Chapter 1
Introduction: The Structure and Composition of Biomembranes

1.1 The Importance and Diversity of Membranes

Membranes play a central role in both the structure and function of all cells, prokaryotic and eukaryotic, plant and animal. Membranes basically define compartments, each membrane associated with an inside and an outside. If this were all they did, membranes would be considerably less interesting than they are. But, membranes not only define compartments, they also determine the nature of all communication between the inside and outside. This may take the form of actual passage of ions or molecules between the two compartments (in and out) or may be in the form of information, transmitted through conformational changes induced in membrane components. In addition, attached to membranes are many cellular enzymes. Some of these enzymes catalyze transmembrane reactions, involving reactants on both sides of the membrane or molecular transport. Others are involved in sequential reactions involving a series of enzymes which are concentrated in the plane of the membrane, thus facilitating efficient interactions. Still other enzymes have membrane-bound substrates and/or are involved in the maintenance or biosynthesis of the membrane. Most of the fundamental biochemical functions in cells involve membranes at some point, including such diverse processes as prokaryotic DNA replication (e.g., refs. 807, 777, 803), protein biosynthesis, protein secretion, bioenergetics, and hormonal responses.

Electron micrographs of mammalian cells reveal the wealth of membranous organelles which comprise a large part of the intracellular volume. It is now clear that the structural principles for all these membranes are basically the same. Furthermore, these structural similarities apply also to plant cell membranes and bacterial membranes. These common features, recognized by Robertson in the late 1950s (1231), allow us to apply lessons learned in one membrane system, such as the erythrocyte membrane, to other systems, tempered with a reasonable

degree of caution. This caution is necessary because, paradoxically, one of the most salient points to be made about membranes is their remarkable diversity. This diversity is due primarily to the different functions of the proteins present in each membrane and to the way in which these proteins interact with each other as well as with cytoplasmic components. These interactions result in distinct morphologies, such as in the microvilli of the intestinal epithelium or the tubular endoplasmic reticulum, and may result in lateral inhomogeneities within a given membrane (see Section 4.5). The main point is that there is a common ground for studying membranes in general, but that an appreciation for the subject lies in large measure in the comprehension of the molecular and biological basis for the diversity in membrane structure and function.

Progress in the study of membranes has come from exploiting the advantages for studying the membranes from a variety of organisms. Bacteria have relatively simple envelopes containing one or two membranes, which can be manipulated genetically or by altering the growth conditions. Enveloped viruses enter animal cells by membrane fusion (Section 9.52) and exit by budding (Section 4.53). The maturation of viral proteins provides an excellent experimental system for studying membrane protein biosynthesis (Section 10.2).

Eukaryotic cells have numerous membranous organelles, and each membrane is unique in composition, structural detail, and function. In order to understand the motivation behind many of the studies described in later chapters it is important to have some background in the biological functions of these various membrane systems. Figure 1.1 shows a schematic indicating the various membranes as they appear in a generic animal and plant cell. Note that the appearance of the organelles will be different in other cell types, and, in addition, some cells, such as the rod cell of the retina or the skeletal muscle cell, have highly specialized membranes which have unique functions.

(1) *Plasma membrane*: The plasma membrane defines the boundaries of the cell and is the point of contact between the cell and its environment. As such, the plasma membrane contains specialized components involved in intercellular contacts and communication, hormonal response, and transport of both small and large molecules into and out of the cell. However, the plasma membrane is itself divided into specialized regions in those cells which are simultaneously in contact with different environments. Figure 1.2 shows the location of the *apical* and *basolateral* plasma membrane domains for a hepatocyte and for a polarized epithelial cell. The apical membrane is that which is in contact with the "external" environment, such as the bile canaliculus in the case of the liver cell or the gastrointestinal lumen for an epithelial cell in the gut. The apical membrane can contain specialized structures such as the *microvilli*, which can be organized to form the *brush border membranes* in some absorptive cells. Microvilli greatly increase the effective surface area of the membrane and facilitate efficient transport. The basolateral membrane is that which is in contact with other cells (lateral or contiguous membrane) or blood sinusoids (sinusoidal membrane). In the hepatocyte, the lateral and sinusoidal membranes are morphologically and biochemically separable (402).

