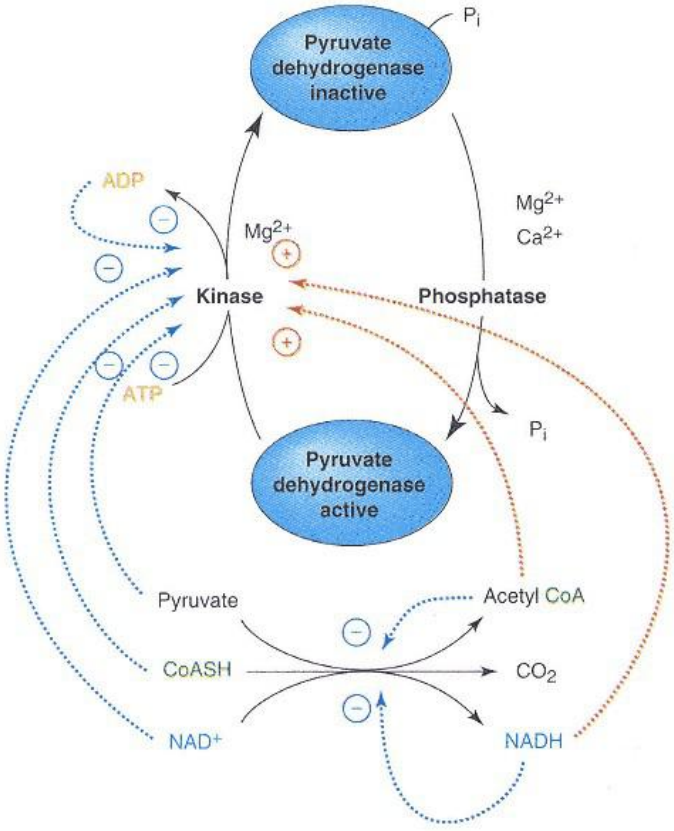


Figure 6.16
Regulation of the pyruvate dehydrogenase multienzyme complex.

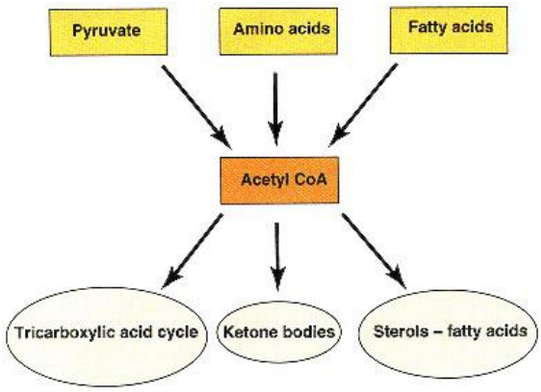


Figure 6.17
Sources and fates of acetyl CoA.

pyruvate dehydrogenase in adipose tissue, and catecholamines, such as **epinephrine,** activate pyruvate dehydrogenase in cardiac tissue. The mechanisms of these hormonal effects are not well understood, but alterations of the intracellular distribution of Ca^{2+}, such that the phosphoprotein phosphatase reaction is stimulated in the mitochondrial matrix, may be involved in these effects. These hormonal effects are not mediated directly by alterations in the tissue cAMP levels because the pyruvate dehydrogenase protein kinase and phosphatase are cAMP-independent or insensitive (see Clin. Corr. 6.1).

Acetyl CoA Is Used by Several Different Pathways

The various fates of acetyl CoA generated in the mitochondrial matrix include (1) complete oxidation of the acetyl group in the tricarboxylic acid cycle for energy generation; (2) in the liver, conversion of excess acetyl CoA into ketone bodies, acetoacetate and β-hydroxybutyrate; and (3) transfer of the acetyl units to the cytosol with subsequent biosynthesis of such molecules as sterols (see Chapter 10) and long-chain fatty acids (see Chapter 9) (Figure 6.17).

6.4— The Tricarboxylic Acid Cycle

The primary metabolic fate of acetyl CoA produced in the various energy-generating catabolic pathways of most cells is its complete oxidation in a cyclic series of reactions termed the **tricarboxylic acid (TCA) cycle.** This metabolic cycle is also commonly referred to as the **citric acid cycle** or the **Krebs cycle** after Sir Hans Krebs who postulated the essential features of this pathway in 1937. Various investigators defined many of the enzymes and di- and tricarboxylic acid intermediates but it was Krebs who pieced them together. The primary location of the enzymes of the TCA cycle is in the mitochondrion, although isozymes of some are found in the cytosol. This type of distribution is appropriate because the pyruvate dehydrogenase multienzyme complex and the fatty acid β-oxidation sequence, the two primary sources for generating acetyl CoA, are also located in the mitochondrion. A primary function of the TCA cycle is to generate reducing equivalents that are utilized to generate energy, that is, ATP, in the **electron transport–oxidative phosphorylation** sequence, another process contained exclusively in the mitochondrion (Figure 6.18). Mitochondrial energy transduction is discussed in Section 6.7.

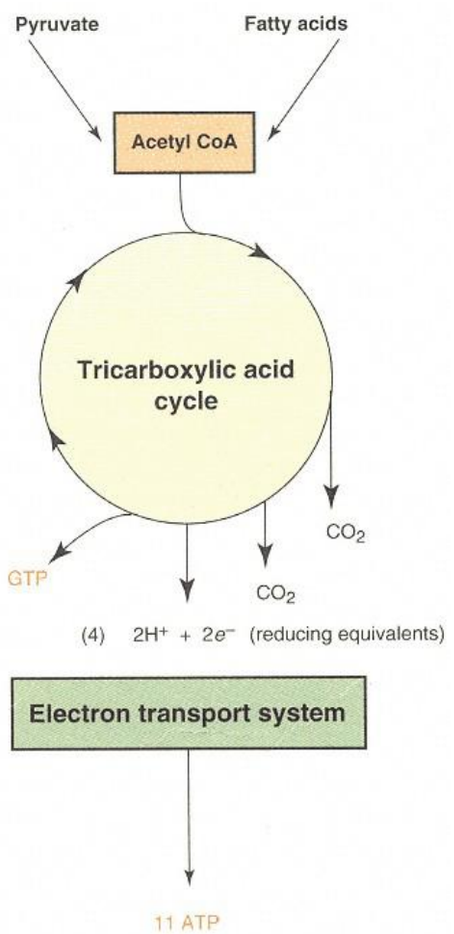


Figure 6.18
General description of mitochondrial ATP synthesis.

Figure 6.18 illustrates the essential process involved in the TCA cycle. The substrate of the cycle is the two-carbon unit acetyl CoA and the products of a complete turn of the cycle are two CO_2 plus one high-energy phosphate bond (as GTP) and four reducing equivalents (i.e., three NADH and one $FADH_2$).

Reactions of the Tricarboxylic Acid Cycle

The individual enzymatic reactions are presented in Figure 6.19. The initial step of the cycle is catalyzed by **citrate synthase.** This is a highly exergonic reaction and commits acetyl groups to **citrate** formation and complete oxidation in the Krebs cycle. As shown below citrate synthase involves condensation of an acetyl moiety and the α-keto function of the dicarboxylic acid **oxaloacetate.** Citrate synthase (mol wt 100,000) is in the mitochondrial matrix.

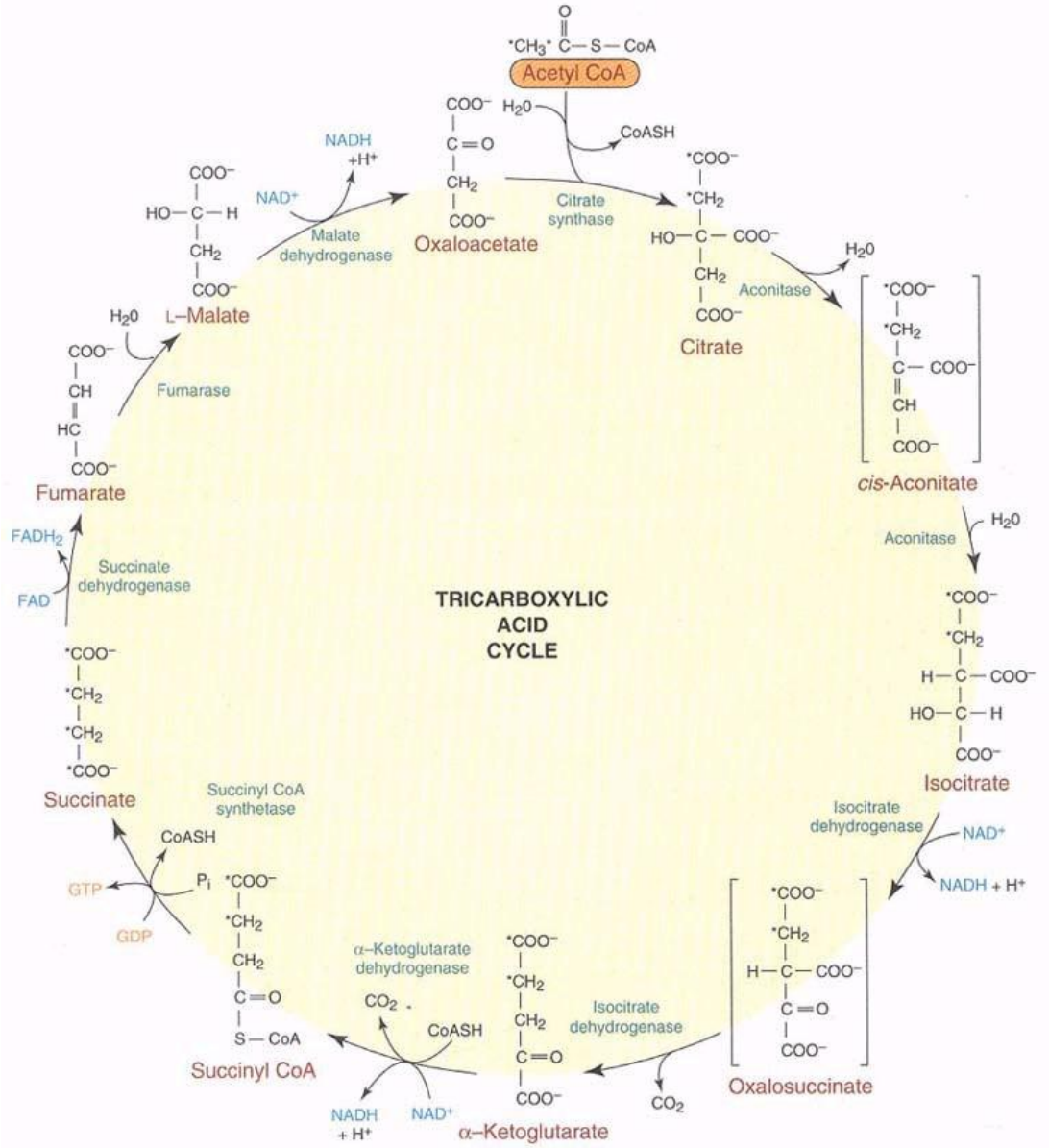
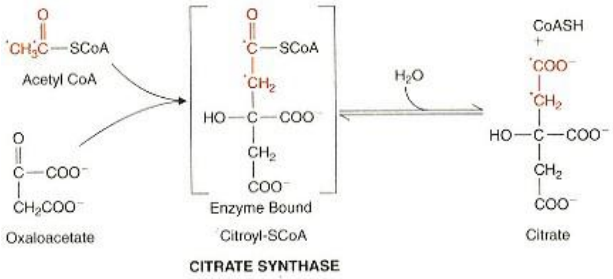


Figure 6.19
The tricarboxylic acid cycle.
Asterisked carbons indicate the fate of the carbons of the acetyl group.



The equilibrium of this reaction is far toward citrate formation with a G° near –9 kcal mol^{-1}. The citroyl-SCoA intermediate is not released from the enzyme during the reaction and remains bound to the catalytic site on citrate synthase. The citrate synthase reaction is considerably displaced from equilibrium under *in situ* conditions, which makes this step a primary candidate for regulatory modulation. The purified enzyme is regulated (inhibited) by ATP, NADH, succinyl CoA, and long-chain acyl CoA derivatives, but these effects have not been demonstrated in intact metabolic systems under physiological conditions.

CLINICAL CORRELATION 6.1

Pyruvate Dehydrogenase Deficiency

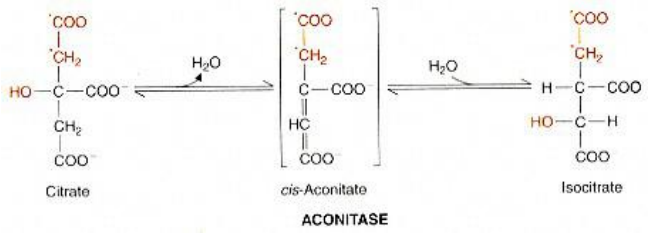
A variety of disorders in pyruvate metabolism have been detected in children. Some involve deficiencies of the different catalytic or regulatory subunits of the pyruvate dehydrogenase multienzyme complex. Children diagnosed with pyruvate dehydrogenase deficiency usually exhibit elevated serum levels of lactate, pyruvate, and alanine, which produce a chronic lactic acidosis. Such patients frequently exhibit severe neurological defects, and in most situations this type of enzymatic defect results in death. The diagnosis of pyruvate dehydrogenase deficiency is usually made by assaying the enzyme complex and/or its various enzymatic sub-units in cultures of skin fibroblasts taken from the patient. In certain instances patients respond to dietary management in which a ketogenic diet is administered and carbohydrates are minimized. Patients in shock have lactic acidosis because decreased delivery of O_2 to tissues inhibits pyruvate dehydrogenase and increases anaerobic metabolism. This situation has been treated with dichloroacetate, an inhibitor of pyruvate dehydrogenase kinase and therefore an activator of the enzyme complex.

Patel, M. S., and Harris, R. A. Mammalian α-keto acid dehydrogenase complexes: gene regulation and genetic defects. *FASEB J.* 9:1164, 1995.

The most probable means for regulating the citrate synthase reaction is availability of its two substrates, acetyl CoA and oxaloacetate. Note the many important fates and effects of citrate in energy and biosynthetic metabolism indicated in Figure 6.20; citrate is a regulatory effector of other metabolic pathways and a source of carbon and reducing equivalents for various synthetic purposes (see Chapters 7 and 9 for further details).

Citrate synthase reacts with **monofluoroacetyl CoA** to form **monofluo-rocitrate,** a potent inhibitor of the next step in the cycle, the **aconitase** reaction. In fact, whether monofluorocitrate is synthesized *in situ* as a result of **fluoro-acetate** poisoning or administered experimentally, a nearly complete block of TCA cycle activity is observed.

Citrate is converted to isocitrate in the aconitase reaction:



This reaction involves generation of an enzyme-bound intermediate, ***cis*-aconitate.** At equilibrium there exist 90% citrate, 3% *cis*-aconitate, and 7% isocitrate; hence the equilibrium of aconitase lies toward citrate formation. Although the aconitase reaction does not require cofactors, it requires ferrous (Fe^{2+}) iron in its catalytic mechanism. This Fe^{2+} is involved in an **iron–sulfur center,** which is an essential component in the hydratase activity of aconitase.

Isocitrate dehydrogenase catalyzes the first dehydrogenase reaction in the TCA cycle. Isocitrate is converted to ***α*-ketoglutarate** in an oxidative decarboxylation reaction. In this step of the cycle the initial (of two) CO_2 is produced and the initial (of three) NADH + H^+ are generated. Isocitrate dehydrogenase

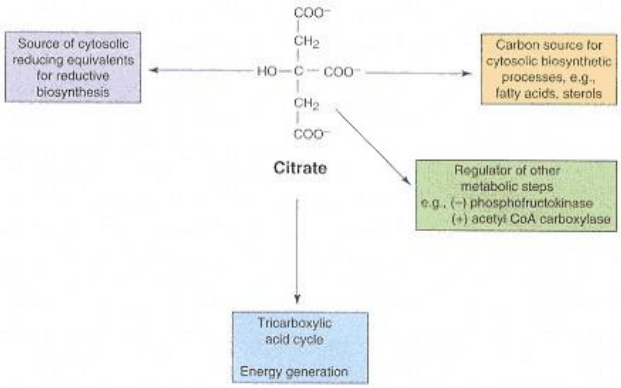
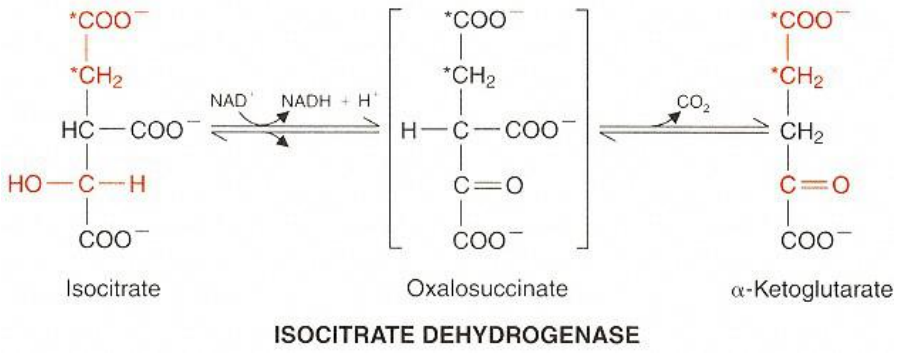


Figure 6.20
Fates and functions of citrate.

