Лекция 3.
Биологические мембраны. Обмен веществом

CHAPTER

Lipids

Lipids play an important role in cell structure and function. In this electron micrograph of the cytoplasm of the photosynthetic alga *Euglena,* the lipid-containing membranes of a chloroplast (upper right) and several mitochondria (surrounding the chloroplast and lower left) are visible. Two lipid droplets, stores of chemical energy, can be seen in the chloroplast. The gray oval structure at the lower right is a lipid-filled inclusion in the cytoplasm.

Biological lipids are a chemically diverse group of compounds, the common and defining feature of which is their insolubility in water. The biological functions of the lipids are equally diverse. Fats and oils are the principal stored forms of energy in many organisms, and phospholipids and sterols make up about half the mass of biological membranes. Other lipids, although present in relatively small quantities, play crucial roles as enzyme cofactors, electron carriers, light-absorbing pigments, hydrophobic anchors, emulsifying agents, hormones, and intracellular messengers. This chapter introduces representative lipids of each type, with emphasis on their chemical structure and physical properties.

Storage Lipids

The fats and oils used almost universally as stored forms of energy in living organisms are highly reduced compounds, derivatives of **fatty acids.** The fatty acids are hydrocarbon derivatives, at about the same low oxidation state (that is, as highly reduced) as the hydrocarbons in fossil fuels. The complete oxidation of fatty acids (to CO_2 and H_2O) in cells, like the explosive oxidation of fossil fuels in internal combustion engines, is highly exergonic.

We will introduce here the structure and nomenclature of the fatty acids most commonly found in living organisms. Two types of fatty acid-containing compounds, triacylglycerols and waxes, are described to illustrate the diversity of structure and physical properties in this family of compounds.

Fatty Acids Are Hydrocarbon Derivatives

Fatty acids are carboxylic acids with hydrocarbon chains of 4 to 36 carbons. In some fatty acids, this chain is fully saturated (contains no double bonds) and unbranched; others contain one or more double bonds (Table 9–1). A few contain three-carbon rings or hydroxyl groups. A simplified nomenclature for these compounds specifies the chain length and number of double bonds, separated by a colon; the 16-carbon saturated palmitic acid is abbreviated 16:0, and the 18-carbon oleic acid, with one double bond, is 18:1. The positions of any double bonds are specified by superscript numbers following Δ (delta); a 20-carbon fatty acid with one double bond between C-9 and C-10 (C-1

Table 9–1 Some naturally occurring fatty acids

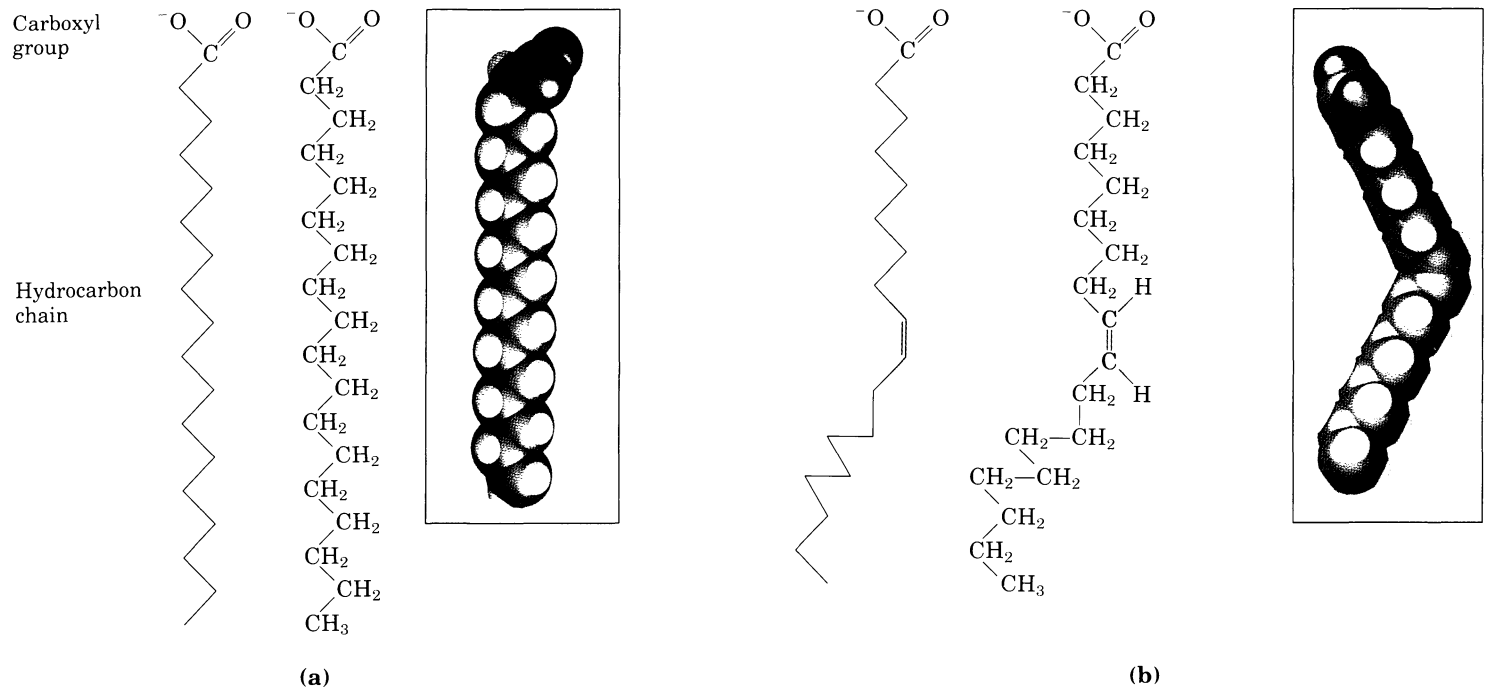
Carbon skeleton	Structure*	Systematic name†	Common name (derivation)	Melting point (°C)	Solubility at 30 °C (mg/g solvent)	
					Water	Benzene
12:0	$CH_3(CH_2)_{10}COOH$	*n*-Dodecanoic acid	Lauric acid (Latin *laurus*, laurel plant)	44.2	0.063	2,600
14:0	$CH_3(CH_2)_{12}COOH$	*n*-Tetradecanoic acid	Myristic acid (Latin *Myristica*, nutmeg genus)	53.9	0.024	874
16:0	$CH_3(CH_2)_{14}COOH$	*n*-Hexadecanoic acid	Palmitic acid (Greek *palma*, palm tree)	63.1	0.0083	348
18:0	$CH_3(CH_2)_{16}COOH$	*n*-Octadecanoic acid	Stearic acid (Greek *stear*, hard fat)	69.6	0.0034	124
20:0	$CH_3(CH_2)_{18}COOH$	*n*-Eicosanoic acid	Arachidic acid (Latin *Arachis*, legume genus)	76.5		
24:0	$CH_3(CH_2)_{22}COOH$	*n*-Tetracosanoic acid	Lignoceric acid (Latin *lignum*, wood + *cera*, wax)	86.0		
16:1(Δ^9)	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$		Palmitoleic acid	−0.5		
18:1(Δ^9)	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$		Oleic acid (Greek *oleum*, oil)	13.4		
18:2($\Delta^{9,12}$)	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$		α-Linoleic acid (Greek *linon*, flax)	−5		
18:3($\Delta^{9,12,15}$)	$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$		Linolenic acid	−11		
20:4($\Delta^{5,8,11,14}$)	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_3COOH$		Arachidonic acid	−49.5		

* All acids are shown in their un-ionized form. At pH 7, all free fatty acids have an ionized carboxylate. Note that numbering of carbon atoms begins at the carboxyl group carbon.

† The prefix *n*- indicates the "normal" unbranched structure. For instance, "dodecanoic" simply indicates 12 carbon atoms, which could be arranged in a variety of branched forms. Thus "*n*-dodecanoic" specifies the linear, unbranched form.

being the carboxyl carbon), and another between C-12 and C-13, is designated 20:2($\Delta^{9,12}$), for example. The most commonly occurring fatty acids have even numbers of carbon atoms in an unbranched chain of 12 to 24 carbons (Table 9–1). As we shall see in Chapter 20, the even number of carbons results from the mode of synthesis of these compounds, which involves condensation of acetate (two-carbon) units.

The position of double bonds is also regular; in most monounsaturated fatty acids the double bond is between C-9 and C-10 (Δ^9), and the other double bonds of polyunsaturated fatty acids are generally Δ^{12} and Δ^{15} (Table 9–1). The double bonds of polyunsaturated fatty acids are almost never conjugated (alternating single and double bonds, as in —CH=CH—CH=CH—), but are separated by a methylene group (—CH=CH—CH_2—CH=CH—). The double bonds of almost all naturally occurring unsaturated fatty acids are in the cis configuration.



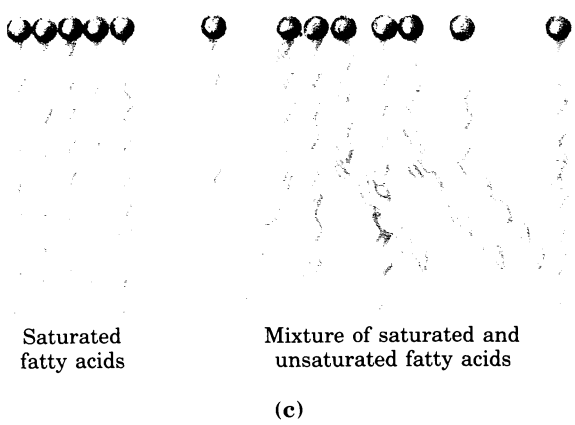
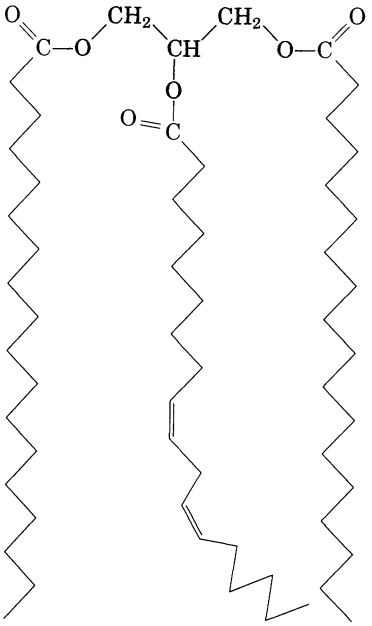


Figure 9–1 The packing of fatty acids depends on their degree of saturation. **(a)** Stearic acid (stearate at pH 7) is shown in its usual extended conformation. **(b)** The cis double bond (shaded) in oleic acid (oleate) does not permit rotation and introduces a rigid bend in the hydrocarbon tail. All the other bonds are free to rotate. **(c)** Fully saturated fatty acids in the extended form pack into nearly crystalline arrays, stabilized by many hydrophobic interactions. The presence of one or more cis double bonds interferes with this tight packing, and results in less stable aggregates.

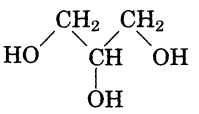
The physical properties of the fatty acids, and of compounds that contain them, are largely determined by the length and degree of unsaturation of the hydrocarbon chain. The nonpolar hydrocarbon chain accounts for the poor solubility of fatty acids in water. Lauric acid (12:0, M_r 200), for example, has a solubility of 0.063 mg/g of water—much less than that of glucose (M_r 180), which is 1,100 mg/g of water. The longer the fatty acyl chain and the fewer the double bonds, the lower the solubility in water (Table 9–1). The carboxylic acid group is polar (and ionized at neutral pH), and accounts for the slight solubility of short-chain fatty acids in water.

The melting points of fatty acids and of compounds that contain them are also strongly influenced by the length and degree of unsaturation of the hydrocarbon chain (Table 9–1). At room temperature (25 °C), the saturated fatty acids from 12:0 to 24:0 have a waxy consistency, whereas unsaturated fatty acids of these lengths are oily liquids. In the fully saturated compounds, free rotation around each of the carbon–carbon bonds gives the hydrocarbon chain great flexibility; the most stable conformation is this fully extended form (Fig. 9–1a), in which the steric hindrance of neighboring atoms is minimized. These molecules can pack together tightly in nearly crystalline arrays, with atoms all along their lengths in van der Waals contact with the atoms of neighboring molecules (Fig. 9–1c). A cis double bond forces a kink in the hydrocarbon chain (Fig. 9–1b). Fatty acids with one or several such kinks cannot pack together as tightly as fully saturated fatty acids (Fig. 9–1c), and their interactions with each other are therefore weaker. Because it takes less thermal energy to disorder these poorly ordered arrays of unsaturated fatty acids, they have lower melting points than saturated fatty acids of the same chain length (Table 9–1).

In vertebrate animals, free fatty acids (having a free carboxylate group) circulate in the blood bound to a protein carrier, serum albumin. However, fatty acids are present mostly as carboxylic acid deriva-



Triacylglycerol (general structure)



Glycerol

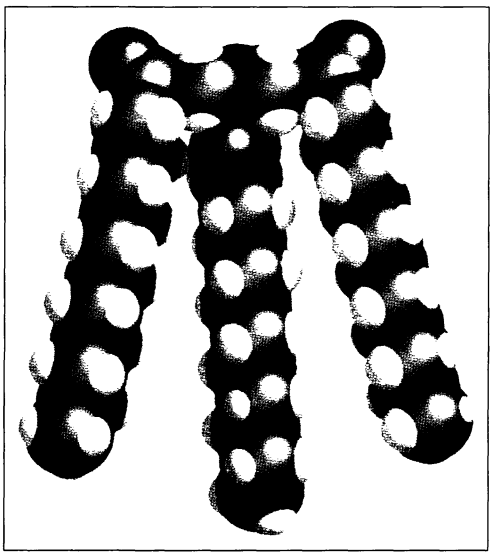


Figure 9–2 Glycerol and triacylglycerols. The triacylglycerol shown here has identical fatty acids (palmitate, 18:0) in positions 1 and 3. When there are two different fatty acids in positions 1 and 3 of the glycerol, C-2 (in red) of glycerol (shaded) becomes a chiral center (see Fig. 3–9). Biological triacylglycerols have the L configuration.

tives such as esters or amides. Lacking the charged carboxylate group, these fatty acid derivatives are generally even less soluble in water than are the free carboxylic acids.

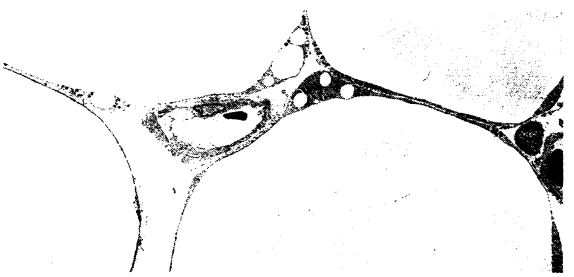
Triacylglycerols Are Fatty Acid Esters of Glycerol

The simplest lipids constructed from fatty acids are the **triacylglycerols,** also referred to as triglycerides, fats, or neutral fats. Triacylglycerols are composed of three fatty acids each in ester linkage with a single glycerol (Fig. 9–2). Those containing the same kind of fatty acid in all three positions are called simple triacylglycerols, and are named after the fatty acid they contain. Simple triacylglycerols of 16:0, 18:0, and 18:1, for example, are tristearin, tripalmitin, and triolein, respectively. Mixed triacylglycerols contain two or more different fatty acids; to name these compounds unambiguously, the name and position of each fatty acid must be specified.

Because the polar hydroxyls of glycerol and the polar carboxylates of the fatty acids are bound in ester linkages, triacylglycerols are nonpolar, hydrophobic molecules, essentially insoluble in water. This explains why oil–water mixtures (oil-and-vinegar salad dressing, for example) have two phases. Because lipids have lower specific gravities than water, the oil floats on the aqueous phase.

Triacylglycerols Provide Stored Energy and Insulation

In most eukaryotic cells, triacylglycerols form a separate phase of microscopic, oily droplets in the aqueous cytosol, serving as depots of metabolic fuel. Specialized cells in vertebrate animals, called adipocytes, or fat cells, store large amounts of triacylglycerols as fat droplets, which nearly fill the cell (Fig. 9–3). Triacylglycerols are also stored in the seeds of many types of plants, providing energy and biosynthetic precursors when seed germination occurs.



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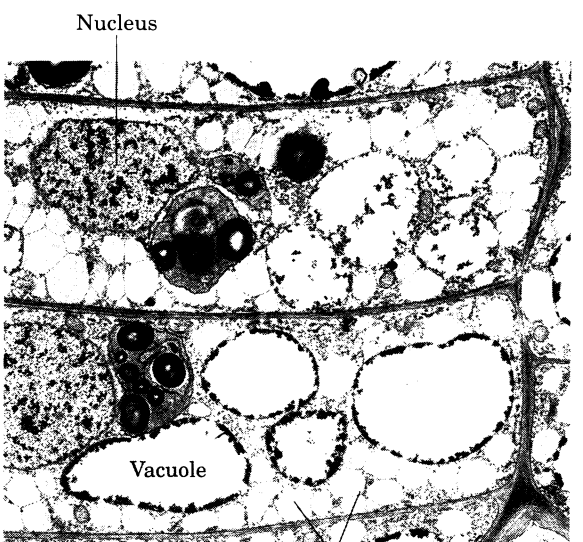


(b)

Figure 9–3 Fat stores in cells. **(a)** Cross-section of four guinea pig adipocytes, showing huge fat droplets that virtually fill the cells. Also visible are several capillaries in cross-section. **(b)** Two cambial cells from the underground stem of the plant *Isoetes muricata,* a quillwort. In winter, these cells store fats as lipid droplets.

BOX 9–1 Sperm Whales: Fatheads of the Deep

Studies of sperm whales have uncovered another way in which triacylglycerols are biologically useful. The sperm whale's head is very large, accounting for over one-third of its total body weight (Fig. 1). About 90% of the weight of the head is made up of the spermaceti organ, a blubbery mass that contains up to 3,600 kg (about 4 tons) of spermaceti oil, a mixture of triacylglycerols and waxes containing an abundance of unsaturated fatty acids. This mixture is liquid at the normal resting body temperature of the whale, about 37 °C, but it begins to crystallize at about 31 °C and becomes solid when the temperature drops several more degrees.

The probable biological function of spermaceti oil has been deduced from research on the anatomy and feeding behavior of the sperm whale. These mammals feed almost exclusively on squid in very deep water. In their feeding dives they descend 1,000 m or more; the record dive is 3,000 m (almost 2 miles). At these depths the sperm whale has no competitors for the very plentiful squid. The sperm whale rests quietly, waiting for schools of squid to pass. For a marine animal to remain at a given depth, without a constant swimming effort, it must have the same density as the surrounding water. The sperm whale can change its buoyancy to match the density of its surroundings—from the tropical ocean surface to great depths where the water is much colder and thus has a greater density.

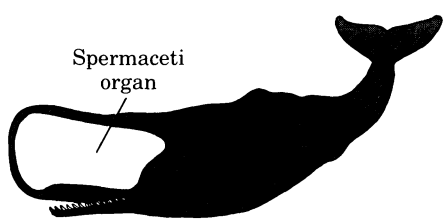


Figure 1 Silhouette of a sperm whale, showing the spermaceti organ, a huge enlargement of the snout that lies above the upper jaw.

The key to the sperm whale's ability to change its buoyancy is the freezing point of spermaceti oil. When the temperature of liquid spermaceti oil is lowered several degrees during a deep dive, it congeals or crystallizes and becomes more dense, thus changing the buoyancy of the whale to match the density of seawater. Various physiological mechanisms promote rapid cooling of the oil during a dive. During the return to the surface, the congealed spermaceti oil is warmed again and melted, decreasing its density to match that of the surface water. Thus we see in the sperm whale a remarkable anatomical and biochemical adaptation, perfected by evolution. The triacylglycerols synthesized by the sperm whale contain fatty acids of the necessary chain length and degree of unsaturation to give the spermaceti oil the proper melting point for the animal's diving habits.

Unfortunately for the sperm whale population, spermaceti oil is commercially valuable as a lubricant. Several centuries of intensive hunting of these mammals have depleted the world's population of sperm whales.

As stored fuels, triacylglycerols have two significant advantages over polysaccharides such as glycogen and starch. The carbon atoms of fatty acids are more reduced than those of sugars, and oxidation of triacylglycerols yields more than twice as much energy, gram for gram, as that of carbohydrates. Furthermore, because triacylglycerols are hydrophobic and therefore unhydrated, the organism that carries fat as fuel does not have to carry the extra weight of water of hydration that is associated with stored polysaccharides. In humans, fat tissue, which is composed primarily of adipocytes, occurs under the skin, in the abdominal cavity, and in the mammary glands. Obese people may have 15 or 20 kg of triacylglycerols deposited in their adipocytes, sufficient to supply energy needs for months. In contrast, the human body can store less than a day's energy supply in the form of glycogen. Carbohydrates such as glucose and glycogen do offer certain advantages as quick sources of metabolic energy, one of which is their ready solubility in water.

In some animals, triacylglycerols stored under the skin serve not only as energy stores but as insulation against very low temperatures. Seals, walruses, penguins, and other warm-blooded polar animals are

amply padded with triacylglycerols. In hibernating animals (bears, for example) the huge fat reserves accumulated before hibernation also serve as energy stores (see Box 16–1). The low density of triacylglycerols is the basis for another remarkable function of these compounds. In sperm whales, a store of triacylglycerols allows the animals to match the buoyancy of their bodies to that of their surroundings during deep dives in cold water (Box 9–1).

Many Foods Contain Triacylglycerols

Most natural fats, such as those in vegetable oils, dairy products, and animal fat, are complex mixtures of simple and mixed triacylglycerols. These contain a variety of fatty acids differing in chain length and degree of saturation (Table 9–2). Vegetable oils such as corn and olive oil are composed largely of triacylglycerols with unsaturated fatty acids, and thus are liquids at room temperature. They are converted industrially into solid fats by catalytic hydrogenation, which reduces some of their double bonds to single bonds. Triacylglycerols containing only saturated fatty acids, such as tristearin, the major component of beef fat, are white, greasy solids at room temperature.

Table 9–2 Fatty acid composition of three natural food fats*

	State at room temperature (25 °C)	Fatty acids (%)†				
		Saturated				Unsaturated
		C_4–C_{12}	C_{14}	C_{16}	C_{18}	C_{16} + C_{18}
Olive oil	Liquid	<2	<2	13	3	80
Butter	Solid (soft)	11	10	26	11	40
Beef fat	Solid (hard)	<2	<2	29	21	46

* These fats consist of mixtures of triacylglycerols, differing in their fatty acid composition and thus in their melting points.

† Values are given as percentage of total fatty acids.

When lipid-rich foods are exposed too long to the oxygen in air, they may spoil and become rancid. The unpleasant taste and smell associated with rancidity result from the oxidative cleavage of the double bonds in unsaturated fatty acids to produce aldehydes and carboxylic acids of shorter chain length and therefore higher volatility.

Hydrolysis of Triacylglycerols Produces Soaps

The ester linkages of triacylglycerols are susceptible to hydrolysis by either acid or alkali. Heating animal fats with NaOH or KOH produces glycerol and the Na^+ or K^+ salts of the fatty acids, known as soaps (Fig. 9–4). The usefulness of soaps is in their ability to solubilize or disperse water-insoluble materials by forming microscopic aggregates (micelles). When used in "hard" water (having high concentrations of Ca^{2+} and Mg^{2+}), soaps are converted into their insoluble calcium or magnesium salts, forming a residue. Synthetic detergents such as sodium dodecylsulfate (SDS; see p. 141) are less prone to precipitation in hard water, and have largely replaced natural soaps in many industrial applications.

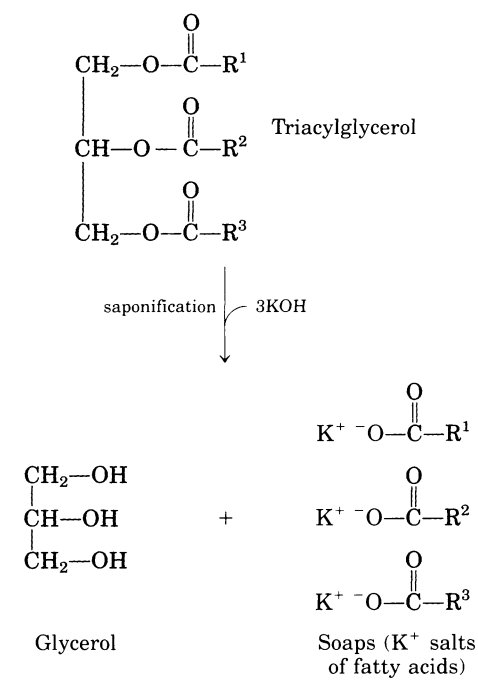


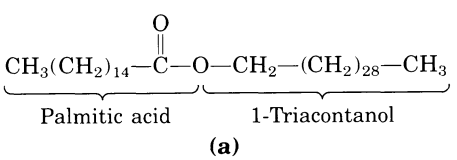
Figure 9–4 Triacylglycerol breakdown by alkaline hydrolysis: the process of saponification. R^1, R^2, R^3 represent long alkyl chains. Household soap is made by hydrolyzing a mixture of triacylglycerols (animal fat, for example) with KOH. The K^+ salts of the fatty acids are collected, washed free of KOH, and pressed into cakes.

At neutral pH, a variety of **lipases** catalyze the enzymatic hydrolysis of triacylglycerols. Lipases in the intestine aid in the digestion and absorption of dietary fats. Adipocytes and germinating seeds contain lipases that break down stored triacylglycerols, releasing fatty acids for export to other tissues where they are required as fuel.

Waxes Serve as Energy Stores and Water-Impermeable Coatings

Biological waxes are esters of long-chain saturated and unsaturated fatty acids (having 14 to 36 carbon atoms) with long-chain alcohols (having 16 to 30 carbon atoms) (Fig. 9–5). Their melting points (60 to 100 °C) are generally higher than those of triacylglycerols. In marine organisms that constitute the plankton, waxes are the chief storage form of metabolic fuel.

Waxes also serve a diversity of other functions in nature, related to their water-repellent properties and their firm consistency. Certain skin glands of vertebrates secrete waxes to protect the hair and skin and to keep them pliable, lubricated, and waterproof. Birds, particularly waterfowl, secrete waxes from their preen glands to make their feathers water-repellent. The shiny leaves of holly, rhododendrons, poison ivy, and many tropical plants are coated with a layer of waxes, which protects against parasites and prevents excessive evaporation of water.



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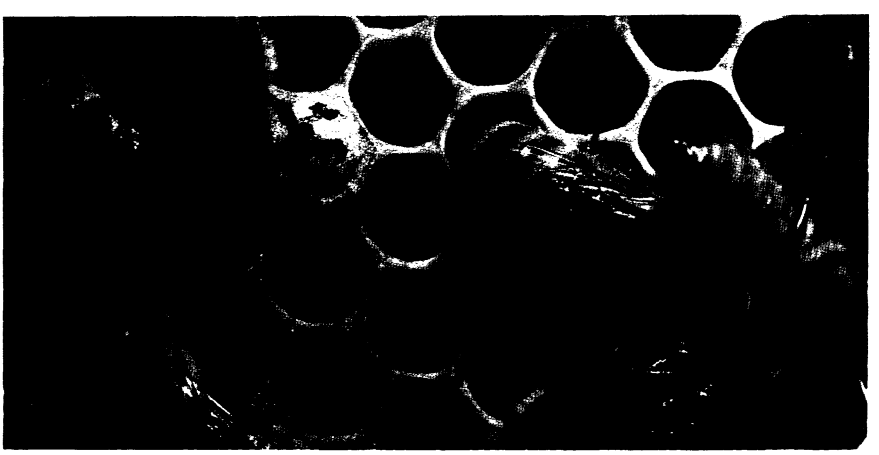


(b)

Figure 9–5 **(a)** Triacontanylpalmitate, the major component of beeswax. It is an ester of palmitic acid with the alcohol triacontanol. **(b)** A honeycomb, constructed of beeswax, is firm at 25 °C and completely impervious to water. The term "wax" originates in the Old English word *weax,* meaning "the material of the honeycomb."

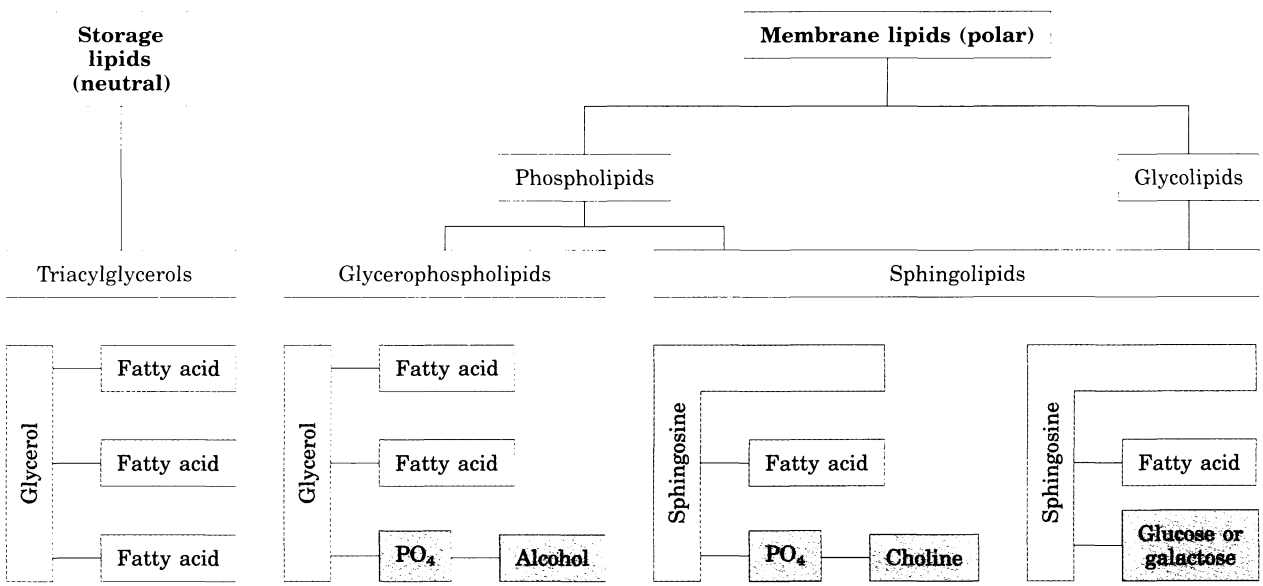
Biological waxes find a variety of applications in the pharmaceutical, cosmetic, and other industries. Lanolin (from lamb's wool), beeswax (Fig. 9–5), carnauba wax (from a Brazilian palm tree), and spermaceti oil (from whales) are widely used in the manufacture of lotions, ointments, and polishes.

Structural Lipids in Membranes

The central architectural feature of biological membranes is a double layer of lipids, which constitutes a barrier to the passage of polar molecules and ions. Membrane lipids are amphipathic; the orientation of their hydrophobic and hydrophilic regions directs their packing into

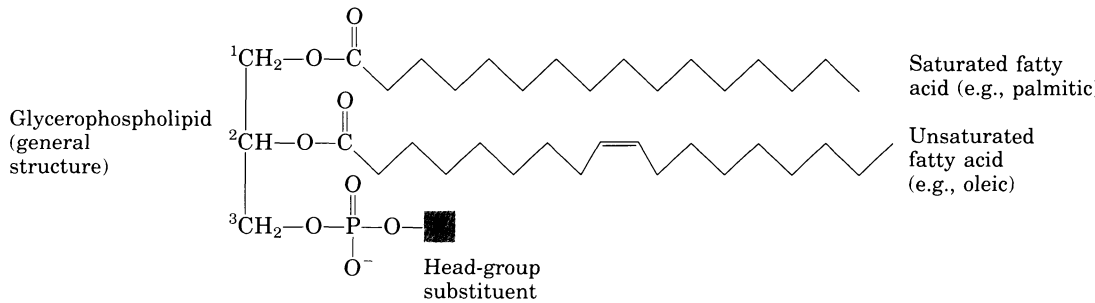
membrane bilayers. Three general types of membrane lipids will be described: glycerophospholipids, in which the hydrophobic regions are composed of two fatty acids joined to glycerol; sphingolipids, in which a single fatty acid is joined to a fatty amine, sphingosine; and sterols, compounds characterized by a rigid system of four fused hydrocarbon rings. The hydrophilic moieties in these amphipathic compounds may be as simple as a single —OH group at one end of the sterol ring system, or they may be more complex. Glycerophospholipids and sphingolipids contain polar or charged alcohols at their polar ends; some also contain phosphate groups (Fig. 9–6). Within these three classes of membrane lipids, enormous diversity results from various combinations of fatty acid "tails" and polar "heads." We describe here a representative sample of the types of membrane lipids found in living organisms. The arrangement of these lipids in membranes, and their structural and functional roles therein, are considered in the next chapter.

Figure 9–6 The principal classes of storage and membrane lipids. All of the classes shown here have either glycerol or sphingosine as the backbone. A third class of membrane lipids, the sterols, is described later (see Fig. 9–13).