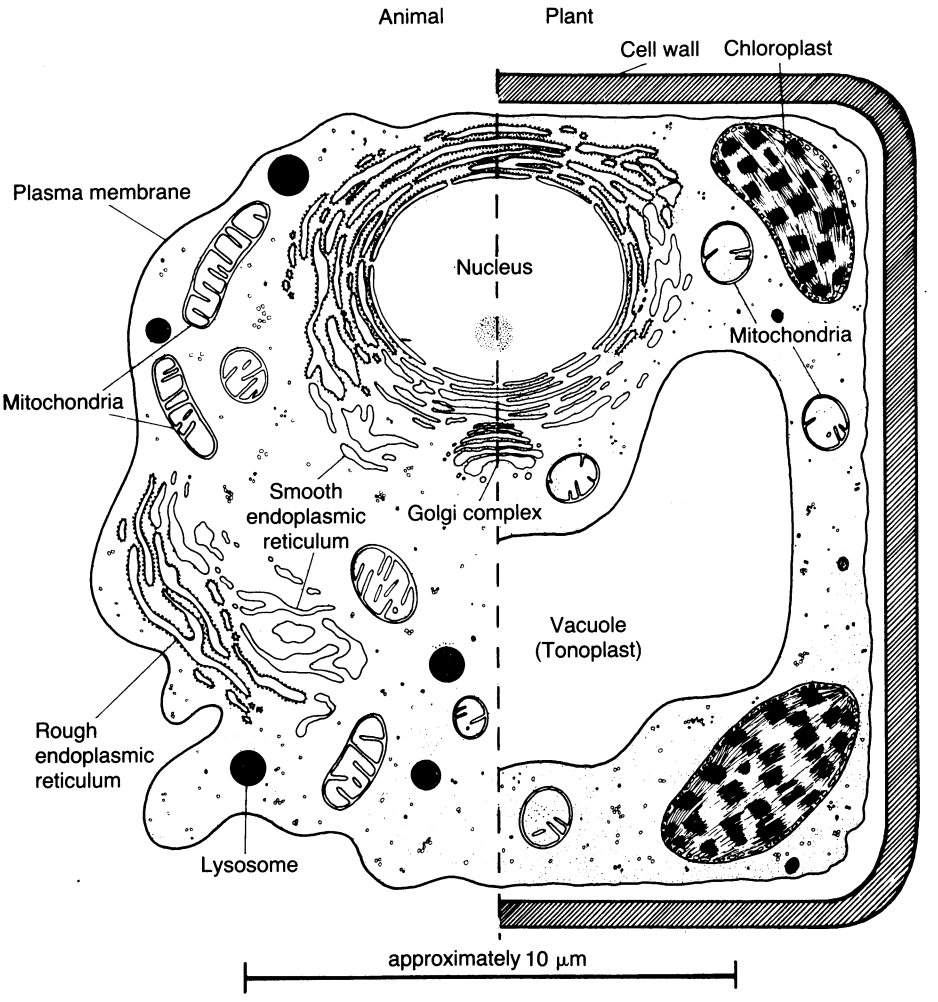


Figure 1.1. Schematic showing organelles of eukaryotic animal and plant cells as revealed by electron microscopy. Adapted from ref. 425a.

The basolateral membrane in the hepatocyte contains several specialized structures for cell–cell adhesion and intercellular transport.

Tight junctions seal the contacts between cells to prevent mixing the contents of the bile and blood vessels.

Gap junctions contain a regular array of pores that allow small molecules to pass through the plasma membranes of two adjacent cells. Electron microscopic and biochemical studies have revealed some molecular detail of these pores, showing each to contain a hexagonal array of protein subunits (see Section 8.21).