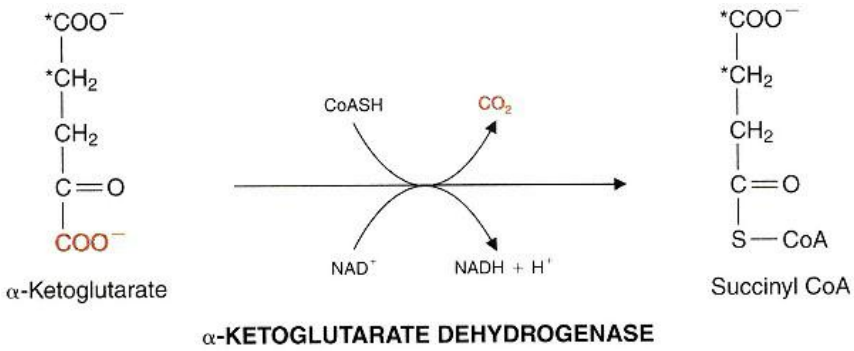
involved in mitochondria from mammalian tissues requires NAD^+ as the acceptor of reducing equivalents.



Mitochondria possess an isocitrate dehydrogenase that requires **$NADP^+$.** The $NADP^+$-linked enzyme is also found in the cytosol, where it is involved in providing reducing equivalents for cytosolic reductive processes. The equilibrium of this reaction lies strongly toward ***α*-ketoglutarate** formation with a G° of nearly –5 kcal mol^{-1}. NAD^+-linked isocitrate dehydrogenase has a molecular weight of 380,000 and consists of eight identical subunits. The reaction requires a divalent metal cation (e.g., Mn^{2+} or Mg^{2+}) in decarboxylation of the β position of oxalosuccinate. NAD^+-linked isocitrate dehydrogenase is stimulated by ADP and in some cases AMP and is inhibited by ATP and NADH. Hence, under high-energy conditions (i.e., high ATP/ADP + P_i and high NADH/NAD^+ ratios), NAD^+-linked isocitrate dehydrogenase of the TCA cycle is inhibited. During periods of low energy the activity of this enzyme is stimulated in order to accelerate energy generation by the TCA cycle.

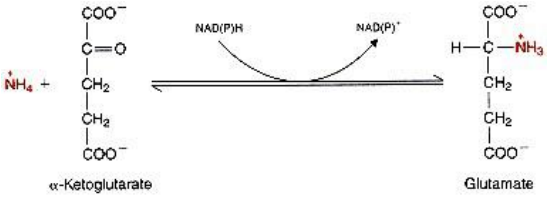
Conversion of α-ketoglutarate to **succinyl CoA** is catalyzed by the ***α*-ketoglutarate dehydrogenase** multienzyme complex, which is nearly identical to the pyruvate dehydrogenase complex in terms of reactions catalyzed and some of its structural features. Again, **thiamine pyrophosphate, lipoic acid,** CoASH, **FAD,** and NAD^+ participate in the catalytic mechanism. The multienzyme complex consists of α-ketoglutarate dehydrogenase, **dihydrolipoyl transsuccinylase,** and dihydrolipoyl dehydrogenase as three catalytic subunits. The equilibrium of the α-ketoglutarate dehydrogenase reaction lies strongly toward succinyl CoA formation with a G° of –8 kcal mol^{-1}. In this reaction the second molecule of CO_2 and the second reducing equivalent (i.e., NADH + H^+) of the cycle are produced. Another product of this reaction, succinyl CoA, is an energy-rich thiol ester compound similar to acetyl CoA. Unlike the pyruvate dehydrogenase complex, α-ketoglutarate dehydrogenase

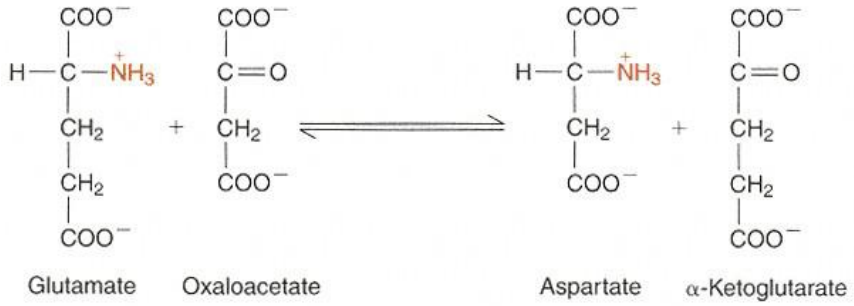
complex is not regulated by a protein kinase-mediated phosphorylation reaction. The nucleoside triphosphates—ATP and GTP—NADH, and succinyl CoA inhibit this enzyme complex while Ca^{2+} has been shown to activate α-ketoglutarate dehydrogenase in certain tissues.



α-KETOGLUTARATE DEHYDROGENASE

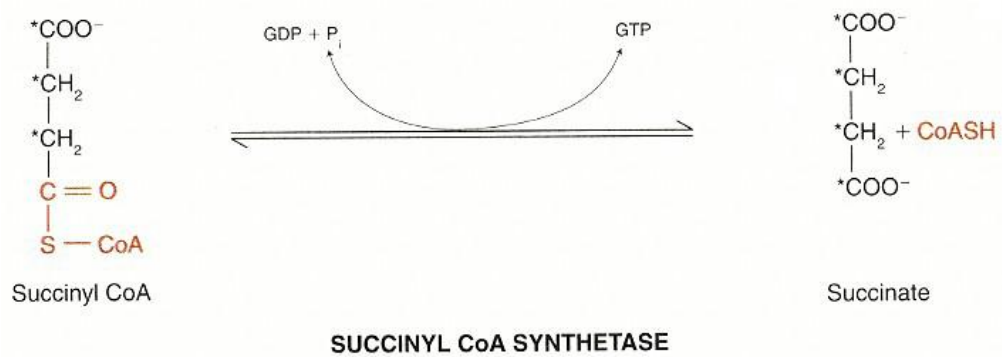
It is at the level of α-ketoglutarate that an intermediate may leave the TCA cycle to be reductively aminated in the glutamate dehydrogenase reaction. This mitochondrial enzyme converts α-ketoglutarate to glutamate in the presence of NADH or NADPH and ammonia. Using various transamination reactions the amino group incorporated into glutamate can be transferred to a variety of amino acids. These enzymes and the relevance of the incorporation or release of ammonia into or from α-keto acids are discussed in Chapter 11.





GLUTAMATE-OXALOACETATE TRANSAMINASE

The energy-rich character of the thiol ester linkage of succinyl CoA is conserved in a substrate-level phosphorylation reaction in the next step of the TCA cycle. **Succinyl-CoA synthetase** (or succinate thiokinase) converts succinyl CoA to succinate and in mammalian tissues results in phosphorylation of GDP to GTP.



SUCCINYL CoA SYNTHETASE

This reaction is freely reversible with $G^{o} = -0.7$ kcal mol^{-1} and the catalytic mechanism involves an enzyme–succinyl phosphate intermediate.

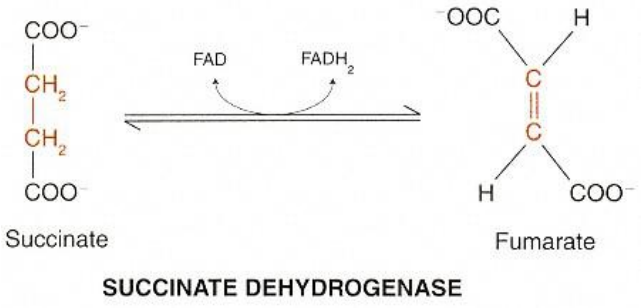
$$\text{Succinyl CoA} + P_i + \text{Enz} \rightleftharpoons \text{Enz–succinyl phosphate} + \text{CoASH}$$

$$\text{Enz–succinyl phosphate} \rightleftharpoons \text{Enz–phosphate} + \text{succinate}$$

$$\text{Enz–phosphate} + \text{GDP} \rightleftharpoons \text{Enz} + \text{GTP}$$

The enzyme is phosphorylated on the 3 position of a histidine residue during the succinyl-CoA synthetase reaction. Hence, in this step of the TCA cycle, a high-energy bond is conserved as GTP. Because of the presence of the nucleoside diphosphate kinase discussed earlier in this chapter, the γ-phosphate of GTP can be transferred to ADP to generate ATP.

Succinyl CoA represents a metabolic branch point in that intermediates may enter or exit the TCA cycle at this point (Figure 6.21). Succinyl CoA may be formed either from α-ketoglutarate in the cycle or from **methylmalonyl CoA** in the final steps of breakdown of odd-chain length fatty acids or the branched-chain amino acids valine and isoleucine. Metabolic fates of succinyl CoA include its conversion to succinate in the succinyl-CoA synthetase reaction of the Krebs cycle and its condensation with glycine to form **δ-aminolevulinate** by **δ-aminolevulinate synthase,** the initial reaction in **porphyrin** biosynthesis (see p. 1011).



Succinate is oxidized to **fumarate** by **succinate dehydrogenase,** which is tightly bound to the inner mitochondrial membrane and is composed of two

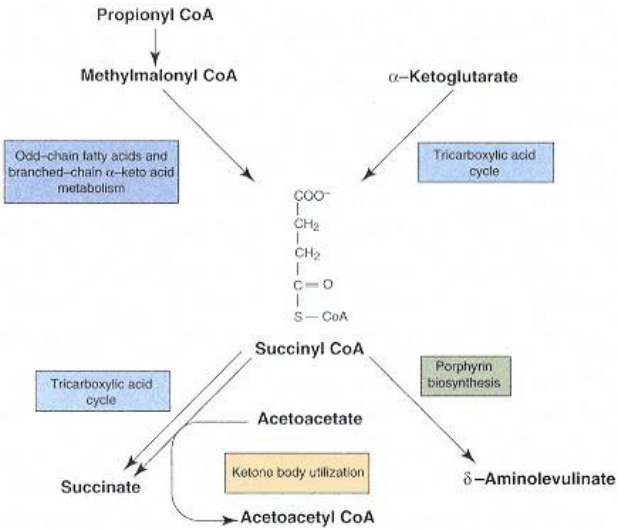
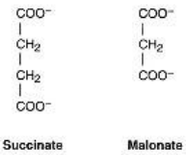


Figure 6.21
Sources and fates of succinyl CoA.

subunits with mol wt 70,000 and 30,000. The 70,000 mol wt subunit contains the substrate-binding site, covalently bound FAD (to a lysine residue), four nonheme iron atoms, and four acid-labile sulfur atoms, whereas the 30,000 mol wt subunit contains four nonheme irons and four acid-labile sulfur atoms. This enzyme is a typical example of an **iron–sulfur protein** in which nonheme iron undergoes valence changes (e.g., Fe^{2+} Fe^{3+}) during removal of electrons and protons from succinate and subsequent transfer of these reducing equivalents through covalently bound FAD to the mitochondrial electron-transfer chain at the coenzyme Q–cytochrome *b* level.

Succinate dehydrogenase is strongly inhibited by malonate and oxaloacetate and is activated by ATP, P_i, and succinate. **Malonate** inhibits succinate dehydrogenase competitively with respect to succinate. This inhibitory characteristic of malonate is due to a very close structural similarity between malonate and succinate (Figure 6.22). Malonate is used experimentally as a very effective inhibitor of the TCA cycle in complex metabolic systems. In fact, the ability of malonate to inhibit the cycle was used by Krebs as evidence for the cyclic nature of this oxidative metabolic pathway.



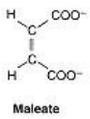
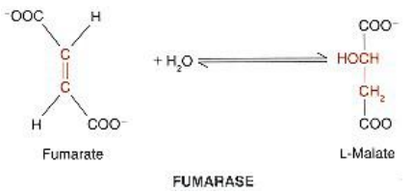


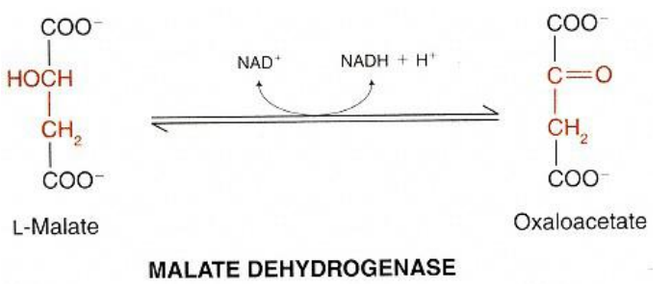
Figure 6.22
Structures of succinate, a TCA cycle intermediate; malonate, a cycle inhibitor; and maleate, a compound not involved in the cycle.

Fumarate is hydrated to form **L-malate** in the next step in the TCA cycle by the enzyme fumarase.



Fumarase is a tetramer (mol wt 200,000) and is stereospecific for the trans form of substrate (the cis form, maleate, is not a substrate; Figure 6.22). The product of the reaction is L-malate and the reaction is freely reversible under physiological conditions. See Clin. Corr. 6.2 concerning a genetic deficiency of fumarase.

The final reaction in the TCA cycle is catalyzed by malate dehydrogenase with the final (of three) reducing equivalents as NADH + H^+ being removed from the cycle intermediates.



The equilibrium of the **malate dehydrogenase** reaction lies far toward L-malate formation, because $G^{\circ} = +7.0$ kcal mol^{-1}. Thus the reaction is an endothermic reaction when considered in the forward direction. However, **citrate synthase** and other reactions of the cycle pull malate dehydrogenase toward **oxaloacetate** formation by removing oxaloacetate. In addition, NADH produced in various cycle NAD^+-linked dehydrogenases is oxidized rapidly to NAD^+ by the mitochondrial respiratory chain.

CLINICAL CORRELATION 6.2

Fumarase Deficiency

Deficiencies of enzymes of the TCA cycle are rarely found. Several cases, however, are on record in which there is a severe deficiency of fumarase in both mitochondria and cytosol of tissues (e.g., blood lymphocytes). The condition is characterized by severe neurological impairment, encephalopathy, and dystonia developing soon after birth. Urine contains abnormal amounts of fumarate and one or more of succinate, α-ketoglutarate, citrate, and malate. Both enzymes are derived from a single gene and both parents had half-normal levels of enzyme activity but are clinically normal, as is appropriate for an autosomal recessive disorder. The first description of a mutation in the gene reported that glutamate at residue 319 was replaced by glutamine.

Bourgeron, T., Chretien, D., Poggi-Bach, J., et al. Mutation of the fumarase gene in two siblings with progressive encephalopathy and fumarase deficiency. *J. Clin. Invest.* 93:2514, 1994.

Conversion of the Acetyl Group of Acetyl CoA to CO_2 and H_2O Conserves Energy

In summary, the TCA cycle (Figure 6.18) serves as a terminal oxidative pathway for most metabolic fuels. Two-carbon moieties as acetyl CoA are taken into the

cycle and are oxidized completely to CO_2 and H_2O. During this process 4 reducing equivalents (3 as NADH + H^+ and 1 as $FADH_2$) are produced, which are used subsequently for energy generation. Oxidation of each NADH + H^+ results in formation of 3 ATP molecules in **oxidative phosphorylation,** while oxidation of $FADH_2$ formed in the succinate dehydrogenase reaction yields 2 molecules of ATP. Also, a high-energy bond is formed as GTP in the succinyl-CoA synthetase reaction. Hence the net yield of ATP or its equivalent (i.e., GTP) for the complete oxidation of an acetyl group in the Krebs cycle is 12.