Glycerophospholipids Are Derivatives of Phosphatidic Acid

Membranes contain several classes of lipids in which two fatty acids are ester-linked to glycerol at C-1 and C-2, and a highly polar or charged (and therefore hydrophilic) head group is attached to C-3 (Fig. 9–6). The most abundant of these polar lipids in most membranes are the **glycerophospholipids,** sometimes called phosphoglycerides (Fig. 9–7). In glycerophospholipids, a polar alcohol is joined to C-3 of glycerol through a phosphodiester bond. All glycerophospholipids are derivatives of phosphatidic acid (Fig. 9–7) and are named for their polar head groups (phosphatidylcholine and phosphatidylethanolamine, for example). All have a negative charge on the phosphate group at pH 7.0. The head-group alcohol may also contribute one or more charges at pH near 7.



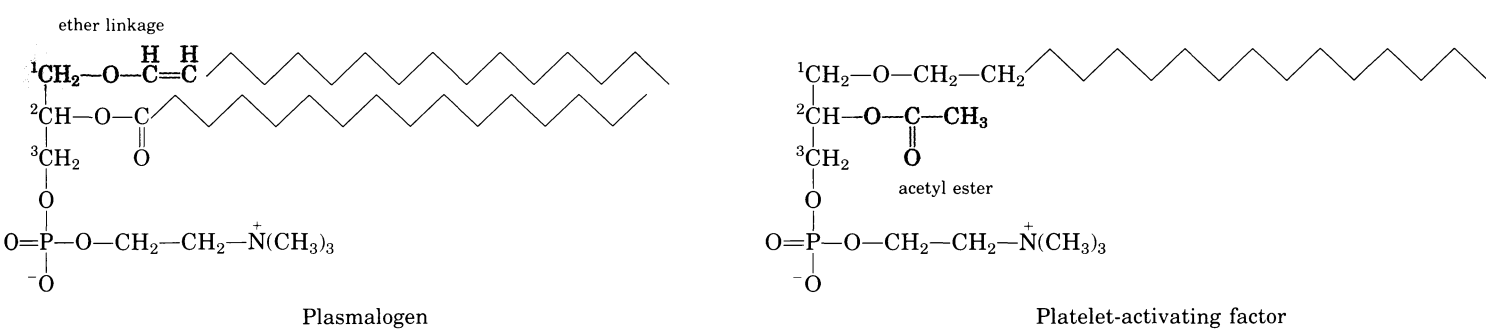
Name of X	Formula of X	Name of glycerophospholipid	Net charge (at pH 7)
—		Phosphatidic acid	−1
Ethanolamine		Phosphatidylethanolamine	0
Choline		Phosphatidylcholine	0
Serine		Phosphatidylserine	−1
Glycerol		Phosphatidylglycerol	−1
Inositol		Phosphatidylinositol	−1
Phosphatidyl glycerol		Cardiolipin	−2

Figure 9–7 The common glycerophospholipids are diacylglycerols linked to head-group alcohols through a phosphodiester bond. Phosphatidic acid is the parent compound, a phosphomonoester. Each derivative is named for the head-group alcohol (X), with the prefix "phosphatidyl." In cardiolipin, two phosphatidic acids share a single glycerol.

The fatty acids in glycerophospholipids can be any of a wide variety. They are different in different species, in different tissues of the same species, and in different types of glycerophospholipids in the same cell or tissue. In general, glycerophospholipids contain a saturated fatty acid at C-1 and an unsaturated fatty acid at C-2, and the fatty acyl groups are commonly 16 or 18 carbons long—but there are many exceptions.

Some Phospholipids Have Ether-Linked Fatty Acids

Some animal tissues and some unicellular organisms are rich in ether lipids, in which one of the two acyl chains is attached to glycerol in ether, rather than ester, linkage. The ether-linked chain may be saturated, as in the alkyl ether lipids, or may contain a double bond between C-1 and C-2, as in **plasmalogens** (Fig. 9–8). Vertebrate heart tissue is uniquely enriched in ether lipids; about half of the heart phospholipids are plasmalogens. The membranes of halophilic bacteria, of ciliated protists, and of certain invertebrates also contain high proportions of ether lipids. Their functional significance in these membranes is unknown; perhaps they confer resistance to phospholipases that cleave ester-linked fatty acids from membrane lipids. At least one ether lipid, **platelet-activating factor** (Fig. 9–8), is an important hormone. It is released from white blood cells called basophils and stimulates platelet aggregation and the release of serotonin from platelets. It exerts a variety of effects on liver, smooth muscle, heart, uterine, and lung tissues, and plays an important role in inflammation and the allergic response.



Plasmalogen

Platelet-activating factor

Figure 9–8 Plasmalogens and platelet-activating factor. Plasmalogens have one *ether*-linked alkenyl chain where most glycerophospholipids have an *ester*-linked fatty acid (compare Fig. 9–7). Platelet-activating factor has a long ether-linked alkyl chain at C-1 of glycerol, but C-2 is ester-linked to a very short fatty acid (acetic acid), which makes the compound much more water-soluble than most glycerophospholipids and plasmalogens. The head group alcohol is choline in plasmalogens and platelet-activating factor.

Sphingolipids Are Derivatives of Sphingosine

Sphingolipids, the second large class of membrane lipids, also have a polar head and two nonpolar tails, but unlike glycerophospholipids they contain no glycerol. Sphingolipids are composed of one molecule of the long-chain amino alcohol sphingosine (4-sphingenine) or one of its derivatives, one molecule of a long-chain fatty acid, a polar head alcohol, and sometimes phosphoric acid in diester linkage at the polar head group (Fig. 9–9).

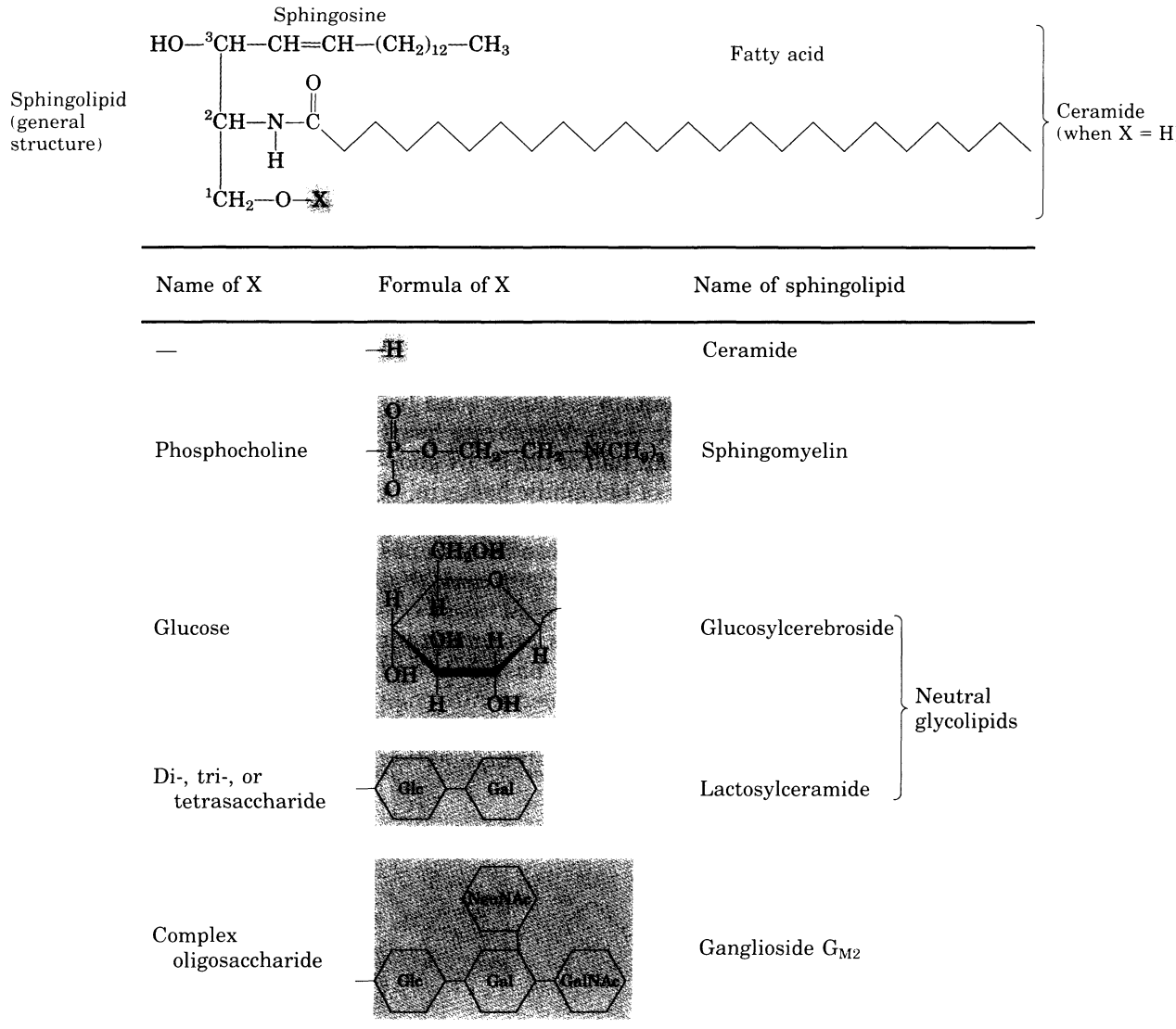
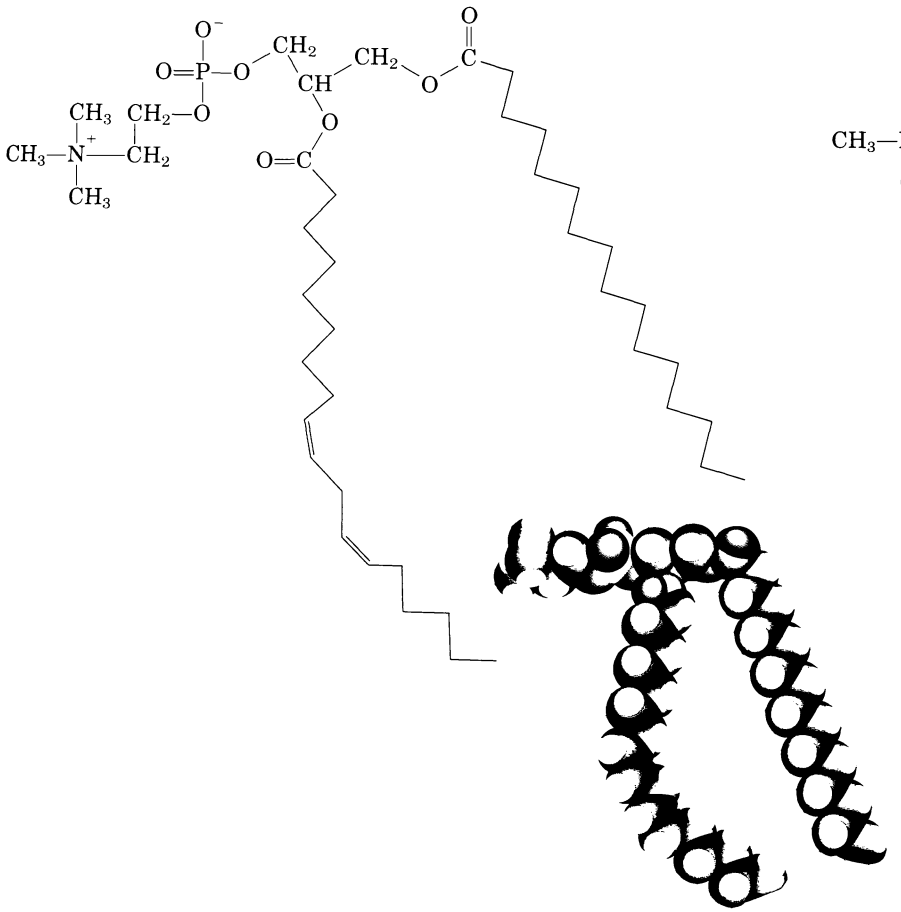


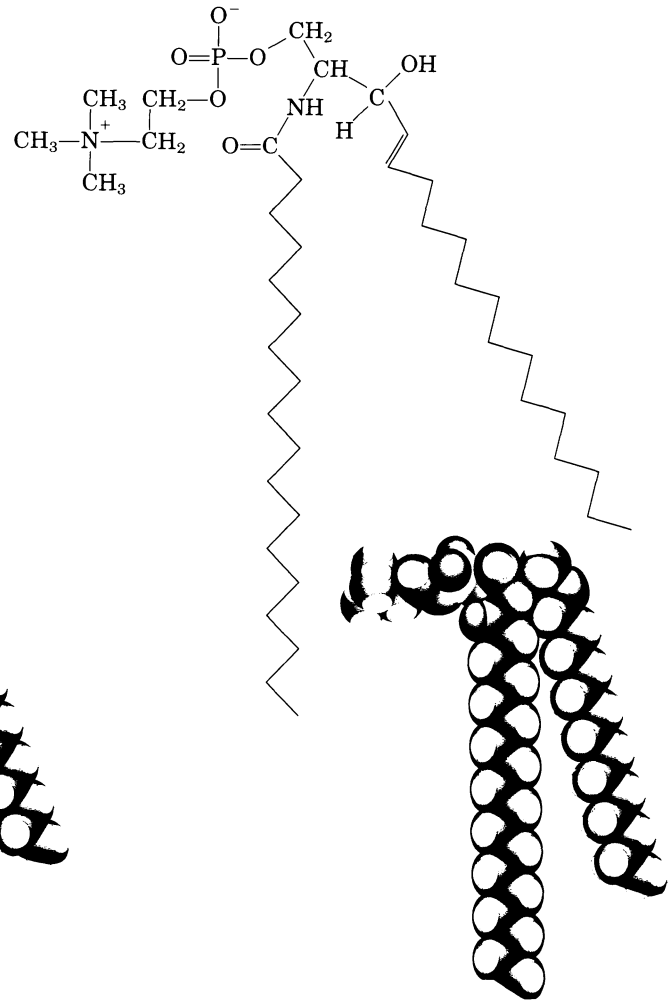
Figure 9–9 Sphingolipids. The first three carbons at the polar end of sphingosine are analogous to the three carbons of glycerol in glycerophospholipids. In ceramide, the parent compound for this group, the amino group at C-2 bears a fatty acid in amide linkage. Individual sphingolipids differ in the polar head group (X) attached at C-1. The fatty acid components of sphingolipids are usually saturated or monounsaturated, and contain 16, 18, 22, or 24 carbon atoms. Gangliosides have very complex oligosaccharide head groups. These compounds are given identifying symbols (e.g., G_{M1}, G_{M2}) that indicate the structure of the head group. At least 15 different classes of gangliosides have been found in higher animals. Standard symbols for sugars are used in this figure: Glc, D-glucose; Gal, D-galactose; GalNAc, *N*-acetyl-D-galactosamine; NeuNAc, *N*-acetylneuraminic acid (sialic acid).

Carbons C-1, C-2, and C-3 of the sphingosine molecule bear functional groups (—OH, $—NH_2$, —OH) that are structurally homologous with the three hydroxyl groups of glycerol in glycerophospholipids. When a fatty acid is attached in amide linkage to the $—NH_2$, the resulting compound is a **ceramide** (Fig. 9–9), which is structurally similar to a diacylglycerol. Ceramide is the fundamental structural unit common to all sphingolipids.

There are three subclasses of sphingolipids, all derivatives of ceramide, but differing in their head groups: sphingomyelins, neutral (uncharged) glycolipids, and gangliosides (Fig. 9–9). **Sphingomyelins** contain phosphocholine or phosphoethanolamine as their polar head group, and are therefore classified as phospholipids, together with glycerophospholipids. Indeed, sphingomyelins resemble phosphatidylcholines in their general properties and three-dimensional structure, and in having no net charge on their head groups (Fig. 9–10). Sphingomyelins are present in plasma membranes of animal cells; the myelin sheath which surrounds and insulates the axons of myelinated neurons is a good source of sphingomyelins, and gives them their name.



Phosphatidylcholine

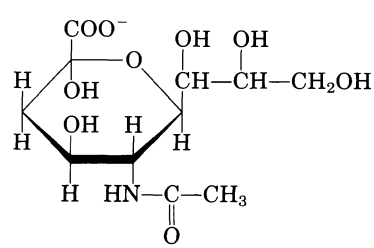


Sphingomyelin

Figure 9–10 The similarities in shape and in molecular structure of phosphatidylcholine (a glycerophospholipid) and sphingomyelin (a sphingolipid) are clear when their space-filling and structural formulas are drawn as here.

Neutral glycolipids and gangliosides have one or more sugars in their head group, connected directly to the —OH at C-1 of the ceramide moiety; they do not contain phosphate. These sugar-containing sphingolipids are sometimes called **glycosphingolipids.** Neutral glycolipids contain one to six (sometimes more) sugar units, which may be D-glucose, D-galactose, or *N*-acetyl-D-galactosamine (Fig. 9–9). These glycosphingolipids occur largely in the outer face of the plasma membrane. **Cerebrosides** have a single sugar linked to ceramide (Fig. 9–9); those with galactose are characteristically found in the plasma membranes of cells in neural tissue, and those with glucose, in the plasma membranes of cells in nonneural tissues.

Gangliosides, the most complex sphingolipids (Fig. 9–9), contain very large polar heads made up of several sugar units. One or more of the terminal sugar units of gangliosides is *N*-acetylneuraminic acid, also called sialic acid, which has a negative charge at pH 7. Gangliosides make up about 6% of the membrane lipids in the gray matter of the human brain, and they are present in lesser amounts in the membranes of most nonneural animal tissues.



N-Acetylneuraminic acid
(sialic acid)

Sphingolipids Are Sites of Biological Recognition

When the sphingolipids were discovered a century ago by the physician-chemist Johann Thudicum, their biological role seemed as enigmatic as the Sphinx, for which he named them. Sphingolipids are now known to be involved in various recognition events at the cell surface. For example, glycosphingolipids are the determinants of the human blood groups A, B, and O (Fig. 9–11). The ganglioside G_{M1}, which doubtless plays some role of value to the animal cell that contains it, is the point of attachment of cholera toxin as it attacks an animal cell, a case of coevolution of a host cell and its pathogenic parasite. The membranes of the human nervous system contain at least 15 different gangliosides for which no function is yet known. However, it is clearly important that the synthesis and breakdown of these compounds be tightly regulated; derangements in the metabolism of cerebrosides and gangliosides underlie the devastating effects of several human genetic diseases, including Tay-Sachs and Niemann-Pick diseases (Box 9–2).

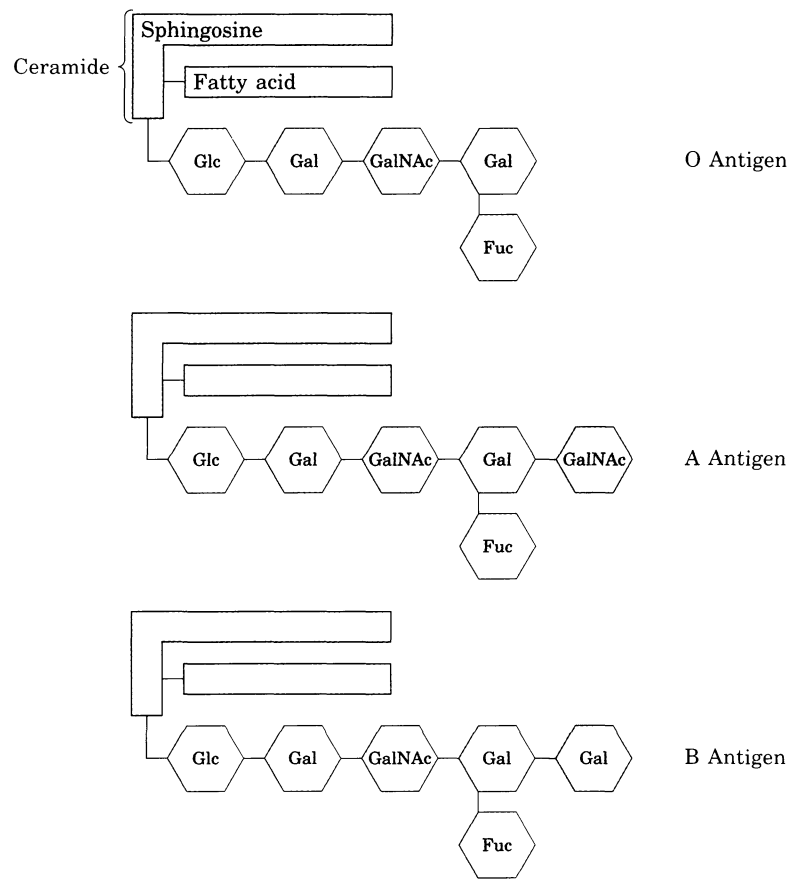


Figure 9–11 The human blood groups (O, A, B) are determined in part by the sugar head groups in these glycosphingolipids. The same three types of complex sugar groups are also found attached to certain blood proteins of individuals of blood types O, A, and B, respectively. The symbol Fuc represents the sugar fucose.

BOX 9–2

Some Inherited Human Diseases Resulting from Abnormal Accumulations of Membrane Lipids

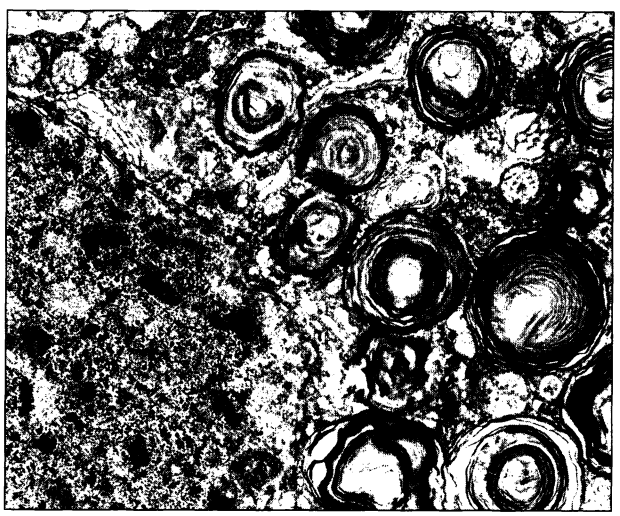
The polar lipids of membranes undergo constant metabolic turnover, the rate of their synthesis normally being counterbalanced by an equal rate of breakdown. The breakdown of lipids is promoted by hydrolytic enzymes, each capable of hydrolyzing a specific covalent bond. For example, the degradation of phosphatidylcholine, a major membrane lipid, takes place by the action of several different phospholipases (see Fig. 9–12).

The metabolism of membrane sphingolipids, including sphingomyelin, cerebrosides, and gangliosides, is prone to genetic defects of enzymes involved in their degradation. When they are synthesized at a normal rate but their degradation is impaired, sphingolipids or their partial breakdown products accumulate in the tissues. For example, in Niemann-Pick disease, sphingomyelin accumulates in the brain, spleen, and liver. The disease first becomes evident in infants, causing mental retardation and early death. Niemann-Pick disease is caused by a rare genetic defect in the hydrolytic enzyme sphingomyelinase, which cleaves phosphocholine from sphingomyelin.

Much more common is Tay-Sachs disease, in which a specific ganglioside accumulates in the brain and spleen owing to the lack of the lysosomal enzyme hexosaminidase A, a degradative enzyme that normally hydrolyzes a specific bond between an *N*-acetyl-D-galactosamine and a D-galactose residue in the polar head of the ganglioside (see Fig. 9–9). As a result, the partially degraded gangliosides accumulate, causing degeneration of the nervous system. The symptoms of Tay-Sachs disease are progressive retardation in development, paralysis, blindness, and death by the age of 3 or 4 yr.

Tay-Sachs disease is rare in the population at large (1 in 300,000 births) but has a very high incidence (1 in 3,600 births) in Ashkenazic Jews (those of Eastern European extraction), who make up more than 90% of the Jewish population of the United States. One in 28 Ashkenazic Jews carries the defective gene in recessive form, which means that when both parents are carriers, there is a one in four probability that a child will develop Tay-Sachs disease. Genetic counseling of parents has become important in averting the occurrence of this disease. Tests have been devised to determine the presence of the recessive gene in prospective parents. These tests involve measuring the level of hexosaminidase A in skin cells. Carriers of the defective gene have a reduced (but for these individuals, functional) level of the enzyme. Tests of the fetus can also be made during pregnancy by taking a sample of amniotic fluid, the fluid surrounding the growing fetus, in a process known as amniocentesis. The activity of hexosaminidase A can be measured in fetal cells contained in this fluid.

(a)



(b) 1 μm

Figure 1 **(a)** A 1-year-old infant with Tay-Sachs disease. **(b)** Electron micrograph of a portion of an affected brain cell, showing the abnormal ganglioside deposits in the lysosomes.

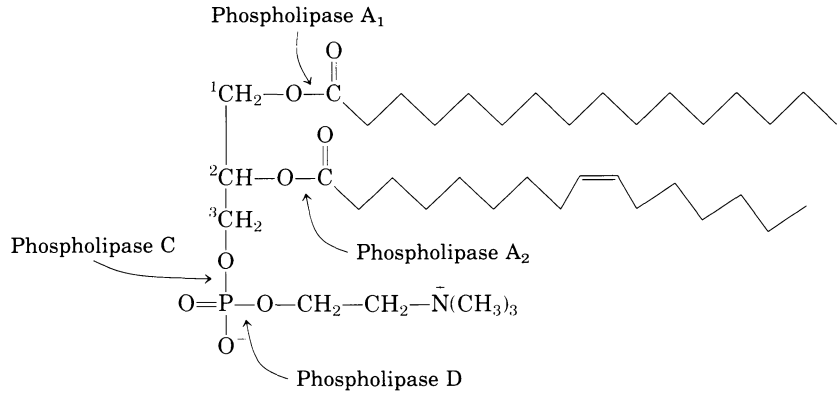


Figure 9–12 The specificities of phospholipases. Phospholipases A_1 and A_2 hydrolyze the ester bonds of intact glycerophospholipids at C-1 and C-2 of glycerol, respectively. Phospholipases C and D each split one of the phosphodiester bonds in the head group, as indicated. Some phospholipases act only on one type of glycerophospholipid, such as phosphatidylinositol or phosphatidylcholine; others are less specific. When one of the fatty acids has been removed by a type-A phospholipase, the second fatty acid is cleaved from the molecule by a lysophospholipase.

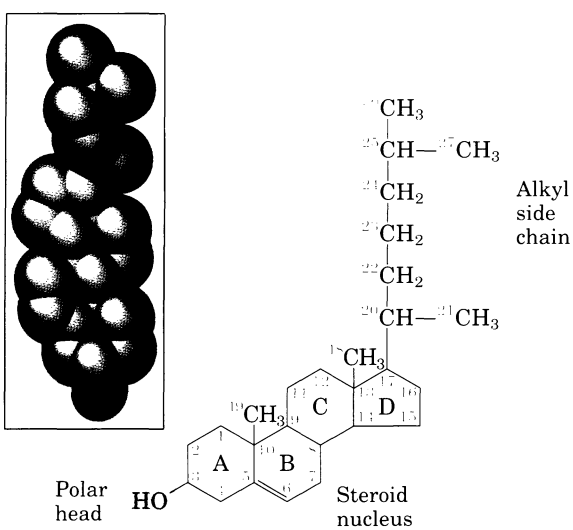
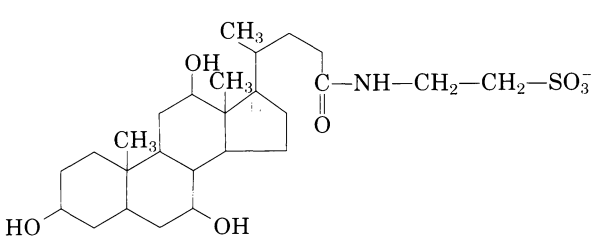


Figure 9–13 Cholesterol. To simplify reference to derivatives of the steroid nucleus, the rings are labeled A through D and the carbon atoms are numbered (in blue) as shown. The hydroxyl group on C-3 represents the polar head group. For storage and transport of the sterol, this hydroxyl group condenses with a fatty acid to form a sterol ester.



Specific Phospholipases Degrade Membrane Phospholipids

Most cells continually degrade and replace their membrane lipids. For each of the bonds in a glycerophospholipid, there is a specific hydrolytic enzyme (Fig. 9–12). Phospholipases of the A type remove one of the two fatty acids, producing a lysophospholipid; these esterases do not attack the ether link in plasmalogens. Lysophospholipases remove the remaining fatty acid.

Phospholipid breakdown is part of at least two signaling processes in animal cells. Extracellular signals (certain hormones, for example) activate a phospholipase C that specifically cleaves phosphatidylinositols, releasing diacylglycerol and inositol phosphates, which serve as intracellular signals. Other extracellular stimuli activate a phospholipase A that releases arachidonic acid from membrane lipids; arachidonate serves as a precursor in the synthesis of one of the eicosanoids that act as intracellular messengers. These messenger roles for lipids are discussed later in this chapter.

Sterols Have Four Fused Hydrocarbon Rings

Sterols are structural lipids present in the membranes of most eukaryotic cells. Their characteristic structure is the steroid nucleus consisting of four fused rings, three with six carbons and one with five (Fig. 9–13). The steroid nucleus is almost planar, and relatively rigid; the fused rings do not allow rotation about C—C bonds. **Cholesterol,** the major sterol in animal tissues, is amphipathic, with a polar head group (the hydroxyl group at C-3) and a nonpolar hydrocarbon body (the steroid nucleus and the hydrocarbon side chain at C-17) about as long as a 16-carbon fatty acid in its extended form. Similar sterols are found in other eukaryotes: stigmasterol in plants and ergosterol in fungi, for example. With rare exceptions, bacteria lack sterols. The sterols of all species are synthesized from simple five-carbon isoprene subunits (as are the fat-soluble vitamins, quinones, and dolichols described below).

In addition to their roles as membrane constituents, the sterols serve as precursors for a variety of products with specific biological activities. Bile acids, in which the side chain at C-17 is hydrophilic, act as detergents in the intestine, emulsifying dietary fats to make them more readily accessible to digestive lipases. A variety of steroid hormones (described below) are also produced from cholesterol by oxidation of the side chain at C-17.

On receiving the Nobel Prize in 1985 for their work on cholesterol metabolism, Michael Brown and Joseph Goldstein recounted in their lecture the extraordinary history of cholesterol:

> Cholesterol is the most highly decorated small molecule in biology. Thirteen Nobel Prizes have been awarded to scientists who devoted major parts of their careers to cholesterol. Ever since it was isolated from gallstones in 1784, cholesterol has exerted an almost hypnotic fascination for scientists from the most diverse areas of science and medicine.

We shall return to cholesterol later, to consider its role in biological membranes, its remarkable biosynthetic pathway, and its role as precursor to the steroid hormones.

Amphipathic Lipids Aggregate

We have noted that glycerophospholipids, sphingolipids, and sterols are virtually insoluble in water. When mixed with water, these amphipathic compounds form microscopic lipid aggregates in a phase separate from their aqueous surroundings. Lipid molecules cluster together with their hydrophobic moieties in contact with each other and their hydrophilic groups interacting with the surrounding water. Recall that lipid clustering reduces the amount of hydrophobic surface exposed to water and thus minimizes the number of molecules in the shell of ordered water at the lipid–water interface (see Fig. 4–7), resulting in an increase in entropy. Hydrophobic interactions among lipid molecules provide the thermodynamic driving force for the formation and maintenance of these structures.

Depending on the precise conditions and the nature of the lipids used, three types of lipid aggregates can form when amphipathic lipids are mixed with water (Fig. 9–14). **Micelles** are relatively small, spherical structures involving a few dozen to a few thousand molecules arranged so that their hydrophobic regions aggregate in the interior, excluding water, and their hydrophilic head groups are at the surface, in contact with water. Micelle formation is favored when the cross-sectional area of the head group is greater than that of the acyl side chain(s) (Fig. 9–14a), as it is in free fatty acids, lysophospholipids (which lack one fatty acid), and the detergent SDS.

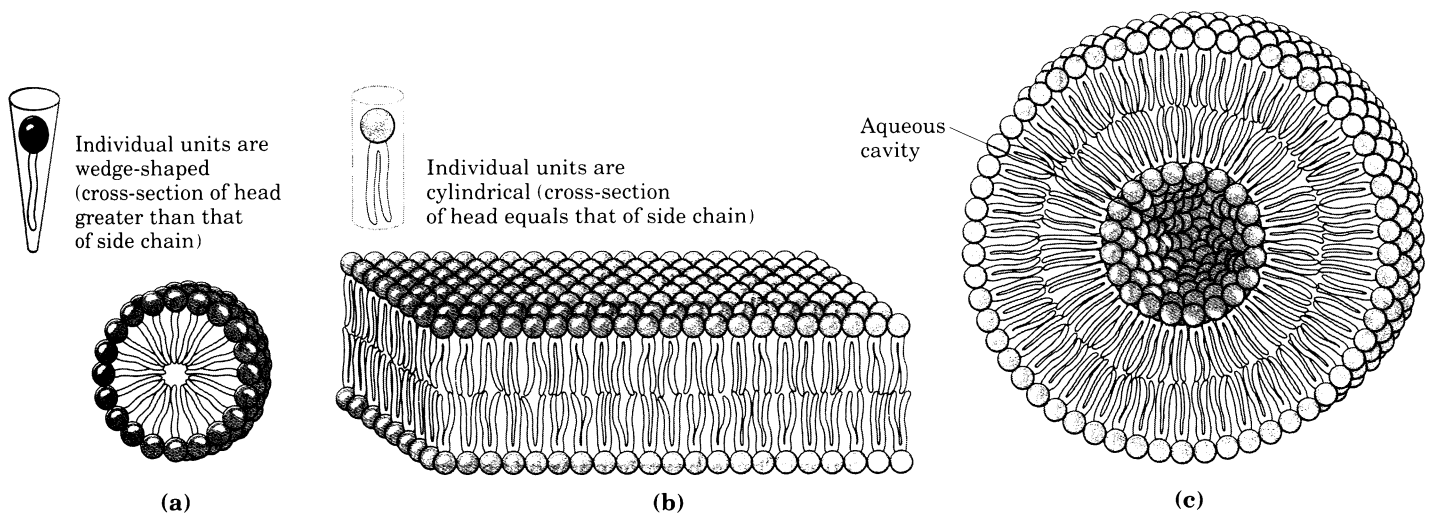


Figure 9–14 Amphipathic lipid aggregates that form in water. **(a)** In spherical micelles, the hydrophobic chains of the fatty acids are sequestered at the core of the sphere. There is virtually no water in the hydrophobic interior of the micelle. **(b)** In a bilayer, all acyl side chains except those at the edges of the sheet are protected from interaction with water. **(c)** When an extensive two-dimensional bilayer folds on itself, it forms a liposome, a three-dimensional hollow vesicle enclosing an aqueous cavity.