Desmosomes also function as adhesion sites between cells and are involved in

contacts between the plasma membrane and cytoskeletal elements (see Section 4.3).

The apical, lateral, and sinusoidal portions of the plasma membranes are morphologically distinct and have unique compositions and functions. If the cells are disrupted gently, these specialized regions of the plasma membrane can be physically separated and purified (402). It is not understood on a molecular level how these specialized domains of the plasma membrane are maintained in the cell, but, clearly, there cannot be free diffusion of all membrane components between them (see Section 4.51).

(2) *Nuclear membrane*: The nuclear envelope which is present in interphase cells appears in electron micrographs as a double membrane, with a narrow space in between called the perinuclear space (459). The nuclear envelope appears to be formed from portions of the endoplasmic reticulum (see below) and these two systems may, in fact, be physically continuous. The most prominent morphologi-

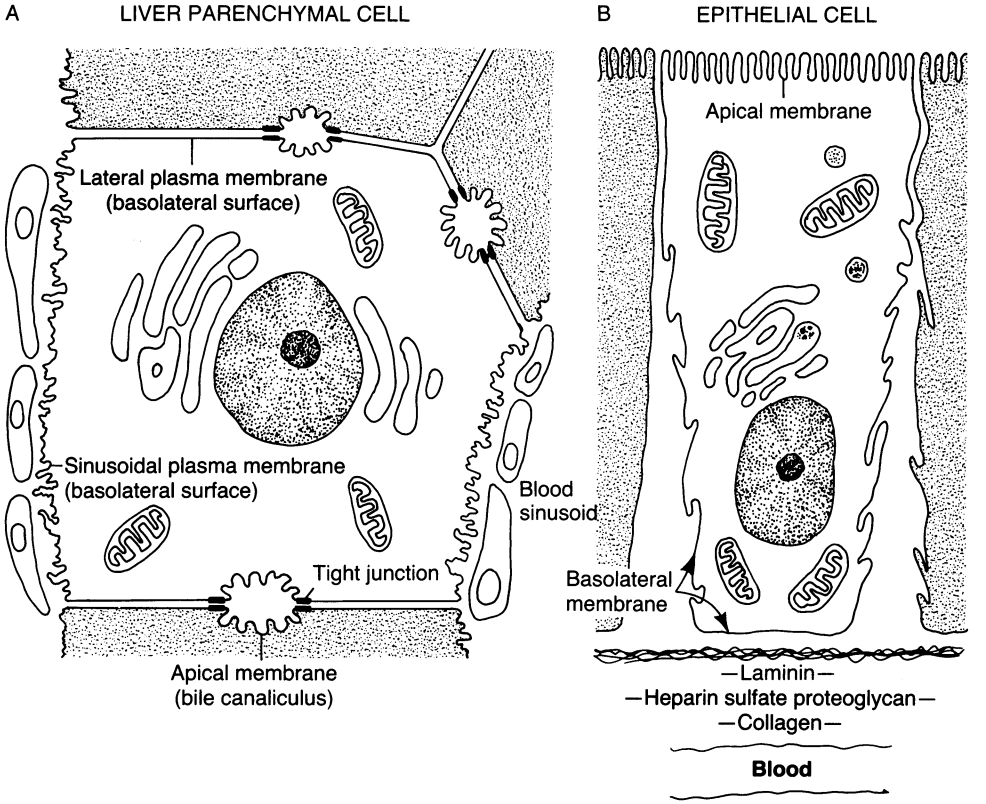


Figure 1.2. Schematic showing the plasma membrane domains of (A) a hepatocyte, and (B) a polarized epithelial cell.

cal features of the nuclear envelope are the pore structures. These nuclear pore complexes have a diameter of about 600 Å and appear to be assembled by morphologically distinct constituents arranged in octagonal symmetry (1483) (see Section 8.22). They are located in regions where the inner and outer nuclear membranes appear to fuse. The pores presumably allow the passage of mRNA–protein complexes from the nucleus to the cytoplasm and of regulatory proteins from the cytoplasm into the nucleus. Little biochemical work has been reported on the nuclear envelope.