During complete oxidation of glucose to CO_2 and H_2O, there is a net formation of (1) 2 molecules of ATP per glucose in the conversion of glucose to 2 molecules of pyruvate; (2) 6 molecules of ATP per glucose as a result of the translocation and subsequent oxidation in the mitochondrial matrix of 2 molecules of NADH + H^+ formed in the glyceraldehyde-3-phosphate dehydrogenase reaction of glycolysis; and (3) 30 molecules of ATP per glucose from the oxidation of the 2 molecules of pyruvate in the pyruvate dehydrogenase reaction and subsequent conversion of 2 molecules of acetyl CoA to CO_2 and H_2O in the TCA cycle. Hence the net ATP yield during the complete oxidation of glucose to 6 CO_2 + 6 H_2O is 38 molecules of ATP.

The Activity of the Tricarboxylic Acid Cycle Is Carefully Regulated

A variety of factors are involved in the regulation of the activity of the TCA cycle. First, the supply of acetyl units, whether derived from pyruvate (i.e., carbohydrate) or fatty acids, is a crucial factor in determining the rate of the cycle. Regulatory influences on the pyruvate dehydrogenase complex have an important effect on the cycle. Likewise, any control exerted on the processes of transport and β-oxidation of fatty acids would be an effective determinant of the cycle activity.

Second, because the primary dehydrogenase reactions of the Krebs cycle are dependent on a continuous supply of both NAD^+ and FAD, their activities are very stringently controlled by the mitochondrial respiratory chain, which is responsible for oxidizing the NADH and $FADH_2$ produced by substrate oxidation in the cycle. Because the activity of the respiratory chain is coupled obligatorily to the generation of ATP in the reactions of oxidative phosphorylation, the activity of the Krebs cycle is very much dependent on a respiratory control, which is strongly affected by the availability of ADP + phosphate and oxygen. Hence an inhibitory agent or metabolic condition that interrupts the supply of oxygen, the continuous supply of ADP, or the source of reducing equivalents (e.g., substrate for the cycle) would shut down cycle activity. This type of control of the cycle is generally considered to be a coarse control of the cycle. There are a variety of postulated effector-mediated regulatory interactions between various intermediates or nucleotides and the individual enzymes of the cycle, which may serve to exert a fine control on the activity of the cycle. Illustrations of these interactions are shown in Figure 6.23 and have been noted during the discussions of individual enzymes of the Krebs cycle. The physiological relevance of many of these types of individual regulatory interactions has not been established rigorously in intact metabolic systems.

6.5—
Structure and Compartmentation by Mitochondrial Membranes

Because the metabolic pathways for oxidation of pyruvate, the end product of glycolysis, and fatty acids are located in mitochondria, a major portion of the energy-generating capacity of most cells resides in the mitochondrial compartment of the cell. The number of mitochondria in various tissues (Figure 6.24*a, b*) reflects the physiological function of the tissue and determines its capacity

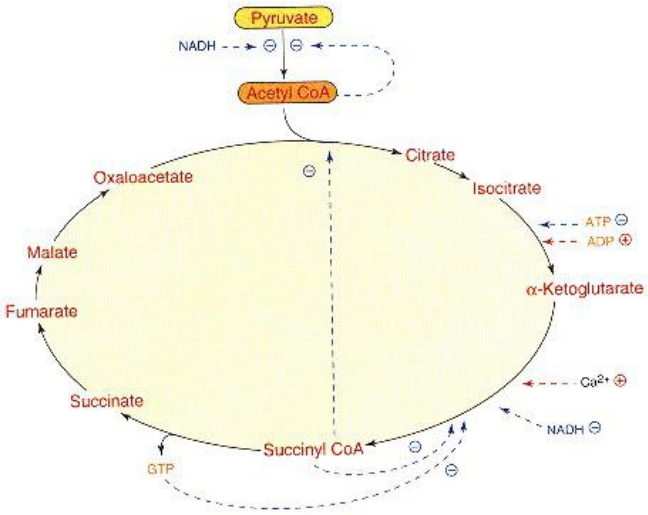


Figure 6.23
Representative examples of regulatory interactions in the TCA cycle.

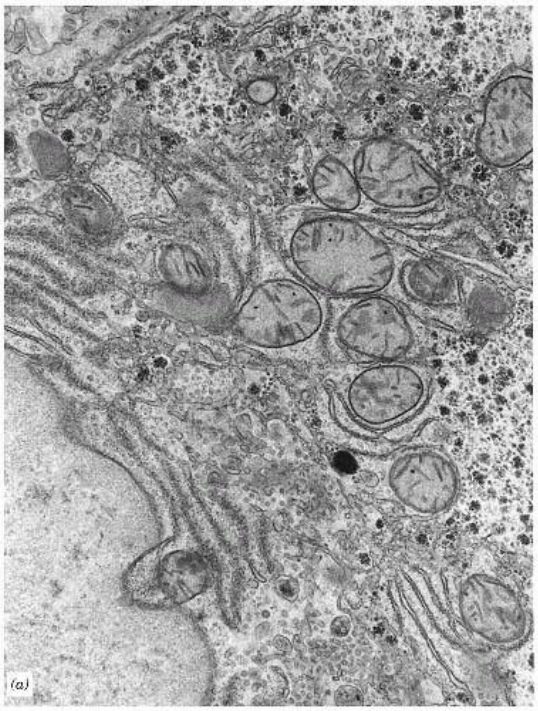


Figure 6.24
(a) Electron micrograph of mitochondria in hepatocytes from rat liver (×39,600).
Courtesy of Dr. W. B. Winborn, Department of Anatomy, The University of Texas Health Science Center at San Antonio, and the Electron Microscopy Laboratory, Department of Pathology, The University of Texas Health Science Center at San Antonio.

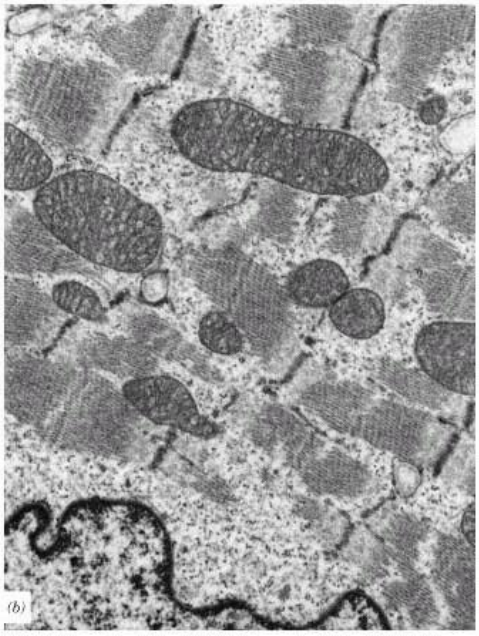


Figure 6.24
(b) Electron micrograph of mitochondria in muscle fibers from rabbit heart (×39,600).
Courtesy of Dr. W. B. Winborn, Department of Anatomy, The University of Texas Health Science Center at San Antonio, and the Electron Microscopy Laboratory, Department of Pathology, The University of Texas Health Science Center at San Antonio.

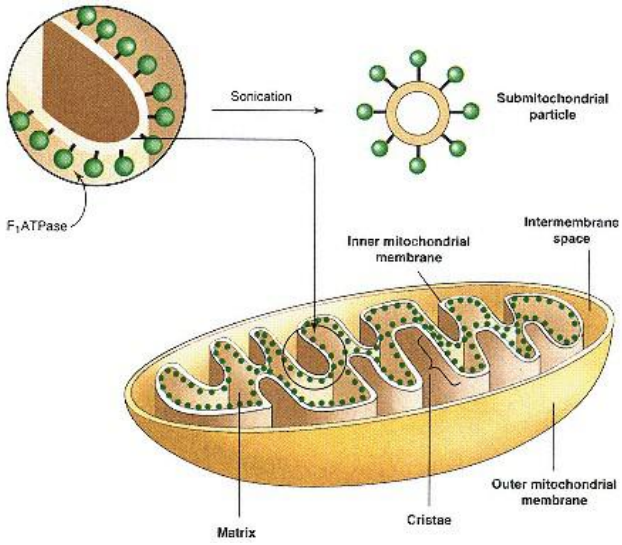


Figure 6.25
Diagram of various submitochondrial compartments.

to perform aerobic metabolic functions. For example, the erythrocyte has no mitochondria and does not possess the capacity to generate energy using oxygen as a terminal electron acceptor. On the other hand, cardiac tissue is a highly aerobic tissue, and it has been estimated that about one-half of the cytoplasmic volume of cardiac cells is composed of mitochondria. The liver is also highly dependent on aerobic metabolic processes for its various functions, and it has been estimated that mammalian hepatocytes contain between 800 and 2000 mitochondria. Mitochondria exist in a variety of different shapes, depending on the cell type from which they are derived. As can be seen in Figure 6.24 mitochondria from liver are nearly spherical in shape, whereas those found in cardiac muscle are oblong or cylindrical.

Inner and Outer Mitochondrial Membranes Have Different Compositions and Functions

Mitochondria are composed of two membranes, an outer and a highly invaginated inner membrane (Figure 6.25). The outer membrane is considered a rather simple membrane, composed of about 50% lipid and 50% protein, with relatively few enzymatic or transport functions. Table 6.5 defines some of the enzymatic components of the outer membrane.

The inner membrane is structurally and functionally much more complex than the outer membrane. Roughly 80% of the inner membrane is protein. It contains most of the enzymes involved in **electron transport** and oxidative phosphorylation, various dehydrogenases and several transport systems, which are involved in transferring substrates, metabolic intermediates and adenine nucleotides between the cytosol and the mitochondrial matrix (Table 6.5).

Some enzymatic components are loosely associated with the inner membrane, whereas others are either tightly bound or are actual structural elements of the membrane. Hence there is a wide variability in the extent to which physical (ultrasonic irradiation or freezing and thawing), chemical (organic solvent or detergent treatment), or enzymatic (protease or lipase) treatments remove, release, or inactivate the enzymes associated with the inner membrane.

TABLE 6.5 Enzymatic Composition of the Various Mitochondrial Subcompartments

Outer Membrane	*Intermembrane Space*	*Inner Membrane*	*Matrix*
Monoamine oxidase	Adenylate kinase	Succinate dehydrogenase	Pyruvate dehydrogenase
Kynurenine hydroxylase	Nucleoside diphosphate kinase	F_1-ATPase	Citrate synthase
Nucleoside diphosphate kinase		NADH dehydrogenase	Isocitrate dehydrogenase
Phospholipase A		β-Hydroxybutyrate dehydrogenase	α-Ketoglutarate dehydrogenase
Fatty acyl-CoA synthetases		Cytochromes b, c_1, c, a, a_3	Aconitase
NADH: cytochrome-*c* reductase (rotenone-insensitive)		Carnitine: acyl-CoA transferase	Fumarase
			Succinyl-CoA synthetase
		Adenine nucleotide translocase	
Choline phosphotransferase			Malate dehydrogenase
		Mono-, di-, and tricarboxylate translocase	
		Glutamate-aspartate translocase	Fatty acid β-oxidation system

Experimental procedures permit separation of inner from outer mitochondrial membranes. The outer membrane may be stripped off and isolated, using digitonin (a detergent), osmotic shock, or ultrasonic irradiation followed by density-gradient ultracentrifugation (Figure 6.26). The resulting inner membrane plus matrix fraction is referred to as a **mitoplast.** The contents of the matrix can be released from the mitoplast, by treatment with a nonionic detergent or vigorous sonication. Once the various subcompartments of the mitochondrion have been separated, analyses may be performed to determine the location of the various characteristic marker enzymes, several of which are listed in Table 6.5. Enzymatic markers have been used effectively to detect the presence of mitochondria or even a particular portion of mitochondria in membrane preparations of diverse derivation.

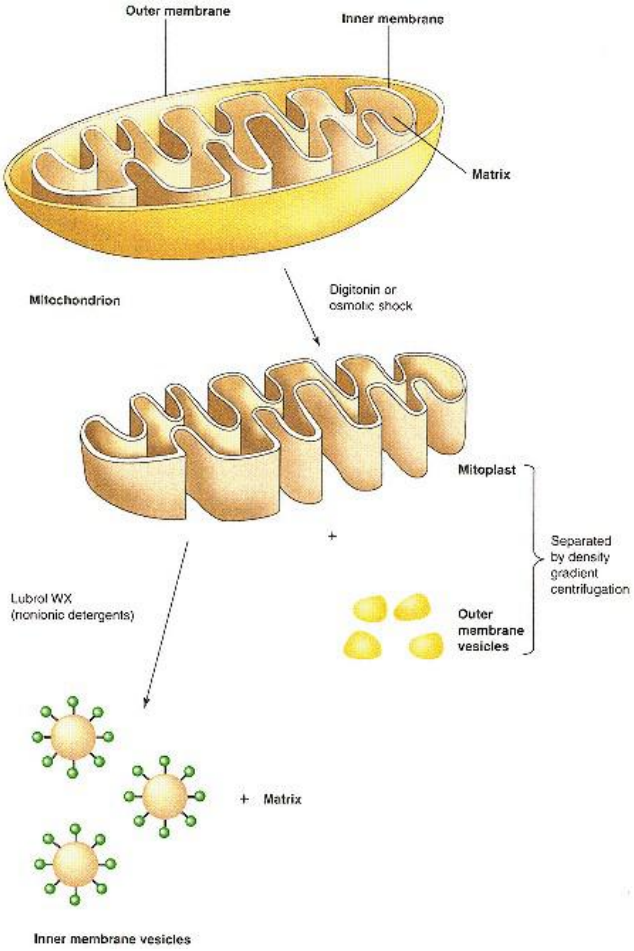


Figure 6.26
Separation of mitochondrial membranes.

Mitochondrial Inner Membranes Contain Substrate Transport Systems

Whereas the outer membrane presents little or no permeability barrier to substrate or nucleotide molecules of interest in energy metabolism, the inner membrane limits the types of substrates, intermediates, and nucleotides that can diffuse from the cytosol into the matrix compartment.

Various transport systems have been described in mitochondria (Figure 6.27), some of which have been thoroughly characterized. The primary function of these transport systems is to facilitate the selective movement of various substrates and intermediates back and forth across the inner mitochondrial membrane from the cytosol to the mitochondrial matrix. Through the action of these transporters, various substrates and other molecules can be accumulated in the mitochondrial matrix since the transporters can facilitate the movement of the substrate against a concentration gradient. The importance of a mitochondrial transporter derives from involvement of the substance transported in a variety of mitochondrial metabolic processes.

Substrate Shuttles Transport Reducing Equivalents across the Inner Mitochondrial Membrane

The various nucleotides involved in cellular oxidation-reduction reactions (e.g., NAD^+, NADH, $NADP^+$, NADPH, FAD, and $FADH_2$) and CoA and its derivatives are not permeable to the inner mitochondrial membrane. Hence, for example, to transport reducing equivalents (e.g., protons and electrons) from cytosol to mitochondrial matrix or vice versa, **"substrate shuttle** mechanisms" involving the reciprocal transfer of reduced and oxidized members of various oxidation-reduction couples are used to accomplish the net transfer of reducing equivalents across the membrane. Two examples of how this transfer of reducing equivalents from the cytosol to the matrix occurs are shown in Figure 6.28. The **malate–aspartate shuttle** and the **α-glycerol phosphate shuttle** are

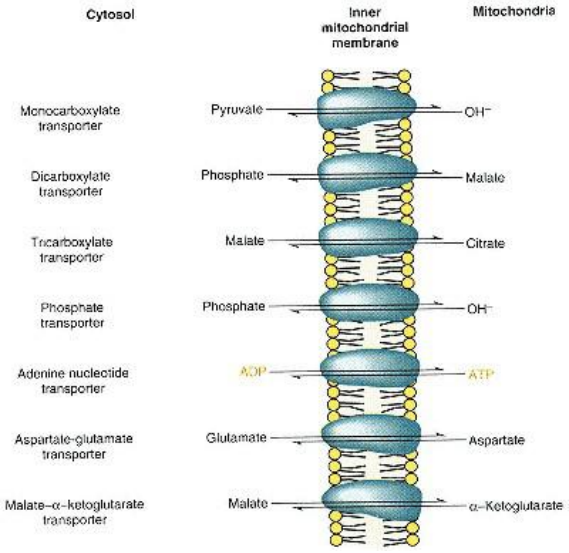


Figure 6.27
Mitochondrial metabolite transporters.