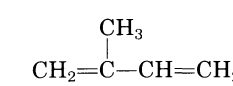
A second type of lipid aggregate in water is the **bilayer,** in which two lipid monolayers combine to form a two-dimensional sheet. Bilayer formation occurs most readily when the cross-sectional areas of the head group and side chain(s) are similar (Fig. 9–14b), as in glycerophospholipids and sphingolipids. The hydrophobic portions in each monolayer interact, excluding water. The hydrophilic head groups interact with water at the two surfaces of the bilayer.

The third type of lipid aggregate is formed when a lipid bilayer folds back on itself to form a hollow sphere called a **liposome** or vesicle (Fig. 9–14c). By forming vesicles, bilayer sheets lose their hydrophobic edge regions, achieving maximal stability in their aqueous environment. These bilayer vesicles enclose water, creating a separate aqueous compartment. It is likely that the first living cells resembled liposomes, their aqueous contents segregated from the rest of the world by a hydrophobic shell. We shall see in the next chapter that lipid bilayers are fundamental to the structure of all biological membranes.

Lipids with Specific Biological Activities

The two classes of lipids considered thus far (storage lipids and structural lipids) are major cellular components; membrane lipids represent 5 to 10% of the dry mass of most cells, and storage lipids, more than 50% of the mass of an adipocyte. With some important exceptions, these lipids play a *passive* role in the cell; fuels are acted on by oxidative enzymes, and lipid membranes form impermeable barriers that separate cellular compartments. Another group of lipids, although relatively minor cellular components on a mass basis, have specific and essential biological activities. These include hundreds of steroids—compounds that share the four-ring steroid nucleus but are more polar than cholesterol—and large numbers of isoprenoids, which are synthesized from five-carbon precursors related to isoprene:

Isoprene



The isoprenoids include vitamins A, D, E, and K, first recognized as fatty materials essential to the normal growth of animals, and numerous biological pigments. Other "active" lipids serve as essential cofactors for enzymes, as electron carriers, or as intracellular signals. To illustrate the range of their structures and biological activities we will briefly describe a few of these compounds. In later chapters, their synthesis and biological roles will be considered in more detail.

Steroid Hormones Carry Messages between Tissues

The major groups of steroid hormones are the male and female sex hormones and the hormones of the adrenal cortex, cortisol and aldosterone (Fig. 9–15). All of these hormones contain an intact steroid nucleus. They are produced in one tissue and carried in the bloodstream to target tissues, where they bind to highly specific receptor proteins and trigger changes in gene expression and metabolism. Because of the very high affinity of receptor for hormone, very low concentrations of hormone (as low as 10^{-9} M) suffice to produce the effect on target tissues. These hormones and their actions are described in more detail in Chapter 22.

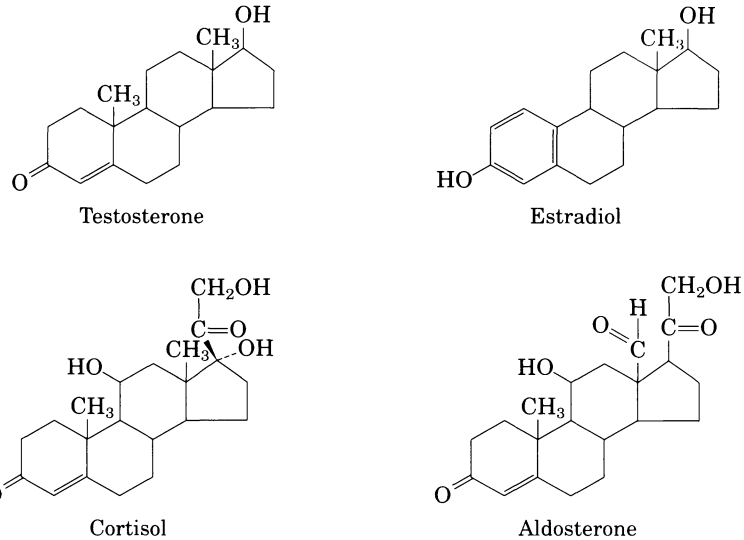


Figure 9–15 Steroids derived from cholesterol. Testosterone, the male sex hormone, is produced in the testes. Estradiol, one of the female hormones, is produced in the ovaries and placenta. Cortisol and aldosterone are hormones produced in the cortex of the adrenal gland; they regulate glucose metabolism and salt excretion, respectively.

Hydrolysis of Phosphatidylinositol Produces Intracellular Messengers

Phosphatidylinositol and its phosphorylated derivatives (Fig. 9–16) are components of the plasma membranes of all eukaryotic cells. They serve as a reservoir of messenger molecules that are released inside the cell when certain extracellular signals interact with specific receptors in the plasma membrane. For example, when the hormone vasopressin binds to receptor molecules in the plasma membranes of cells in the kidney and the blood vessels, a specific phospholipase in the membrane is activated. This phospholipase breaks the bond between glycerol and phosphate in phosphatidylinositol-4,5-bisphosphate (Fig. 9–16), releasing two products: inositol-1,4,5-trisphosphate and diacylglycerol. Inositol-1,4,5-trisphosphate causes the release of Ca^{2+} sequestered in membrane-bounded compartments of the cell, triggering the activation of a variety of Ca^{2+}-dependent enzymes and hormonal responses. Diacylglycerol binds to and activates an enzyme, protein kinase C, that transfers phosphate groups from ATP to several cytosolic proteins, thereby altering their enzymatic activities.

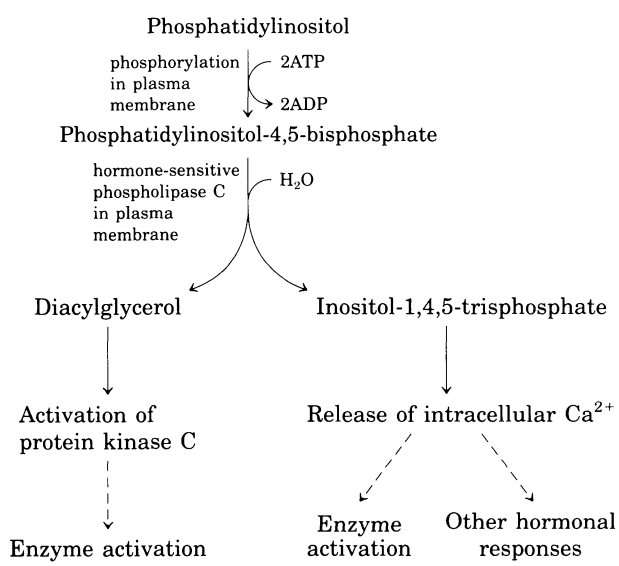


Figure 9–16 Phosphatidylinositol-4,5-bisphosphate, formed in the plasma membrane by phosphorylation of phosphatidylinositol, is hydrolyzed by a specific phospholipase C in response to hormonal signals. Both of the products of hydrolysis act as intracellular messengers.

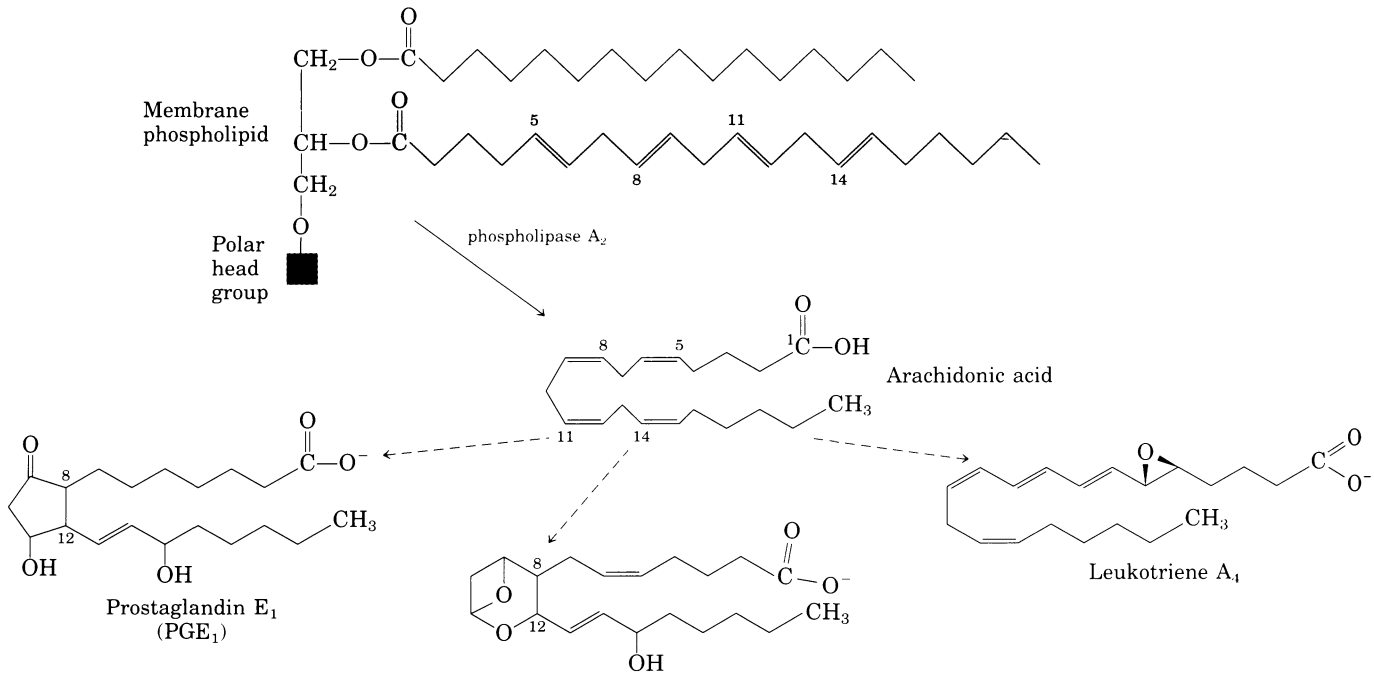


Figure 9–17 Arachidonic acid and some of its eicosanoid derivatives. In response to certain hormonal signals, phospholipase A_2 releases arachidonic acid (arachidonate at pH 7) from membrane phospholipids; arachidonic acid then serves as a precursor to various eicosanoids. These include prostaglandins such as PGE_1, in which carbon atoms 8 and 12 of arachidonic acid are joined to form the characteristic five-membered ring; thromboxane A_2, in which carbons 8 and 12 are joined and an oxygen atom is added to form the six-membered ring; and leukotriene A, containing a series of three conjugated double bonds. Aspirin and ibuprofen block the formation of prostaglandins and thromboxanes from arachidonic acid.

Eicosanoids Are Potent Biological Effectors

Eicosanoids (Fig. 9–17) are fatty acid derivatives with a variety of extremely potent hormonelike actions on various tissues of vertebrate animals. Unlike hormones, they are not transported between tissues in the blood, but act on the tissue in which they are produced. This family of compounds is known to be involved in reproductive function; in the inflammation, fever, and pain associated with injury or disease; in the formation of blood clots and the regulation of blood pressure; in gastric acid secretion; and in a variety of other processes important in human health or disease. More roles for the eicosanoids doubtless remain to be discovered.

Eicosanoids are all derived from the 20-carbon polyunsaturated fatty acid arachidonic acid, $20:4(\Delta^{5,8,11,14})$ (Fig. 9–17), from which they take their general name (Greek *eikosi*, "twenty"). There are three classes of eicosanoids: prostaglandins, thromboxanes, and leukotrienes. Various eicosanoids are produced in different cell types by different synthetic pathways, and have different target cells and biological actions.

The **prostaglandins** (PG) (Fig. 9–17) all contain a five-membered ring of carbon atoms originally part of the chain of arachidonic acid. They derive their name from the tissue in which they were first recognized (the prostate gland). Two groups were originally defined: PGE, for ether-soluble, and PGF, for phosphate buffer-soluble (*fosfat* in Swedish). Each contains numerous subtypes, named PGE_1, PGE_2, etc. Prostaglandins are now known to act in many tissues by regulating the synthesis of the intracellular messenger molecule 3′,5′-cyclic AMP (cAMP). Because cAMP mediates the action of many hormones, the prostaglandins affect a wide range of cellular and tissue functions. Some prostaglandins stimulate contraction of the smooth muscle of the uterus during labor or menstruation. Others affect blood flow to specific organs, the wake–sleep cycle, and the responsiveness of certain tissues to hormones such as epinephrine and glucagon. Prostaglandins in a third group elevate body temperature (producing fever) and cause inflammation, resulting in pain.

The **thromboxanes,** first isolated from blood platelets (also known as thrombocytes), have a six-membered ring containing an ether (Fig. 9–17). They are produced by platelets and act in formation of blood clots and the reduction of blood flow to the site of a clot.

Leukotrienes, found first in leukocytes, contain three conjugated double bonds (Fig. 9–17). They are powerful biological signals; for example, they induce contraction of the muscle lining the airways to the lung. Overproduction of leukotrienes causes asthmatic attacks. The strong contraction of the smooth muscles of the lung that occurs during anaphylactic shock is part of the potentially fatal allergic reaction in individuals hypersensitive to bee stings, penicillin, or various other agents.

Vitamins A, D, E, and K Are Fat-Soluble

During the first third of this century, a major focus of research in physiological chemistry was the identification of **vitamins**—compounds essential to the health of humans and other vertebrate animals that cannot by synthesized by these animals and must therefore be obtained in the diet. Early nutritional studies identified two general classes of such compounds: those soluble in nonpolar organic solvents (fat-soluble vitamins) and those that could be extracted from foods with aqueous solvents (water-soluble vitamins). Eventually the fat-soluble group was resolved into the four vitamins A, D, E, and K, all of which are isoprenoid compounds. Isoprenoids are synthesized by the condensation of isoprene units.

Vitamin A (retinol) (Fig. 9–18) is a pigment essential to vision. It was first recognized as an essential nutritional factor for laboratory animals, and was later isolated from fish liver oils. Vitamin A itself

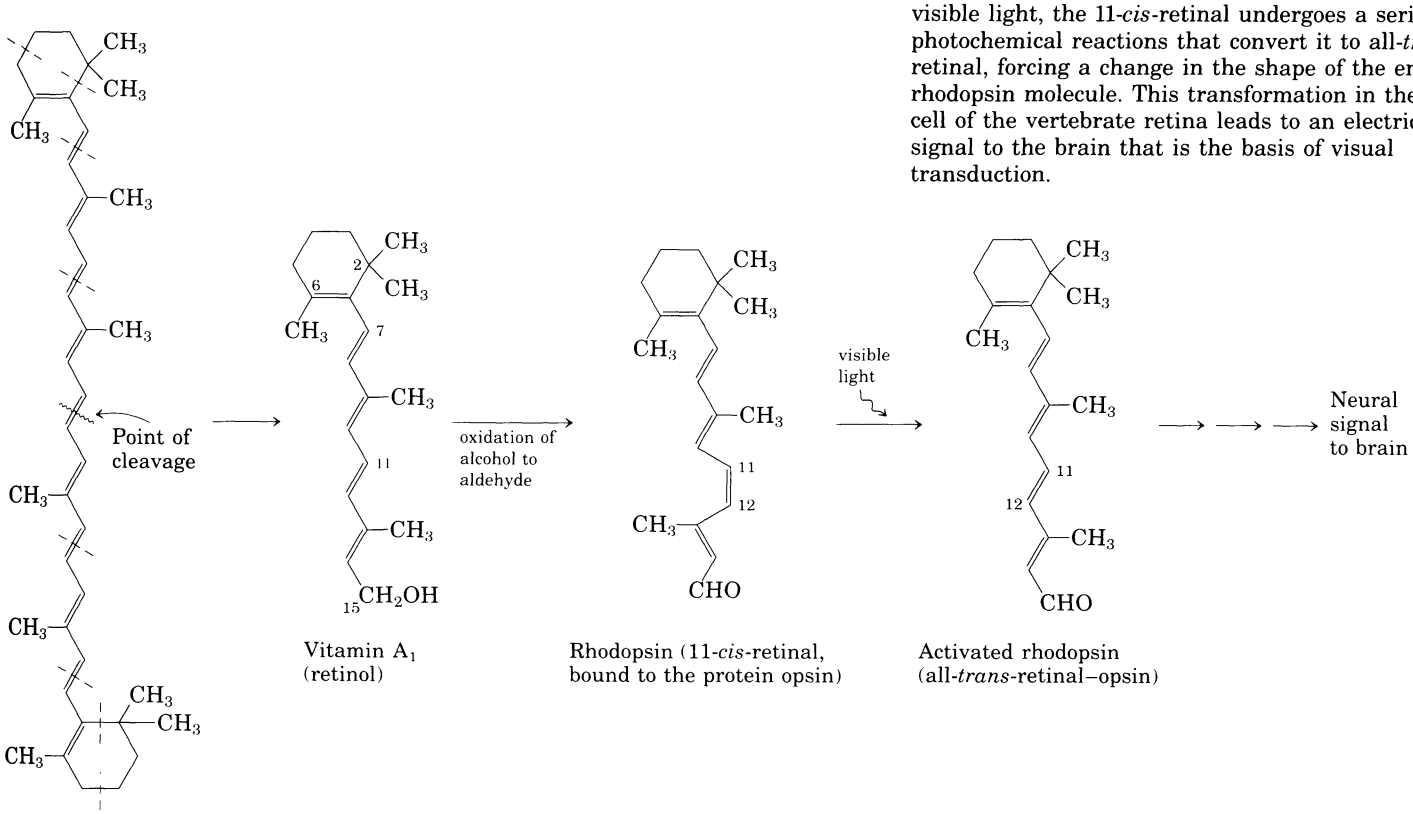


Figure 9–18 Vitamin A_1 and its precursor, β-carotene. The isoprene structural units are set off by dashed red lines. Cleavage of β-carotene yields two molecules of vitamin A_1 (retinol). Oxidation at C-15 converts retinol to the aldehyde, retinal. Rhodopsin, a visual pigment widely employed in nature, consists of retinal and the protein opsin. In the dark, retinal of rhodopsin is in the 11-cis form. When a rhodopsin molecule is excited with visible light, the 11-*cis*-retinal undergoes a series of photochemical reactions that convert it to all-*trans*-retinal, forcing a change in the shape of the entire rhodopsin molecule. This transformation in the rod cell of the vertebrate retina leads to an electrical signal to the brain that is the basis of visual transduction.

does not occur in plants, but many plants contain carotenoids, light-absorbing pigments that can be enzymatically converted into vitamin A by most animals. Figure 9–18 shows, for example, how vitamin A can be formed by cleavage of β-carotene, the pigment that gives carrots, sweet potatoes, and other yellow vegetables their characteristic color. Deficiency of vitamin A leads to a variety of symptoms in humans and experimental animals, which include dry skin, xerophthalmia (dry eyes), dry mucous membranes, retarded development and growth, sterility in male animals, and night blindness, an early symptom commonly used in the medical diagnosis of vitamin A deficiency.

Vitamin D is a derivative of cholesterol and the precursor to a hormone essential in calcium and phosphate metabolism in vertebrate animals. Vitamin D_3, also called cholecalciferol, is normally formed in the skin in a photochemical reaction driven by the ultraviolet component of sunlight (Figure 9–19). It is also abundant in fish liver oils, and is added to commercial milk as a nutritional supplement. Vitamin D_3 itself is not biologically active, but it is the precursor of 1,25-dihydroxycholecalciferol, a potent hormone that regulates the uptake of calcium in the intestine and the balance of release and deposition of bone calcium and phosphate. Deficiency of vitamin D leads to defective bone formation, resulting in the disease rickets.

Vitamin E (Fig. 9–20) is the collective name for a group of closely related lipids called tocopherols, all of which contain a substituted aromatic ring and a long hydrocarbon side chain. Tocopherols are found in hens' eggs and vegetable oils, and are especially abundant in wheat germ. Deficiency of vitamin E is very rare in humans, but when laboratory animals are fed diets depleted of vitamin E, they develop scaly skin, muscular weakness and wasting, and sterility. Tocopherols can undergo oxidation–reduction reactions on the aromatic ring. The vitamin activity of tocopherols likely results from their ability to prevent oxidative damage to the lipids of cellular membranes. Recall the reactions of unsaturated fatty acids with oxygen that cause rancidity in foods. If such reactions were to occur in living cells, the resulting defects in membrane function might cause cell death. Tocopherols react with and destroy the most reactive forms of oxygen, protecting unsaturated fatty acids from oxidation. Tocopherols are used commercially to retard spoilage of certain foods.

Vitamin K is a lipid cofactor required for normal blood clotting. Vitamin K_1 (phylloquinone; Fig. 9–20) is found in green plant leaves,

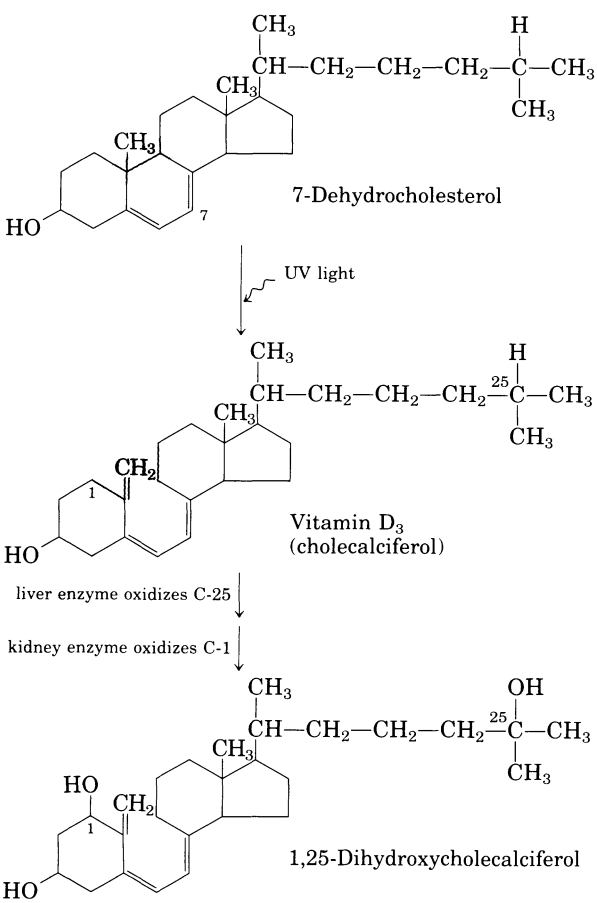
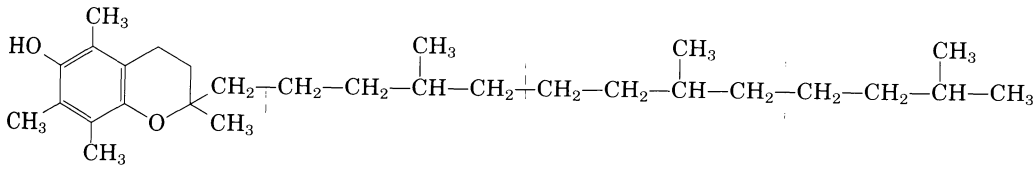
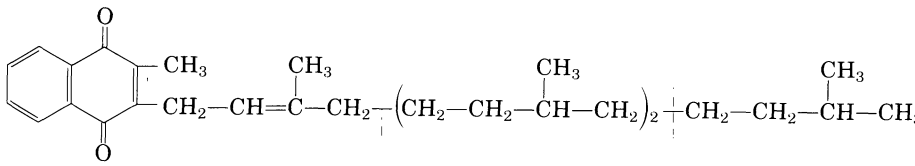


Figure 9–19 Vitamin D_3 production and metabolism. Vitamin D_3 is produced by irradiation of 7-dehydrocholesterol in the skin, and in the kidney is converted into the active hormone, 1,25-dihydroxycholecalciferol, which regulates the metabolism of Ca^{2+} and PO_4^{3-}. Dietary vitamin D prevents rickets, a disease once common in cold climates, where heavy clothing blocked the UV component of sunlight necessary to vitamin D_3 production in skin.


Vitamin E: an antioxidant


Vitamin K_1: a blood-clotting cofactor (phylloquinone)

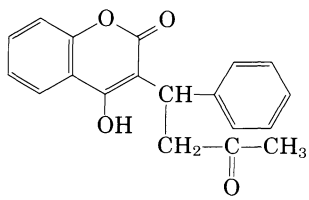
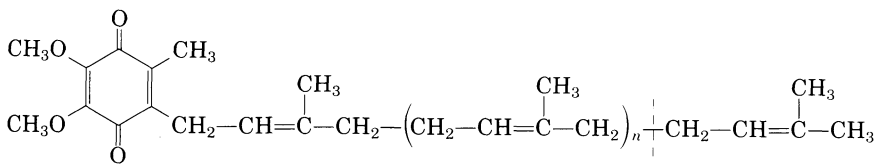
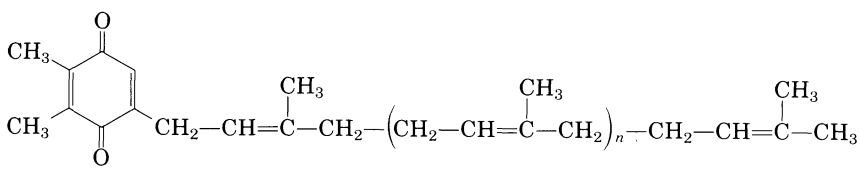
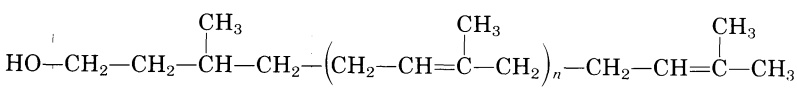

Warfarin: a blood anticoagulant

Figure 9–20 Some other biologically active isoprenoid compounds or derivatives. Note that the values of *n* exclude the first and last isoprene unit in each isoprenoid side chain as represented here. Warfarin does not occur naturally. It is an analog of vitamin K that lacks an isoprenoid side chain.


Ubiquinone: a mitochondrial electron carrier (coenzyme Q) (n = 4–8)

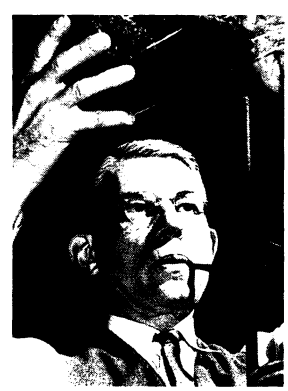

Plastoquinone: a chloroplast electron carrier (n = 4–8)


Dolichol: a sugar carrier (n = 9–22)

and a related form, vitamin K_2 (menaquinone), is formed by bacteria residing in the animal intestine. The vitamin acts in the formation of prothrombin, a blood plasma protein essential in blood-clot formation. Prothrombin is a proteolytic enzyme that splits specific peptide bonds in the blood protein fibrinogen, converting it to fibrin, the insoluble, fibrous protein that holds blood clots together. Deficiency of vitamin K results in slowed blood clotting, which can be fatal to a wounded animal. Henrik Dam and Edward A. Doisy are given credit for having independently discovered the antihemorrhaghic action of vitamin K.

Warfarin (Fig. 9–20) is a synthetic analog of vitamin K, which acts as a competitive inhibitor of prothrombin formation. It is extremely poisonous to rats, causing death by internal bleeding. Ironically, this potent rodenticide is also a valuable anticoagulant drug for the treatment of human patients in whom excessive blood clotting is dangerous—surgical patients and victims of coronary thrombosis.


Henrik Dam
1895–1976


Edward A. Doisy
1893–1986

Lipid Quinones Carry Electrons

Ubiquinone and plastoquinone (Fig. 9–20), also isoprenoid derivatives, function as electron carriers in the production of ATP in mitochondria and chloroplasts. In most mammalian tissues, ubiquinone (also called coenzyme Q) has ten isoprene units. Plastoquinone is the plant equivalent of ubiquinone. In their roles as electron carriers, both ubiquinone and plastoquinone can accept either one or two electrons and either one or two protons to be reduced, as shown in Figure 18–2.

Dolichols Form Activated, Hydrophobic Sugar Derivatives

During the assembly of the complex carbohydrates of bacterial cell walls, and during the addition of polysaccharide units to certain proteins (glycoproteins) in eukaryotes, the sugar units to be added are chemically activated by attachment to **dolichols** (Fig. 9–20), another group of isoprenoids. Dolichols from animals have between 17 and 21 isoprene units (85 to 105 carbon atoms), bacterial dolichols have 11 units, and those of plants and fungi have 14 to 24 isoprene units. These very hydrophobic compounds have strong hydrophobic interactions with membrane lipids, anchoring the attached sugars to the membrane where they participate in sugar-transfer reactions.

Resolution and Analysis of Lipids

In exploring the role of lipids in a biological process, it is often useful to know which lipids are present, and in what proportions. Because lipids are insoluble in water, their extraction from tissues and subsequent fractionation require the use of organic solvents and some techniques not commonly used in the purification of water-soluble molecules such as proteins and carbohydrates. In general, complex mixtures of lipids are separated by differences in their polarity or solubility in nonpolar solvents. Lipids that contain ester- or amide-linked fatty acids can be hydrolyzed (saponified) by treatment with acid or alkali, to yield their component parts for analysis.

Lipid Extraction Requires Organic Solvents

Neutral lipids (triacylglycerols, waxes, pigments, etc.) are readily extracted from tissues with ethyl ether, chloroform, or benzene, solvents in which lipid clustering driven by hydrophobic interactions does not occur. Membrane lipids are more effectively extracted by more polar organic solvents, such as ethanol or methanol, which reduce the hydrophobic interactions among lipid molecules but also weaken the hydrogen bonds and electrostatic interactions that bind membrane lipids to membrane proteins. A commonly used extractant is a mixture of chloroform, methanol, and water, initially in proportions that are miscible, producing a single phase (1:2:0.8, v/v/v). After homogenizing tissue in this solvent to extract all lipids, more water is added to the resulting extract, and it separates into two phases, methanol/water (top phase) and chloroform (bottom phase). The lipids remain in the chloroform, and more polar molecules (proteins, sugars) partition into the polar phase of methanol/water (Fig. 9–21).

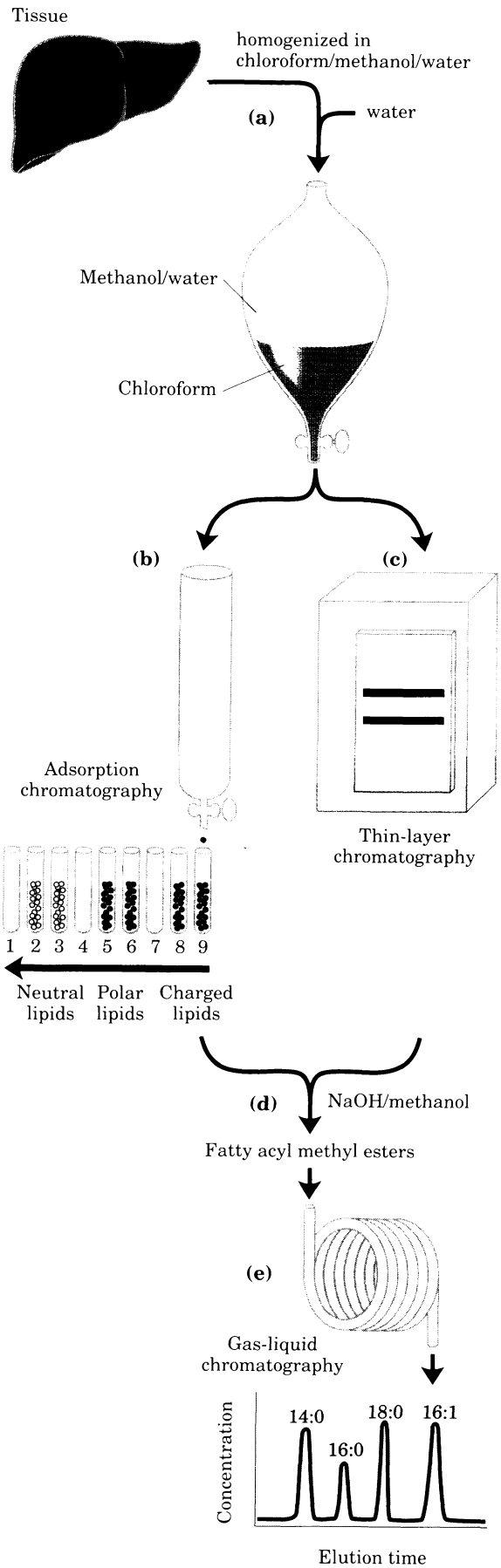


Figure 9–21 Some common procedures used in the extraction, separation, and identification of cellular lipids. **(a)** Tissue is homogenized in a chloroform/methanol/water mixture, which on addition of water and removal of unextractable sediment by centrifugation yields two phases. Different types of extracted lipids in the chloroform phase may be separated by **(b)** adsorption chromatography on a column of silica gel, through which solvents of increasing polarity are passed, or **(c)** thin-layer chromatography (TLC), in which lipids are carried by a rising solvent front, less polar lipids traveling farther than more polar or charged lipids. TLC with appropriate solvents also can be used to separate individual lipid species from a single class; for example, the charged lipids phosphatidylserine, phosphatidylglycerol, and phosphatidylinositol are easily separated by TLC. For the determination of fatty acid composition, a lipid fraction containing ester-linked fatty acids is **(d)** transesterified in a warm aqueous solution of NaOH and methanol, producing a mixture of fatty acyl methyl esters, which are then **(e)** separated on the basis of chain length and degree of saturation by gas-liquid chromatography. Precise determination of molecular mass, by mass spectroscopy (not shown), allows unambiguous identification of individual lipids. The lipid is ionized and volatilized by heat and the resulting molecular ion is passed through an electromagnetic field, which deflects ions to a degree dependent on their size. By comparison with standard ions of known molecular mass, the mass of the unknown molecular ion is determined with such great accuracy that the structure of the lipid can be deduced.