(3) *Endoplasmic reticulum* (ER): This is a complex network of cisternae or tube-like structures which occupies a considerable portion of the internal volume of a typical animal cell. The primary purpose is to provide a site for the biosynthesis of proteins destined for secretion, for internalization into lysosomes, or for incorporation into the plasma membrane. Potentially lethal hydrolytic enzymes which are to be secreted outside the cell or sequestered in the lysosome are processed to mature forms inside the ER. Ribosomes are frequently associated with the ER membrane, giving it a rough appearance in electron micrographs (rough ER). The complex processes by which membrane, secretory, or lysosomal proteins are synthesized, matured, and properly delivered to their destinations are described in Chapter 10.

Other portions of the ER are devoid of ribosomes (smooth ER), and these are apparently sites for reactions involving sterol biosynthesis, detoxification reactions, and fatty acid desaturation, all of which involve a complex interactive electron transport system containing cytochrome b_5 and the cytochrome P450 enzymes (see Chapter 6).

(4) *Golgi apparatus*: This organelle appears as a series of tubules and stacked, disk-shaped structures called cisternae. The primary function is the post-translational modification of glycoproteins synthesized initially in the endoplasmic reticulum and destined eventually for secretion or for incorporation in the plasma membrane or for delivery to the lysosome. The organelle contains a number of glycosidases and glycosyltransferases which act sequentially as the protein being processed is passed, probably via vesicles, from one end of the Golgi stack where it enters (*cis*-Golgi), to the other end where it exits (*trans*-Golgi). The Golgi, therefore, is really composed of a series of distinct membranes making up the cisternae, In fact, different sub-fractions can be physically separated which are enzymatically distinct (1248) (see Section 10.2; Figure 10.4). The manner in which membrane and secretory proteins are transported through this system is discussed in Chapter 10.

(5) *Lysosome*: This organelle is responsible for macromolecular degradation, and contains a number of hydrolytic enzymes such as proteases and lipases (316). Materials taken into the cell by endocytosis and phagocytosis which are to be degraded are delivered via vesicles to the lysosome. Also, the breakdown involved in the normal turnover of cellular components is accomplished within the lysosome. The manner in which lysosomal enzymes are synthesized, marked for delivery to the lysosome, and then delivered is one in which some of the steps are understood reasonably well, and is discussed in Chapter 10.

(6) *Peroxisome*: This organelle contains oxidative enzymes involved in the breakdown of small molecules, such as amino acids, xanthine, and, in particular, fatty acids (933). The name derives from the presence of catalase, which breaks down peroxide, a byproduct of the oxidative reactions.

(7) *Mitochondrion*: This organelle is the site of oxidative phosphorylation where ATP is produced at the expense of the oxidation of substrates such as NADH and succinate. The mitochondrion contains two membranes and an intermembranous space. The interior is called the matrix (see Figure 10.1). The inner membrane is invaginated to form a series of septa called cristae, and contains the enzymes involved in electron transport and ATP synthesis. The role of diffusion within the plane of the membrane of the components of the electron transport chain and its functional significance are discussed in Chapter 6. The manner in which proteins which are synthesized in the cytoplasm but end up within one of the mitochondrial compartments or membranes is covered in Chapter 10.

(8) *Chloroplast*: This is the organelle containing the photosynthetic apparatus. It has an outer envelope consisting of two membranes and an interior called the stroma. Within the stroma are the thylakoid membranes, in which the photosynthetic components reside. The thylakoid membranes are closely stacked or appressed in some regions and are unstacked and exposed to the stroma in other places (see Figure 4.8). The compositions of the appressed and stroma-exposed domains of the thylakoid membrane are different, demonstrating lateral heterogeneity (see Section 4.52). The enzymology of the photosynthetic electron transport chain is discussed in Section 6.6.