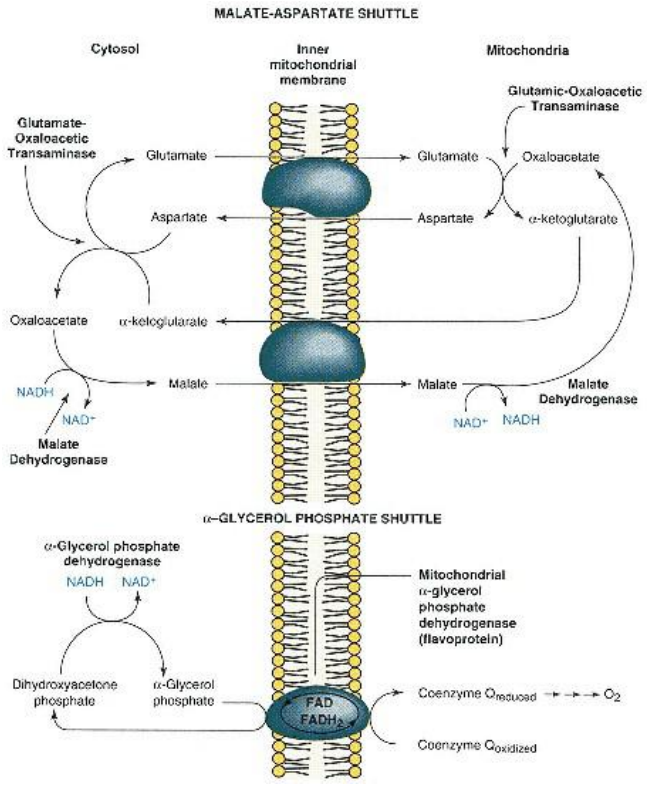


Figure 6.28
Transport shuttles for reducing equivalents.

employed in various tissues to translocate reducing equivalents from the cytosol to the mitochondrial matrix, where they are oxidized to yield energy. The operation of such substrate shuttles requires that the appropriate enzymes are localized on the correct side of the membrane and that appropriate transporters or translocases be present on/in the membrane. The operation of the malate–aspartate shuttle depends on the fact that NADH, NAD^+, and oxaloacetate are not permeable to the inner mitochondrial membrane, on the distribution of malate dehydrogenase and aspartate aminotransferase on both sides of the inner mitochondrial membrane, and on the existence of membrane transporters that exchange intramitochondrial aspartate for cytosolic glutamate and cytosolic malate for intramitochondrial α-ketoglutarate.

Acetyl Units Are Transported by Citrate

Acetyl CoA is impermeable to the inner mitochondrial membrane but the acetyl group can be transferred from the mitochondrial compartment to the cytosol, where acetyl moieties are required for fatty acid or sterol biosynthesis (Figure 6.29).

Intramitochondrial acetyl CoA is converted to citrate by citrate synthase of the TCA cycle. Subsequently, the citrate is exported to the cytosol by a

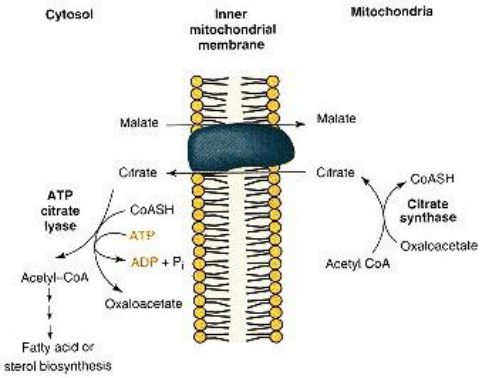


Figure 6.29
Export of intramitochondrially generated citrate to the cytosol to serve as a source of acetyl CoA for biosynthesis of fatty acids or sterols.

tricarboxylate transporter in exchange for a dicarboxylate such as malate. Cytosolic citrate is then cleaved to acetyl CoA and oxaloacetate at the expense of an ATP by ATP: citrate lyase (see p. 371). Substrate shuttle mechanisms in liver are involved in movement of appropriate substrates and intermediates in both directions across the inner mitochondrial membranes during periods of active **gluconeogenesis** (see p. 302) and **ureagenesis** (see p. 454).

Transport of Adenine Nucleotides and Phosphate

Adenine nucleotides are transported across the inner mitochondrial membrane by a very specific **adenine nucleotide translocator.** Nucleotide species such as the guanine, uridine, or cytosine nucleotides are neither exchanged across the inner membrane on the adenine nucleotide-specific translocator nor transported by a comparable carrier specific for nonadenine nucleotides. Cytosolic ADP, formed during energy-consuming reactions, is exchanged for mitochondrial ATP, generated in the process of oxidative phosphorylation (Figure 6.30). At pH 7 ADP has three negative charges and ATP has four, so that a 1:1 exchange of ADP:ATP would cause a charge imbalance across the membrane. Hence the ADP for ATP exchange across the mitochondrial membrane is an electrogenic process, requiring that the charge imbalance be compensated for by the movement of a proton or another charged species. The adenine nucleotide carrier was isolated due to its capacity to bind very tightly to atractyloside, a specific inhibitor of the carrier. It is a dimer with a subunit molecular weight of 30,000. It is unlikely that the rate of transport of adenine nucleotides across the mitochondrial membrane is ever limiting to the overall process of mitochondrial ATP synthesis. Low concentrations of long-chain fatty acyl CoA derivatives inhibit (i.e., K_i = 1 μM) the transport of ATP and ADP in isolated liver mitochondria. However, experimental results obtained under *in vivo* conditions in intact liver cells indicate that there occurs little, if any, inhibition of the adenine nucleotide transporter under metabolic conditions in which a large concentration of long-chain fatty acyl CoA accumulates.

A specific transporter transports cytosolic phosphate into the mitochondrial matrix for negatively charged hydroxyl ions in an electroneutral exchange (Figure 6.30). Also, phosphate transport may be accomplished in a proton-compensated mechanism; for example, phosphate and protons are transported in a 1:1 ratio. Phosphate transport is strongly inhibited by mersalyl and various mercurial reagents.

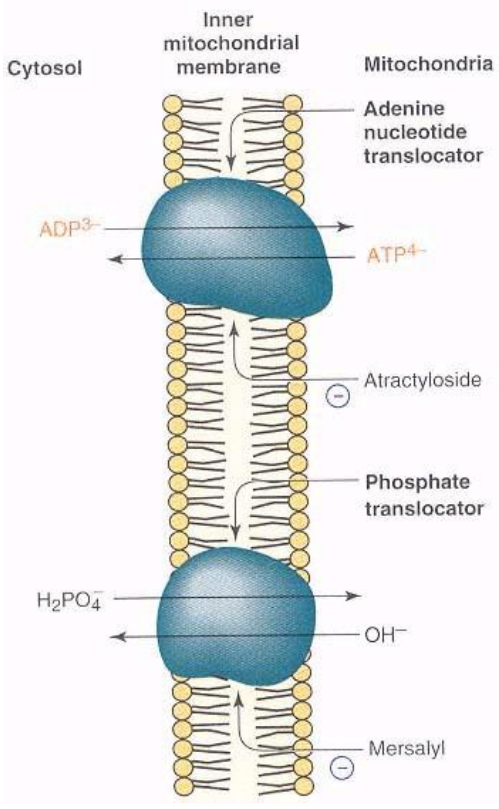


Figure 6.30
The adenine nucleotide and phosphate translocators.

Mitochondria Have a Specific Calcium Transport Mechanism

Mitochondria from most tissues possess a transport system for translocating Ca^{2+} across the mitochondrial inner membrane. It is difficult to overestimate the importance of the distribution/redistribution of cellular calcium pools in different cell functions, such as muscle contraction, neural transmission, secretion, and hormone action. Calcium exists in distinct pools in the cell. The cytosol, mitochondria, endoplasmic reticulum, nuclei, and Golgi complex have their own pools of calcium. Some of the intracellular calcium is bound to nucleotides, metabolites, or membrane ligands, while a portion of the intracellular calcium is free in solution. A gradient of Ca^{2+} exists from outside to inside a cell. Estimates of intracellular cytosolic calcium are in the range of 10^{-7} M, whereas extracellular calcium is at least four orders of magnitude greater. Total intramitochondrial calcium has been estimated to be ~10^{-4} M but the free ionic calcium concentration in the mitochondrion is in the range of 10^{-7} M. Hence processes involved in the alternate sequestering and release of an intracellular store of calcium can greatly influence intracellular calcium pools and various cell functions. Mitochondria accumulate rather large quantities of calcium at the expense of ATP hydrolysis, respiration, or the electrochemical gradient created across the mitochondrial membrane. Mitochondrial calcium transport is inhibited by low concentrations of lanthanides (trivalent metal cations) and by ruthenium red. Mg^{2+} competes with Ca^{2+} for the carrier in certain types of mitochondria. The current view is that there is a specific carrier in the inner mitochondrial membrane, which is likely a glycoprotein (Figure 6.31). The mitochondrial calcium carrier exhibits saturation kinetics, has a high affinity for calcium, and is highly specific for calcium. Permeant counterions such as phosphate or acetate stimulate calcium transport and allow the cation to be retained in the matrix. The most probable utility of the ability of mitochondria to accumulate calcium occurs during cellular injury when extracellular calcium enters the cell. Mitochondria can sequester the calcium to minimize the change in the cytosolic calcium level. Certain hormones may affect intracellular calcium distribution (e.g., epinephrine or vasopressin) as part of the mechanism of the hormone response; it is unlikely that the mitochondrial calcium pool contributes to the hormone-sensitive pool of calcium.

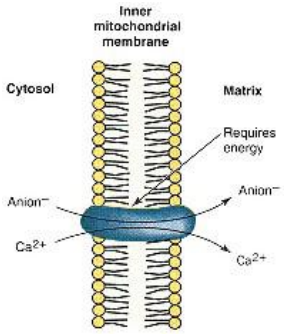


Figure 6.31
Mitochondrial calcium carrier.
The energy requirement can be met from ATP, pH, or membrane potential.

In summary, the inner mitochondrial membrane possesses a variety of transport systems involved in the movement of nucleotides, substrates, metabolites, and metal cations into and out of the mitochondrial matrix. These transport functions are essential for the complex cellular metabolic pathways and their regulation (see Clin. Corr. 6.3).

6.6— Electron Transfer

During the enzymatic reactions involved in glycolysis, fatty acid oxidation, and the TCA cycle, reducing equivalents are derived from the sequential breakdown of the initial metabolic fuel. In glycolysis, NADH is produced by glyceraldehyde-3-phosphate dehydrogenase and must be reoxidized in the cytosol (e.g., by lactate dehydrogenase as is the case in the red blood cell) or the reducing equivalents of NADH must be transported to the mitochondrial matrix via one of the substrate shuttles. The latter mechanism will yield the maximum energy from the metabolism of glucose. In fatty acid oxidation and the TCA cycle, reducing equivalents as both NADH and $FADH_2$ are produced in the mitochondrial matrix. To transduce this reducing power into utilizable energy, mitochondria have a system of electron carriers in or associated with the inner mitochondrial membrane, which in the presence of oxygen convert reducing equivalents into utilizable energy. This process is called **electron transport.** As will be seen, NADH and $FADH_2$ oxidation in this process results in production of

3 and 2 mol of ATP per mole of reducing equivalent transferred to oxygen, respectively.

Oxidation–Reduction Reactions

Prior to the presentation of a description of the many components and the mechanism of the electron transport sequence, it is important to discuss some basic information concerning oxidation–reduction reactions. The mitochondrial electron transport system is little more than a sequence of linked oxidation–reduction reactions, for example,

$$AH_2 + B \rightleftharpoons A + BH_2$$

Oxidation–reduction reactions occur when there is a transfer of electrons from a suitable electron donor (the reductant) to a suitable electron acceptor (the oxidant). In some oxidation–reduction reactions only electrons are transferred from the reductant to the oxidant (i.e., electron transfer between cytochromes),

$$\text{Cytochrome } c\ (Fe^{2+}) + \text{cytochrome } a\ (Fe^{3+}) \rightleftharpoons \text{cytochrome } c\ (Fe^{3+}) + \text{cytochrome } a\ (Fe^{2+})$$

whereas in other types of reactions, both electrons and protons (hydrogen atoms) are transferred (e.g., electron transfer between NADH and FAD).

$$NADH + H^+ + FAD \rightleftharpoons NAD^+ + FADH_2$$

Oxidized and reduced forms of compounds or groups operating in oxidation-reduction reactions are referred to as **redox couples** or pairs. The facility with which a given electron donor (reductant) gives up its electrons to an electron acceptor (oxidant) is expressed quantitatively as the oxidation-reduction potential of the system. An **oxidation–reduction potential** is measured in volts as an **electromotive force** (emf) of a half-cell made up of both members of an oxidation–reduction couple when compared to a standard reference half-cell (usually the hydrogen electrode reaction). The potential of the standard hydrogen electrode is set by convention at 0.0 V at pH 0.0. However, when this standard potential is corrected for pH 7.0 the reference electrode potential becomes –0.42 V. The oxidation–reduction potentials for a variety of important biochemical reactions are tabulated in Table 6.6. The reductant of an oxidation–reduction pair with large negative potential will give up its electrons more readily than pairs with smaller negative or positive redox potentials. On the

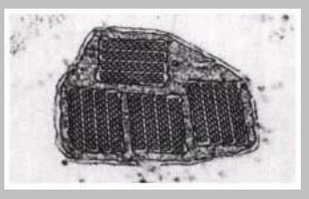
CLINICAL CORRELATION 6.3

Mitochondrial Myopathies

Diseases that involve defects in various metabolic functions of muscle have been described. Clinically, patients with myopathy complain of weakness and cramping of the affected muscles; infants have difficulty feeding and crawling; severe fatigue results from minimal exertion; and there is usually evidence of muscle wasting. On the basis of electron microscopic examination and enzymatic characterization of muscle biopsy material, many myopathies have been found that have a primary lesion in mitochondrial function.

Deficiencies in mitochondrial transport functions (i.e., carnitine: palmitoyl-CoA transferase) and in components of the mitochondrial electron transport chain (NADH dehydrogenase, cytochrome *b*, cytochrome *a, a*$_3$, or the mitochondrial F_1F_0-ATPase) have been described. In many mitochondrial myopathies large paracrystalline inclusions occur within the mitochondrial matrix (see figure). It is not known whether this crystalline material is inorganic or organic in composition. In certain mitochondrial myopathies electron transport is only loosely coupled to ATP production; in other cases these processes exhibit normal tight coupling. Because some of these disorders involve defects in enzymes encoded by mitochondrial genes, they have the unique pattern of inheritance from the mother, since all mitochondria are derived from mitochondria in the ovum.

Petty, R. K. H., Harding, A. E., and Morgan-Hughes, J. A. The clinical features of mitochondrial myopathy. *Brain* 109:915, 1986; and Shoffner, J. M., and Wallace, D. C. Oxidative phosphorylation diseases and mitochondrial mutations: diagnosis and treatment. *Annu. Rev. Nutr.* 14:535,1994.



Example of paracrystalline inclusions in mitochondria from muscles of ocular myopathic patients (×36,000). Courtesy of Dr. D. N. Landon, Institute of Neurology, University of London.

TABLE 6.6 Standard Oxidation-Reduction Potentials for Various Biochemical Reactions

Oxidation-Reduction System	*Standard Oxidation-Reduction Potential E_0' (V)*
Acetate + $2H^+ + 2e^- \rightleftharpoons$ acetaldehyde	–0.60
$2H^+ + 2e^- \rightleftharpoons H_2$	–0.42
Acetoacetate + $2H^+ + 2e^- \rightleftharpoons$ β-hydroxybutyrate	–0.35
$NAD^+ + 2H^+ + 2e^- \rightleftharpoons NADH + H^+$	–0.32
Acetaldehyde + $2H^+ + 2e^- \rightleftharpoons$ ethanol	–0.20
Pyruvate + $2H^+ + 2e^- \rightleftharpoons$ lactate	–0.19
Oxaloacetate + $2H^+ + 2e^- \rightleftharpoons$ malate	–0.17
Coenzyme Q_{ox} + $2e^- \rightleftharpoons$ coenzyme Q_{red}	+0.10
Cytochrome *b* $(Fe^{3+}) + e^- \rightleftharpoons$ cytochrome *b* (Fe^{2+})	+0.12
Cytochrome *c* $(Fe^{3+}) + e^- \rightleftharpoons$ cytochrome *c* (Fe^{2+})	+0.22
Cytochrome *a* $(Fe^{3+}) + e^- \rightleftharpoons$ cytochrome *a* (Fe^{2+})	+0.29
$\frac{1}{2}O_2 + 2H^+ + 2e^- \rightleftharpoons H_2O$	+0.82

other hand, a strong oxidant (e.g., characterized by a large positive potential) has a very high affinity for electrons.