Adsorption Chromatography Separates Lipids of Different Polarity

The complex mixture of tissue lipids can be fractionated further by chromatographic procedures based on the different polarities of each class of lipid. In adsorption chromatography (Fig. 9–21), an insoluble, polar material such as silica gel (a form of silicic acid, $Si(OH)_4$), is packed into a long, thin glass column, and the lipid mixture (in chloroform solution) is applied to the top of the column. The polar lipids bind tightly to the polar silicic acid, but the neutral lipids pass directly through the column and emerge in the first chloroform wash. The polar lipids are then eluted, in order of increasing polarity, by washing the column with solvents of progressively higher polarity. Uncharged but polar lipids (cerebrosides, for example) are eluted with acetone, and very polar or charged lipids (such as glycerophospholipids) are eluted with methanol.

Thin-layer chromatography on silicic acid (Fig. 9–21) employs the same principle. A thin layer of silica gel (silicic acid) is spread onto a glass plate, to which it adheres. A small sample of lipids dissolved in chloroform is applied near one edge of the plate, which is dipped in a

shallow container of an organic solvent within a closed chamber saturated with the solvent vapor. As the solvent rises on the plate by capillary action, it carries lipids with it. The less polar lipids move farthest, as they have less tendency to bind to the polar silicic acid. The lipids can be detected after their separation by spraying the plate with a dye (rhodamine), which fluoresces when associated with lipids, or by exposing the plate to iodine fumes. Iodine reacts with the double bonds in fatty acids, giving the lipids that contain them a yellow or brown color. For subsequent analysis, regions containing separated lipids can be scraped from the plate and the lipids recovered by extraction with an organic solvent.

Gas-Liquid Chromatography Resolves Mixtures of Volatile Lipid Derivatives

Gas-liquid chromatography separates volatile components of a mixture according to their relative tendencies to dissolve in the inert material packed in the chromatography column, and to volatilize and move through the column, carried by a current of an inert gas such as helium. Some lipids are naturally volatile, but most must first be derivatized to increase their volatility (that is, lower their boiling point). For the analysis of the fatty acids present in a sample of phospholipids, the lipids are first heated in a methanol/HCl or methanol/NaOH mixture, which converts fatty acids esterified to glycerol into their methyl esters (transesterification). These fatty acyl methyl esters are then loaded onto the gas-liquid chromatography column, and the column is heated to volatilize the compounds. Those fatty acyl esters most soluble in the column material partition into (dissolve in) that material; those less soluble are carried by the stream of helium and emerge first from the column (Fig. 9–21). The order of elution depends on the nature of the solid adsorbant in the column, and on the boiling point of the components of the lipid mixture. Using these techniques, mixtures of fatty acids with various chain lengths and various degrees of unsaturation can be completely resolved.

Specific Hydrolysis Aids in Determination of Lipid Structure

Certain classes of lipids are susceptible to degradation under specific conditions. For example, all ester-linked fatty acids in triacylglycerols, phospholipids, and sterol esters are released by mild acid or alkaline treatment, and somewhat harsher hydrolysis conditions release amide-bound fatty acids from sphingolipids. Enzymes that specifically hydrolyze certain lipids are also useful in the determination of lipid structure. Phospholipases A, C, and D (see Fig. 9–12) each split specific bonds in phospholipids and yield products with characteristic solubilities and chromatographic behaviors. Phospholipase C, for example, releases a water-soluble phosphoryl alcohol (phosphocholine from phosphatidylcholine) and a chloroform-soluble diacylglycerol, each of which can be characterized separately to determine the structure of the intact phospholipid. The combination of specific hydrolysis with characterization of the products by thin-layer chromatography or gas-liquid chromatography often allows determination of the structure of a lipid. To establish unambiguously the length of a hydrocarbon chain, or the position of double bonds, mass spectral analysis of lipids or their volatile derivatives is invaluable.

Summary

Lipids are water-insoluble components of cells that can be extracted by nonpolar solvents. Some lipids serve as structural components of membranes and others as storage forms of fuel. Fatty acids, which provide the hydrocarbon components of lipids, usually have an even number (12 to 24) of carbon atoms and may be saturated or unsaturated; unsaturated fatty acids have double bonds in the cis configuration. In most unsaturated fatty acids, one double bond is at the Δ^9 position (between C-9 and C-10).

Triacylglycerols contain three fatty acid molecules esterified to the three hydroxyl groups of glycerol. Simple triacylglycerols contain only one type of fatty acid; mixed triacylglycerols contain at least two different types. Triacylglycerols are primarily storage fats; they are present in many types of foods.

The polar lipids, which have polar heads and nonpolar tails, are major components of membranes. The most abundant are the glycerophospholipids, which contain two fatty acid molecules esterified to two hydroxyl groups of glycerol, and a second alcohol, the head group, esterified to the third hydroxyl of glycerol via a phosphodiester bond. Glycerophospholipids differ in the structure of the head group; common glycerophospholipids are phosphatidylethanolamine and phosphatidylcholine. The polar heads of the glycerophospholipids carry electric charges at pH near 7. The sphingolipids, also membrane components, contain sphingosine, a long-chain aliphatic amino alcohol, but no glycerol. Sphingomyelin possesses, in addition to phosphoric acid and choline, two long hydrocarbon chains, one contributed by a fatty acid and the other by sphingosine. Two other classes of sphingolipids are neutral glycolipids and gangliosides, which contain various sugar components. Cholesterol, a sterol, is a precursor of many steroids and is also an important component of plasma membranes of animal cells. All polar lipids are amphipathic; they have polar or charged heads and nonpolar hydrocarbon tails. They spontaneously form micelles, bilayers, and liposomes, stabilized by hydrophobic interactions.

Some types of lipids, although present in relatively small quantities, play critical roles as cofactors or signals. Steroid hormones are derived from sterols. Phosphatidylinositol is hydrolyzed to yield two intracellular messengers, diacylglycerol and inositol trisphosphate. Prostaglandins, thromboxanes, and leukotrienes are extremely potent hormonelike molecules derived from arachidonic acid. Vitamins A, D, E, and K are fat-soluble compounds made up of isoprene units. All play essential roles in the metabolism or physiology of animals. Vitamin A furnishes the visual pigment of the vertebrate eye. Vitamin D is parent to a hormone that regulates calcium and phosphate metabolism. Vitamin E probably functions in the protection of membrane lipids from oxidative damage, and vitamin K is essential in the blood-clotting process. Ubiquinones and plastoquinones, also isoprenoid derivatives, function as electron carriers in animals and plants, respectively. Dolichols activate and anchor sugars on cellular membranes for use in the synthesis of certain complex carbohydrates and glycoproteins.

In the determination of lipid composition, lipids are extracted from tissues with organic solvents and separated by thin-layer or gas-liquid chromatography. Individual lipids are identified by their chromatographic behavior, their susceptibility to hydrolysis by specific enzymes, or by mass spectral determination of their molecular masses.

Further Reading

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Problems

1. *Melting Points of Fatty Acids* The melting points of a series of 18-carbon fatty acids are stearic acid, 69.6 °C; oleic acid, 13.4 °C; linoleic acid, −5 °C; and linolenic acid, −11 °C. What structural aspect of these 18-carbon fatty acids can be correlated with the melting point? Provide a molecular explanation for the trend in melting points.

2. *Spoilage of Cooking Fats* Some fats used in cooking, such as olive oil, spoil rapidly upon exposure to air at room temperature, whereas others, such as solid shortening, remain unchanged. Why?

3. *Preparation of Béarnaise Sauce* During the preparation of béarnaise sauce, egg yolks are incorporated into melted butter to stabilize the sauce

and avoid separation. The stabilizing agent in the egg yolks is lecithin (phosphatidylcholine). Suggest why this works.

4. *Hydrolysis of Lipids* Name the products of mild hydrolysis of the following lipids with dilute NaOH:

(a) 1-stearoyl-2,3-dipalmitoylglycerol

(b) 1-palmitoyl-2-oleoylphosphatidylcholine

5. *Number of Detergent Molecules per Micelle* When a small amount of sodium dodecyl sulfate ($Na^+ CH_3(CH_2)_{11}OSO_3^-$) is dissolved in water, the detergent ions go into solution as monomeric species. As more detergent is added, a point is reached (the critical micelle concentration) at which the monomers associate to form micelles. The critical micelle concentration of SDS is 8.2 mM. An examination of the micelles shows that they have an average particle weight (the sum of the molecular weights of the constituent monomers) of 18,000. Calculate the number of detergent molecules in the average micelle.

6. *Hydrophobic and Hydrophilic Components of Membrane Lipids* A common structural feature of membrane lipid molecules is their amphipathic nature. For example, in phosphatidylcholine, the two fatty acid chains are hydrophobic and the phosphocholine head group is hydrophilic. For each of the following membrane lipids, name the components that serve as the hydrophobic and hydrophilic units:

(a) phosphatidylethanolamine

(b) sphingomyelin

(c) galactosylcerebroside

(d) ganglioside

(e) cholesterol

7. *Properties of Lipids and Lipid Bilayers* Lipid bilayers formed between two aqueous phases have this important property: they form two-dimensional sheets, the edges of which close upon each other, and undergo self-sealing to form liposomes.

(a) What properties of lipids are responsible for this property of bilayers? Explain.

(b) What are the biological consequences of this property with regard to the structure of biological membranes?

8. *Chromatographic Separation of Lipids* A mixture of the following lipids is applied to a silica gel column, and the column is then washed with progressively more polar solvents. The mixture consists of: phosphatidylserine, cholesteryl palmitate (a sterol ester), phosphatidylethanolamine, phosphatidylcholine, sphingomyelin, palmitic acid, *n*-tetradecanol, triacylglycerol, and cholesterol. In what order do you expect the lipids to elute from the column?

9. *Storage of Fat-Soluble Vitamins* In contrast to water-soluble vitamins, which must be a part of our daily diet, fat-soluble vitamins can be stored in the body in amounts sufficient for many months. Suggest an explanation for this difference based on solubilities.

10. *Alkali Lability of Triacylglycerols* A common procedure for cleaning the grease trap in a sink is to add a product that contains sodium hydroxide. Explain why this works.

11. *Dependence of Melting Point on Fatty Acid Unsaturation* Draw all of the possible triacylglycerols that you could construct from glycerol, palmitic acid, and oleic acid. Rank them in order of increasing melting point.

12. *Operational Definition of Lipids* How is the definition of "lipid" different from the definitions of other types of biomolecules that we have considered, such as amino acids, nucleic acids, and proteins?

13. *Effect of Polarity on Solubility* Rank, in order of increasing solubility in water, a triacylglycerol, a diacylglycerol, and a monoacylglycerol, all containing only palmitic acid.

14. *Intracellular Messengers from Phosphatidylinositols* When the hormone vasopressin stimulates cleavage of phosphatidylinositol-4,5-bisphosphate by hormone-sensitive phospholipase C, two products are formed. Compare their properties and solubilities in water, and predict whether either would be expected to diffuse readily through the cytosol.

15. *Identification of Unknown Lipids* Johann Thudichum, who practiced medicine in London about 100 years ago, also dabbled in lipid chemistry in his spare time. He isolated a variety of lipids from neural tissue, and characterized and named many of them. His carefully sealed and labeled vials of isolated lipids were rediscovered many years later. How would you confirm, using techniques available to you but not to him, that the vials he labeled "sphingomyelin" and "cerebroside" actually contain these compounds?

16. *Analysis of Choline-Containing Phospholipids* How would you distinguish sphingomyelin from phosphatidylcholine by chemical, physical, or enzymatic tests?

CHAPTER

Biological Membranes and Transport

The first living cell probably came into being when a membrane formed, separating that cell's precious contents from the rest of the universe. Membranes define the external boundary of cells and regulate the molecular traffic across that boundary; they divide the internal space into discrete compartments to segregate processes and components (Fig. 10–1); they organize complex reaction sequences; and they are central to both biological energy conservation and cell-to-cell communication. The biological activities of membranes flow from their remarkable physical properties. Membranes are tough but flexible, self-sealing, and selectively permeable to polar solutes. Their flexibility permits the shape changes that accompany cell growth and movement (such as amoeboid movement). Their ability to seal over temporary breaks in their continuity allows two membranes to fuse, as in exocytosis, or a single membrane-enclosed compartment to undergo fission, yielding two sealed compartments, as in endocytosis or cell division, without creating gross leaks through the cell surface. Because membranes are selectively permeable, they retain certain compounds and ions within cells and within specific cellular compartments, and exclude others.

Membranes are not merely passive barriers. They include an array of proteins specialized for promoting or catalyzing a variety of molecular events. Pumps move specific organic solutes and inorganic ions across the membrane against a concentration gradient; energy transducers convert one form of energy into another; receptors on the plasma membrane sense extracellular signals, converting them into molecular changes within the cell.

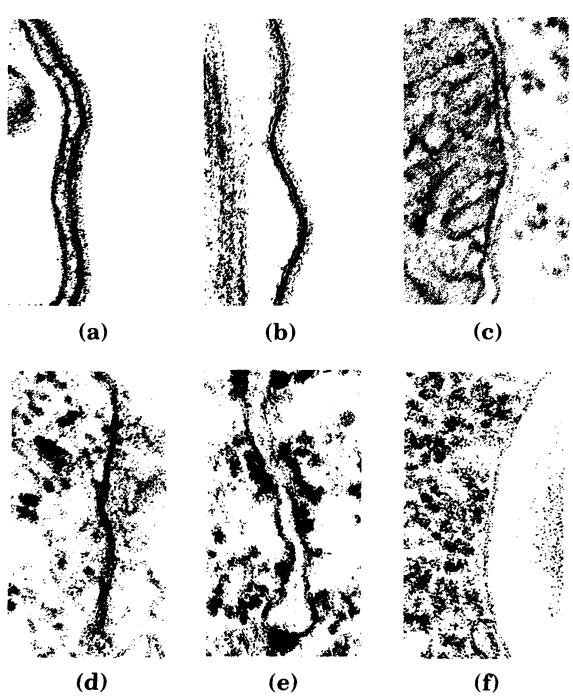


Figure 10–1 Viewed in cross section, all intracellular membranes share a characteristic trilaminar appearance. The protozoan *Paramecium* contains a variety of specialized membrane-bounded organelles. When a thin section of a *Paramecium* is stained with osmium tetroxide to highlight membranes, each of the membranes appears as a three-layer structure, 5 to 8 nm thick. The trilaminar images consist of two electron-dense layers on the inner and outer surfaces separated by a less dense central region. At left are high-magnification views of the membranes of **(a)** a cell body (plasma and alveolar membranes tightly apposed), **(b)** a cilium, **(c)** a mitochondrion, **(d)** a digestive vacuole, **(e)** the endoplasmic reticulum, and **(f)** a secretory vesicle.

Membranes are composed of just two layers of molecules, and are therefore very thin; they can be thought of as essentially two-dimensional. A large number of cellular processes are associated with membranes (such as the synthesis of lipids and certain proteins, and the energy transductions in mitochondria and chloroplasts). Because intermolecular collisions are far more probable in this two-dimensional space than in three-dimensional space, the efficiency of certain enzyme-catalyzed pathways organized within a two-dimensional membrane is vastly increased.

In this chapter we first describe the composition of cellular membranes and their chemical architecture—the physical structure that underlies their biological functions. We then turn to membrane transport, the protein-mediated transmembrane passage of solutes. In later chapters we will discuss the role of membranes in energy transduction, lipid synthesis, signal transduction, and protein synthesis.

The Molecular Constituents of Membranes

One approach to understanding membrane function is to study membrane composition—to determine, for example, which components are commonly present in membranes and which are unique to membranes with specific functions. Knowledge of composition is also invaluable in studies of membrane structure, as any viable model for membrane structure must conform to and explain the known composition. Before describing membrane structure and function, we therefore consider the molecular components of membranes.

Proteins and polar lipids account for almost all of the mass of biological membranes; the small amount of carbohydrate present is generally part of glycoproteins or glycolipids. The relative proportions of protein and lipid differ in different membranes (Table 10–1), reflecting the diversity of biological roles. The myelin sheath, which serves as a passive electrical insulator wrapped around certain neurons, consists primarily of lipids, but the membranes of bacteria, mitochondria, and chloroplasts, in which many enzyme-catalyzed metabolic processes take place, contain more protein than lipid.

Table 10–1 Major components of plasma membranes of different species*

	Protein (%)	Phospholipid (%)	Other lipids	Sterol (%)	Sterol type
Mouse liver	45	27	—	25	Cholesterol
Corn leaf	47	26	Galactolipids	7	Sitosterol
Yeast	52	7	Triacylglycerols Steryl esters	4	Ergosterol
Paramecium (ciliate protist)	56	40	—	4	Stigmasterol
E. coli	75	25	—	0	—

* Values are given as weight percentages.

Each Membrane Has a Characteristic Lipid Composition

For studies of membrane composition, it is essential first to isolate the membrane of interest. When eukaryotic cells are subjected to mechani-

Table 10–2 Lipid composition of organelle membranes of a rat liver cell*

	Chol	PC	PE	PS	PI	PG	CL	SM
Plasma membrane	30	18	11	9	4	0	0	14
Golgi complex	8	40	15	4	6	0	0	10
Smooth endoplasmic reticulum	10	50	21	0	7	0	2	12
Rough endoplasmic reticulum	6	55	16	3	8	0	0	3
Nuclear membrane	10	55	20	3	7	0	0	3
Lysosomal membrane	14	25	13	0	7	0	5	24
Mitochondrial membrane								
Inner	3	45	24	1	6	2	18	3
Outer	5	45	23	2	13	3	4	5

* Values are given as weight percentages. Chol designates cholesterol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PG, phosphatidylglycerol; CL, cardiolipin; SM, sphingomyelin.

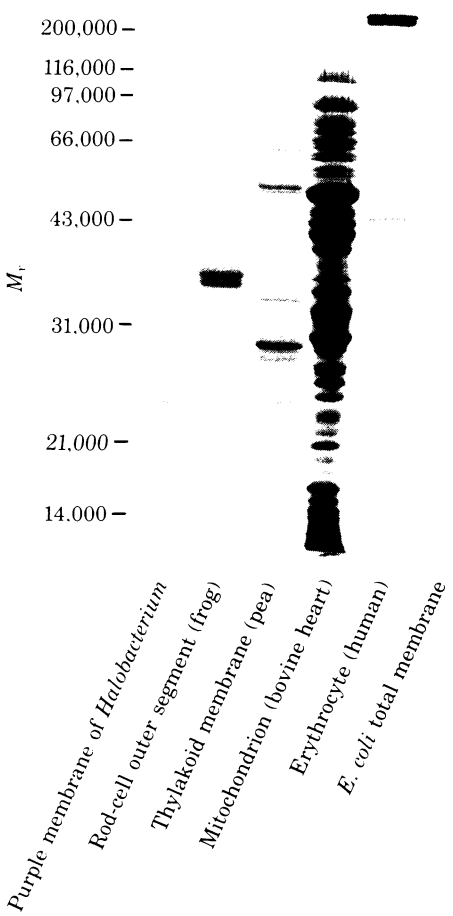


Figure 10–2 Membranes with specialized functions differ in protein composition, as revealed by electrophoretic separation on a polyacrylamide gel in the presence of the detergent SDS (p. 141). The purple membrane of *Halobacterium* and the rod-cell outer segment membrane are very rich in bacteriorhodopsin and rhodopsin, respectively. The myelin sheath also contains relatively few kinds of proteins. The other membranes shown have more complex functions, reflected in a wider variety of membrane proteins.

cal shear, their plasma membranes are torn and fragmented, releasing cytosolic components and membrane-bounded organelles: mitochondria, chloroplasts, lysosomes, nuclei, and others. The plasma membrane fragments and intact organelles can be isolated by centrifugal techniques described in Chapter 2 (see Fig. 2–24).

Chemical analysis of membranes isolated from various sources reveals certain common properties. Membrane lipid composition is characteristic for each kingdom, each species, each tissue, and each organelle within a given cell type (Table 10–2). Cells clearly have mechanisms to control the kinds and amounts of membrane lipids synthesized and to target specific lipids to particular organelles. These distinct combinations doubtless confer advantages on cells and organisms during evolution, but in most cases the functional significance of these characteristic lipid compositions remains to be discovered.

Membranes with Different Functions Have Different Proteins

The protein composition of membranes from different sources (Fig. 10–2) varies even more widely than their lipid composition, reflecting functional specialization. The outer segment of the rod cells of the vertebrate retina is highly specialized for the reception of light; more than 90% of its membrane protein is the light-absorbing protein rhodopsin (see Fig. 9–18). The less-specialized plasma membrane of the erythrocyte has about 20 prominent proteins as well as dozens of minor ones; many of these serve as transporters, each responsible for moving a specific solute across the membrane. The inner (plasma) membrane of *E. coli* contains hundreds of different proteins, various transporters, as well as many enzymes involved in energy-conserving metabolism, lipid synthesis, protein export, and cell division. The outer membrane of *E. coli* has a different function (protection) and a different set of proteins. Some membrane proteins have more or less complex arrays of covalently bound carbohydrates, which may make up from 1 to 70% of the total mass of these glycoproteins. In the rhodopsin of the vertebrate eye, a single hexasaccharide makes up 4% of the mass; in glycophorin, a glycoprotein of the plasma membrane of erythrocytes, 60% of the mass consists of complex polysaccharide units covalently attached to specific amino acid residues. Ser, Thr, and Asn residues are often the points of attachment (see Fig. 11–23). In general, plasma

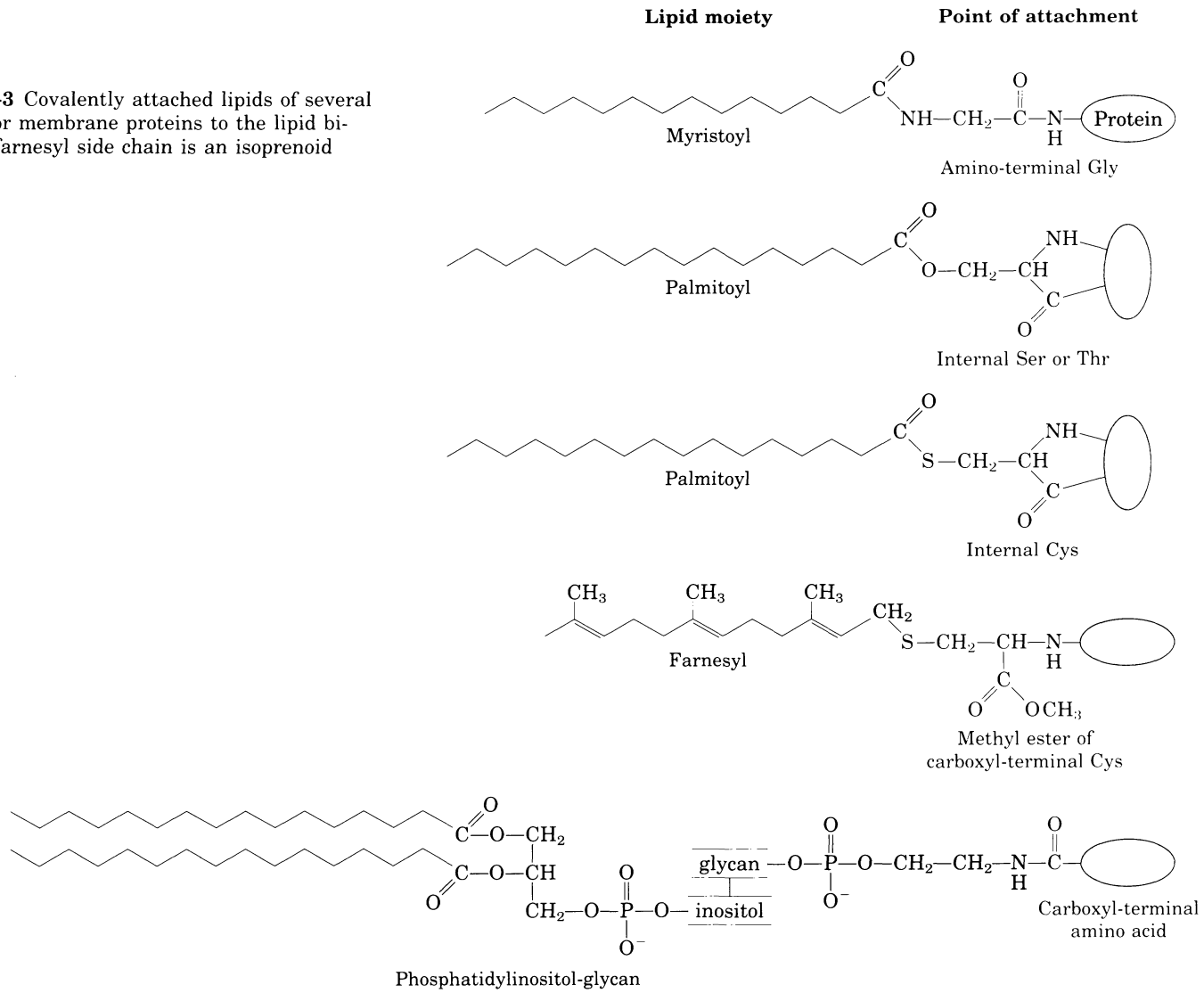


Figure 10–3 Covalently attached lipids of several types anchor membrane proteins to the lipid bilayer. The farnesyl side chain is an isoprenoid (p. 256).

membranes contain many glycoproteins, but intracellular membranes such as those of mitochondria and chloroplasts rarely contain covalently bound carbohydrates. The sugar moieties of surface glycoproteins influence the protein folding, transport to the cell surface, and receptor functions of these glycoproteins.

Certain membrane proteins are covalently attached to one or more lipids, which probably serve as hydrophobic anchors, holding the proteins to the membrane. The lipid moiety on some membrane proteins is a fatty acid, attached in amide or ester linkage; other proteins have a long-chain isoprenoid covalently attached, and others are joined through a complex polysaccharide (a glycan; see Chapter 11) to a molecule of phosphatidylinositol (Fig. 10–3).

The Supramolecular Architecture of Membranes

All biological membranes share certain fundamental properties. They are impermeable to most polar or charged solutes, but permeable to nonpolar compounds; are 5 to 8 nm thick; appear trilaminar (three-layered) when viewed in cross section with the electron microscope (see Fig. 10–1). The combined evidence from electron microscopy, chemical composition, and physical studies of permeability and of the motion of

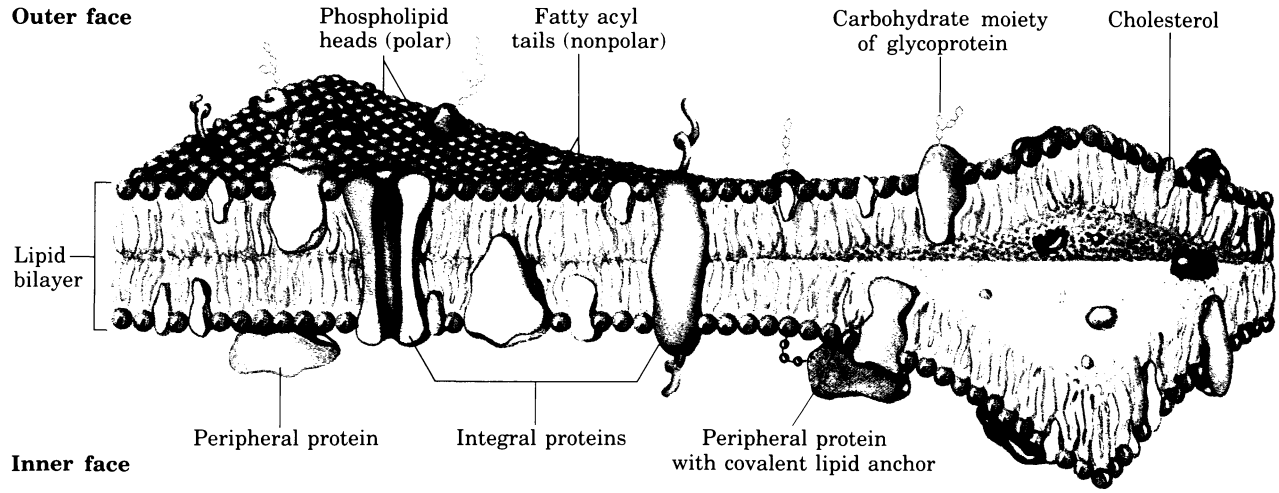


Figure 10–4 The fluid mosaic model for membrane structure. The fatty acyl chains in the interior of the membrane form a fluid, hydrophobic region. Integral membrane proteins float in this sea of lipid, held by hydrophobic interactions with their nonpolar amino acid side chains. Both proteins and lipids are free to move laterally in the plane of the bilayer, but movement of either from one face of the bilayer to the other is restricted. The carbohydrate moieties attached to some proteins and lipids of the plasma membrane are invariably exposed on the extracellular face of the membrane.

individual protein and lipid molecules within membranes supports the **fluid mosaic model** for the structure of biological membranes (Fig. 10–4). Amphipathic phospholipids and sterols form a lipid bilayer, with the nonpolar regions of lipids facing each other at the core of the bilayer and their polar head groups facing outward. In this lipid bilayer, globular proteins are embedded at irregular intervals, held by hydrophobic interactions between the membrane lipids and hydrophobic domains in the proteins. Some proteins protrude from one or the other face of the membrane; most span its entire width. The orientation of proteins in the bilayer is asymmetric, giving the membrane "sidedness"; the protein domains exposed on one side of the bilayer are different from those exposed on the other side, reflecting functional asymmetry. The individual lipid and protein subunits in a membrane form a fluid mosaic; its pattern, unlike a mosaic of ceramic tile and mortar, is free to change constantly. The membrane mosaic is fluid because the interactions among lipids, and between lipids and proteins, are noncovalent, leaving individual lipid and protein molecules free to move laterally in the plane of the membrane.

We will now look at some of these features of the fluid mosaic model in more detail, and consider the experimental evidence that supports it.

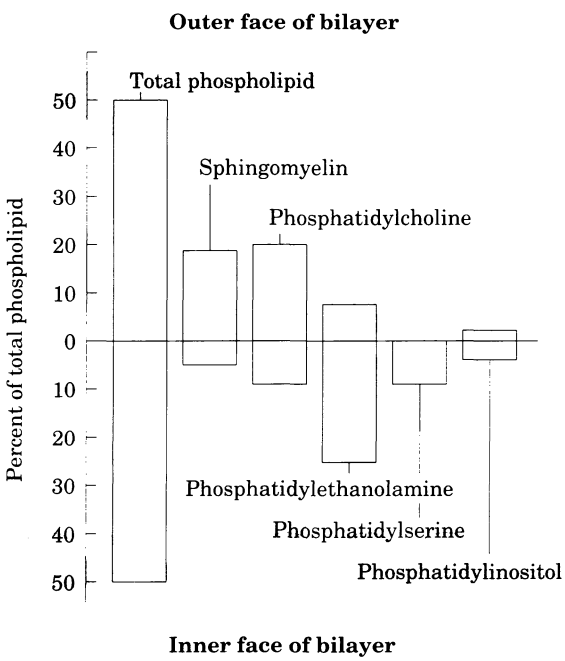


Figure 10–5 The distribution of specific erythrocyte membrane lipids between the inner and outer face is asymmetric.

A Lipid Bilayer Is the Basic Structural Element

We saw in Chapter 9 that lipids, when suspended in water, spontaneously form bilayer structures that are stabilized by hydrophobic interactions (see Fig. 9–14). The thickness of biological membranes (5 to 8 nm, measured by electron microscopy) is about that expected for a lipid bilayer 3 nm thick with proteins protruding on each side. X-ray diffraction by membranes shows the distribution of electron density expected for a bilayer structure. Liposomes (lipid vesicles) formed in the laboratory show the same relative impermeability to polar solutes as is seen in biological membranes (although the latter are permeable to solutes for which they have specific transporters). In short, all evidence indicates that biological membranes are constructed of lipid bilayers.