The **Nernst equation** characterizes the relationship between the standard oxidation–reduction potential of a particular redox pair (E_0'), the observed potential (E), and the ratio of the concentrations of oxidant and reductant in the system:

$$E = E_0' + \frac{2.3\,RT}{nF} \log \left(\frac{[\text{oxidant}]}{[\text{reductant}]} \right)$$

E is the observed potential with all concentrations at 1 M. E_0' is the standard potential at pH 7.0. R is the gas constant of 8.3 J deg^{-1} mol^{-1}. T is the absolute temperature in kelvin units (K). n is the number of electrons being transferred. F is the Faraday constant of 96,500 J V^{-1}.

When an observed potential is equal to the standard potential, a potential is defined that is referred to as the midpoint potential. At the midpoint potential the concentration of oxidant is equal to that of reductant. Knowing standard oxidation–reduction potentials of a diverse variety of biochemical reactions allows one to predict the direction of electron flow or transfer when more than one redox pair is linked together by the appropriate enzyme that causes a reaction to occur. For example, as shown in Table 6.6 the NAD^+–NADH pair has a standard potential of –0.32 V, and the pyruvate–lactate pair possesses a potential of –0.19. This means that electrons will flow from the NAD^+–NADH system to the pyruvate–lactate system as long as the enzyme (lactate dehydrogenase) is present; for example,

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \rightleftharpoons \text{lactate} + \text{NAD}^+$$

Hence in the mitochondrial electron-transfer system electrons or reducing equivalents are produced in NAD^+- and FAD-linked dehydrogenase reactions, which have standard potentials at or close to that of NAD^+–NADH and are passed through the electron-transfer chain, which has as its terminal acceptor the oxygen–water couple.

Free-Energy Changes in Redox Reactions

Oxidation–reduction potential differences between two redox pairs are similar to free-energy changes in chemical reactions, in that both quantities depend on the concentration of reactants and products of the reaction and the following relationship exists:

$$\Delta G^{\circ\prime} = -nF\,\Delta E_0'$$

Using this expression, the free-energy change for electron-transfer reactions can be calculated if the potential difference between two oxidation–reduction pairs is known. Hence, for the mitochondrial electron-transfer process in which electrons are transferred between the NAD^+–NADH couple $(E_0' = +0.82\ \text{V})$, the free-energy change for this process can be calculated:

$$\Delta G^\circ = -nF\Delta E_0' = -2 \times 23.062 \times 1.14\ \text{V}$$
$$\Delta G^\circ = -52.6\ \text{kcal mol}^{-1}$$

where 23.062 is the Faraday constant in kcal V^{-1} and n is the number of electrons transferred; for example, in the case of NADH O_2, $n = 2$. The free energy available from the potential span between NADH and oxygen in the electron-transfer chain is capable of generating more than enough energy to synthesize three molecules of ATP per two reducing equivalents or two electrons trans-

ported to oxygen. In addition, because of the negative sign of the free energy available in the mitochondrial electron transfer, this process is exergonic and will proceed provided that the necessary enzymes are present.

Mitochondrial Electron Transport Is a Multicomponent System

Before defining the mechanistic details of the mitochondrial electron transport chain it is necessary to describe the various components that participate in the transfer of electrons in this system. The major enzymes or proteins functioning as electron-transfer components involved in the mitochondrial electron-transfer system are as follows: (1) NAD^+-linked dehydrogenases, (2) flavin-linked dehydrogenases, (3) iron–sulfur proteins, and (4) **cytochromes.**

NAD-Linked Dehydrogenases

The initial stage in the mitochondrial electron transport sequence consists of the generation of reducing equivalents in the TCA cycle, the fatty acid β-oxidation sequence, and various other dehydrogenase reactions. The NAD-linked dehydrogenase reactions of these pathways reduce NAD^+ to NADH while converting the reduced member of an oxidation–reduction couple to the oxidized form; for example, for the isocitrate dehydrogenase reaction,

$$\text{Isocitrate} + NAD^+ \rightleftharpoons \alpha\text{-ketoglutarate} + CO_2 + NADH + H^+$$

Two nicotinamide nucleotides are involved in various metabolic reactions, NAD and NADP (Figure 6.32). Nicotinamide adenine dinucleotide phosphate has a phosphate esterified to the 2 position of the ribose in the adenosine portion of

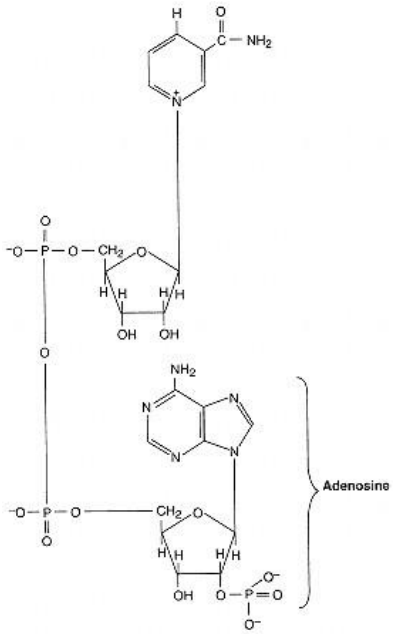
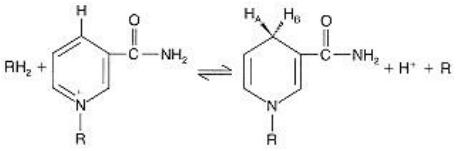


Figure 6.32
Structure of nicotinamide adenine dinucleotide phosphate: NADP.

TABLE 6.7 The Stereospecificity of NAD(P)- Linked Dehydrogenases

NAD(P)-Linked Dehydrogenase	*Specificity*
Alcohol dehydrogenase	A
Malate dehydrogenase	A
Lactate dehydrogenase	A
Isocitrate dehydrogenase ($NADP^+$)	A
Hydroxyacyl-CoA dehydrogenase	B
Glyceraldehyde-3-phosphate dehydrogenase	B
Glucose-6-phosphate dehydrogenase ($NADP^+$)	B

the dinucleotide. Each NAD(P)-linked dehydrogenase catalyzes a stereospecific transfer of the reducing equivalent from the substrate to the nucleotide (see p. 143).



NAD(P)-linked dehydrogenases are either A specific or B specific. Table 6.7 lists examples of the stereospecificity of NAD(P)-linked dehydrogenases. Once formed, NAD(P)H is released from the dehydrogenase and serves as the substrate for the mitochondrial electron transport system. NADPH is not a substrate for the mitochondrial respiratory chain but is used in reductive biosynthetic reactions of such processes as fatty acid and sterol synthesis. When $NAD(P)^+$ is converted to NAD(P)H, there is a characteristic change in the absorbance and fluorescence properties of these nucleotides, which occurs as a result of the reduction of $NAD(P)^+$. The reduced form of the nicotinamide coenzyme has an absorbance maximum at 340 nm (Figure 6.33) not present in the oxidized $NAD(P)^+$ form. Furthermore, when the reduced form of the nicotinamide coenzyme is excited by light at 340 nm a fluorescence emission maximum is seen at 465 nm. These absorbance and fluorescence properties of the nicotinamide coenzymes have been employed extensively in developing assays for dehydrogenase reactions (see p. 168) and have been utilized to monitor the oxidation-reduction state of a tissue or a preparation of intact mitochondria. With an appropriate spectrophotometer (e.g., dual wavelength), capable of measuring small absorbance changes in turbid cell or mitochondrial suspensions, the relative changes in the oxidized-reduced nicotinamide coenzymes may be determined as a function of the metabolic condition of the cell or subcellular suspension (e.g., changes in substrate, oxygen concentration, or upon drug or hormone addition). This type of spectrophotometric technique and more sophisticated techniques—in which a light guide is used to direct a beam of excitation light to the surface of an intact organ or tissue, and another light guide is employed to observe the reflected fluorescence emission at a longer wavelength—have been valuable tools in understanding the very complicated relationships that exist between the mitochondrial respiratory chain and the metabolic characteristics of various tissues.

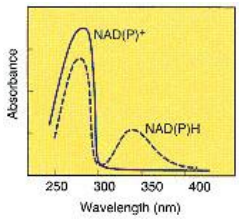


Figure 6.33
Absorbance properties of NAD^+ and NADH.

Another effective method for monitoring the oxidation–reduction state of the cytosolic or mitochondrial compartments is to measure the oxidized and reduced members of various redox couples in tissue extracts, in the bathing solution of a tissue, or in the effluent perfusate of an isolated, perfused organ. Because lactate dehydrogenase is exclusively a cytosolic enzyme the pyruvate/lactate ratio in the tissue or organ perfusate should accurately reflect the cytosolic NAD^+/NADH ratio under a variety of metabolic conditions. Similarly, the ***β*-hydroxybutyrate dehydrogenase** is exclusively mitochondrial, and hence the ratio of acetoacetate/*β*-hydroxybutyrate should reflect the oxidation–reduction state of the mitochondrial NAD^+–NADH system. If the ratio of acetoacetate/*β*-hydroxybutyrate and the equilibrium constant for *β*-hydroxybutyrate dehydrogenase are known, the NAD^+/NADH ratio under any condition can be calculated:

$$\text{Acetoacetate} + \text{NADH} + \text{H}^+ \rightleftharpoons \beta\text{-hydroxybutyrate} + \text{NAD}^+$$

$$K_{eq} = \frac{[\beta\text{-hydroxybutyrate}][\text{NAD}^+]}{[\text{acetoacetate}][\text{NADH}][\text{H}^+]}$$

Flavin-Linked Dehydrogenases

The second type of oxidation–reduction reaction essential in mitochondrial electron transport employs a **flavin** (e.g., derived from riboflavin) as electron acceptor as part of flavin-linked dehydrogenases. The two flavins commonly utilized in oxidation–reduction reactions are FAD (flavin adenine dinucleotide) and FMN (flavin mononucleotide) (Figure 6.34).

Five flavin-containing enzymes play an essential role in energy metabolism in mammalian mitochondria (Table 6.8). In the pyruvate and α-ketoglutarate dehydrogenase multienzyme complexes, the final reaction catalyzed involves the flavoprotein enzyme, dihydrolipoyl dehydrogenase, in which the bound FAD moiety accepts electrons from reduced lipoamide on the transacylase subunit and then transfers the reducing equivalents to NAD^+. Also, in the TCA cycle, succinate dehydrogenase is a flavin-linked protein, which oxidizes succinate to fumarate and converts FAD to $FADH_2$. The first dehydrogenation reaction in β-oxidation of fatty acids is catalyzed by the acyl-CoA dehydrogenase, another flavin-linked enzyme. Finally, oxidation of NADH in the mitochondrial respiratory chain is catalyzed by a FMN-containing enzyme, NADH dehydrogenase, and the reducing equivalents are transferred to another flavoprotein called the electron-transferring flavoprotein.

The flavins FAD and FMN either may be bound very tightly noncovalently (i.e., with dissociation constants in the range of 10^{-10} M) to their respective enzymes, as is the case for NADH dehydrogenase, or may be bound covalently to the protein (e.g., to a histidine residue), as is the case with succinate dehydrogenase. Flavoproteins are classified into two groups: (1) dehydrogenases in which the reduced flavin is reoxidized by electron carriers other than oxygen (e.g., coenzyme Q and other flavins, or *in vitro* with chemical agents such as ferricyanide, methylene blue, or phenazine methosulfate) and (2) oxidases in which the flavin may be reoxidized using molecular oxygen, O_2, as the electron acceptor, and yielding H_2O_2 as the product. The H_2O_2 may then be broken down to water and oxygen by the enzyme **catalase,**

$$2H_2O_2 \xrightleftharpoons{\text{catalase}} 2H_2O + O_2$$

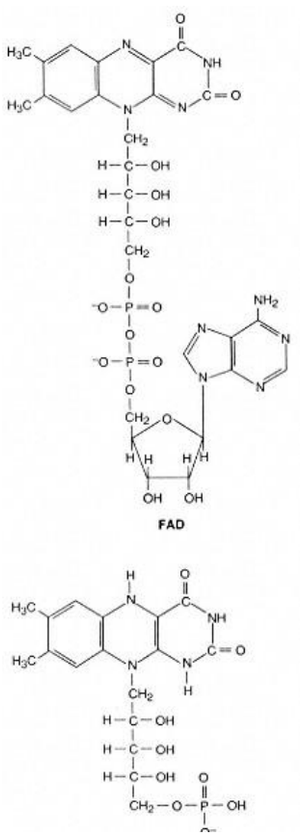


Figure 6.34
Structures of flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN).

Iron-Sulfur Centers

A number of flavin-linked enzymes contain nonheme iron (i.e., an iron-sulfur center; see p. 1004) involved in the catalytic mechanism. In these enzymes iron is converted from the oxidized (Fe^{3+}) to reduced (Fe^{2+}) form during the transfer of reducing equivalents on and off the flavin moiety. Both succinate dehydroge-

TABLE 6.8 Various Flavin-Linked Dehydrogenases

Enzyme	*Function*	*Flavin Nucleotide*
Succinate dehydrogenase	Tricarboxylic acid cycle	FAD
Dihydrolipoyl dehydrogenase	Component in pyruvate and α-ketoglutarate dehydrogenase complexes	FAD
NADH dehydrogenase	Electron transport chain	FMN
Electron-transferring flavoprotein	Electron transport chain	FAD
Acyl-CoA dehydrogenase	Fatty acid β-oxidation	FAD
D-Amino acid oxidase	Amino acid oxidation	FAD
Monoamine oxidase	Oxidation of monoamines	FAD

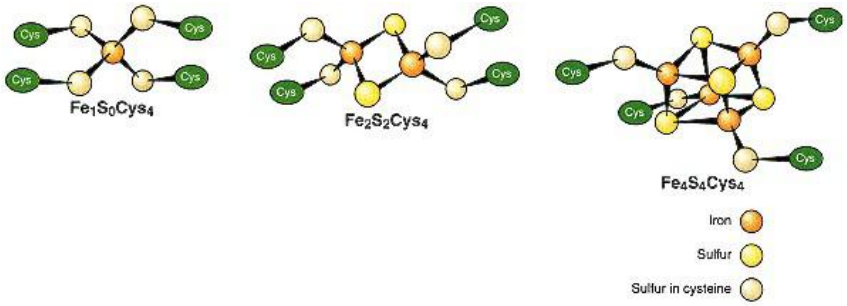


Figure 6.35
The structures of iron–sulfur centers.
White, sulfur; gray, sulfur in cysteine; and black, iron.

nase and NADH dehydrogenase contain iron–sulfur centers. The iron component of the iron–sulfur center is bound in various arrangements to cysteine residues in the protein and to acid-labile sulfur, for example, $Fe_4S_4Cys_4$, $Fe_2S_2Cys_4$, and $Fe_1S_0Cys_4$ (Figure 6.35). Iron-sulfur proteins are found in abundance in all species from the simplest microorganism to mammals. Certain flavin-linked enzymes (e.g., xanthine oxidase) contain one or two molybdenum atoms associated with their catalytic mechanism. The tightly bound molybdenum undergoes a valence change during transfer of electrons: Mo^{6+} Mo^{5+}.

Cytochromes

Organisms that require oxygen (i.e., aerobic organisms) in their energy-generating functions possess various cytochromes that are involved in electron-transfer systems. Cytochromes are a class of proteins characterized by the presence of an iron-containing heme group bound to the protein. Unlike the heme group in hemoglobin or myoglobin in which the heme iron remains in the Fe^{2+} state,

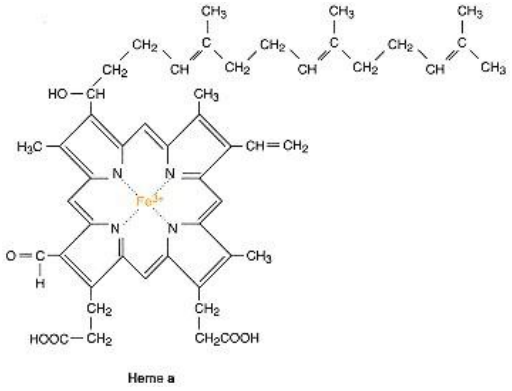


Figure 6.36
Structures of heme a and heme c.

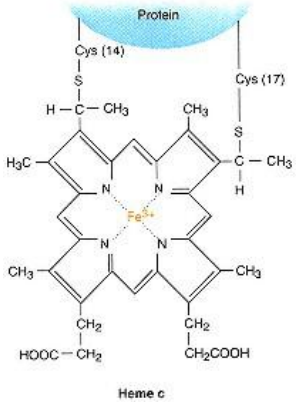


Figure 6.36
Continued.

the iron in the heme of a cytochrome is alternately oxidized (Fe^{3+}) or reduced (Fe^{2+}) as it functions in the electron transport chain.

Cytochromes of mammalian mitochondria are designated as *a*, *b*, and *c* on the basis of the α band of their absorption spectrum and the type of heme group (Figure 6.36). Cytochrome *c* is a small protein (104 amino acid residues) with mol wt = 13,000. Amino acid sequences of cytochrome *c* from a great many species have been described and show that 20 out of 104 amino acid residues are invariant. The iron of the heme group in cytochrome *c* is coordinated between the four nitrogen atoms of the tetrapyrrole structure of the porphyrin group, whereas the fifth and sixth coordination positions are occupied by the methionine residue at position 80 and the histidine residue at position 18 of the protein (Figure 6.37). Since all six coordination positions are filled in most of the cytochromes, binding of oxygen directly to the iron is prevented as is binding of respiratory inhibitors such as cyanide, azide, and carbon monoxide. The notable exception is cytochrome a_3, which is involved in the terminal step in mitochondrial electron transport. The heme group in cytochrome *c* is attached to the protein, not only by the fifth and sixth coordination positions of the heme iron, but also by the vinyl side chains of the protoporphyrin IX structure, from which hemes in cytochromes *a* and *c* are derived. These vinyl side chains are reduced by the addition of reduced sulfhydryls from cysteine residues at positions 14 and 17 in cytochrome c apoprotein. Hence the heme is covalently linked to the protein as well as being coordinated through the Fe^{2+} group in the heme. The three-dimensional structure of cytochrome c is shown in Figure 6.38.

Coenzyme Q

Coenzyme Q, also called **ubiquinone,** is neither a nucleotide nor a protein but a lipophilic electron carrier. Like the nicotinamide coenzymes and to a certain extent cytochrome *c*, coenzyme Q serves as a "mobile" electron transport component that operates between the various flavin-linked dehydrogenases (e.g., NADH dehydrogenase, succinate dehydrogenase, and fatty acyl-CoA dehydrogenase) and cytochrome *b* of the electron transport chain. The quinone portion of the coenzyme Q molecule is alternately oxidized and reduced by

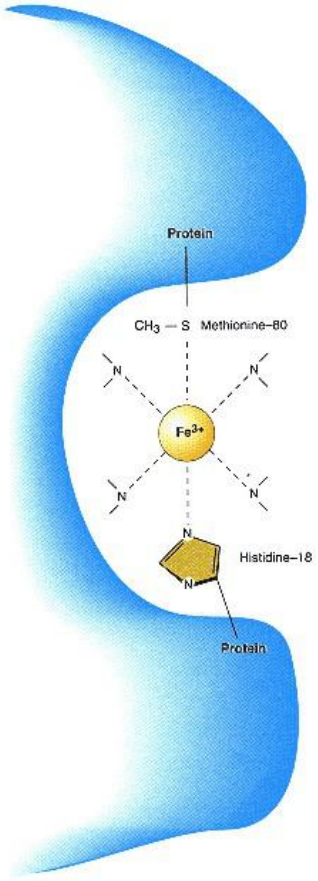


Figure 6.37
The six coordination positions of cytochrome c.

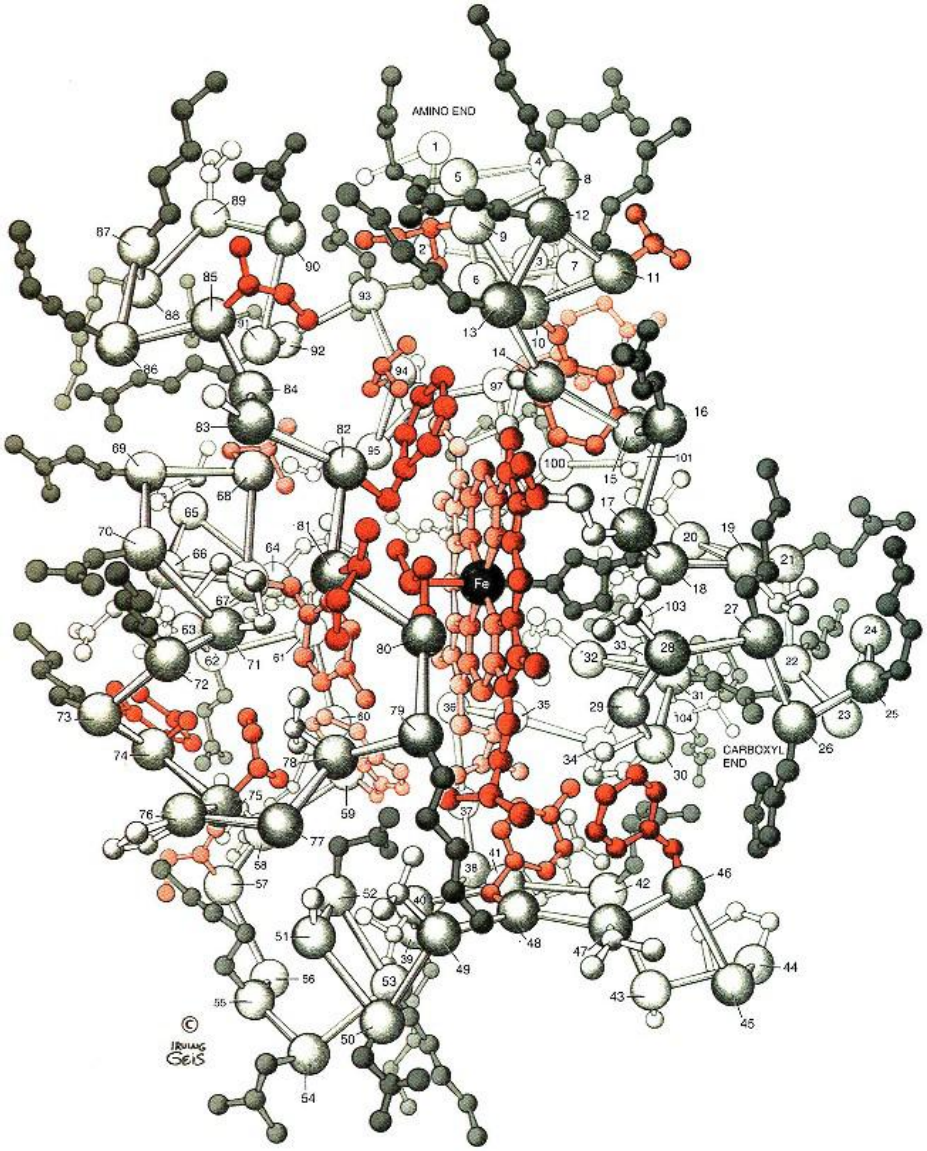


Figure 6.38
The three-dimensional structure of cytochrome c.
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the addition of two reducing equivalents, that is 2 H^+, and 2 e^- (Figure 6.39). The number (n) of isoprene units in the side chain varies between 6 and 10, depending on the source of the coenzyme Q. The side chain renders the coenzyme Q lipid soluble and facilitates the accessibility of this electron carrier to the lipophilic portions of the inner mitochondrial membrane.

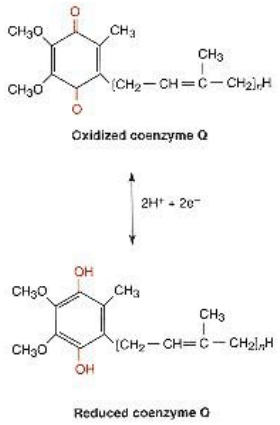


Figure 6.39
Oxidation–reduction of coenzyme Q.

The Mitochondrial Electron Transport Chain Is Located in the Inner Membrane in a Specific Sequence

The various electron-transferring proteins and other electron carriers that comprise the mitochondrial electron-transfer chain are arranged in a sequential pattern in the inner mitochondrial membrane. Reducing equivalents are extracted from substrates in the TCA cycle, the fatty acid β-oxidation sequence, and indirectly from glycolysis and passed sequentially through the electron transport chain to molecular oxygen. The arrangement of carriers is illustrated in Figure 6.40. Electrons or reducing equivalents are fed into the electron transport chain at the level of NADH or coenzyme Q from the primary NAD^+- and FAD-linked dehydrogenase reactions and are transported to molecular oxygen through the cytochrome chain. This electron transport system is constructed so that the reduced member of one redox couple is oxidized by the oxidized member of the next component in the system:

$$NADH + H^+ + FMN \leftrightarrow FMNH_2 + NAD^+$$

or

$$\text{Cytochrome } b\,(Fe^{2+}) + \text{cytochrome } c_1\,(Fe^{3+}) \leftrightarrow \text{cytochrome } b\,(Fe^{3+}) + \text{cytochrome } c_1\,(Fe^{2+})$$

Note that electron transfer from NADH through coenzyme Q involves 2 e^-, whereas the reactions between coenzyme Q and oxygen involving the various cytochromes are 1 e^- transfer reactions.

The components of the respiratory chain have characteristic absorption spectra that can be determined in suspensions of isolated mitochondria or submitochondrial particles using a dual-beam spectrophotometer. The different absorption bands are shown in Figure 6.41. One of the light beams of the spectrophotometer was passed through a suspension of liver mitochondria, which was maintained under fully reduced conditions (e.g., substrate plus no oxygen), and the other beam was passed through an identical suspension in the presence of oxygen. The resulting spectrum is a difference spectrum of the reduced minus the oxidized states of the mitochondrial respiratory chain.

During transfer of electrons from the NADH–NAD^+ couple $(E_0' = +0.82)$ there occurs an oxidation-reduction potential decrease of 1.14 V. This drop in potential occurs in discrete steps as reducing equivalents or electrons are passed between the different segments of the chain (Figure 6.42). There is at least a 0.3-V decrease in potential between each of the three coupling or phosphorylation sites. A potential drop of 0.3 V is more than sufficient to accommodate synthesis of a high-energy phosphate bond of ATP. For example,

$$\Delta E_0' = 0.3\ \text{V}$$
$$\Delta G^\circ = -nF\Delta E_0'$$
$$\Delta G^\circ = -2 \times 23.062 \times 0.3$$
$$\Delta G^\circ = -13.8\ \text{kcal mol}^{-1}$$

Various components of the electron transport chain are located asymmetrically in the mitochondrial membrane. **Cytochrome-*c* oxidase,** which catalyzes the

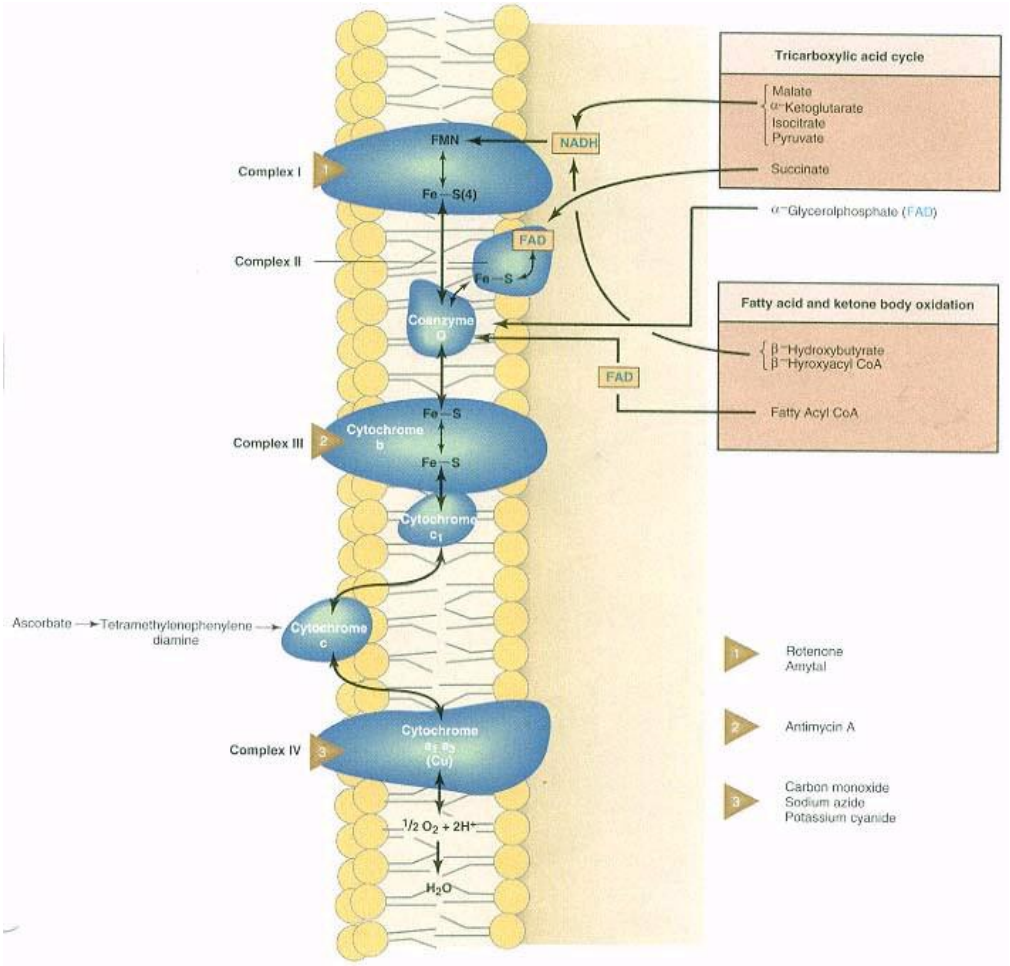


Figure 6.40
Mitochondrial electron transport chain.

terminal step in the electron-transfer chain, spans the membrane between the matrix and the intermembrane space (Figure 6.43). This protein is a dimeric complex of 13 polypeptides that contains heme a, heme a_3, and three copper atoms. Cytochrome *c* binds to the oxidase from the cytosolic side of the membrane, whereas oxygen binds from the matrix side of the membrane during the electron-transferring event.

Figure 6.44 depicts the organization of the entire electron transport sequence in the inner mitochondrial membrane. The initial reaction is catalyzed by the NADH dehydrogenase complex, designated **Complex I,** which accepts

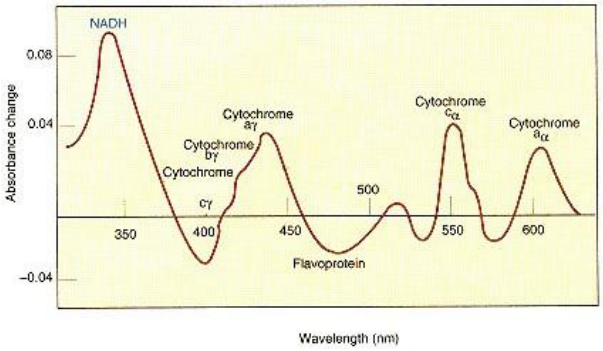


Figure 6.41
Difference spectrum of liver mitochondrial suspension (oxidized – reduced).

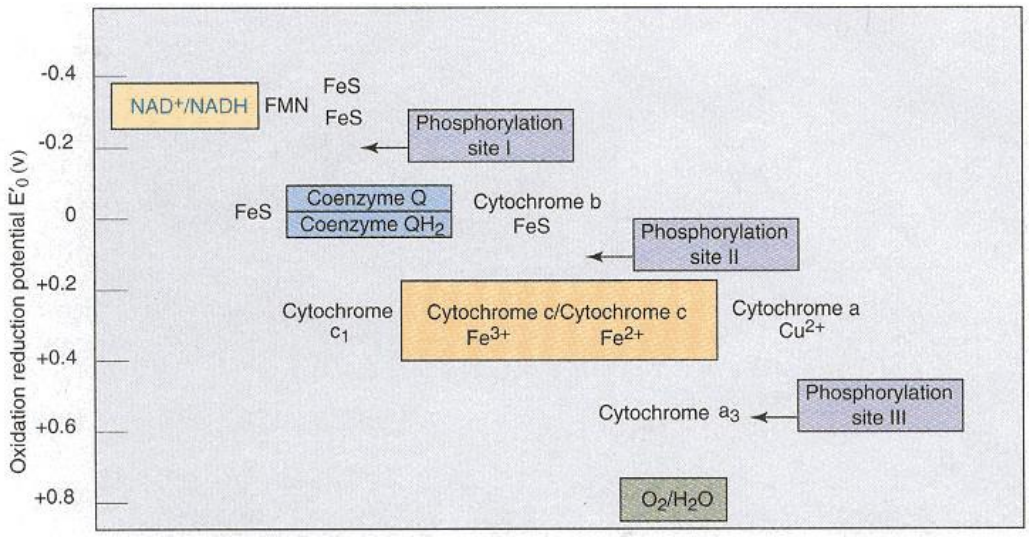


Figure 6.42
Oxidation–reduction potentials of the mitochondrial electron transport chain carriers.