Membrane lipids are asymmetric in their distribution on the two faces of the bilayer, although the asymmetry, unlike that of membrane proteins, is not absolute. In the plasma membrane, for example, certain lipids are typically found primarily in the outer face of the bilayer, and others in the inner (cytoplasmic) face (Fig. 10–5).

Membrane Lipids Are in Constant Motion

Although the lipid bilayer structure itself is stable, the individual phospholipid and sterol molecules have great freedom of motion within the plane of the membrane (Fig. 10–6). They diffuse laterally so fast that an individual lipid molecule can circumnavigate an erythrocyte in a few seconds. The interior of the bilayer is also fluid; individual hydrocarbon chains of fatty acids are in constant motion produced by rotation about the carbon–carbon bonds of the long acyl side chains.

The degree of fluidity depends on lipid composition and temperature. At low temperature, relatively little lipid motion occurs and the bilayer exists as a nearly crystalline (paracrystalline) array. Above a temperature that is characteristic for each membrane, lipids can undergo rapid motion. The temperature of the transition from paracrystalline solid to fluid depends upon the lipid composition of the membrane. Saturated fatty acids pack well into a paracrystalline array, but the kinks in unsaturated fatty acids (see Fig. 9–1) interfere with this packing, preventing the formation of a paracrystalline solid state. The higher the proportion of saturated fatty acids, the higher is the solid-to-fluid transition temperature of the membrane.

The sterol content of a membrane also is an important determinant of this transition temperature. The rigid planar structure of the steroid nucleus, inserted between fatty acyl side chains, has two effects on fluidity: below the temperature of the solid-to-fluid transition, sterol insertion prevents the highly ordered packing of fatty acyl chains, and thus fluidizes the membrane. Above the thermal transition point, the rigid ring system of the sterol reduces the freedom of neighboring fatty acyl chains to move by rotation about carbon–carbon bonds, and thus reduces the fluidity in the core of the bilayer. Sterols therefore tend to moderate the extremes of solidity and fluidity of the membranes that contain them.

Both microorganisms and cultured animal cells regulate their lipid composition so as to achieve a constant fluidity under various growth conditions. For example, when cultured at low temperatures, bacteria synthesize more unsaturated fatty acids and fewer saturated ones than when cultured at higher temperatures (Table 10–3). As a result of this adjustment in lipid composition, membranes of bacteria cultured at high or low temperature have about the same degree of fluidity.

Table 10–3 Fatty acid composition of *E. coli* cells cultured at different temperatures

	Percentage of total fatty acids*			
Fatty acid	10 °C	20 °C	30 °C	40 °C
Myristic (14:0)	4	4	4	8
Palmitic (16:0)	18	25	29	48
Palmitoleic (16:1)	26	24	23	9
Oleic (18:1)	38	34	30	12
Hydroxymyristic	13	10	10	8
Ratio of unsaturated: saturated†	2.9	2.0	1.6	0.38

Source: Data from Marr, A.G. & Ingraham, J.L. (1962) Effect of temperature on the composition of fatty acids in *Escherichia coli*. *J. Bacteriol.* **84,** 1260.

* The exact fatty acid composition depends not only on growth temperature, but also on growth stage and growth medium composition.

† The total percentage of 16:1 plus 18:1 divided by total percentage of 14:0 plus 16:0. Hydroxymyristic acid was omitted from this calculation.

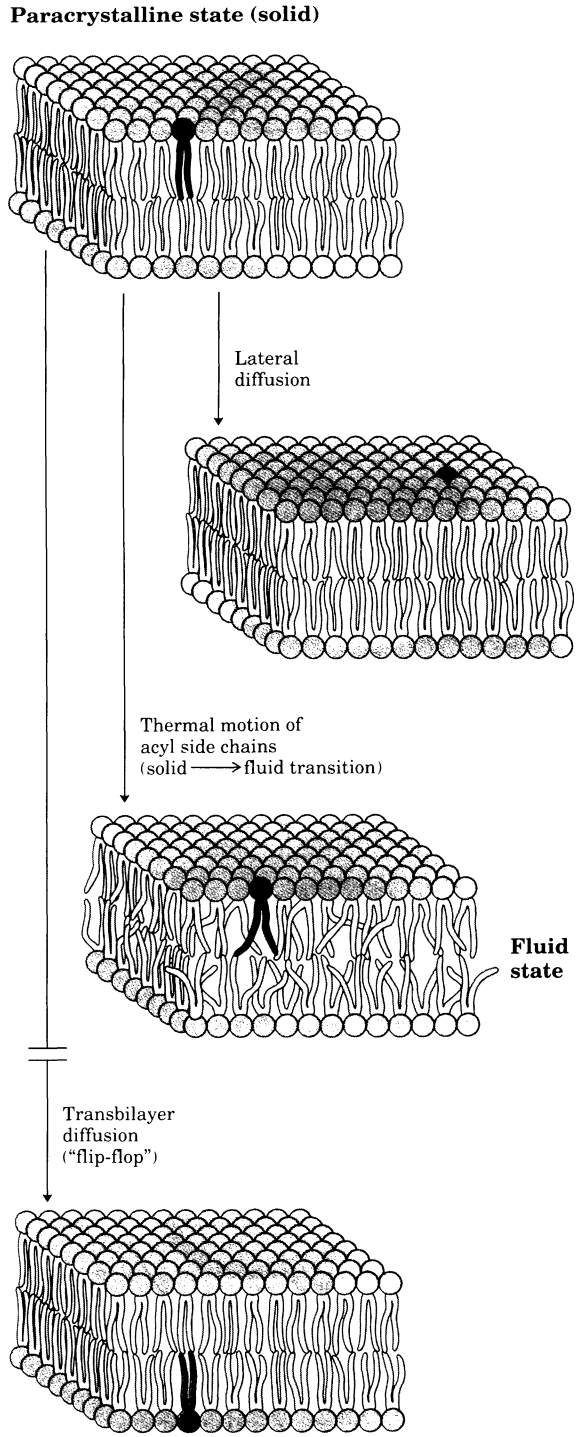
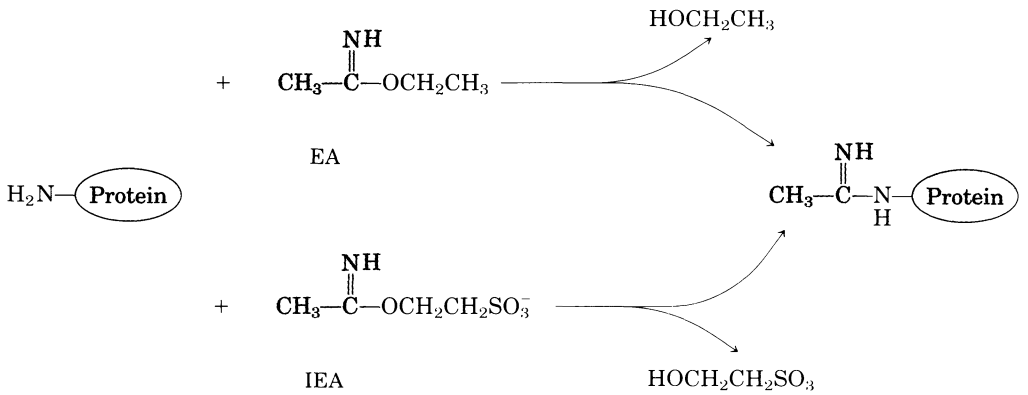
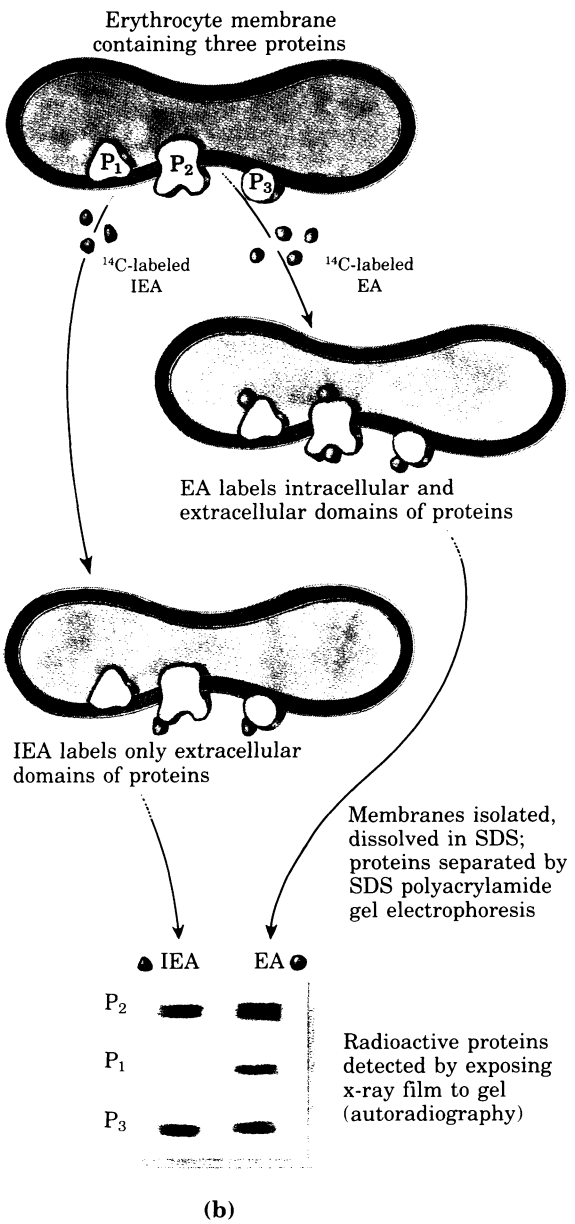


Figure 10–6 Lipid motion within a bilayer includes lateral diffusion of individual molecules within one face of the bilayer, and thermal motion of the fatty acyl groups in the bilayer interior. Because of the thermal motion, the bilayer is fluid above a certain temperature; as the temperature is lowered, lipids become paracrystalline. Flip-flop diffusion is a very uncommon event.



(a)



(b)

Although lateral migration of membrane components and thermal flexing of the acyl chains clearly occurs, a third kind of motion is restricted: transbilayer or "flip-flop" diffusion, the movement of a lipid from one face of the bilayer to the other (Fig. 10–6). For such motion to occur, the lipid head group, which is polar and may be charged, must leave its aqueous environment and move into the hydrophobic interior of the bilayer, a process with a large, positive free-energy change (highly endergonic). During synthesis of the bacterial plasma membrane, for example, phospholipids produced on the inside surface of the membrane must undergo flip-flop diffusion to enter the outer face of the bilayer. There are proteins that facilitate flip-flop diffusion, providing a transmembrane path that is energetically more favorable.

Membrane Proteins Penetrate and Span the Lipid Bilayer

When individual protein molecules and multiprotein complexes in freeze-fractured biological membranes are visualized with the electron microscope (Box 10–1), some proteins appear on only one face of the membrane; most span the full thickness of the bilayer, and protrude from both inner and outer membrane surfaces. Among the latter are some proteins that conduct solutes or signals across the membrane.

Membrane protein localization has also been investigated with reagents that react with protein side chains but cannot cross membranes (Fig. 10–7). The human erythrocyte is convenient for such studies, because the plasma membrane is the only membrane present.

Figure 10–7 Experiments to determine the transmembrane arrangement of membrane proteins. **(a)** Both ethylacetimidate (EA) and isoethionylacetimidate (IEA) react with free amino groups in proteins, but only the ethyl derivative (EA) diffuses freely through the membrane. **(b)** Comparison of the labeling patterns with the two reagents reveals whether a given protein is exposed only on the outer surface or only on the inner surface. Proteins labeled by both reagents, but more heavily by the permeant reagent, are exposed on both sides, and thus span the membrane.

BOX 10–1 Electron Microscopy of Membranes

Combined with different staining procedures and tissue-preparation methods, electron microscopy has revealed important details of membrane structure. Shown here are three different aspects of the erythrocyte membrane, visualized by electron microscopy after three different ways of preparing the cells for examination.

Figure 1 is a **transmission electron micrograph** of a section through the erythrocyte membrane, showing the two dense lines visible after osmium tetroxide staining of cells, corresponding to the outer and inner polar layers of the membrane-lipid head groups. The clear zone between the lines is the hydrophobic portion of the lipid bilayer, which contains the nonpolar fatty acyl tails.

Figure 2 shows the glycocalyx on the outer surface of the erythrocyte, visualized by a special staining procedure. This "fuzzy coat," which consists of hydrophilic oligosaccharide groups of membrane glycoproteins and glycolipids, is over 100 nm thick, more than ten times the thickness of the lipid bilayer itself.

A view of the inside of the erythrocyte membrane (illustrated in Figure 3), is produced by the **freeze-fracture** method. In this procedure the cells are frozen and the frozen block is shattered or split. The fracture lines sometimes split a membrane along a plane between the two lipid layers. The exposed surface is coated with a very thin layer of carbon, then visualized with the electron microscope (Fig. 4). The inside surface of one lipid layer forms the smooth background; the clusters of globular bodies are molecules of integral membrane proteins.

Figure 1

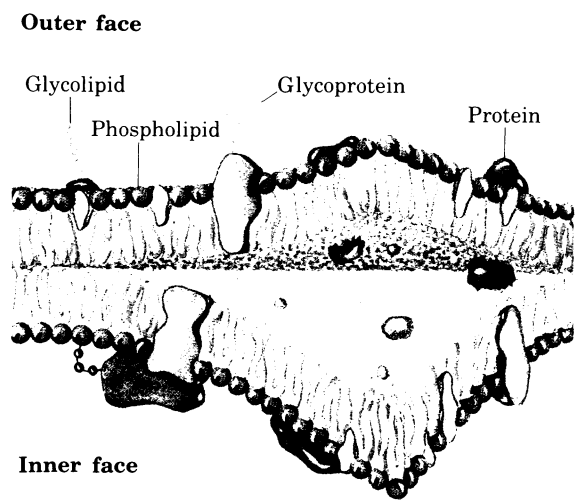


Figure 3

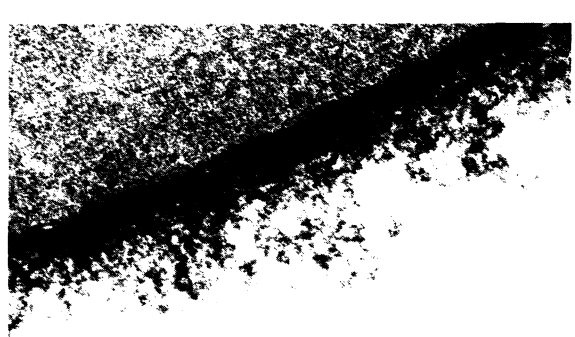


Figure 2

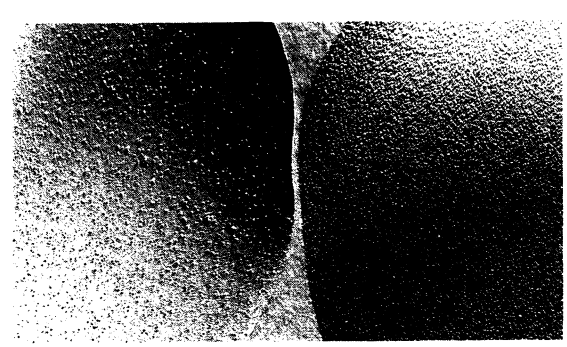


Figure 4

Experiments like those described in Figure 10–7 show that the glycophorin molecule spans the erythrocyte membrane. Its amino-terminal domain (bearing the carbohydrate) is on the outer surface of the erythrocyte, and its carboxyl terminus protrudes on the inside of the cell. The amino-terminal and carboxyl-terminal domains contain many polar or charged amino acid residues, and are therefore quite hydrophilic. However, a long segment in the center of the protein contains mainly hydrophobic amino acid residues. These findings suggest the transmembrane arrangement of glycophorin shown in Figure 10–8.

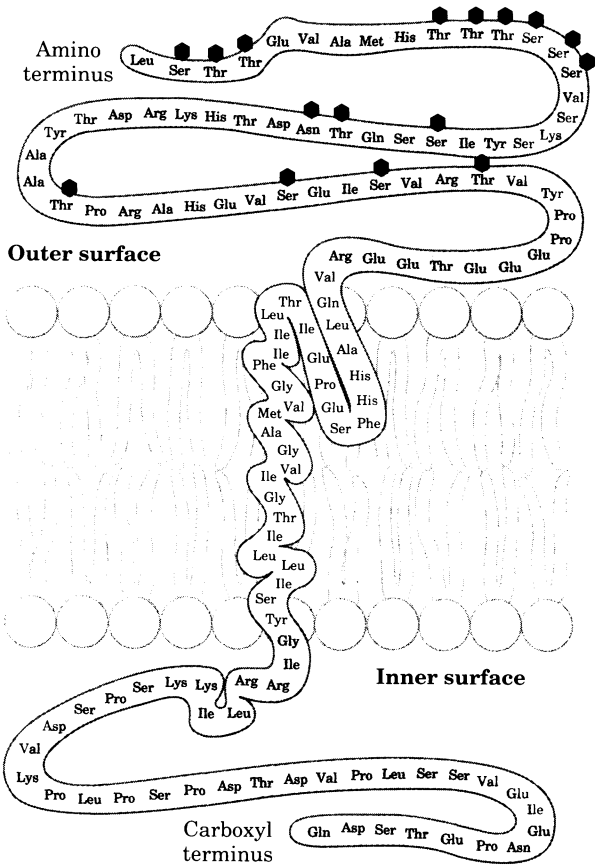


Figure 10–8 Transbilayer disposition of glycophorin in the erythrocyte. One hydrophilic domain, containing all the sugar residues, is on the outer surface, and another hydrophilic domain protrudes on the inner surface. The red hexagons represent a tetrasaccharide (containing two NeuNAc, Gal, and GalNAc) O-linked to a serine or threonine residue; the blue hexagon represents an oligosaccharide chain N-linked to an asparagine residue. A segment of relatively hydrophobic residues forms an α helix that traverses the membrane bilayer.

Membrane Proteins Are Oriented Asymmetrically

One further fact may be deduced from the results of the experiments with glycophorin: it does not move from one face of the bilayer to the other; its disposition in the membrane is asymmetric. Similar studies of other membrane proteins show that each has a specific orientation in the bilayer, and that protein reorientation by flip-flop diffusion occurs seldom, if ever. Furthermore, glycoproteins of the plasma membrane are invariably situated with their sugar residues on the outer surface of the cell. As we shall see, the asymmetric arrangement of membrane proteins results in functional asymmetry; all the molecules of a given ion pump, for example, have the same orientation and therefore all pump in the same direction.

Integral Membrane Proteins Are Insoluble in Water

Membrane proteins may be divided operationally into two groups: **integral** (intrinsic) **proteins,** which are very firmly bound to the membrane, and **peripheral** (extrinsic) **proteins,** which are bound more loosely, or reversibly. Peripheral membrane proteins can be released from membranes by relatively mild treatments (Fig. 10–9), and once released from the membrane they are generally water soluble. In contrast, the release of integral proteins from membranes requires the action of agents (detergents, organic solvents, or denaturants) that interfere with hydrophobic interactions. Even after integral proteins have been solubilized, removal of the detergent may cause the protein to precipitate as an insoluble aggregate. The insolubility of integral membrane proteins results from the presence of domains rich in hydrophobic amino acids; hydrophobic interactions between the protein and the lipids of the membrane account for the firm attachment of the protein.

Some Integral Proteins Have Hydrophobic Transmembrane Anchors

Integral membrane proteins generally have domains rich in hydrophobic amino acids. In some proteins, there is a single hydrophobic sequence in the middle of the protein (as in glycophorin) or at the amino or carboxyl terminus. Other membrane proteins have multiple hydrophobic sequences, each long enough to span the lipid bilayer when in the α-helical conformation.

One of the best-studied membrane-spanning proteins, bacteriorhodopsin, contains seven very hydrophobic internal sequences, and crosses the lipid bilayer seven times. Bacteriorhodopsin is a light-driven proton pump that is densely packed in regular arrays in the purple membrane of the bacterium *Halobacterium halobium*. When these arrays are viewed with the electron microscope from several angles, the resulting images allow a three-dimensional reconstruction of the bacteriorhodopsin molecule (Fig. 10–10). Seven α-helical segments, each traversing the lipid bilayer, are connected by nonhelical loops at the inner or outer face of the membrane. The amino acid sequence of bacteriorhodopsin has seven segments with about 20 hydrophobic residues, each segment just long enough to make a helix that spans the lipid bilayer. Hydrophobic interactions between the nonpolar amino acids and the acyl side chains of the membrane lipids firmly anchor the protein in the membrane, providing a transmembrane pathway for proton translocation.

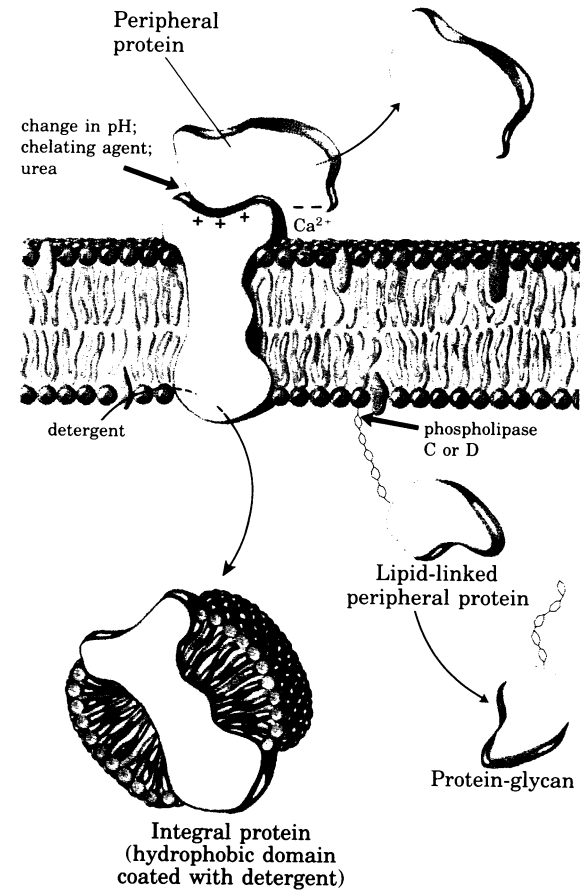


Figure 10–9 Membrane proteins can be distinguished by the conditions required to release them from the membrane. Most peripheral proteins can be released by changes in pH or ionic strength, removal of Ca^{2+} by a chelating agent, or addition of urea, which breaks hydrogen bonds. Peripheral proteins covalently attached to a membrane lipid, such as through a phosphatidylinositol–glycan anchor (see Fig. 10–3), are released by phospholipase C or D. Integral proteins can be extracted with detergents, which disrupt the hydrophobic interactions with the lipid bilayer, forming micelles with individual protein molecules.

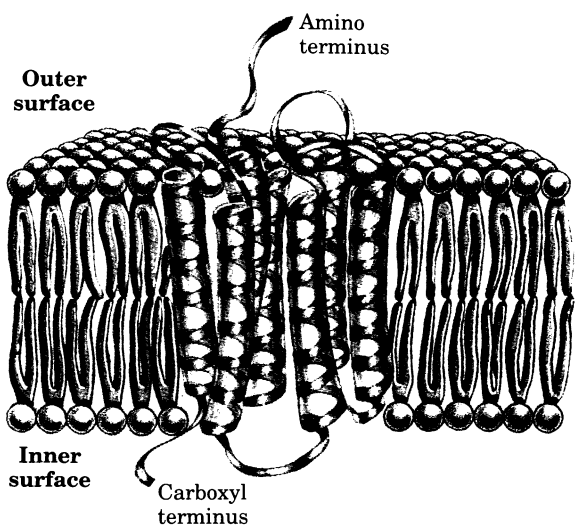


Figure 10–10 The single polypeptide chain of bacteriorhodopsin folds into seven hydrophobic α helices, each of which traverses the lipid bilayer and is roughly perpendicular to the plane of the membrane. The seven transmembrane helices are clustered, and the space around and between them is filled with the acyl chains of membrane lipids.

BOX 10–2 Predicting the Topology of Membrane Proteins

It is generally much easier to determine the amino acid sequence of a membrane protein (by sequencing the protein itself or its gene) than to determine its three-dimensional structure. Consequently, only a few three-dimensional structures are known, but hundreds of sequences are available for membrane proteins. The sequences of most integral proteins contain one or more regions rich in hydrophobic residues and long enough to span the 3 nm thick lipid bilayer. An α-helical peptide of 20 residues is just long enough to span the bilayer (the length per residue is 0.15 nm). Because a polypeptide chain surrounded by lipids has no water molecules with which to form hydrogen bonds, it will tend to fold into α helices or β sheets, in which intrachain hydrogen bonding is maximized. If the side chains of all amino acids in a helix are nonpolar, hydrophobic interactions with the surrounding lipids further stabilize the helices.

Several simple methods of analyzing amino acid sequences have been found to yield reasonably accurate predictions of secondary structure for transmembrane proteins. The relative polarity of each of the 20 amino acids has been determined experimentally by measuring the free-energy change of moving a given residue from a hydrophobic solvent into water. This free energy of transfer ranges from very exergonic for charged or polar residues to very endergonic for amino acids with aromatic or aliphatic hydrocarbon side chains (Table 1). To estimate the overall hydrophobicity of a sequence of amino acids, one sums the free energies of transfer for those residues, obtaining a **hydropathy index** for that region. To search a sequence for potential membrane-spanning segments, one calculates the hydropathy index for successive segments of a given size (a "window," which may be from 7 to 20 residues). For a window of 7 residues, the indexes for residues 1 to 7, 2 to 8, 3 to 9, and so on, are plotted as in Figure 1. A region of about 20 residues of high hydropathy index is presumed to be a transmembrane segment. When the sequences of membrane proteins of known three-dimensional structure are scanned in this way, a reasonably good correspondence is found between predicted and known membrane-spanning segments. Hydropathy analysis predicts a single hydrophobic helix for glycophorin (Fig. 1a), five for the M subunit of the photosynthetic reaction center protein (Fig. 1b), seven transmembrane segments for bacteriorhodopsin (Fig. 1c), and twelve segments for the chloride–bicarbonate exchanger (Fig. 1d).

Many of the transport proteins described in this chapter are believed, on the basis of their amino acid sequences and hydropathy plots, to have multiple membrane-spanning helical regions. These assignments of topology should be considered tentative until confirmed by direct structural determination.

Table 1 Residue hydrophobicity

Amino acid	Free energy of transfer (kJ/mol)
Ile	3.1
Phe	2.5
Val	2.3
Leu	2.2
Trp	1.5
Met	1.1
Ala	1.0
Gly	0.67
Cys	0.17
Tyr	0.08
Pro	−0.29
Thr	−0.75
Ser	−1.1
His	−1.7
Glu	−2.6
Asn	−2.7
Gln	−2.9
Asp	−3.0
Lys	−4.6
Arg	−7.5

Source: From Eisenberg, D., et al. (1982) Hydrophobic moments in protein structure. *Faraday Symp. Chem. Soc.* **17,** 109–120.

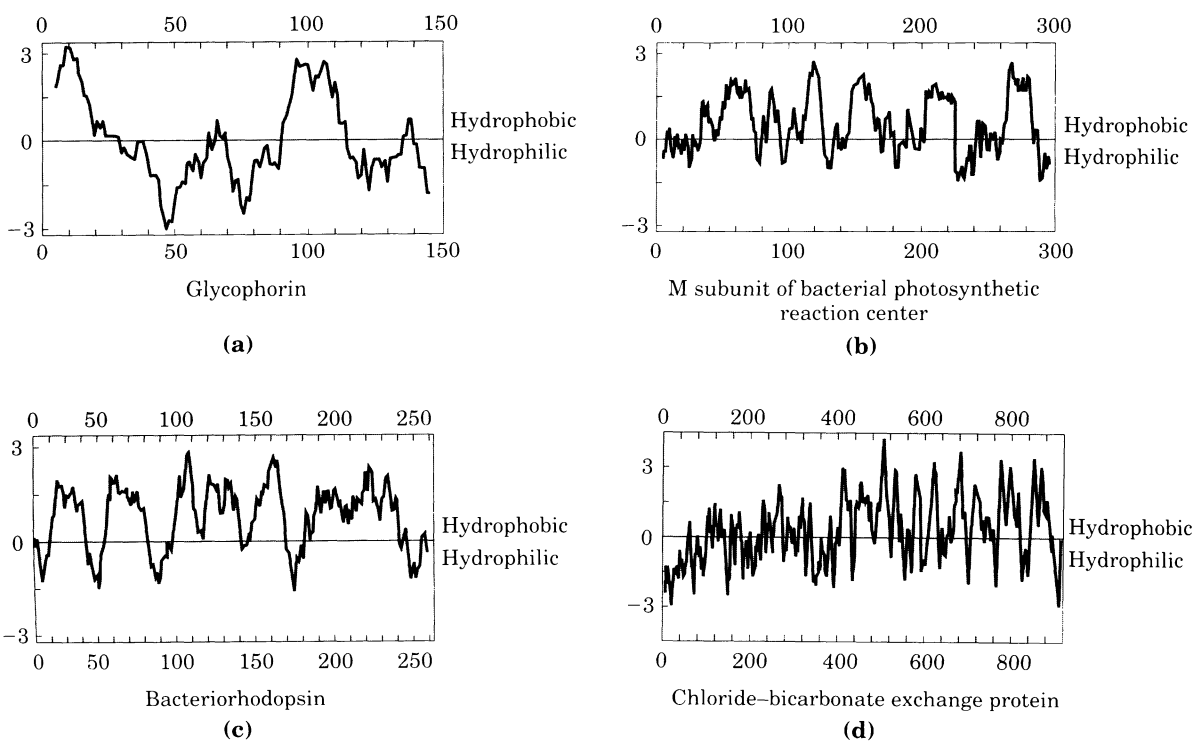


Figure 1 Plots of hydropathy index against residue number for four integral membrane proteins.

This pattern of seven hydrophobic membrane-spanning helices has proven to be a common motif in membrane structure, seen in at least ten other membrane proteins, all involved in signal reception. Although no information is yet available on the three-dimensional structures of these proteins, it seems likely that they will prove to be structurally similar to bacteriorhodopsin. The presence of long hydrophobic regions along the amino acid sequence of a membrane protein is generally taken as evidence that such sequences traverse the lipid bilayer, acting as hydrophobic anchors or forming transmembrane channels; virtually all integral membrane proteins have at least one such sequence (Box 10–2). When sequence information yields predictions consistent with chemical studies of protein localization (such as those described above for glycophorin and bacteriorhodopsin), the assumption that hydrophobic regions correspond to membrane-spanning domains is better justified.

The Structure of a Crystalline Integral Membrane Protein Has Been Determined

The same techniques that have allowed determination of the three-dimensional structures of many soluble proteins can in principle be applied to membrane proteins. However, very few membrane proteins have been crystallized; they tend instead to form amorphous aggregates. One instructive exception is the photosynthetic reaction center from a purple bacterium (Fig. 10–11). The protein has four subunits, three of which contain α-helical segments that span the membrane. These segments are rich in nonpolar amino acids, and their hydrophobic side chains are oriented toward the outside of the protein, interacting with the hydrocarbon side chains of membrane lipids. The architecture of the reaction center protein is therefore the inverse of that seen in most water-soluble proteins, which have their hydrophobic residues buried within the protein core and their hydrophilic residues on the surface available for polar interactions with water (recall the structures of myoglobin and hemoglobin, for example). The structures of only a few membrane proteins are known, but the hydrophobic exterior of the reaction center protein seems to be typical of integral membrane proteins.

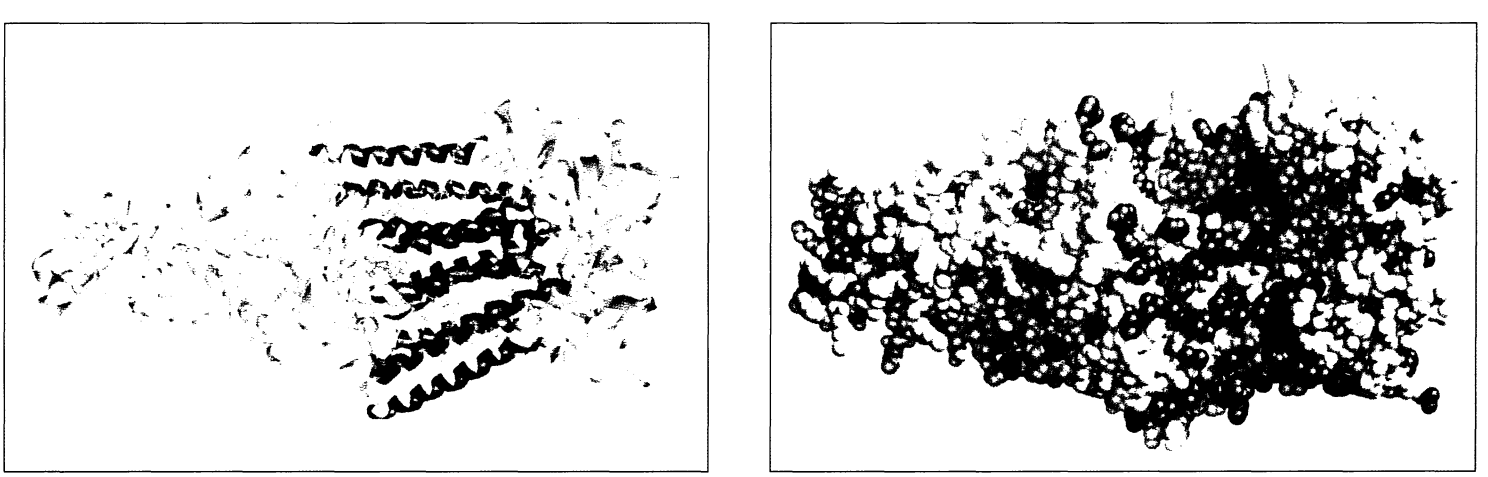


Figure 10–11 Three-dimensional structure of the photosynthetic reaction center of a purple bacterium, *Rhodopseudomonas viridis*. This was the first integral membrane protein to have its atomic structure determined by x-ray diffraction methods. Eleven α-helical segments from three of the four subunits span the lipid bilayer, forming a cylinder 4.5 nm long, with hydrophobic residues on the exterior, interacting with lipids of the bilayer. In the ribbon representation at left, the residues that are part of the transmembrane helices are shown in purple. The high density of nonpolar residues in the region of the bilayer is illustrated in the space-filling model on the right, in which the four very hydrophobic residues (Phe, Val, Ile, Leu) are shown in purple. In both views, the prosthetic groups (light-absorbing pigments and electron carriers; see Fig. 18–47) are shown in yellow.

Peripheral Proteins Associate Reversibly with the Membrane

Many peripheral proteins are held to the membrane by electrostatic interactions and hydrogen bonding with the hydrophilic domains of integral membrane proteins, and perhaps with the polar head groups of membrane lipids. They can be released by relatively mild treatments that interfere with electrostatic interactions or break hydrogen bonds (see Fig. 10–9). These peripheral proteins may serve as regulators of membrane-bound enzymes, or as tethers that connect integral membrane proteins to intracellular structures or limit the mobility of certain membrane proteins.

Lipids attached covalently to certain membrane proteins (see Fig. 10–3) anchor these proteins to the lipid bilayer by hydrophobic interactions. Proteins thus held can be released from the membrane by the breakage of a single bond; the action of phospholipase C or D, for example, frees the membrane protein from the hydrophobic portion of a phosphatidylinositol "anchor" (see Fig. 10–9). It seems likely that this type of quick-release mechanism gives cells the capacity to change their membrane surface architecture rapidly, or to alter the subcellular localization of proteins that shuttle between membrane and cytosol.

Although these proteins with lipid anchors resemble integral membrane proteins in that they can be solubilized by detergent treatment, they are generally considered peripheral membrane proteins on the basis of their other properties: their association with the membrane is often weak and reversible, they do not contain long hydrophobic sequences, and once solubilized (by phospholipase action, for example), they behave like typical soluble proteins.

Membrane Proteins Diffuse Laterally in the Bilayer

Many membrane proteins behave as though they were afloat in a sea of lipids. We noted earlier that membrane lipids are free to diffuse laterally in the plane of the bilayer, and are in constant motion. The experiment diagrammed in Figure 10–12 shows that this is also true of some membrane proteins. Other experimental techniques confirm that many but not all membrane proteins undergo rapid lateral diffusion, but there are many exceptions to this generalization. Some membrane proteins associate with adjacent membrane proteins to form large aggregates ("patches") on the surface of a cell or organelle, in which indi-

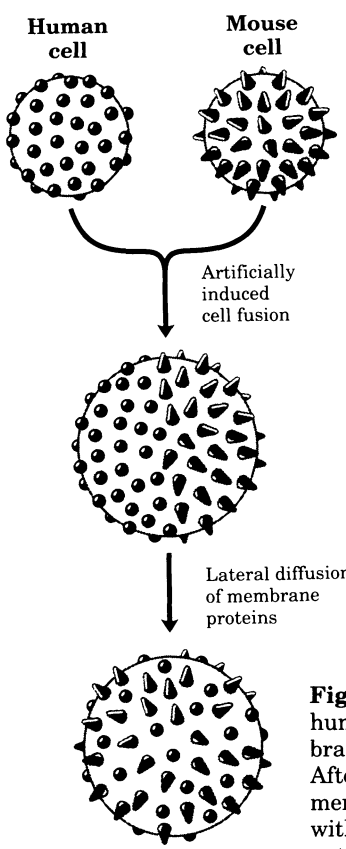


Figure 10–12 The fusion of a mouse cell with a human cell results in the randomization of membrane proteins from the mouse and the human cell. After fusion of the cells, the location of each type of membrane protein is determined by staining cells with species-specific antibodies. Anti-mouse and anti-human antibodies are specifically tagged with molecules that fluoresce with different colors. Observed with the fluorescence microscope, the colored antibodies are seen to mix on the surface of the hybrid cell within minutes after fusion, indicating rapid diffusion of the membrane proteins throughout the lipid bilayer.

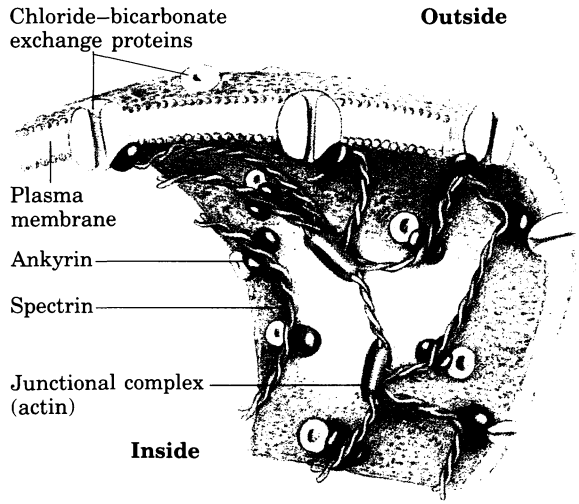


Figure 10–13 The chloride–bicarbonate exchange protein of the erythrocyte spans the membrane and is tethered to the cytoskeletal protein spectrin by ankyrin, limiting lateral mobility. Ankyrin contains a covalently bound palmitoyl side chain (Fig. 10–3), which may hold to the membrane. Spectrin is a long, filamentous protein that forms a network attached to the cytoplasmic face of the membrane, thereby stabilizing it against deformation in shape.

vidual protein molecules do not move relative to one another. Acetylcholine receptors (p. 292) form dense patches at synapses. Other membrane proteins are anchored to internal structures that prevent their free diffusion in the membrane bilayer. In the erythrocyte membrane, both glycophorin and the chloride–bicarbonate exchanger (p. 286) are tethered from the inside to a filamentous cytoskeletal protein, spectrin (Fig. 10–13).

Membrane Fusion Is Central to Many Biological Processes

Although membranes are stable, they are by no means static. The fluid mosaic structure is dynamic and flexible enough to allow fusion of two membranes. Within the endomembrane system described in Chapter 2 (see Fig. 2–10) there is constant reorganization of the membranous compartments, as small vesicles bud from the Golgi complex carrying newly synthesized lipids and proteins to other organelles and to the plasma membrane. Exocytosis, endocytosis, fusion of egg and sperm cells, and cell division all involve membrane reorganization in which the fundamental operation is fusion of two membrane segments without loss of continuity (Fig. 10–14).

To fuse, two membranes must first approach each other within molecular distances (a few nanometers). Much evidence suggests that an increase in intracellular Ca^{2+} concentration is the signal for certain fusion events such as exocytosis. **Annexins** are a family of proteins located just beneath the plasma membrane. They bind avidly to the head groups of phospholipids in bilayers, but only in the presence of Ca^{2+}. Some annexins also associate with specific intracellular vesicles fated for exocytosis. These proteins cause clumping of liposomes in vitro, presumably by cross-linking lipid molecules of two different vesi-

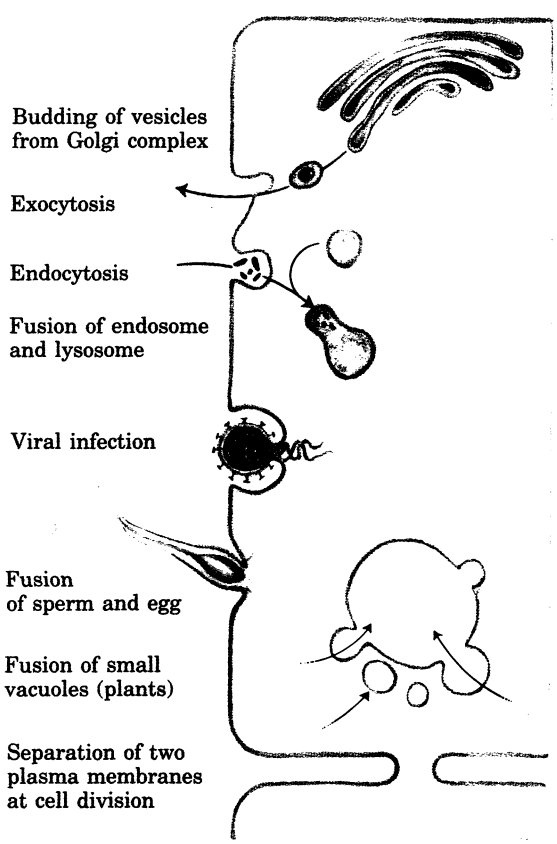


Figure 10–14 Membrane fusion is central to a variety of cellular processes, involving both organelles and the plasma membrane.

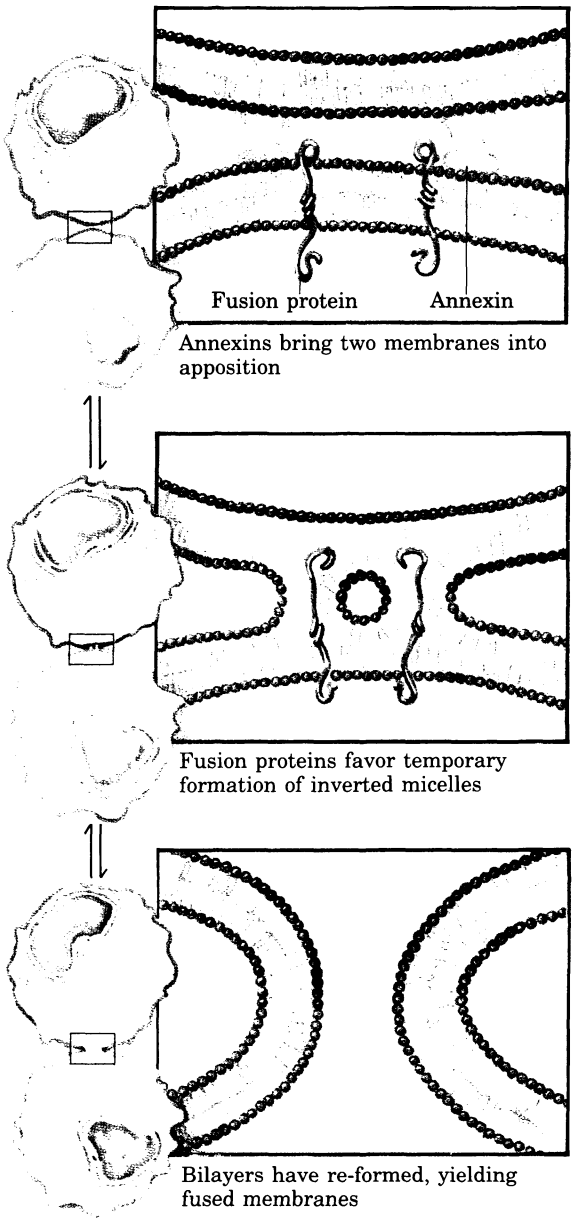


Figure 10–15 One plausible model for membrane fusion. The inverted micelles shown here are only one kind of nonbilayer structure that might be assumed by membrane lipids. When the lipids revert to bilayer structures, either the original structure (top) or the fusion product (bottom) can form. The fusion proteins may act by favoring the formation of the nonbilayer intermediate.

cles. In one simple model of membrane fusion (Fig. 10–15), annexins hold two membranes in close and stable apposition in the first step of fusion.

Another family of proteins believed to act in fusion is the **fusion proteins,** which are typified by the integral membrane protein HA, essential for the entry of the influenza virus into host cells (Fig. 10–14). (Note that these "fusion proteins" are unrelated to the products of two fused genes, also called fusion proteins, discussed in Chapter 28.) Like most other fusion proteins, the HA protein contains two regions rich in nonpolar amino acids, one a typical membrane-spanning domain, the other (the "fusion peptide") rich in Ala and Gly residues. The fusion protein may bridge the two membranes, with its membrane-spanning domain in one membrane and the fusion peptide inserted into the other (Fig. 10–15).

Fusion proteins may bring about transient distortions of the bilayer structure in the region of fusion. Physical studies of pure phospholipids in vitro have shown that several nonbilayer structures, such as inverted micelles, can form under some circumstances. In the model illustrated in Figure 10–15, an alternative structure exists in equilibrium with the bilayer, and represents the transition structure between unfused and fused membranes. Fusion proteins are believed to favor the formation of the transition structure, thus easing the phospholipid reorganization that results in fusion. In addition to annexins and fusion proteins, several, perhaps many, other proteins are probably involved; the machinery for fusion may be much more complex than implied by the simple model described here.

Solute Transport across Membranes

Every living cell must acquire from its surroundings the raw materials for biosynthesis and for energy production, and must release to its environment the byproducts of metabolism. The plasma membrane contains proteins that specifically recognize and carry into the cell such necessities as sugars, amino acids, and inorganic ions. In some cases, these components are brought into the cell against a concentration gradient—"pumped" in. Certain other species are pumped out, to keep their cytosolic concentrations lower than those in the surrounding medium. With few exceptions, the traffic of small molecules across the plasma membrane occurs by protein-mediated processes, via transmembrane channels, carriers, or pumps. Within the eukaryotic cell, different compartments have different concentrations of metabolic intermediates and products, and these, too, must move across intracellular membranes in tightly regulated, protein-mediated processes. Table 10–4 summarizes the properties of membrane transport systems.

Table 10–4 Summary of transport types

Type of transport	Protein carrier?	Saturable with substrate?	Produces concentration gradient?	Energy-dependent?	Energy source (if any)	Examples
Simple diffusion	No	No	No	No	—	H_2O, O_2, N_2, CH_4
Passive transport (facilitated diffusion)	Yes	Yes	No	No	—	Glucose permease of erythrocytes
Active transport						
Primary	Yes	Yes	Yes	Yes	ATP, light, substrate oxidation	H^+ ATPase (plant plasma membrane); Na^+K^+ ATPase (animal plasma membrane)
Secondary	Yes	Yes	Yes	Yes	Ion gradient	Amino acids and sugars (Na^+-driven; intestine); lactose (H^+-driven; bacteria)
Ion channels	Yes	No	No	No*	—	Na^+ channel of acetylcholine receptor (plasma membrane of neuron)

* Although the mechanism of transport via ion channels is not directly energy dependent, the direction of ion flow is determined by the transmembrane differences in electrochemical potential. Ions always move *down* their electrochemical gradient through ion channels.

Passive Transport Is Downhill Diffusion Facilitated by Membrane Proteins

When two aqueous compartments containing unequal concentrations of a soluble compound or ion are separated by a permeable divider, the solute moves by **simple diffusion** from the region of higher concentration, through the divider, to the region of lower concentration, until the two compartments have equal solute concentrations (Fig. 10–16). This behavior of solutes is in accord with the second law of thermodynamics: molecules will tend spontaneously to assume the distribution of greatest randomness, i.e., entropy will increase.

In living organisms, simple diffusion is impeded by selectively permeable barriers—the membranes that separate intracellular compartments and surround cells. To pass through the bilayer, a polar or charged solute must give up its interactions with the water molecules in its hydration shell, then diffuse about 3 nm through a solvent in which it is poorly soluble (the central region of the lipid bilayer), before reaching the other side and regaining its water of hydration (Fig. 10–17). The energy used to strip away the hydration shell and move a polar compound from water into lipid is regained as the compound leaves the membrane on the other side and is rehydrated. However, the intermediate stage of transmembrane passage represents a high-

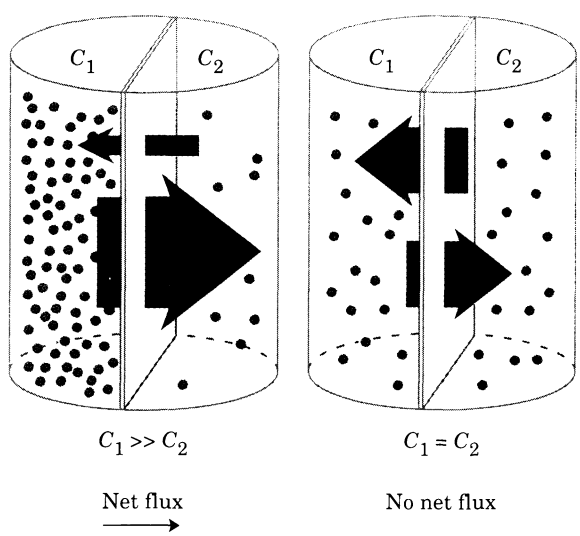


Figure 10–16 The rate of net movement of a solute across a permeable membrane depends upon the size of the concentration gradient. C_1 and C_2 are the solute concentrations on the left and right sides of the membrane.

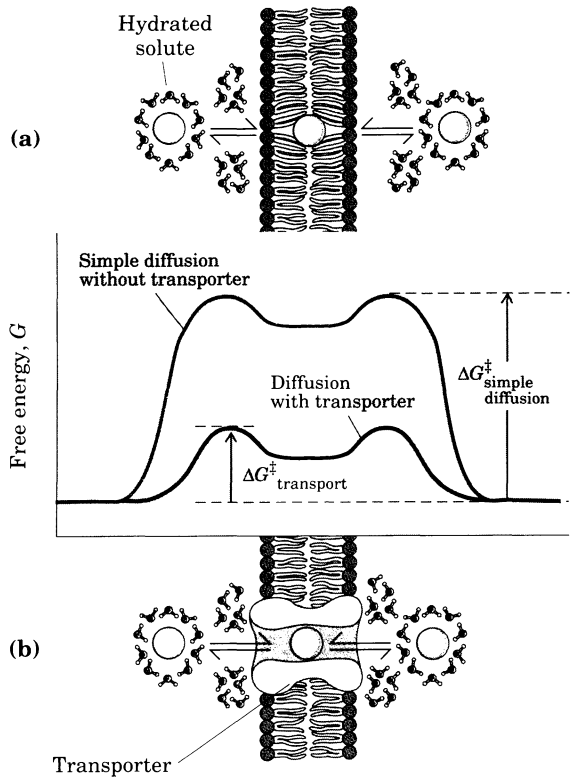


Figure 10–17 Energy changes that occur as a solute in aqueous solution passes through the lipid bilayer of a biological membrane. **(a)** In simple diffusion, the removal of the hydration shell is highly endergonic, and the energy of activation ($\Delta G^{\ddagger}$) for diffusion through the bilayer is very high. **(b)** A transporter protein—by forming noncovalent interactions with the dehydrated solute to replace its hydrogen bonds with water, and by providing a hydrophilic transmembrane passageway—reduces the $\Delta G^{\ddagger}$ for transmembrane diffusion of the solute.

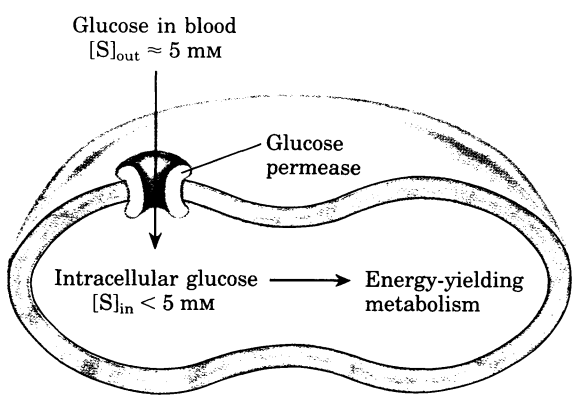


Figure 10–18 The glucose permease of erythrocytes facilitates the passage of glucose into the cell, down its concentration gradient. The 12 transmembrane segments of the permease form a hydrophilic path through the hydrophobic center of the membrane.

energy state comparable to the transition state in an enzyme-catalyzed chemical reaction. In both cases, an activation barrier must be overcome to reach the intermediate stage (Fig. 10–17; compare with Fig. 8–4). The energy of activation for translocation of a polar solute across the bilayer is so large that pure lipid bilayers are virtually impermeable to polar and charged species over the periods of time important to cells.

Water itself is an exception to this generalization. Although polar, it diffuses rapidly across biological membranes by mechanisms not fully understood. When the *solute* concentrations on two sides of a membrane are very different, there is a concentration gradient of *solvent* (water) molecules, and this osmotic imbalance results in the transmembrane flux of water until the osmotic strength equalizes on both sides of the membrane. A few biologically important gases also cross membranes by simple diffusion: molecular oxygen (O_2), nitrogen (N_2), and methane (CH_4), all of which are relatively nonpolar.

Transmembrane passage of polar compounds and ions is made possible by membrane proteins that lower the activation energy for transport by providing an alternative path for specific solutes through the lipid bilayer. Proteins that bring about this **facilitated diffusion** or **passive transport** are not enzymes in the usual sense; their "substrates" are moved from one compartment to another, but are not chemically altered. Membrane proteins that speed the movement of a solute across a membrane by facilitating diffusion are called **transporters** or **permeases.**

The kind of detailed structural information obtained for many soluble enzymes by x-ray crystallography is not yet available for most membrane transporters; as a group, these proteins are both difficult to purify and difficult to crystallize. However, from studies of the specificity and kinetics of transporters it is clear that their action is closely analogous to that of enzymes. Like enzymes, transporters bind their substrates through many weak, noncovalent interactions and with stereochemical specificity. The negative free-energy change that occurs with these weak interactions, $\Delta G_{\text{binding}}$, counterbalances the positive free-energy change that accompanies loss of the water of hydration from the substrate, $\Delta G_{\text{dehydration}}$, thereby lowering the activation energy, $\Delta G^{\ddagger}$, for transmembrane passage (Fig. 10–17). Transporter proteins span the lipid bilayer at least once, and usually several times, forming a transmembrane channel lined with hydrophilic amino acid side chains. The channel provides an alternative path for its specific substrate to move across the lipid bilayer, without having to dissolve in it, further lowering $\Delta G^{\ddagger}$ for transmembrane diffusion. The result is an increase of orders of magnitude in the rate of transmembrane passage of the substrate.

The Glucose Permease of Erythrocytes Mediates Passive Transport

Energy-yielding metabolism in the erythrocyte depends on a constant supply of glucose from the blood plasma, where its concentration is maintained at about 5 mM. Glucose enters the erythrocyte by facilitated diffusion via a specific glucose permease (Fig. 10–18). This integral membrane protein (M_r 45,000) has 12 hydrophobic segments, and probably spans the membrane 12 times. It allows glucose entry into the cell at a rate about 50,000 times greater than its unaided diffusion through a lipid bilayer. Because glucose transport into erythrocytes is a typical example of passive transport, we will look at it in some detail.

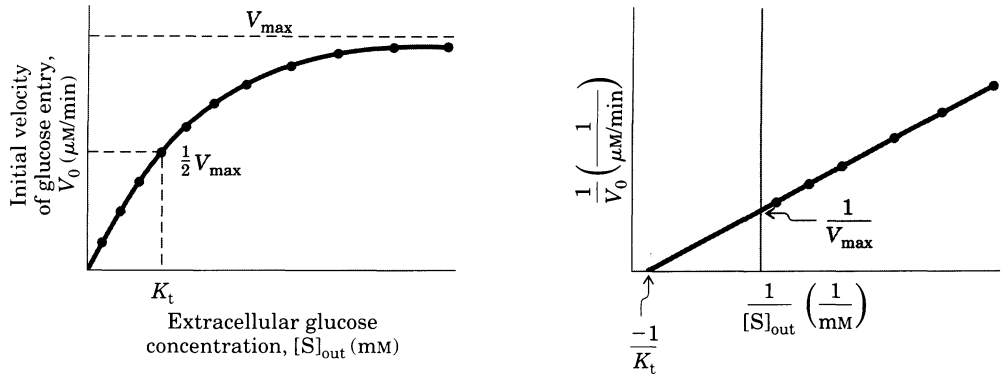


Figure 10–19 The initial rate of glucose entry into an erythrocyte depends upon the initial concentration of glucose on the outside, $[S]_{out}$. The kinetics of facilitated diffusion are analogous to the kinetics of an enzyme-catalyzed reaction. Compare these plots with Fig. 8–11, and Fig. 1 in Box 8–1. Note that K_t is analogous to K_m, the Michaelis–Menten constant.

The process of glucose transport can be described by analogy with an enzymatic catalysis in which the "substrate" is glucose outside the cell (S_{out}), the "product" is glucose inside (S_{in}), and the "enzyme" is the transporter, T. When the rate of glucose uptake is measured as a function of external glucose concentration (Fig. 10–19), the resulting plot is hyperbolic; at high external glucose concentrations the rate of uptake approaches V_{max}. Formally, such a transport process can be described by the equations

$$S_{out} + T \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} S_{out} \cdot T \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} S_{in} \cdot T \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} S_{in} + T$$

in which k_1, k_{-1}, etc., are the forward and reverse rate constants for each step. The first step is the binding of glucose to a stereospecific site on the transporter protein on the exterior surface of the membrane; step 2 is the transmembrane passage of the substrate; and step 3 is the release of the substrate (product), now on the inner surface of the membrane, from the transporter into the cytoplasm.

The rate equations for this process can be derived exactly as for enzyme-catalyzed reactions (Chapter 8), yielding an expression analogous to the Michaelis–Menten equation:

$$V_0 = \frac{V_{max}[S]_{out}}{K_t + [S]_{out}}$$

in which V_0 is the initial velocity of accumulation of glucose inside the cell when its concentration in the surrounding medium is $[S]_{out}$, and K_t ($K_{transport}$) is a constant, analogous to the Michaelis–Menten constant, a combination of rate constants characteristic of each transport system. This equation describes the *initial* velocity—the rate observed when $[S]_{in} = 0$.

Because no chemical bonds are made or broken in the conversion of S_{out} into S_{in}, neither "substrate" nor "product" is intrinsically more stable, and the process of entry is therefore fully reversible. As $[S]_{in}$ approaches $[S]_{out}$, the rates of entry and exit become equal. Such a system is therefore incapable of accumulating the substrate (glucose) within cells at concentrations above that in the surrounding medium; it simply achieves equilibration of glucose on the two sides of the membrane at a much higher rate than would occur in the absence of a specific transporter. The glucose transporter is specific for D-glucose, for which the measured K_t is 1.5 mM. For the close analogs D-mannose and D-galactose, which differ only in the position of one hydroxyl group, the values of K_t are 20 and 30 mM, respectively, and for L-glucose, K_t exceeds 3,000 mM! (Recall that a high K_t generally reflects a low affinity of transporter for substrate.) The glucose transporter of the erythrocyte therefore shows the three hallmarks of passive transport: high rates of diffusion down a concentration gradient, saturability, and specificity.

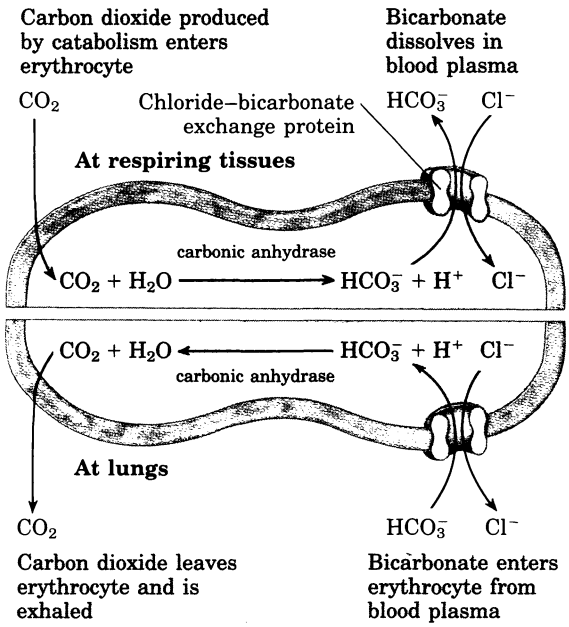


Figure 10–20 The chloride–bicarbonate exchanger of the erythrocyte membrane allows the entry and exit of HCO_3^- without changes in the transmembrane electrical potential. The role of this shuttle system is to increase the CO_2-carrying capacity of the blood.

Chloride and Bicarbonate Are Cotransported across the Erythrocyte Membrane

The erythrocyte contains another facilitated diffusion system, an anion exchanger, which is essential in CO_2 transport from tissues such as muscle and liver to the lungs. Waste CO_2 released from respiring tissues into the blood plasma enters the erythrocyte, where it is converted into bicarbonate (HCO_3^-) by the enzyme carbonic anhydrase (Fig. 10–20). The HCO_3^- reenters the blood plasma for transport to the lungs. Because HCO_3^- is much more soluble in blood plasma than is CO_2, this roundabout route increases the blood's capacity to carry carbon dioxide from the tissues to the lungs. In the lungs, HCO_3^- reenters the erythrocyte and is converted to CO_2, which is eventually exhaled. For this shuttle to be effective, very rapid movement of HCO_3^- across the erythrocyte membrane is required.

The **chloride–bicarbonate exchanger,** also called the **anion exchange protein,** or (for historical reasons) band 3, increases the permeability of the erythrocyte membrane to HCO_3^- by a factor of more than a million. Like the glucose transporter, it is an integral membrane protein that probably spans the membrane 12 times. Unlike the glucose transporter, this protein mediates a bidirectional exchange; for each HCO_3^- ion that moves in one direction, one Cl^- ion must move in the opposite direction (Fig. 10–20). The result of this paired movement of two monovalent anions is no net change in the charge or electrical potential across the erythrocyte membrane; the process is not electrogenic. The coupling of Cl^- and HCO_3^- movement is obligatory; in the absence of chloride, bicarbonate transport stops. In this respect, the anion exchanger resembles many other systems that simultaneously carry two solutes across a membrane, all of which are called **cotransport systems.** When, as in this case, the two substrates move in opposite directions, the process is **antiport.** In **symport,** two substrates are moved simultaneously in the same direction (Fig. 10–21). Transporters that carry only one substrate, such as the glucose permease, are sometimes called **uniport** systems.

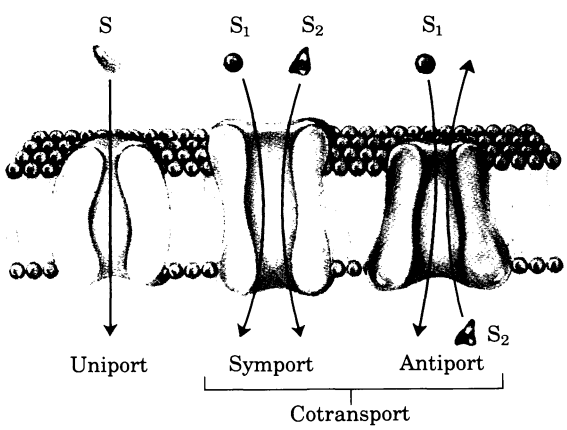


Figure 10–21 The three general classes of transport systems differ in the number of solutes (substrates) transported and the direction in which each is transported. Examples of all three types of transporters are discussed in the text. Note that this classification tells us nothing about whether these are energy-requiring (active transport) or energy-independent (passive transport) processes.

Active Transport Results in Solute Movement against a Concentration Gradient

In passive transport, the transported species always moves down its concentration gradient, and no net accumulation occurs. **Active transport,** by contrast, results in the accumulation of a solute on one

side of a membrane. Active transport is thermodynamically unfavorable (endergonic), and occurs only when coupled (directly or indirectly) to an exergonic process such as the absorption of sunlight, an oxidation reaction, the breakdown of ATP, or the concomitant flow of some other chemical species down its concentration gradient. In primary active transport, solute accumulation is coupled *directly* to an exergonic reaction (e.g., conversion of ATP to ADP + P_i). Secondary active transport occurs when endergonic (uphill) transport of one solute is coupled to the exergonic (downhill) flow of a different solute that was originally pumped uphill by primary active transport.