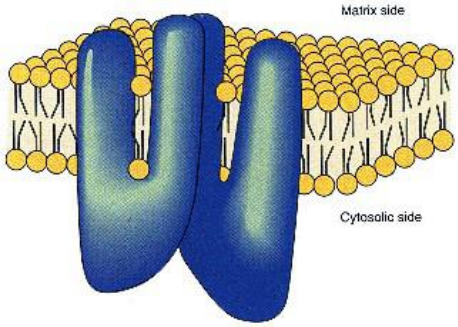


Figure 6.43
Model of cytochrome-*c* oxidase dimer in the mitochondrial inner membrane.
Redrawn with permission
from Frey, T. G., Costello, M. J., Karlsson, B., Haselgrove, J.C., and Leigh, J.S. J. *Mol. Biol.* 162:113, 1982.

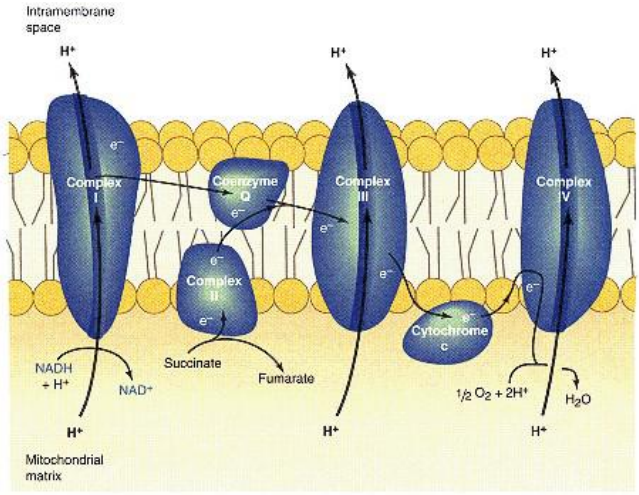


Figure 6.44
The four electron transport complexes of the mitochondrial electron transport sequence.

protons and electrons from NADH + H^+ and transfers them to coenzyme Q. **Complex II** consists of the succinate dehydrogenase flavoprotein component, which accepts reducing equivalents from succinate, again for passage to coenzyme Q. Being a highly lipophilic molecule, coenzyme Q is quite mobile in the mitochondrial membrane, which facilitates its ability to transfer electrons from both Complex I and Complex II to the cytochrome bc_1 complex **(Complex III).** Cytochrome *c* then accepts electrons from Complex III for transport to cytochrome oxidase **(Complex IV)** where molecular oxygen is the terminal electron acceptor. Protons (e.g., H^+) are ejected from the mitochondrial matrix into the intramembrane space at three points in this sequence of reactions (Figure 6.44). As described below, these protons will be translocated back into the matrix by the F_1F_0-ATPase present in the mitochondrial inner membrane as part of the oxidative phosphorylation phase of this energy-transducing system. Clinical Correlation 6.4 describes clinical conditions in which there are genetic dysfunctions of some of the Complexes.

CLINICAL CORRELATION 6.4

Subacute Necrotizing Encephalomyelopathy

This condition is also called Leigh disease. It manifests in infants and young children as severe lactic acidosis and neurological abnormalities. It is characterized by symmetrical lesions in basal ganglia, brain stem, and spinal cord that are detectable by computerized tomography (CT) scanning. The condition is frequently fatal. Dysfunction in oxidative phosphorylation especially in Complex IV (cytochrome-*c* oxidase) is common. Dysfunction in Complex I (NADH dehydrogenase), Complex II (succinate dehydrogenase), F_1F_0-ATPase, or pyruvate dehydrogenase complex can also produce the same clinical picture. It is clear that the condition is genetically heterogeneous and can arise from a variety of mutations either in nuclear genes that code for proteins of the mitochondrial matrix or inner membrane, or in mitochondrial genes. Leigh disease may occur without a family history of a similar disease or be transmitted as an autosomal recessive defect when the mutation is in a nuclear gene or by maternal inheritance when the mutation is in a mitochondrial gene.

Shoffner, J. M., and Wallace, D. C. In: C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle (Eds.), *The Metabolic and Molecular Bases of Inherited Disease*, 7th ed. New York: McGraw-Hill, 1995, p. 1535.

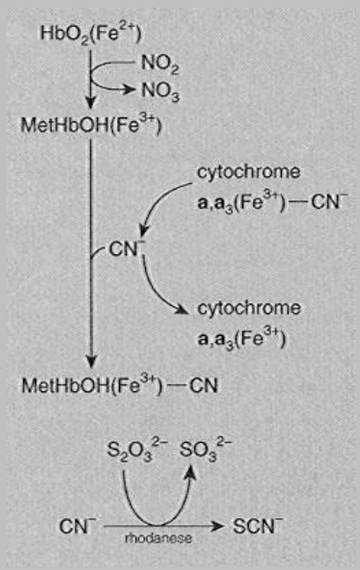
Electron Transport Can Be Inhibited at Specific Sites

The illustration of the mitochondrial respiratory chain shown in Figure 6.40 indicates that a number of compounds specifically inhibit electron transfer at different points. The fish poison **rotenone** and the barbiturate **amytal** (Figure 6.45) inhibit at the level of the flavoprotein, NADH dehydrogenase. Hence electrons or reducing equivalents derived from NAD^+-linked dehydrogenases are not oxidized by the respiratory chain in the presence of rotenone, whereas those derived from flavin-linked dehydrogenases are freely oxidized. The antibiotic **antimycin A** (Figure 6.45) inhibits electron transfer at the level of cytochrome *b*, whereas the terminal step in the respiratory chain catalyzed by cytochrome oxidase is inhibited by cyanide, azide, or carbon monoxide (see Clin. Corr. 6.5). Cyanide and azide combine with the oxidized heme iron (Fe^{3+}) in cytochromes *a* and a_3 and prevent the reduction of heme iron by electrons derived from reduced cytochrome *c*. Carbon monoxide binds to the reduced iron (Fe^{2+}) of cytochrome oxidase. Hence inhibition of mitochondrial electron transport results in an impairment of normal energy-generating function and death of the organism.

CLINICAL CORRELATION 6.5

Cyanide Poisoning

Inhalation of hydrogen cyanide gas or ingestion of potassium cyanide causes a rapid and extensive inhibition of the mitochondrial electron transport chain at the cytochrome oxidase step. Cyanide is one of the most potent and rapidly acting poisons known. Cyanide binds to the Fe^{3+} in the heme of the cytochrome a,a_3 component of the terminal step in the electron transport chain and prevents oxygen from reacting with cytochrome a,a_3 and serving as the final electron acceptor. Mitochondrial respiration and energy production cease, and cell death occurs rapidly. Death due to cyanide poisoning occurs from tissue asphyxia, most notably of the central nervous system. If cyanide poisoning is diagnosed very rapidly, a patient who has been exposed to cyanide is given various nitrites that convert oxyhemoglobin to methemoglobin, which merely involves converting the Fe^{2+} of hemoglobin to Fe^{3+} in methemoglobin. Methemoglobin (Fe^{3+}) competes with cytochrome a,a_3 (Fe^{3+}) for cyanide, forming a methemoglobin–cyanide complex. Administration of thiosulfate causes the cyanide to react with the enzyme rhodanese, forming the nontoxic compound thiocyanate.



Holland, M. A., and Kozlowski, L. M. Clinical features and management of cyanide poisoning. *Clin. Pharmacol*. 5:737, 1986.

Electron Transport Is Reversible

The various events in the mitochondrial electron transport system and the closely coupled reactions of oxidative phosphorylation are reversible, provided an appropriate amount of energy is supplied to drive the system. In mitochondrial systems, reducing equivalents derived from succinate can be transferred to NADH with the concomitant hydrolysis of ATP (Figure 6.46). Electron transport across the other two phosphorylation sites can be reversed in a similar fashion.

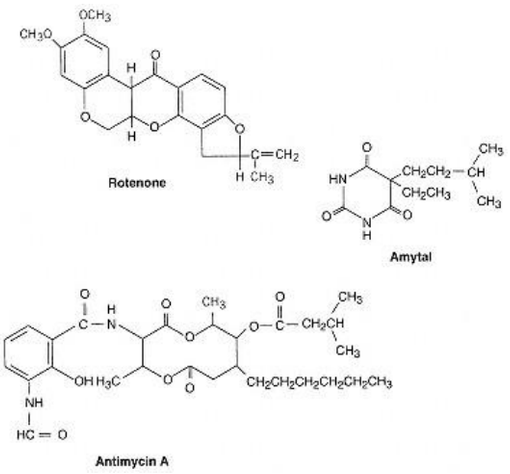


Figure 6.45
Structures of respiratory chain inhibitors.

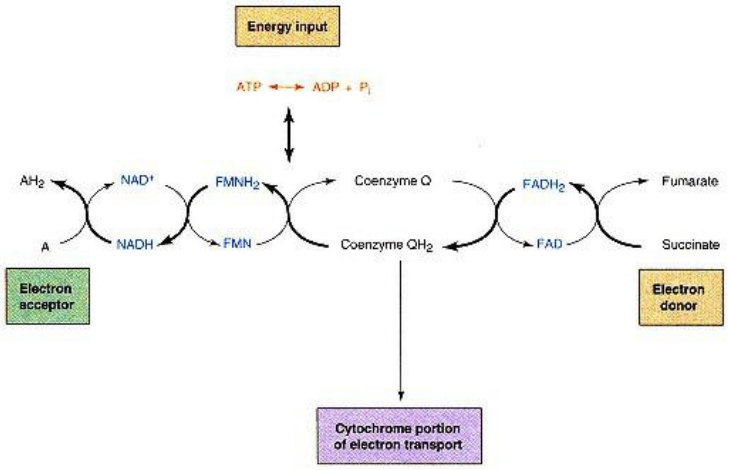


Figure 6.46
Reversal of mitochondrial electron transfer.

Oxidative Phosphorylation Is Coupled to Electron Transport

The obligatory coupling between the electron-transferring reactions and oxidative phosphorylation can best be illustrated in the experiment shown in Figure 6.47. Mitochondrial electron transport monitored by measuring the rate of oxygen consumption by a suspension of liver mitochondria can occur at a rapid rate only following the addition of an oxidizable substrate (the electron donor) and ADP (a phosphate acceptor) plus P_i. The "active" state in the presence of substrate and ADP has been designated State 3 and is a situation in which there occurs rapid electron transfer, oxygen consumption, and rapid synthesis of ATP. Following conversion of all the added ADP to ATP, the rate of electron transfer subsides back to the rate observed prior to ADP addition. Hence respiration is tightly coupled to ATP synthesis and this relationship has been termed respiratory control or phosphate acceptor control. The ratio of the active **(State 3)** rate to the resting **(State 4)** rate of respiration is referred to as the respiratory control ratio and is a measure of the "tightness" of coupling between electron transfer and oxidative phosphorylation. Damaged mitochondrial preparations and preparations to which various uncoupling compounds (see below) have been added exhibit low respiratory control ratios, indicating that the integrity of the mitochondrial membrane is required for tight coupling.

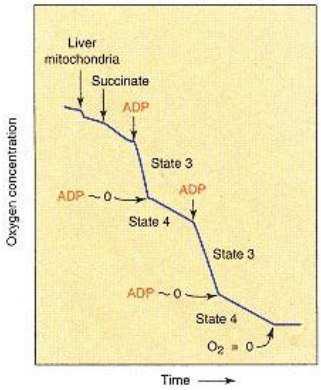
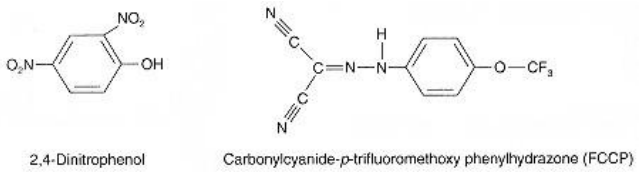


Figure 6.47
Demonstration of the coupling of electron transport to oxidative phosphorylation in a suspension of liver mitochondria.
State 3/state 4 = respiratory control ratio.

The effect of **uncouplers** and inhibitors of the electron transport–oxidative phosphorylation sequence is illustrated in Figure 6.48. Following the addition of ADP, which initiates a rapid State 3 rate of respiration, an inhibitor of the oxidative phosphorylation sequence (actually the mitochondrial F_1F_0-ATPase), **oligomycin,** is added. Oligomycin stops ATP synthesis, and because electron transport and ATP synthesis are tightly coupled, respiration or electron transport is inhibited nearly completely. Following inhibition of both oxygen consumption and ATP synthesis, addition of an uncoupler of these two processes such as **2,4-dinitrophenol** or **carbonylcyanide-*p*-trifluoromethoxy phenylhy-**

drazone (FCCP),



2,4-Dinitrophenol Carbonylcyanide-*p*-trifluoromethoxy phenylhydrazone (FCCP)

causes a rapid initiation of oxygen consumption. Because respiration or electron transport is now uncoupled from ATP synthesis, electron transport may continue but without ATP synthesis.

Note that regulation of the respiration rate of a tissue by provision of a phosphate acceptor, ADP, is a normal physiological situation. For example, when a muscle is exercised, ATP is broken down to ADP and P_i, and creatine phosphate is converted to creatine as the high-energy phosphate bond is transferred to ATP by creatine phosphokinase (see p. 957). As ADP accumulates during the muscular activity, respiration or oxygen consumption is activated, and the energy generated in this fashion allows the ATP and creatine phosphate levels to be replenished (see Clin. Corr. 6.6).

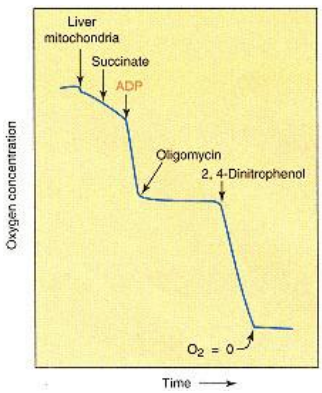


Figure 6.48
Inhibition and uncoupling of oxidative phosphorylation in liver mitochondria.

6.7— Oxidative Phosphorylation

One of the most vexing problems that confronted biochemists during the last four decades was the delineation of the mechanism of oxidative phosphorylation. After years of experimental consideration were expended to define the mechanism of mitochondrial energy conservation, consensus was reached on many of the details of the mechanism by which energy derived from the passage of electrons sequentially along the electron transport chain is transduced into the chemical energy involved in the phosphoanhydride bonds of ATP.

Several hypotheses for the mechanism of oxidative phosphorylation were tested including the **chemical-coupling hypothesis** developed in the early

CLINICAL CORRELATION 6.6

Hypoxic Injury

Acute hypoxic tissue injury has been studied in a variety of human tissues. The occlusion of a major coronary artery during myocardial infarction produces a large array of biochemical and physiological sequelae. When a tissue is deprived of its oxygen supply, the mitochondrial electron transport–oxidative phosphorylation sequence is inhibited, resulting in the decline of cellular levels of ATP and creatine phosphate. As cellular ATP levels diminish, anaerobic glycolysis is activated in an attempt to maintain normal cellular functions. Glycogen levels are rapidly depleted and lactic acid levels in the cytosol increase, reducing the intracellular pH. Hypoxic cells in such an energy deficit begin to swell as they can no longer maintain their normal intracellular ionic environments. Mitochondria swell and begin to accumulate calcium, which may be deposited in the matrix compartment as calcium phosphate. The cell membranes of swollen cells become more permeable, leading to the leakage of various soluble enzymes, coenzymes, and other cell constituents from the cell. As the intracellular pH falls, damage occurs to lysosomal membranes, which release various hydrolytic proteases, lipases, glucosidases, and phosphatases into the cell. Such lysosomal enzymes begin an autolytic digestion of cellular components.

Cells that have been exposed to short periods of hypoxia can recover, without irreversible damage, upon reperfusion with an oxygen-containing medium. The exact point at which hypoxic cell damage becomes irreversible is not precisely known. This process is of great practical importance for transplantation of organs (heart, kidney, and liver), which always undergo a period of hypoxia between the time they are removed from the donor and implanted into the recipient.