The amount of energy needed for the transport of a solute against a gradient can easily be calculated from the initial concentration gradient. The general equation for the free-energy change in the chemical process that converts S into P is

$$\Delta G = \Delta G^{\circ\prime} + RT \ln [P]/[S] \qquad (10\text{–}1)$$

where R is the gas constant 8.315 J/mol • K and T is the absolute temperature. When the "reaction" is simply transport of a solute from a region where its concentration is C_1 to another region where its concentration is C_2, no bonds are made or broken and the standard free-energy change, $\Delta G^{\circ\prime}$, equals zero. The free-energy change for transport, ΔG_t, is then

$$\Delta G_t = RT \ln (C_2/C_1) \qquad (10\text{–}2)$$

For a tenfold gradient, the cost of moving 1 mol of an uncharged solute across the membrane separating two compartments at 25 °C is therefore

$$\Delta G_t = (8.315 \text{ J/mol} \cdot \text{K})(298 \text{ K})(\ln 10/1) = 5{,}705 \text{ J/mol}$$

or 5.7 kJ/mol. Equation 10–2 holds for all uncharged solutes. When the solute is an ion, its movement without an accompanying counterion results in the endergonic separation of positive and negative charges. The energetic cost of moving an ion therefore depends on the difference of electrical potential across the membrane as well as the difference in the chemical concentrations (that is, the **electrochemical potential**):

$$\Delta G_t = RT \ln (C_2/C_1) + Z\mathcal{F}\Delta\psi \qquad (10\text{–}3)$$

where Z is the charge on the ion, $\mathcal{F}$ is the Faraday constant (96,480 J/V • mol), and $\Delta\psi$ is the transmembrane electrical potential (in volts). Eukaryotic cells typically have electrical potentials across their plasma membranes of the order of 0.05 to 0.1 V, so the second term of Equation 10–3 can be a significant contribution to the total free-energy change for transporting an ion. Most cells maintain ion gradients larger than tenfold across their plasma or intracellular membranes, and for many cells and tissues, active transport is therefore a major energy-consuming process.

The mechanism of active transport is of fundamental importance in biology. As we shall see in Chapter 18, the formation of ATP in mitochondria and chloroplasts occurs by a mechanism that is essentially ATP-driven ion transport operating in reverse. The energy made available by the spontaneous flow of protons across a membrane is calculable from Equation 10–3; remember that for flow *down* a concentration gradient, the sign of ΔG is opposite to that for transport *against* the gradient.

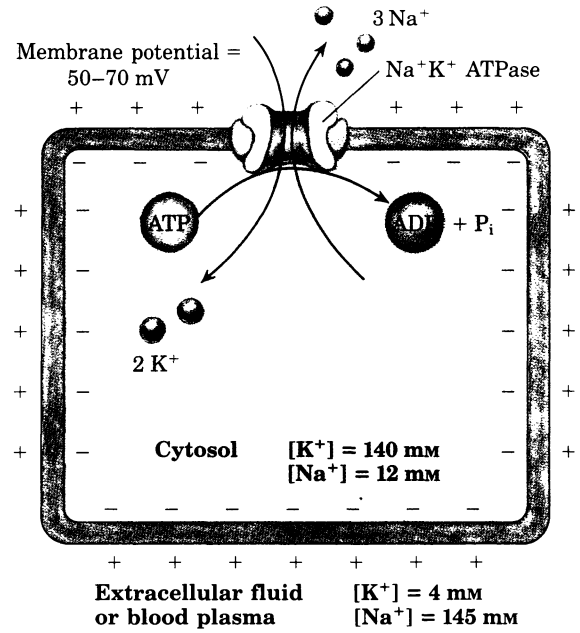


Figure 10–22 The Na^+K^+ ATPase is primarily responsible for setting and maintaining the intracellular concentrations of Na^+ and K^+ and for generating the transmembrane electrical potential, which it does by moving 3 Na^+ out of the cell for every 2 K^+ it moves in. The electrical potential is central to electrical signaling in neurons, and the gradient of Na^+ is used to drive uphill cotransport of various solutes in a variety of cell types.

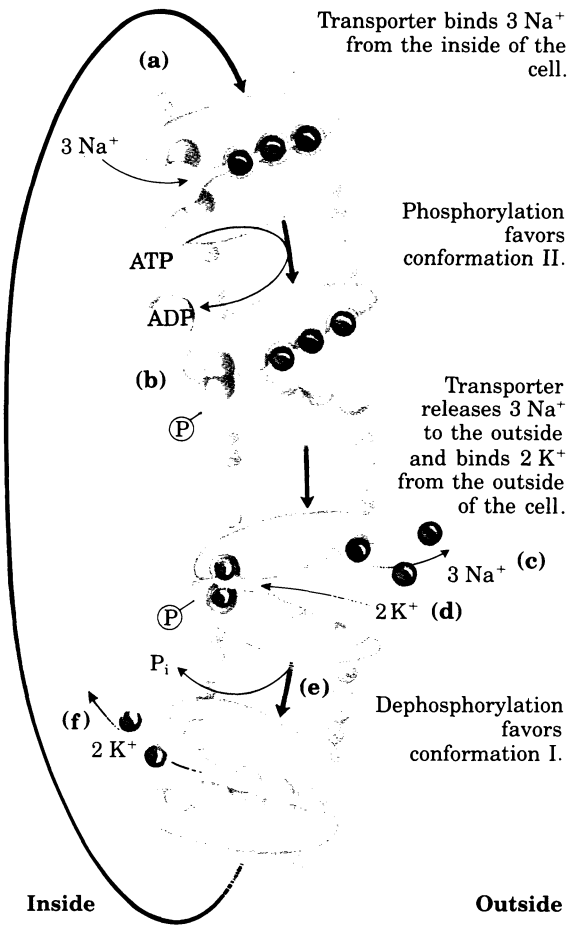


Figure 10–23 Postulated mechanism of Na^+ and K^+ transport by the Na^+K^+ ATPase. The process begins with the binding of three Na^+ to high-affinity sites on the large subunit of the transport protein on the inner surface of the membrane **(a).** This same part of the large subunit also has the ATP-binding site. Phosphorylation of the transporter changes its conformation **(b)** and decreases its affinity for Na^+, leading to Na^+ release on the outer surface **(c).** Next, K^+ on the outside binds to high-affinity sides on the extracellular portion of the large subunit **(d),** the enzyme is dephosphorylated, reducing its affinity for K^+ **(e),** and K^+ is discharged on the inside **(f).** The transport protein is now ready for another cycle of Na^+ and K^+ pumping.

Active Cotransport of Na^+ and K^+ Is Energized by ATP

Virtually every animal cell maintains a lower concentration of Na^+ and a higher concentration of K^+ than is found in its surrounding medium (in vertebrates, extracellular fluid or the blood plasma) (Fig. 10–22). This imbalance is established and maintained by a primary active transport system in the plasma membrane, involving the enzyme **Na^+K^+ ATPase,** which couples breakdown of ATP to the simultaneous movement of both Na^+ and K^+ against their concentration gradients. For each molecule of ATP converted to ADP and P_i, this transporter moves two K^+ ions inward and three Na^+ ions outward, across the plasma membrane. The Na^+K^+ ATPase is an integral membrane protein with two subunits ($M_r \sim 50{,}000$ and ~110,000), both of which span the membrane.

The detailed mechanism by which ATP hydrolysis is coupled to transport remains to be established, but a current working model (Fig. 10–23) supposes that the ATPase cycles between two conformations: conformation II, a phosphorylated form (designated $P\text{–}Enz_{II}$) with high affinity for K^+ and low affinity for Na^+, and conformation I, a dephosphorylated form (Enz_I) with high affinity for Na^+ and low affinity for K^+. The conversion of ATP to ADP and P_i occurs in two steps catalyzed by the enzyme:

(1) formation of phosphoenzyme:

$$ATP + Enz_I \longrightarrow ADP + P\text{–}Enz_{II}$$

(2) hydrolysis of phosphoenzyme:

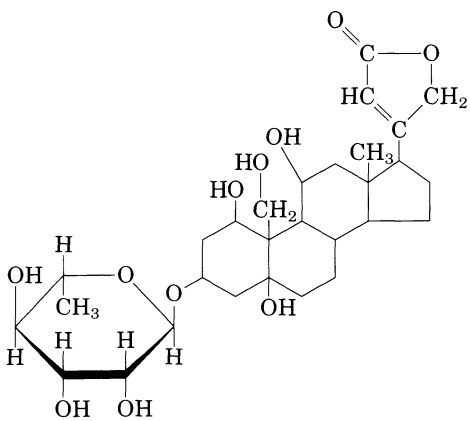
$$P\text{–}Enz_{II} + H_2O \longrightarrow Enz_I + P_i$$

which sum to the hydrolysis of ATP: $ATP + H_2O \longrightarrow ADP + P_i$.

Because three Na^+ ions move outward for every two K^+ ions that move inward, the process is **electrogenic**—it creates a net separation of charge across the membrane, making the inside of the cell negative relative to the outside. The resulting transmembrane potential of −50

to −70 mV (inside negative relative to outside) is essential to the conduction of action potentials in neurons, and is also characteristic of most nonneuronal animal cells. The activity of this Na^+K^+ ATPase in extruding Na^+ and accumulating K^+ is an essential cell function; about 25% of the energy-yielding metabolism of a human at rest goes to support the Na^+K^+ ATPase.

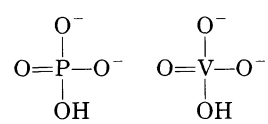
Ouabain (pronounced 'wä-bān), a steroid derivative extracted from the seeds of an African shrub, is a potent and specific inhibitor of the Na^+K^+ ATPase. Ouabain is a powerful poison used to tip hunting arrows; its name is derived from *waba yo,* meaning "arrow poison."



Ouabain

There Are Three General Types of Transport ATPases

The Na^+K^+ ATPase is the prototype for a class of transporters (Table 10–5), all of which are reversibly phosphorylated as part of the transport cycle—thus the name, **P-type ATPase.** All P-type transport ATPases share amino acid sequence homology, especially near the Asp residue that undergoes phosphorylation, and all are sensitive to inhibition by the phosphate analog **vanadate.** Each is an integral membrane protein having multiple membrane-spanning regions. P-type transporters are very widely distributed. In higher plants, a P-type H^+ ATPase pumps protons out of the cell, establishing a difference of as much as 2 pH units and 250 mV across the plasma membrane. For each proton transported, one ATP is consumed. A similar P-type ATPase is responsible for pumping protons from the bread mold *Neurospora,* and for pumping H^+ and K^+ across the plasma membranes of cells that line the mammalian stomach, acidifying its contents (Table 10–5).



Phosphate Vanadate

A distinctly different class of transport ATPases is responsible for acidifying intracellular compartments in many organisms. Within the vacuoles of higher plants and of fungi, for example, the pH is maintained well below that of the surrounding cytoplasm by the action of **V-type ATPase–proton pumps.** V-type (for vacuole) ATPases are also responsible for the acidification of lysosomes, endosomes, the Golgi complex, and secretory vesicles in animal cells. Unlike P-type ATPases, these proton-pumping ATPases (Table 10–5) do not undergo cyclic phosphorylation and dephosphorylation, and are not inhibited by vanadate or ouabain. The mechanism by which they couple ATP hydrolysis to the concentrative transport of protons is not yet known.

Table 10–5 Three classes of ion transport ATPases

Transported ion(s)	Organism	Type of membrane	Role of the ATPase
P-type ATPases			
Na^+K^+	Higher eukaryotes	Plasma	Maintains low $[Na^+]$, high $[K^+]$ inside cell; creates transmembrane electrical potential
H^+K^+	Acid-secreting cells of mammals	Plasma	Acidifies contents of stomach
H^+	Fungi *(Neurospora)*	Plasma	Creates low pH in compartment; activating proteases and other hydrolytic enzymes
H^+	Higher plants	Plasma	
Ca^{2+}	Higher eukaryotes	Plasma	Maintains low $[Ca^{2+}]$ in cytosol
Ca^{2+}	Muscle cells of animals	Sarcoplasmic reticulum (endoplasmic reticulum)	Sequesters intracellular Ca^{2+}, keeping cytosolic $[Ca^{2+}]$ low
V-type ATPases			
H^+	Animals	Lysosomal, endosomal, secretory vesicles	Creates low pH in compartment, activating proteases and other hydrolytic enzymes
H^+	Higher plants	Vacuolar	
H^+	Fungi	Vacuolar	
F-type ATPases			
H^+	Eukaryotes	Inner mitochondrial	Catalyzes formation of ATP from ADP + P_i
H^+	Higher plants	Thylakoid	
H^+	Prokaryotes	Plasma	

A third family of ATP-splitting proton pumps plays the central role in energy-conserving reactions in bacteria, mitochondria, and chloroplasts. This group of related enzymes, the **F-type ATPases** (Table 10–5), will be discussed when we describe ATP formation in mitochondria and chloroplasts (Chapter 18). (The *F* in their name originated in their identification as energy-coupling *f*actors.) They catalyze the reversible transmembrane passage of protons, driven by ATP hydrolysis. Flow of protons across the membrane *down* their concentration gradient is accompanied by ATP synthesis from ADP and P_i, the reversal of ATP hydrolysis. In this role, the F-type ATPase is more appropriately named **ATP synthase.** In some cases, the formation of the proton gradient in energy-conserving processes is driven by an energy source other than ATP, such as substrate oxidation or sunlight, as we will discuss in Chapter 18.

Ion Gradients Provide the Energy for Secondary Active Transport

The ion gradients formed by primary transport of Na^+ or H^+ driven by light, oxidation, or ATP hydrolysis can themselves provide the driving force for the cotransport of other solutes (Fig. 10–24). Many cells contain transport systems that couple the spontaneous, downhill flow of H^+ or Na^+ to the simultaneous uphill pumping of another ion, sugar, or amino acid (Table 10–6). The galactoside permease of *E. coli* allows the accumulation of the disaccharide lactose to levels 100 times that in the surrounding growth medium (Fig. 10–25). *E. coli* normally has a proton gradient across its plasma membrane, produced by energy-yielding metabolism; protons tend spontaneously to flow back into the cell, down this gradient. The lipid bilayer is impermeable to protons, but the galactoside permease provides a route for proton reentry, and lactose is simultaneously carried into the cell on the symporter protein (permease). The endergonic accumulation is thereby coupled to the exergonic flow of protons; the total free-energy change for the coupled process is negative.

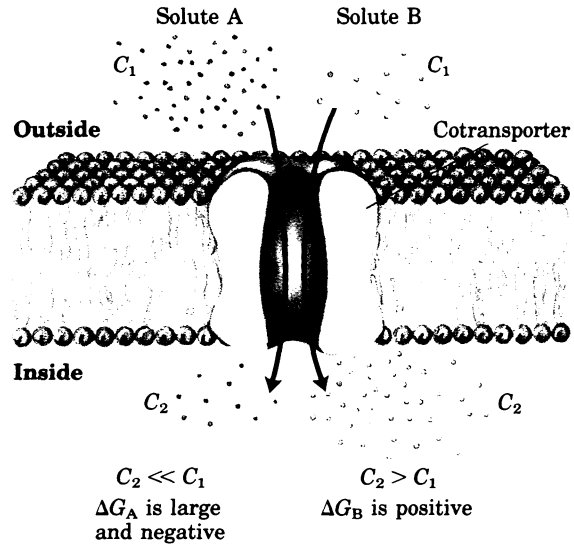
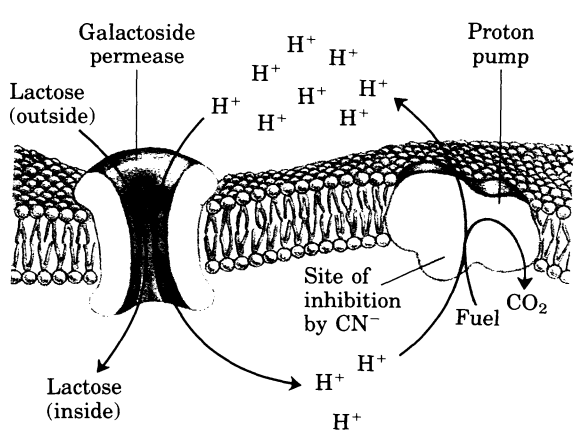


Figure 10–24 In secondary active transport, a single cotransporter couples the flow of one solute (solute 1; e.g., H^+ or Na^+) *down* its concentration gradient to the pumping of a second solute (solute 2; e.g., lactose or glucose) *against* its concentration gradient. When $\Delta G_1 + \Delta G_2 < 0$, cotransport occurs spontaneously. A specific example of this general process is shown in Fig. 10–25.

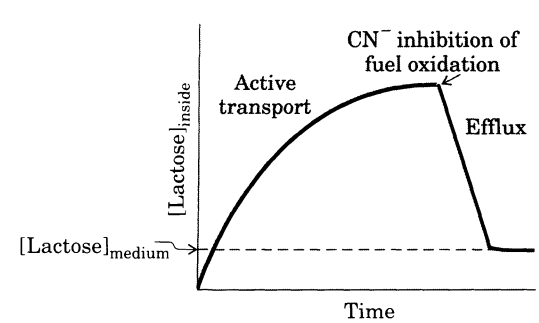
Table 10–6 Cotransport systems driven by gradients of Na^+ or H^+

Organism or tissue	Transported solute (symport or antiport)	Cotransported solute
E. coli	Lactose (symport)	H^+
	Proline (symport)	H^+
	Dicarboxylic acids (symport)	H^+
Intestine, kidney	Glucose (symport)	Na^+
	Amino acids (symport)	Na^+
Vertebrate cells (many types)	Ca^{2+} (antiport)	Na^+
Higher plants	K^+ (antiport)	H^+
Fungi *(Neurospora)*	K^+ (antiport)	H^+



(a)

Figure 10–25 Lactose uptake in *E. coli*. **(a)** The primary transport of H^+ out of the cell, driven by the oxidation of a variety of fuels, establishes a proton gradient. Secondary active transport of lactose into the cell involves symport of H^+ and lactose by the galactoside permease. The uptake of lactose against its concentration gradient is entirely dependent on this inflow of H^+. **(b)** When the energy-yielding oxidation reactions are blocked by cyanide (CN^-), there is an efflux of lactose from the cell, and no further accumulation occurs. The broken line represents the concentration of lactose in the surrounding medium. When active transport is blocked by cyanide, the galactoside permease allows equilibration of lactose inside and outside the cell (passive transport).



(b)

In intestinal epithelial cells, glucose and certain amino acids are accumulated by symport with Na^+, using the Na^+ gradient established by the Na^+K^+ ATPase. Most cells of vertebrate animals also have an antiport system that simultaneously pumps one Ca^{2+} ion out of a cell and allows three Na^+ ions in, thereby maintaining the very low intracellular Ca^{2+} concentration necessary for normal function. The role of Na^+ in symport and antiport systems such as these requires the continued outward pumping of Na^+ to maintain the transmembrane Na^+ gradient. Clearly, the Na^+K^+ ATPase is a central element in many cotransport processes.

Because of the essential role of ion gradients in active transport and energy conservation, natural products and drugs that collapse the ion gradients across cellular membranes are poisons, and may serve as antibiotics. Valinomycin is a small, cyclic peptide that folds around K^+ and neutralizes its positive charge (Fig. 10–26). The peptide then acts as a shuttle, carrying K^+ across membranes down its concentration gradient and deflating that gradient. Compounds that shuttle ions across membranes in this way are called **ionophores,** literally "ion-bearers." Both valinomycin and monensin (a Na^+-carrying ionophore) are antibiotics; they kill microbial cells by disrupting secondary transport processes and energy-conserving reactions.

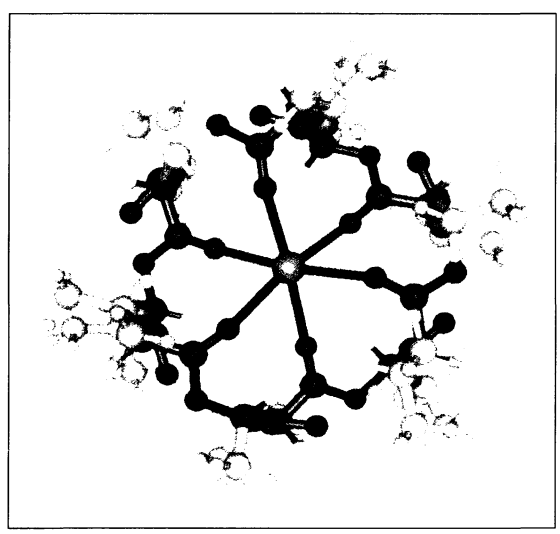
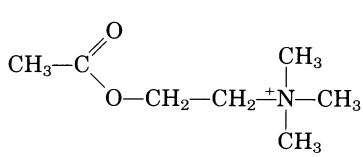


Figure 10–26 Valinomycin, a peptide ionophore that binds K^+. The oxygen (red) atoms that bind K^+ (the green atom at the center) are part of a central hydrophilic cavity. Hydrophobic amino acid side chains (yellow) coat the outside of the molecule. Because the exterior of the K^+-valinomycin complex is hydrophobic, it readily diffuses through membranes, carrying K^+ down its concentration gradient. The resulting dissipation of the transmembrane ion gradient kills cells, making valinomycin a potent antibiotic.

Ion-Selective Channels Act in Signal Transductions

A third transmembrane path for ions, distinct from both transporters and ionophores, is provided by **ion channels,** found in the plasma membranes of neurons, muscle cells, and many other cells, both prokaryotic and eukaryotic. Various stimuli cause rapid changes in the electrical potential across the plasma membranes of neurons and muscle cells, the result of the rapid opening and closing of ion channels. One of the best-studied ion channels is the **acetylcholine receptor** of the vertebrate synapse (the point of connection between two neurons; Fig. 10–27), which plays an essential role in the passage of a signal from one neuron to the next. When the electrical signal carried by the presynaptic neuron reaches the synaptic end of the cell, the neurotransmitter acetylcholine is released into the synaptic cleft. Acetylcholine rapidly diffuses across the cleft to the postsynaptic neuron, where



Acetylcholine

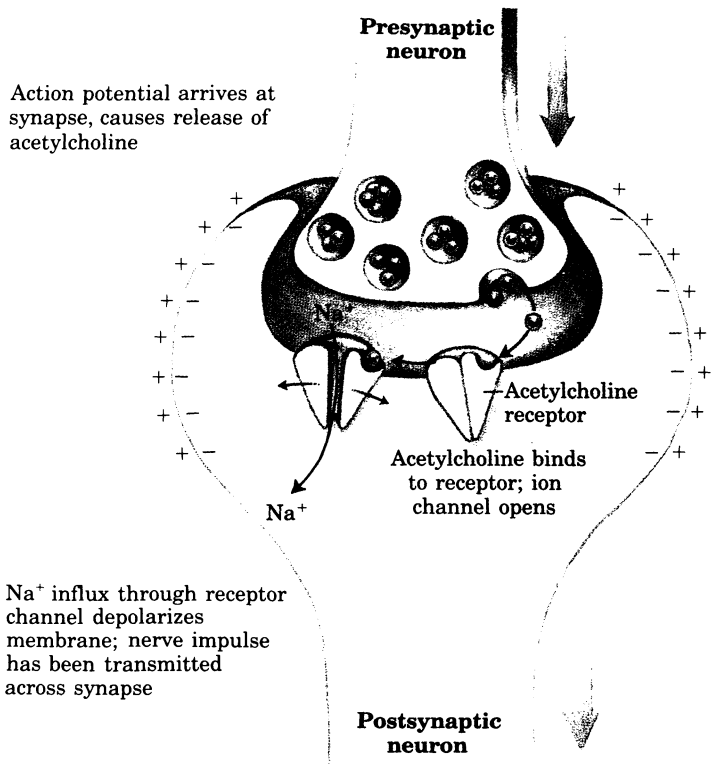


Figure 10–27 Communication occurs across the synaptic cleft between adjoining neurons as acetylcholine released by the presynaptic cell diffuses to specific receptors on the postsynaptic cell. The binding of acetylcholine changes the conformation of the receptor, and results in the opening of a receptor-associated ion channel. Na^+ flows in, down its concentration gradient, carrying positive charge and reducing the membrane electrical potential (depolarizing the cell). Depolarization initiates an electrical signal (action potential) that sweeps through the postsynaptic neuron at very high speed and is conducted to the next synapse, where these events may be repeated.

it binds to high-affinity sites on the acetylcholine receptor. This binding induces a change in receptor structure, opening a transmembrane channel in the receptor protein. Cations in the extracellular fluid, present at higher concentration than in the cytosol of the postsynaptic neuron, flow through the opened channel into the cell, down their concentration gradient, thereby depolarizing (decreasing the transmembrane electrochemical gradient of) the postsynaptic cell. The acetylcholine receptor allows Na^+ and K^+ to pass with equal ease, but other cations and all anions are unable to pass through it.

The rate of Na^+ movement through the acetylcholine receptor ion channel is linear with respect to extracellular Na^+ concentration; the process is not saturable in the way that transporter-catalyzed translocation is saturable with substrate (see Fig. 10–19). This kinetic property distinguishes ion channels from passive transporters. Na^+ movement through the channel is very fast—almost at the rate expected for unhindered diffusion of the ion. Under physiological conditions of ion concentrations and membrane potential, about 2×10^7 Na^+ ions can pass through a single channel in 1 s. A comparison of this figure with the turnover numbers for typical enzymes, which are in the range of 10 to 10^5 s^{-1}, shows the efficiency of transmembrane diffusion through an ion channel. In short, the ion channel of the acetylcholine receptor behaves as though it provided a hydrophilic pore through the lipid bilayer through which an ion of the right size, charge, and geometry can diffuse very rapidly down its electrochemical gradient. This receptor/channel is typical of many ion channels in cells that produce or respond to electrical signals: it has a "gate" that opens in response to stimulation by acetylcholine, and an intrinsic timing mechanism that closes the gate after a split second. Thus the acetylcholine signal is transient—an essential feature of electrical signal conduction.

Summary

Biological membranes are central to life. They define cellular boundaries, divide cells into discrete compartments, organize complex reaction sequences, and act in signal reception and energy transformations. Membranes are composed of lipids and proteins in varying combinations that are specific to each species, cell type, and organelle. The fluid mosaic model describes certain features common to all biological membranes. The lipid bilayer is the basic structural unit. Fatty acyl chains of phospholipids and the steroid nucleus of sterols are oriented toward the interior of the bilayer; their hydrophobic interactions stabilize the bilayer but allow the structure to be flexible. Lipids and most proteins are free to diffuse laterally within the membrane, and the hydrophobic moieties of the lipids undergo rapid thermal motion, making the interior of the bilayer fluid. Fluidity is affected by temperature, fatty acid composition, and sterol content. Cells strive to maintain a constant fluidity when external circumstances change.

Peripheral membrane proteins are loosely associated with the membrane through electrostatic interactions and hydrogen bonds or by covalently attached lipid anchors. Integral membrane proteins associate with the lipid bilayer by hydrophobic interactions with their nonpolar amino acid side chains, which are oriented toward the outside of the protein molecule. Some membrane proteins span the lipid bilayer several times, with hydrophobic sequences of about 20 amino acids, each capable of forming a transmembrane α helix. Such hydrophobic sequences can be detected and used to predict the structure and transmembrane disposition of these proteins. The lipids and proteins of the membrane are inserted into the bilayer with specific sidedness; the membrane is structurally and functionally asymmetric. Many membrane proteins contain covalently attached polysaccharides of various degrees of complexity. Plasma membrane glycoproteins are always oriented with the carbohydrate-bearing domain on the extracellular surface. Annexins and fusion proteins mediate the fusion of two membranes, which accompanies processes such as endocytosis and exocytosis.

The lipid bilayer is impermeable to polar substances. Water is an important exception; it is able to diffuse passively across the bilayer. Other polar species cross biological membranes only by way of specific membrane proteins. Ion channels provide hydrophilic pores through which select ions can diffuse, moving down their electrical or chemical concentration gradients.

The movement of many ions and compounds across cellular membranes is catalyzed by specific transport proteins (transporters), which, like enzymes, show saturation and substrate specificity. Transport via these systems may be passive (down the electrochemical gradient, hence independent of metabolic energy) such as glucose transport into erythrocytes, or active (against the gradient, and dependent on metabolic energy). The energy input for active transport may come from light, oxidation reactions, ATP hydrolysis, or cotransport of some other solute. Some transporters carry out symport, the simultaneous passage of two species in the same direction; others mediate antiport, in which two species move in opposite directions, but simultaneously. An example of antiport is the chloride–bicarbonate exchanger of erythrocytes. In animal cells, the differences in cytosolic and extracellular concentrations of Na^+ and K^+ are established and maintained by active transport via the Na^+K^+ ATPase, and the resulting Na^+ gradient is used as an energy source by a variety of symport and antiport systems.

There are three general types of ion-pumping ATPases. P-type ATPases undergo reversible phosphorylation during their catalytic cycle, and are inhibited by the phosphate analog vanadate. V-type ATPases produce gradients of protons across the membranes of a variety of intracellular organelles, including plant vacuoles. F-type proton pumps (ATP synthases) are central to energy-conserving mechanisms in mitochondria and chloroplasts. Ion-selective channels such as the acetylcholine receptor act in the passage of electrical signals in neurons, muscle cells, and other cells sensitive to a variety of stimuli.

Further Reading

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Harold, F.M. (1986) *The Vital Force: A Study of Bioenergetics,* W.H. Freeman and Company, New York.

Chapters 9 and 10 of this excellent book concern the energetics and mechanisms of transport, and Chapter 4 is a discussion of the energy of ion gradients.

Jain, M.K. (1988) *Introduction to Biological Membranes,* 2nd edn, John Wiley & Sons, Inc., New York.

A textbook of membranology, longer and more advanced than Finean et al.

Martonosi, A.N. (ed) (1985) *The Enzymes of Biological Membranes,* 2nd edn, Plenum Press, New York.

This four-volume set has 61 individual reviews covering many of the topics in this chapter, including the electron microscopy of membranes, protein–lipid interactions in membranes, the energetics of active transport, and the Na^+K^+ ATPase.

Stein, W.D. (1986) *Transport and Diffusion Across Cell Membranes,* Academic Press, Inc., New York.

This excellent textbook on biological transport covers all the transport systems described in this chapter, at a more advanced level.

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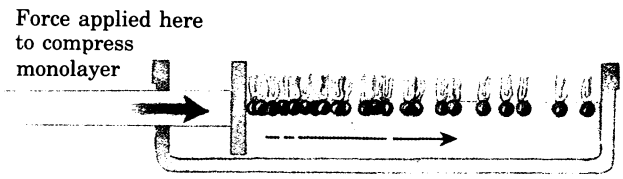
Problems



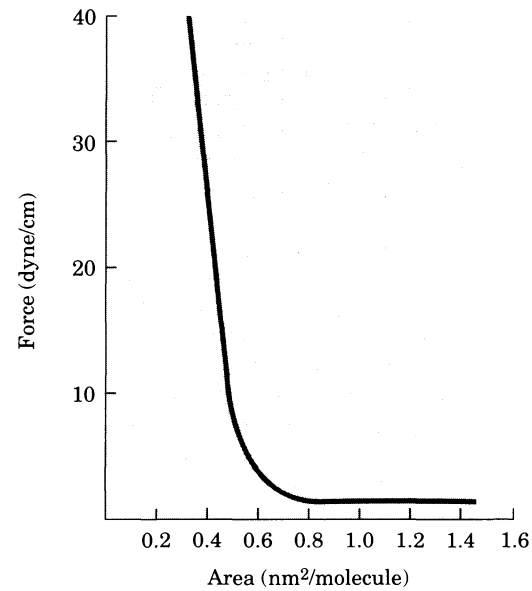
(a)

1. *Determining the Cross-Sectional Area of a Lipid Molecule* When phospholipids are layered gently onto the surface of water, they orient at the air–water interface with their head groups in the water and their hydrophobic tails in the air. The experimental apparatus pictured above **(a)** pushes these lipids together by reducing the surface area available to them. By measuring the force necessary to push the lipids together, it is possible to determine when the molecules are packed tightly together in a continuous monolayer; when that area is approached, the pressure needed to further reduce the surface area increases sharply **(b).** How would you use such an experimental apparatus to determine the average area occupied by a single lipid molecule in a lipid monolayer?

2. *Evidence for Lipid Bilayer* In 1925, E. Gorter and F. Grendel used an apparatus like that described in Problem 1 to determine the surface area of a lipid monolayer formed by lipids extracted from erythrocytes of several animal species. They used a microscope to measure the dimensions of individual cells, from which they calculated the average surface area of one erythrocyte. They obtained the data shown below. Were these investigators justified in concluding that "chromocytes [erythrocytes] are covered by a layer of fatty substances that is two molecules thick" (i.e., a lipid bilayer)?

Animal	Volume of packed cells (mL)	Number of cells (per mm^3)	Total surface area of lipid monolayer from cells (m^2)	Total surface area of one erythrocyte (μm^2)
Dog	40	8,000,000	62	98
Sheep	10	9,900,000	2.95	29.8
Human	1	4,740,000	0.47	99.4

Source: Data from Gorter, E. & Grendel, F. (1925) On bimolecular layers of lipoids on the chromocytes of the blood. *J. Exp. Med.* **41,** 439–443.



(b)

3. *Length of a Fatty Acid Molecule* The carbon–carbon bond distance for single-bonded carbons such as those in a saturated fatty acyl chain is about 0.15 nm. Estimate the length of a single molecule of palmitic acid in its fully extended form. If two molecules of palmitic acid were laid end to end, how would their total length compare with the thickness of the lipid bilayer in a biological membrane?

4. *Temperature Dependence of Lateral Diffusion* The experiment described in Figure 10–12 was done at 37 °C. If, instead, the whole experiment were carried out at 10 °C, what effect would you predict on the rate of cell–cell fusion, and the rate of membrane protein mixing? Why?

5. *Synthesis of Gastric Juice: Energetics* Gastric juice (pH 1.5) is produced by pumping HCl from blood plasma (pH 7.4) into the stomach. Calculate the amount of free energy required to concentrate the H^+ in 1 L of gastric juice at 37 °C. Under cellular conditions, how many moles of ATP must be hydrolyzed to provide this amount of free energy? (The free-energy change for ATP hydrolysis under cellular conditions is about −58 kJ/mol, as we will explain in Chapter 13.)

6. *Energetics of the Na^+K^+ ATPase* The concentration of Na^+ inside a vertebrate cell is about 12 mM, and the cell is bathed in blood plasma containing about 145 mM Na^+. For a typical cell with a transmembrane potential of −0.07 V (inside nega-

tive relative to outside), what is the free-energy change for transporting 1 mol of Na^+ out of the cell at 37 °C?

7. *Action of Ouabain on Kidney Tissue* Ouabain specifically inhibits the Na^+K^+ ATPase activity of animal tissues but is not known to inhibit any other enzyme. When ouabain is added in graded concentrations to thin slices of living kidney tissue, it inhibits oxygen consumption by 66%. Explain the basis of this observation. What does it tell us about the use of respiratory energy by kidney tissue?

8. *Membrane Protein Topology* The receptor for the hormone epinephrine in animal cells is an integral membrane protein (M_r 64,000) that is believed to span the membrane seven times. Show that a protein of this size is capable of spanning the membrane seven times. If you were given the amino acid sequence of this protein, how would you go about predicting which regions of the protein form the membrane-spanning helices?

9. *Energetics of Symport* Suppose that you determined experimentally that a cellular transport system for glucose, driven by symport of Na^+, could accumulated glucose to concentrations 25 times greater than in the external medium, while the external [Na^+] was only ten times greater than the intracellular [Na^+]. Is this a violation of the laws of thermodynamics? If not, how do you explain this observation?

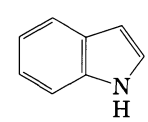
10. *Location of a Membrane Protein* An unknown membrane protein, X, can be extracted from disrupted erythrocyte membranes into a concentrated salt solution. Isolated X can be cleaved into fragments by proteolytic enzymes. But treatment of erythrocytes, first with proteolytic enzymes, followed by disruption and extraction of membrane components, yields intact X. In contrast, treatment of erythrocyte "ghosts" (which consist of only membranes, produced by disrupting the cells and washing out the hemoglobin) with proteolytic enzymes, followed by disruption and extraction, yields extensively fragmented X. What do these experiments indicate about the location of X in the plasma membrane? On the basis of this information, do the properties of X resemble those of glycophorin or those of ankyrin?

11. *Membrane Self-Sealing* Cell membranes are self-sealing—if they are punctured or disrupted mechanically, they quickly and automatically reseal. What properties of membranes are responsible for this important feature?

12. *Lipid Melting Temperatures* Membrane lipids in tissue samples obtained from different parts of the leg of a reindeer show different fatty acid compositions. Membrane lipids from tissue near the hooves contain a larger proportion of unsaturated fatty acids than lipids from tissue in the upper part of the leg. What is the significance of this observation?

13. *Flip-Flop Diffusion* The inner face of the human erythrocyte membrane consists predominantly of phosphatidylethanolamine and phosphatidylserine. The outer face consists predominantly of phosphatidylcholine and sphingomyelin. Although the phospholipid components of the membrane can diffuse in the fluid bilayer, this sidedness is preserved all all times. How?

14. *Membrane Permeability* At pH 7, tryptophan crosses a lipid bilayer membrane about 1,000 times more slowly than does the closely related substance indole:

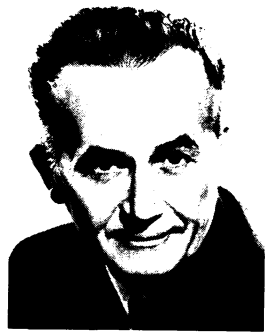


Suggest an explanation for this observation.

Konrad Bloch

Feodor Lynen
1911–1979

John Cornforth



George Popják

animal cells, this cyclization results in the formation of **lanosterol,** which contains the four rings characteristic of the steroid nucleus. Lanosterol is finally converted into cholesterol in a series of about 20 reactions, including the migration of some methyl groups and the removal of others. Elucidation of this extraordinary biosynthetic pathway, one of the most complex known, was accomplished by Konrad Bloch, Feodor Lynen, John Cornforth, and George Popják in the late 1950s.

Cholesterol is the sterol characteristic of animal cells, but plants, fungi, and protists make other, closely related sterols instead of cholesterol, using the same synthetic pathway as far as squalene-2,3-epoxide. At this point the synthetic pathways diverge slightly, yielding other sterols: stigmasterol in many plants and ergosterol in fungi, for example (Fig. 20–35).

Cholesterol Has Several Fates

Most of the cholesterol synthesis in vertebrates takes place in the liver. A small fraction of the cholesterol made there is incorporated into the membranes of hepatocytes, but most of it is exported in one of three forms: biliary cholesterol, bile acids, or cholesteryl esters. **Bile acids** and their salts are relatively hydrophilic cholesterol derivatives that are synthesized in the liver and aid in lipid digestion (p. 480). **Cholesteryl esters** are formed in the liver through the action of **acyl-CoA–cholesterol acyl transferase (ACAT).** This enzyme catalyzes the transfer of a fatty acid from coenzyme A to the hydroxyl group of cholesterol (Fig. 20–36), converting the cholesterol into a more hydrophobic form. Cholesteryl esters are transported in secreted lipoprotein particles to other tissues that use cholesterol, or are stored in the liver.

All growing animal tissues need cholesterol for membrane synthesis, and some organs (adrenal gland and gonads, for example) use cholesterol as a precursor for steroid hormone production (discussed later). Cholesterol is also a precursor of vitamin D (see Fig. 9–19).

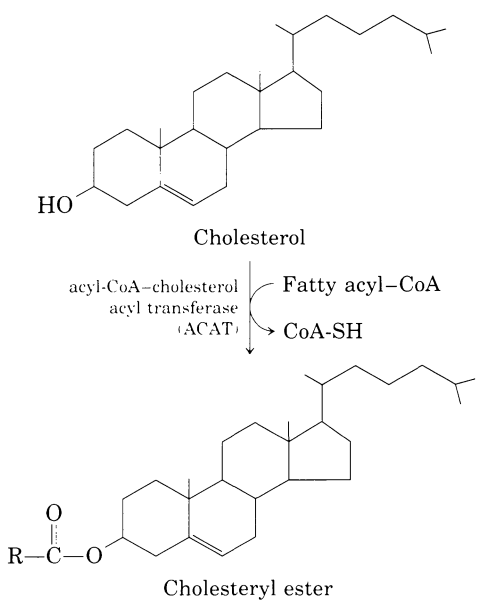
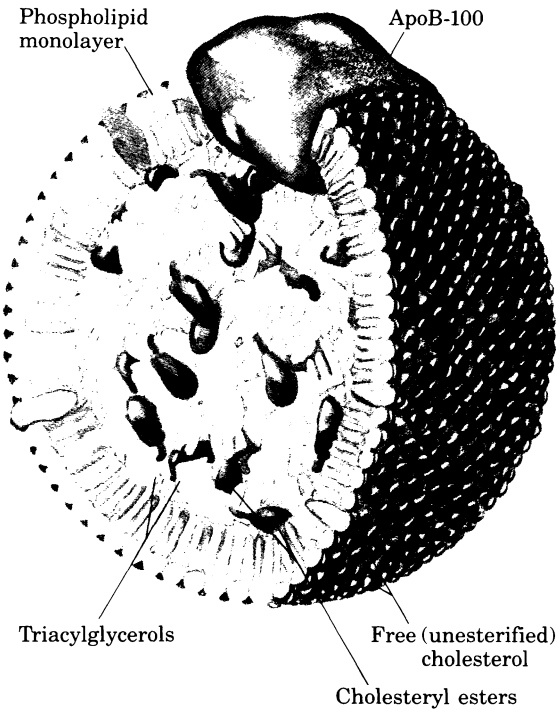


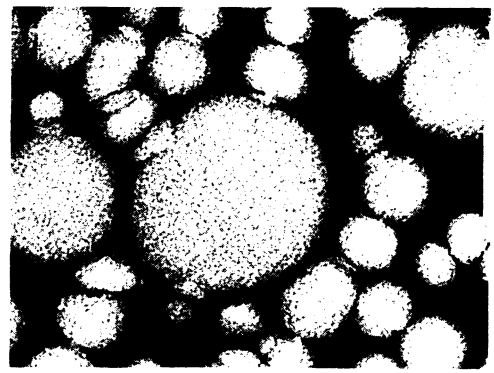
Figure 20–36 Synthesis of cholesteryl esters converts cholesterol into an even more hydrophobic form for storage and transport.

Cholesterol and Other Lipids Are Carried on Plasma Lipoproteins

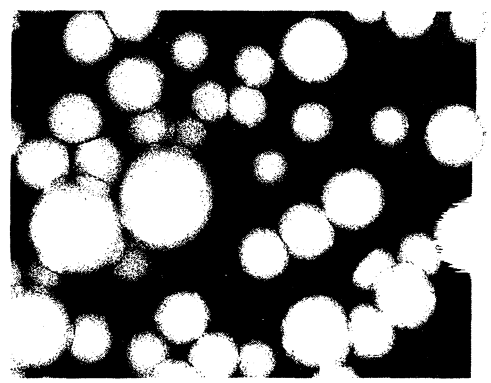
Cholesterol and cholesteryl esters, like triacylglycerols and phospholipids, are essentially insoluble in water. These lipids must, however, be moved from the tissue of origin (liver, where they are synthesized, or intestine, where they are absorbed) to the tissues in which they will be stored or consumed. They are carried in the blood plasma from one



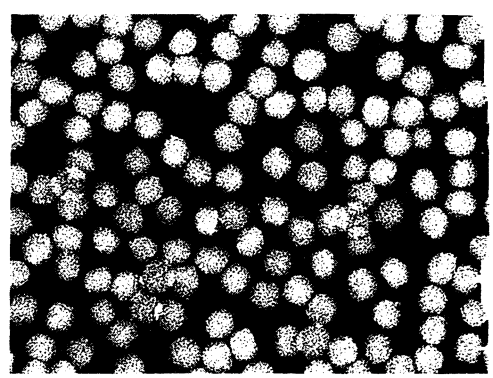
(a)



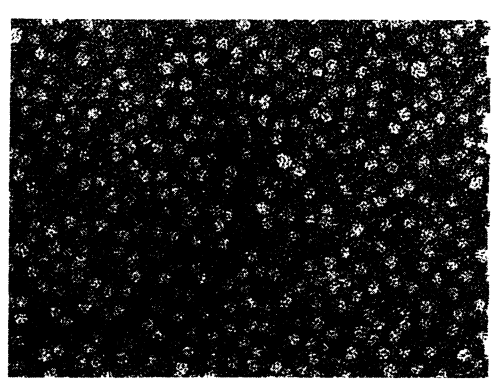
Chylomicrons (×60,000)



VLDL (×180,000)



LDL (×180,000)



HDL (×180,000)

(b)

Figure 20–37 (a) Structure of a low-density lipoprotein (LDL). Apolipoprotein B-100 (apoB-100) is one of the largest single polypeptide chains known, with 4,636 amino acid residues (M_r 513,000). **(b)** Four classes of lipoproteins visualized in the electron microscope after negative staining. From top to bottom: chylomicrons (50–200 nm in diameter); VLDL (28–70 nm); LDL (20–25 nm); and HDL (8–11 nm). For properties of lipoproteins, see Table 20–2.

tissue to another as **plasma lipoproteins,** macromolecular complexes of specific carrier proteins called **apolipoproteins** with various combinations of phospholipids, cholesterol, cholesteryl esters, and triacylglycerols.

Apolipoproteins ("apo" designates the protein in its lipid-free form) combine with lipids to form several classes of lipoprotein particles, spherical complexes with hydrophobic lipids in the core and the hydrophilic side chains of protein amino acids at the surface (Fig. 20–37a). Differing combinations of lipids and proteins produce particles of different densities, ranging from very low-density lipoproteins (VLDL) to high-density lipoproteins (HDL), which may be separated by ultracentrifugation (Table 20–2, p. 676) and visualized by electron microscopy (Fig. 20–37b).

Each class of lipoprotein has a specific function, determined by its point of synthesis, lipid composition, and apolipoprotein content. At least nine different apolipoproteins are found in the lipoproteins of human plasma (Table 20–3); they can be distinguished by their size, their reactions with specific antibodies, and their characteristic distribution in the lipoprotein classes. These protein components act as signals, targeting lipoproteins to specific tissues or activating enzymes that act on the lipoproteins.

Table 20–2 Major classes of human plasma lipoproteins: some properties

Lipoprotein	Density (g/mL)	Composition (wt %)				
		Protein	Phospholipids	Free cholesterol	Cholesteryl esters	Triacylglycerols
Chylomicrons	<1.006	2	9	1	3	85
VLDL	0.95–1.006	10	18	7	12	50
LDL	1.006–1.063	23	20	8	37	10
HDL	1.063–1.210	55	24	2	15	4

Source: Modified from Kritchevsky, D. (1986) Atherosclerosis and nutrition. *Nutr. Int.* **2,** 290–297.

We discussed **chylomicrons** in Chapter 16, in connection with the movement of dietary triacylglycerols from the intestine to other tissues. They are the largest of the lipoproteins and the least dense, containing a high proportion of triacylglycerols (see Fig. 16–2). Chylomicrons are synthesized in the endoplasmic reticulum of epithelial cells that line the small intestine, then move through the lymphatic system, entering the bloodstream through the left subclavian vein. The apolipoproteins of chylomicrons include apoB-48 (unique to this class of lipoproteins), apoE, and apoC-II (Table 20–3). ApoC-II activates lipoprotein lipase in the capillaries of adipose, heart, skeletal muscle, and lactating mammary tissues, allowing the release of free fatty acids to these tissues. Chylomicrons thus carry fatty acids obtained in the diet to the tissues in which they will be consumed or stored as fuel. The remnants of chylomicrons, depleted of most of their triacylglycerols but still containing cholesterol, apoE, and apoB-48, move through the bloodstream to the liver, where they are taken up, degraded in lysosomes, and their constituents recycled.

Table 20–3 Apolipoproteins of the human plasma lipoproteins

Apolipoprotein	Molecular weight	Lipoprotein association	Function (if known)
ApoA-I	28,331	HDL	Activates LCAT
ApoA-II	17,380	HDL	
ApoA-IV	44,000	Chylomicrons, HDL	
ApoB-48	240,000	Chylomicrons	
ApoB-100	513,000	VLDL, LDL	Binds to LDL receptor
ApoC-I	7,000	VLDL, HDL	
ApoC-II	8,837	Chylomicrons, VLDL, HDL	Activates lipoprotein lipase
ApoC-III	8,751	Chylomicrons, VLDL, HDL	Inhibits lipoprotein lipase
ApoD	32,500	HDL	
ApoE	34,145	Chylomicrons, VLDL, HDL	Triggers clearance of VLDL and chylomicron remnants

Source: Modified from Vance, D.E. & Vance, J.E. (eds) (1985) *Biochemistry of Lipids and Membranes.* The Benjamin/Cummings Publishing Company, Menlo Park, CA.

When the diet contains more fatty acids than are needed immediately as fuel, they are converted into triacylglycerols in the liver and packaged with specific apolipoproteins into **very low-density lipoprotein, VLDL.** Excess carbohydrate in the diet can also be converted into triacylglycerols in the liver and exported as VLDLs. In addition to triacylglycerols, VLDLs contain some cholesterol and cholesteryl esters, as well as apoB-100, apoC-I, apoC-II, apoC-III, and apo-E (Table 20–3). These lipoproteins are transported in the blood from the liver to muscle and adipose tissue, where activation of lipoprotein lipase by apoC-II causes the release of free fatty acids from the triacylglycerols of the VLDL. Adipocytes take up these fatty acids, resynthesize triacylglycerols from them, and store the products in intracellular lipid droplets, whereas myocytes mostly oxidize them to supply energy. Most VLDL remnants are removed from circulation by hepatocytes, via receptor-mediated uptake and lysosomal degradation.

The loss of triacylglycerols converts some VLDL to **low-density lipoprotein, LDL** (Table 20–2). Very rich in cholesterol and cholesteryl esters and containing apoB-100 as their major apoprotein, LDLs carry cholesterol to peripheral tissues (in addition to the liver) that have specific surface receptors that recognize apoB-100. These receptors mediate the uptake of cholesterol and cholesteryl esters in a process described below.

The fourth major lipoprotein type, **high-density lipoprotein, HDL,** begins in the liver and small intestine as small, protein-rich particles containing relatively little cholesterol and no cholesteryl esters. HDLs contain apoC-I and apoC-II, among other apolipoproteins (Table 20–3), as well as the enzyme **lecithin-cholesterol acyl transferase** (LCAT), which catalyzes the formation of cholesteryl esters from lecithin (phosphatidylcholine) and cholesterol (Fig. 20–38). LCAT on the surface of nascent (newly forming) HDL particles converts the cholesterol and phosphatidylcholine of chylomicron and VLDL rem-

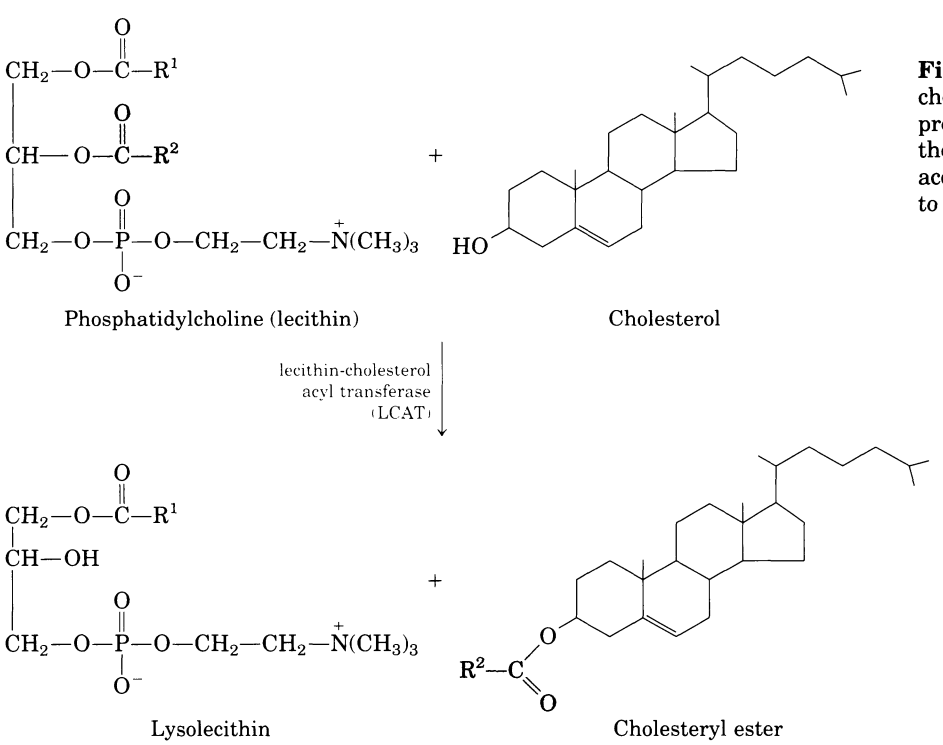


Figure 20–38 The reaction catalyzed by lecithin-cholesterol acyl transferase (LCAT). This enzyme is present on the surface of HDL and is stimulated by the HDL component apoA-I. The cholesteryl esters accumulate within nascent HDLs, converting them to mature HDLs.

nants to cholesteryl esters, which begin to form a core, transforming the disc-shaped nascent HDL to a mature, spherical HDL particle. This cholesterol-rich lipoprotein now returns to the liver, where the cholesterol is unloaded. Some of this cholesterol is converted into bile salts.

Cholesteryl Esters Enter Cells by Receptor-Mediated Endocytosis

Each LDL particle circulating in the bloodstream contains apoB-100, which as noted above is recognized by specific surface receptor proteins, **LDL receptors,** on cells that need to take up cholesterol. The binding of LDL to an LDL receptor initiates endocytosis (see Fig. 2–10), which brings the LDL and its associated receptor into the cell within an endosome (Fig. 20–39). This endosome eventually fuses with a lysosome, which contains enzymes that hydrolyze the cholesteryl es-

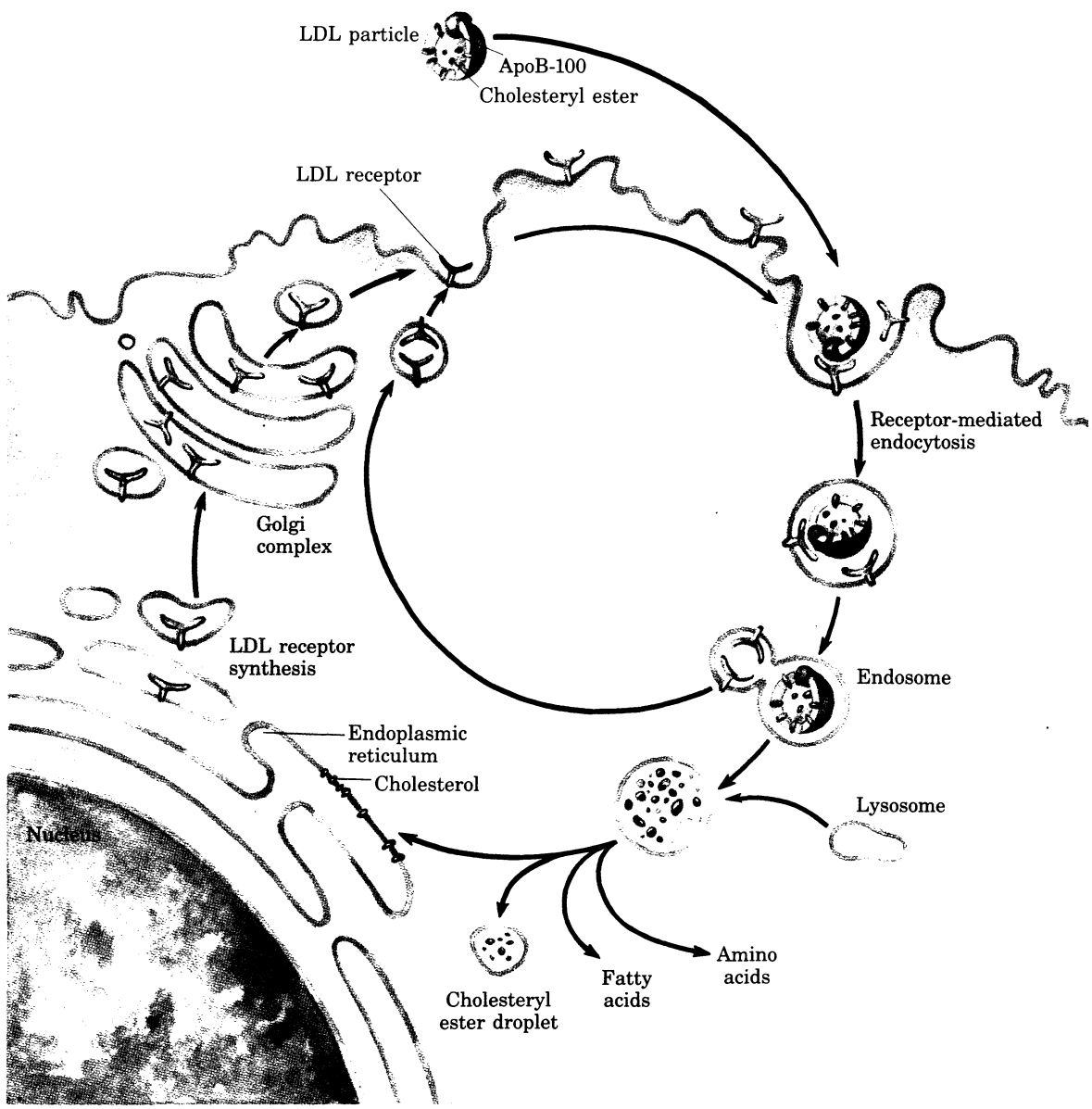


Figure 20–39 Uptake of cholesterol by receptor-mediated endocytosis. Endocytosis is also described in Chapter 2 (p. 32).

ters, releasing cholesterol and fatty acid into the cytosol. The apoB-100 of LDL is also degraded to amino acids, which are released to the cytosol, but the LDL receptor escapes degradation and returns to the cell surface, where it can again function in LDL uptake. This pathway for the transport of cholesterol in blood and its **receptor-mediated endocytosis** by target tissues was elucidated by Michael Brown and Joseph Goldstein.

Cholesterol entering cells by this path may be incorporated into membranes or may be reesterified by ACAT (Fig. 20–36) for storage within cytosolic lipid droplets. The accumulation of excess intracellular cholesterol is prevented by reducing the rate of cholesterol synthesis when sufficient cholesterol is available from LDL in the blood.

Michael Brown and Joseph Goldstein

Cholesterol Biosynthesis Is Regulated by Several Factors

Cholesterol synthesis is a complex and energy-expensive process, and it is clearly advantageous to an organism to be able to regulate the synthesis of cholesterol so as to complement the intake of cholesterol in the diet. In mammals, cholesterol production is regulated by intracellular cholesterol concentration and by the hormones glucagon and insulin. The rate-limiting step in the pathway to cholesterol is the conversion of β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) into mevalonate (Fig. 20–32), and the enzyme that catalyzes this reaction, HMG-CoA reductase, is a complex regulatory enzyme whose activity is modulated over a 100-fold range. It is allosterically inhibited by as yet unidentified derivatives of cholesterol and of the key intermediate mevalonate (Fig. 20–40). HMG-CoA reductase is also hormonally regulated. The enzyme exists in phosphorylated (inactive) and dephosphorylated (active) forms. Glucagon stimulates phosphorylation (inactivation), and insulin promotes dephosphorylation, activating the enzyme and favoring cholesterol synthesis.

In addition to its immediate inhibition of existing HMG-CoA reductase, high intracellular cholesterol also slows the synthesis of new molecules of the enzyme. Furthermore, high intracellular concentrations of cholesterol also activate ACAT (Fig. 20–40), increasing esterification of cholesterol for storage. Finally, high intracellular cholesterol causes reduced production of the LDL receptor, slowing the uptake of cholesterol from the blood.

Unregulated cholesterol production can lead to serious disease. When the sum of the cholesterol synthesized and obtained in the diet exceeds the amount required for the synthesis of membranes, bile salts, and steroids, pathological accumulations of cholesterol in blood vessels (atherosclerotic plaques) can develop in humans, resulting in obstruction of blood vessels (**atherosclerosis**). Heart failure from occluded coronary arteries is a leading cause of death in industrialized societies. Atherosclerosis is linked to high levels of cholesterol in the blood, and particularly to high levels of LDL-bound cholesterol; there is a *negative* correlation between HDL levels and arterial disease.

In the human genetic disease known as familial hypercholesterolemia, blood levels of cholesterol are extremely high, and afflicted individuals develop severe atherosclerosis in childhood. The LDL receptor is defective in these individuals, and the receptor-mediated uptake of cholesterol carried by LDL does not occur. Consequently, cholesterol obtained in the diet is not cleared from the blood; it accumulates and contributes to the formation of atherosclerotic plaques. Endogenous

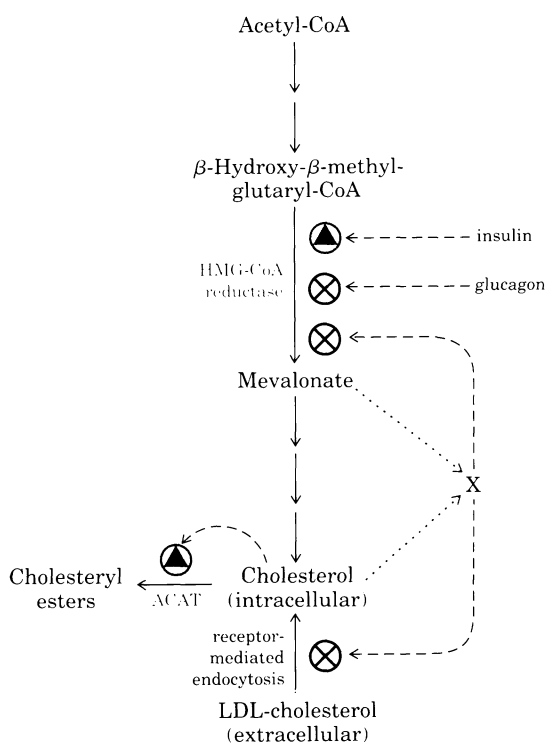
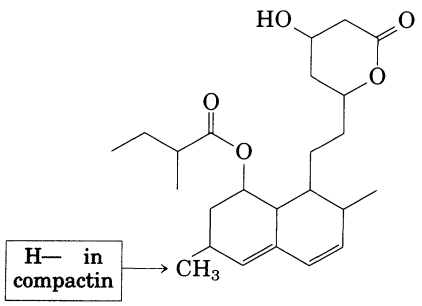


Figure 20–40 Regulation of cholesterol biosynthesis balances synthesis with dietary uptake. Glucagon acts by promoting phosphorylation of HMG-CoA reductase, insulin by promoting dephosphorylation. X represents unidentified metabolites of cholesterol and mevalonate, or other unidentified second messengers.

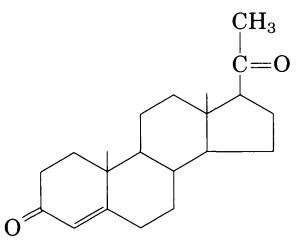


Lovastatin

cholesterol synthesis continues even in the presence of excessive cholesterol in the blood, because the extracellular cholesterol cannot enter the cytosol to regulate intracellular synthesis. Two natural products derived from fungi, **lovastatin** and **compactin,** have shown promise in treating patients with familial hypercholesterolemia. Both are competitive inhibitors of HMG-CoA reductase and thus inhibit cholesterol synthesis. Lovastatin treatment lowers serum cholesterol by as much as 30% in individuals who carry one copy of the gene for familial hypercholesterolemia. When combined with an edible resin that binds bile acids and prevents their reabsorption from the intestine, the drug is even more effective.

Steroid Hormones Are Formed by Side Chain Cleavage and Oxidation

All steroid hormones in humans are derived from cholesterol (Fig. 20–41). Two classes of steroid hormones are synthesized in the cortex of the adrenal gland: **mineralocorticoids,** which control the reabsorption of inorganic ions (Na^+, Cl^-, and HCO_3^-) by the kidney, and **glucocorticoids,** which help regulate gluconeogenesis and also reduce the inflammatory response. The sex hormones are produced in male and female gonads and the placenta. They include **androgens** (e.g., testosterone) and **estrogens** (e.g., estradiol), which influence the development of secondary sexual characteristics in males and females, respectively, and **progesterone,** which regulates the reproductive cycle in females. The steroid hormones are effective at very low concentrations, and they are therefore synthesized in relatively small quantities. In comparison with the bile salts, their production consumes relatively little cholesterol.



Progesterone

The synthesis of these hormones requires removal of some or all of the carbons in the "side chain" that projects from C-17 of the D ring of cholesterol. Side chain removal takes place in the mitochondria of tissues that make steroid hormones. It involves first the hydroxylation of two adjacent carbons in the side chain (C-20 and C-22) then cleavage of the bond between them (Fig. 20–42). Formation of the individual hormones also involves the introduction of oxygen atoms. All of the hydroxylation and oxygenation reactions in steroid biosynthesis are catalyzed by mixed-function oxidases (Box 20–1) that use NADPH, O_2, and mitochondrial cytochrome P-450.

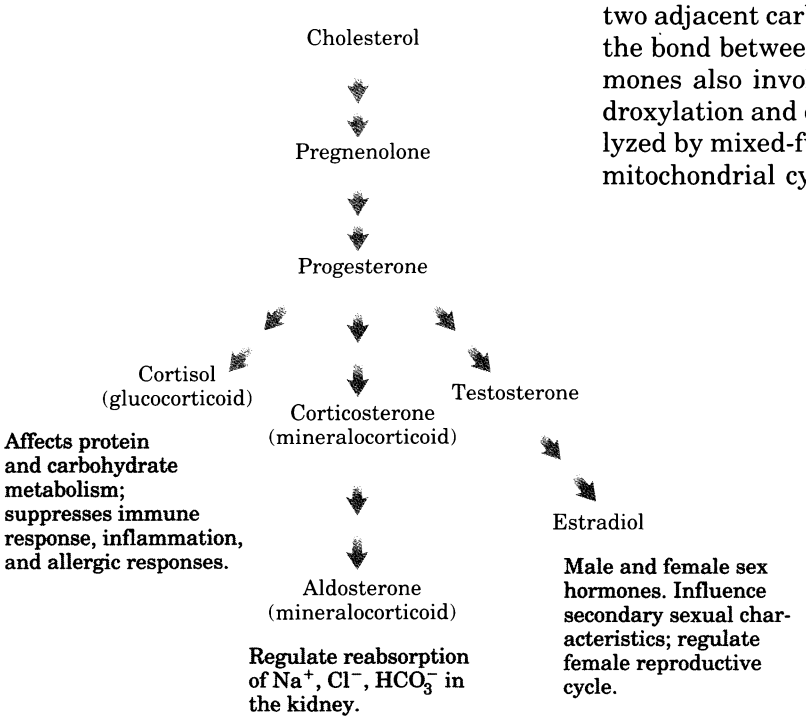


Figure 20–41 Some steroid hormones derived from cholesterol. The structures of some of these compounds are shown in Fig. 9–15.