Kehrer, J. P. Concepts related to the study of reactive oxygen and cardiac reperfusion injury. *Free Radic Res. Commun.* 5:305, 1986; and Granger, D. N. Role of xanthine oxidase and granulocytes in ischemia—reperfusion injury. *Am. J. Physiol.* 255:H1269, 1988.

1950s. This mechanism was based on an analogy with the mechanism of **substrate-level phosphorylation** in the glyceraldehyde-3-phosphate dehydrogenase reaction (see p. 276) of glycolysis. In this reaction glyceraldehyde 3-phosphate is oxidized and a high-energy phosphoric–carboxylic acid anhydride bond is generated in the product of the reaction, 1,3-bisphosphoglycerate. An enzyme-bound high-energy intermediate is generated in this reaction, which is utilized to form the intermediate high-energy compound 1,3-bisphosphoglycerate and ultimately to form ATP in the next reaction in the glycolytic pathway, that of phosphoglycerate kinase (see p. 276). Another example of a substrate-level phosphorylation reaction, which was defined in the 1960s, is the succinyl-CoA synthetase reaction of the TCA cycle. Here the high-energy character of succinyl CoA is converted to the phosphoric acid anhydride bond in GTP with the intermediate participation of a high-energy, phosphorylated histidine moiety on the enzyme. Because of these types of substrate-level phosphorylation reactions, it was proposed that the mechanism of mitochondrial energy transduction involved a series of high-energy intermediates generated in the mitochondrial membrane as a consequence of electron transport. No high-energy intermediates have ever been defined or isolated.

A second proposal for the mechanism of oxidative phosphorylation was the **conformational-coupling hypothesis.** This hypothesis has an analogy in the process of muscle contraction in which ATP hydrolysis is used to drive conformational changes in myosin head groups, which result in the disruption of cross-bridges to actin thin filaments. The conformational-coupling hypothesis proposed that as a consequence of electron transport in the inner mitochondrial membrane a conformational change in a membrane protein occurred. ATP could be synthesized by a mechanism that allowed the membrane protein in its high-energy conformation to revert to its low-energy or random state, with the resultant formation of ATP from ADP and P_i. Hence the high-energy state of the membrane protein is transduced into the bond energy of the γ-phosphate group of ATP. There are various experimental observations indicating that mitochondrial membrane proteins undergo conformational changes during the process of active electron transport. However, there is relatively little evidence demonstrating conclusively that such conformational changes are actually involved in the mechanism of ATP synthesis.

The Chemiosmotic-Coupling Mechanism Involves the Generation of a Proton Gradient and Reversal of an ATP-Dependent Proton Pump

The **chemiosmotic-coupling mechanism** proposed by Peter Mitchell is the mechanism for energy transduction in mitochondria, as well as other biological systems. Mitchell's original proposition compared the energy-generating systems in biological membranes to a common storage battery. Just as energy can be stored in batteries because of the separation of positive and negative charges in the different components of the battery, energy may be generated as a consequence of the separation of charges in complex membrane systems. In the chemiosmotic mechanism (Figure 6.49) an electrochemical gradient (protons) is established across the inner mitochondrial membrane during electron transport. This proton gradient is formed by pumping protons from the mitochondrial matrix side of the inner membrane to the cytosolic side of the membrane. Once a substantial electrochemical gradient is established, the subsequent dissipation of the gradient is coupled to the synthesis of ATP by the **mitochondrial F_1F_0-ATPase.** The electron transport carriers and the F_1F_0-ATPase are localized in such a fashion in the inner mitochondrial membrane that protons are pumped out of the matrix compartment during the electron transport phase of the process and allowed back through the membrane during the ATP synthetase aspect of the process.

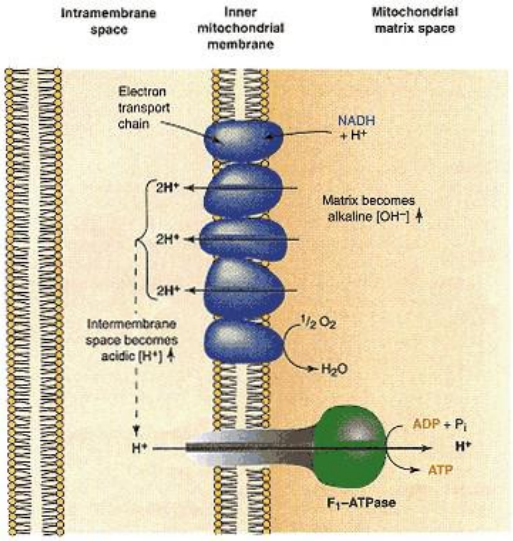


Figure 6.49
The mechanism of chemiosmotic coupling of electron transport and oxidative phosphorylation.

Uncouplers of the processes of respiration and phosphorylation are relatively lipophilic weak acids and act to dissipate the proton gradient by transporting protons through the membrane from the intermembrane space to the matrix. This short-circuits the normal flow of protons through the F_1F_0-ATPase. F_1F_0-ATPase can be purified and when incorporated into artificial membrane vesicles is able to synthesize ATP when an electrochemical gradient is established across the membrane. Proton-translocating ATPases are present and can be purified from a variety of mammalian tissues, bacteria, and yeast. The ATPase is a multicomponent complex with a suggested molecular weight of 480,000–500,000 (Figure 6.50). These ATPases can be incorporated into artificial membranes and can catalyze ATP synthesis. The F_1F_0-ATPase complex consists of a water-soluble portion called F_1 and a hydrophobic portion called F_0. The F_1 consists of five nonidentical subunits (α, β, γ, δ, and ε) with a subunit stoichiometry of $\alpha_3\beta_3\gamma\delta\varepsilon$ and a molecular weight of 350,000–380,000. Nucleotide-binding sites of the enzyme have been localized on the α and β subunits. The γ subunit has been proposed to function as a gate to the proton-translocating activity of the complex, while the δ subunit has been suggested to be necessary for the attachment of the F_1 portion to the membrane. The ε subunit has been proposed to regulate the F_1-ATPase. The F_0 portion consists of three or four nonidentical subunits that are an integral part of the membrane from which the ATPase is derived. When purified F_0 is incorporated into an artificial membrane, it renders the membrane permeable to protons. In addition, the F_0 contains a subunit called the oligomycin-sensitivity-conferring protein, which, as the name implies, causes the ATPase complex to be sensitive to the inhibitory action of oligomycin.

A number of questions relating to the details of the mechanism by which this important biochemical process occurs have not been resolved. Such questions relate to the mechanism by which protons are pumped out of the mitochondrial matrix during electron transport, the stoichiometry of protons translocated per ATP synthesized, and the mechanism by which protons are pumped back into the matrix through the F_1F_0-ATPase.

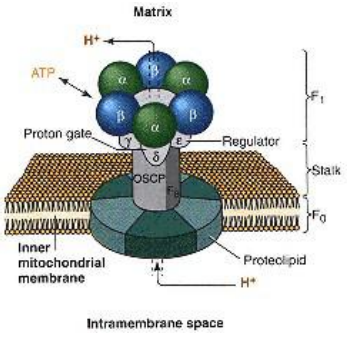


Figure 6.50
A model for the mitochondrial F_1F_0-ATPase.

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Questions

J. Baggott and C. N. Angstadt

1. At 37°C, $-2.303RT = -1.42$ kcal mol^{-1}. For the reaction A B, if $G^\circ = -7.1$ kcal mol^{-1}, what is the equilibrium ratio of B/A?

   A. 10,000,000/1

   B. 100,000/1

   C. 1000/1

   D. 1/1000

   E. 1/100,000

2. A bond may be "high energy" for any of the following reasons EXCEPT:

   A. products of its cleavage are more resonance stabilized than the original compound.

   B. the bond is unusually stable, requiring a large energy input to cleave it.

   C. electrostatic repulsion is relieved when the bond is cleaved.

   D. a cleavage product may be unstable, tautomerizing to a more stable form.

   E. the bond may be strained.

3. The active form of pyruvate dehydrogenase is favored by the influence of all of the following on pyruvate dehydrogenase kinase EXCEPT:

   A. low [Ca^{2+}].

   B. low acetyl CoA/CoASH.

   C. high [pyruvate].

   D. low NADH/NAD^+.

4. At which of the following enzyme-catalyzed steps of the tricarboxylic acid cycle does net incorporation of the elements of water into an intermediate of the cycle occur?

A. aconitase

B. citrate synthase

C. malate dehydrogenase

D. succinate dehydrogenase

E. succinyl-CoA synthase

5. A freely reversible reaction of the tricarboxylic acid cycle is catalyzed by:

A. citrate synthase.

B. isocitrate dehydrogenase.

C. α-ketoglutarate dehydrogenase.

D. pyruvate dehydrogenase.

E. succinyl-CoA synthetase.

6. All of the following tricarboxylic acid cycle intermediates may be added or removed by other metabolic pathways EXCEPT:

A. citrate.

B. fumarate.

C. isocitrate.

D. α-ketoglutarate.

E. oxaloacetate.

7. Regulation of tricarboxylic acid cycle activity *in vivo* may involve the concentration of all of the following EXCEPT:

A. acetyl CoA.

B. ADP.

C. ATP.

D. CoA.

E. oxygen.

8. The mitochondrial membrane contains a transporter for:

A. NADH.

B. acetyl CoA.

C. GTP.

D. ATP.

E. NADPH.

9. Which line of the accompanying table correctly describes the indicated properties of BOTH the malate shuttle and the α-glycerophosphate shuttle?

Table for Question 9

Property	***Malate Shuttle***	***α-Glycerophosphate Shuttle***
A. Location	Inner mitochondrial membrane	Outer mitochondrial membrane
B. ATP generated per cytoplasmic NADH	3	2
C. Transporter	Malate dehydrogenase	α-Glycerophosphate dehydrogenase
D. Species transported	Malate	αGlycerophosphate
E. Matrix electron acceptor	Oxaloacetate	Cytochrome *b*

Refer to the following for Questions 10–12.

A. FAD

B. FMN

C. NAD^+

D. NADPH

E. none of the above

10. Prosthetic group of NADH dehydrogenase.

11. The usual source of reducing equivalents for anabolic processes.

12. Irradiation with light of 340-nm wavelength causes fluorescence emission at 465 nm.

13. If rotenone is added to the mitochondrial electron transport chain:

A. the P/O ratio of NADH is reduced from 3:1 to 2:1.

B. the rate of NADH oxidation is diminished to two-thirds of its initial value.

C. succinate oxidation remains normal.

D. oxidative phosphorylation is uncoupled at site I.

E. electron flow is inhibited at site II.

14. If cyanide is added to tightly coupled mitochondria that are actively oxidizing succinate:

A. subsequent addition of 2,4-dinitrophenol will cause ATP hydrolysis.

B. subsequent addition of 2,4-dinitrophenol will restore succinate oxidation.

C. electron flow will cease, but ATP synthesis will continue.

D. electron flow will cease, but ATP synthesis can be restored by subsequent addition of 2,4-dinitrophenol.

E. subsequent addition of 2,4-dinitrophenol and the phosphorylation inhibitor oligomycin will cause ATP hydrolysis.

15. The heme iron of which of the following is bound to the protein by only one coordination linkage?

A. cytochrome *a*

B. cytochrome a_3

C. cytochrome *b*

D. cytochrome *c*

E. none of the above

16. In substrate level phosphorylation:

A. the substrate reacts to form a product containing a high-energy bond.

B. ATP synthesis is linked to dissipation of a proton gradient.

C. high-energy intermediate compounds cannot be isolated.

D. oxidation of one molecule of substrate is linked to synthesis of more than one ATP molecule.

E. mitochondria participate, but not cytoplasm.

17. The chemiosmotic hypothesis involves all of the following EXCEPT:

A. a membrane impermeable to protons.

B. electron transport by the respiratory chain pumps protons out of the mitochondrion.

C. proton flow into the mitochondria depends on the presence of ADP and P_i.

D. ATPase activity is reversible.

E. only proton transport is strictly regulated; other positively charged ions can diffuse freely across the mitochondrial membrane.

Answers

1. B $G^{\circ} = -2.3RT \log K$. $\log 100{,}000 = 5$. Substitution gives $G^{\circ} = -7.1$ (p. 221).

2. B A "high-energy" bond is so designated because it has a high free energy of hydrolysis. This could arise for reasons A, C, D, or E. High-energy does not refer to a high energy of formation (bond stability) (p. 222).

3. A High $[Ca^{2+}]$ favors the active dehydrogenase but by activating the phosphatase. NADH and acetyl CoA activate pyruvate dehydrogenase kinase, thus inactivating pyruvate dehydrogenase. Pyruvate inhibits the kinase, favoring the active dehydrogenase (p. 230, Figure 6.16).

4. B Water is required to hydrolyze the thioester bond of acetyl CoA. A: Aconitase removes water, then adds it back. C and D: The dehydrogenases remove two protons and two electrons E. Here the thioester undergoes phosphorolysis, not hydrolysis, the phosphate is subsequently transferred from the intermediate succinyl phosphate to GDP. (See p. 232, Figure 6.19).

5. E There are high-energy compounds on both sides of the reaction, namely, GTP and succinyl CoA (p.235). A is irreversible due to cleavage of the thioester link, a high-energy bond. In B and C, CO_2 is released. D: Pyruvate dehydrogenase is not a part of the tricarboxylic acid cycle; it is, however, irreversible.

6. C A: Citrate is transported out of the mitochondria to be used as a source of cytoplasmic acetyl CoA. B: Fumarate is produced during phenylalanine and tyrosine degradation. D can be formed from glutamate. E: Oxaloacetate is produced by pyruvate carboxylase and is used in gluconeogenesis. Clearly, most of the tricarboxylic acid cycle intermediates play multiple roles in the body.

7. D CoA is not a regulator, though admittedly there is a reciprocal relationship between CoA and acetyl CoA concentrations in the short term. A is the substrate (p. 232). B activates isocitrate dehydrogenase, and C inhibits it (p. 239, Figure 6.23). E: The cycle requires oxygen to oxidize NADH and ADP to be converted to ATP (respiratory control) (p. 231).

8. D A and B: Reducing equivalents from NADH are shuttled across the membrane, as is the acetyl group of acetyl CoA, but NADH and acetyl CoA themselves cannot cross (p. 243, Figures 6.28 and 6.29). C and D: Of the nucleotides, only ATP and ADP are transported. The translocator is inhibited by atractyloside (p. 245). E: Like NADH, NADPH does not cross the membrane.

9. B A: Both shuttles operate across the inner membrane. C: Two transporters are used by the malate shuttle, the malate α-ketoglutarate antiporter and the aspartate–glutamate antiporter. D: α-Glycerophosphate is not translocated; only reducing equivalents are. E: Oxaloacetate is a reaction product. NAD^+ is the electron acceptor (p. 244, Figure 6.28).

10. B See Figure 6.40, p. 256.

11. D NADPH is not a substrate for mitochondrial electron transport (p. 250).

12. D Fluorescence excitation of the reduced pyridine ring occurs in a wavelength range where it absorbs light, about 340 nm. Absorbance is a minimum at 300 nm (p. 250, Figure 6.33).

13. C Rotenone inhibits at the level of NADH dehydrogenase (site I), preventing all electron flow and all ATP synthesis from NADH. Flavin-linked dehydrogenases feed in electrons below site I and are unaffected by site I inhibitors (pp. 259, 256, Figure 6.40).

14. A Cyanide inhibits electron transport at site III, blocking electron flow throughout the system. In coupled mitochondria, ATP synthesis ceases too. Addition of an uncoupler permits the mitochondrial ATPase (which is normally driven in the synthetic direction) to operate, and it catalyzes the favorable ATP hydrolysis reaction unless it is inhibited by a phosphorylation inhibitor such as oligomycin (p. 260).

15. B Fe^{2+} has six coordination positions. In heme, four are filled by the porphyrin ring. In cytochromes *a, b*, and *c* the other two are filled by the protein. But in cytochrome a_3, one position must be left vacant to provide an oxygen-binding site (p. 252).

16. A A good example of substrate-level phosphorylation is seen in the glyceraldehyde-3-phosphate dehydrogenase reaction, where a phosphoric–carboxylic acid anhydride intermediate forms (p. 262). This is a cytoplasmic process, part of glycolysis. The intermediate can be isolated.

17. E If the charge separation could be dissipated by free diffusion of other ions, the energy would be lost, and no ATP could be synthesized.