Each of the membrane systems mentioned above, along with other specialized membranes from animal cells, plant cells, or bacteria, poses a different set of important and interesting research questions and provides opportunities for biochemical research. Other systems will be described in subsequent chapters in the text.

1.2 Historical Perspective

It was recognized in the mid-nineteenth century that the plasma membrane at the surface of cells is a discrete structure. At the turn of the century, Overton noted a correlation between the rate at which various small molecules penetrate plant cells and their partition coefficients between oil and water (1112), leading him to speculate on the lipid nature of the membrane. In 1925 Gorter and Grendel (532) proposed that lipids in the erythrocyte membrane are arranged in the form of a bimolecular leaflet, or lipid bilayer. This conclusion resulted from elegantly simple experiments. The erythrocyte lipids were extracted in acetone and then dispersed in water in a Langmuir trough (see Figure 2.23) so as to form a thin layer at the surface of the water. A thin thread was drawn across the surface, thus compressing the lipid molecules at the air–water interface. At a well defined point, the surface layer offered resistance, and this was interpreted as the point where

the lipid layer was a closely packed monomolecular layer. When the measured area occupied by the lipids was compared with the computed area of the erythrocytes from which the lipids were extracted, a 2:1 ratio was found. Thus, it was concluded that the membrane consists of lipids arranged in two layers. Although it is likely that the conclusions of Gorter and Grendel were correct only due to fortuitously offsetting errors (64), this work is historically significant since the concept of the lipid bilayer as the structural basis of the membrane has been dominant ever since and is certainly correct.

The bimolecular lipid membrane was further elaborated in 1935 in the Davson–Danielli or "paucimolecular" model, in which it was postulated that proteins coat the surfaces of the lipid bilayer [279] (Figure 1.3). This was a remarkably successful model, and during the next 30 years numerous experimental results, notably from X-ray diffraction and electron microscopy (see next section), provided overall support. During the same period of time, however, the enormous diversity of membrane functions was becoming clear, and the basic Davson–Danielli model was modified by numerous other workers to account for this functional diversity (e.g., 1387, 425, 880, 1229, 1500).

The rapid development leading to our current view of membranes has largely been due to the progress in characterizing membrane proteins. Freeze-fracture electron microscopy (see next section) revealed globular particles apparently embedded within the membrane (135, 136, 162). Biochemists, meanwhile, were successful at using detergents to dissociate membranes into functional "particles" (542, 543, 1501). Spectroscopic evidence also indicated that membrane proteins had an appreciable amount of α-helix and that they were likely to be globular rather than spread out in a monolayer on the lipid bilayer surface (836, 1543). The non-polar characteristics of membrane proteins (e.g., 543, 1216) also encouraged speculation on hydrophobic contacts between the proteins and the lipid bilayer interior. At the same time, experimental techniques were developed which revealed the fluid nature of the lipid bilayer (468). Singer and Nicolson amalgamated these ideas into the fluid mosaic model, which basically pictures the membrane as a fluid-like phospholipid bilayer into which freely diffusing globular proteins are embedded to varying degrees (1348, 1349). The earlier Davson–Danielli model was a static structural model largely successful at explaining the rather low-resolution structural data available at the time (see next section). In contrast, much of the focus in membrane research since 1970 has been directed at the dynamics of the membrane, and the relationship between this dynamics and membrane function. The emphasis of the fluid mosaic model has undergone modification and will continue to do so. In particular, it is now clear that membrane proteins do not all diffuse freely in the fluid lipid bilayer (690; see Chapter 5), and that there is evidence for differentiated lateral domains within membranes (693; see Chapter 4). The role of the cytoskeleton is under increasing scrutiny (see Chapter 4). There is also a growing belief that some regions of biological membranes may not be arranged in the traditional bilayer (265). Nevertheless, in various modified forms the fluid mosaic model will clearly continue to provide the conceptual backdrop for much of membrane research in the foreseeable future.

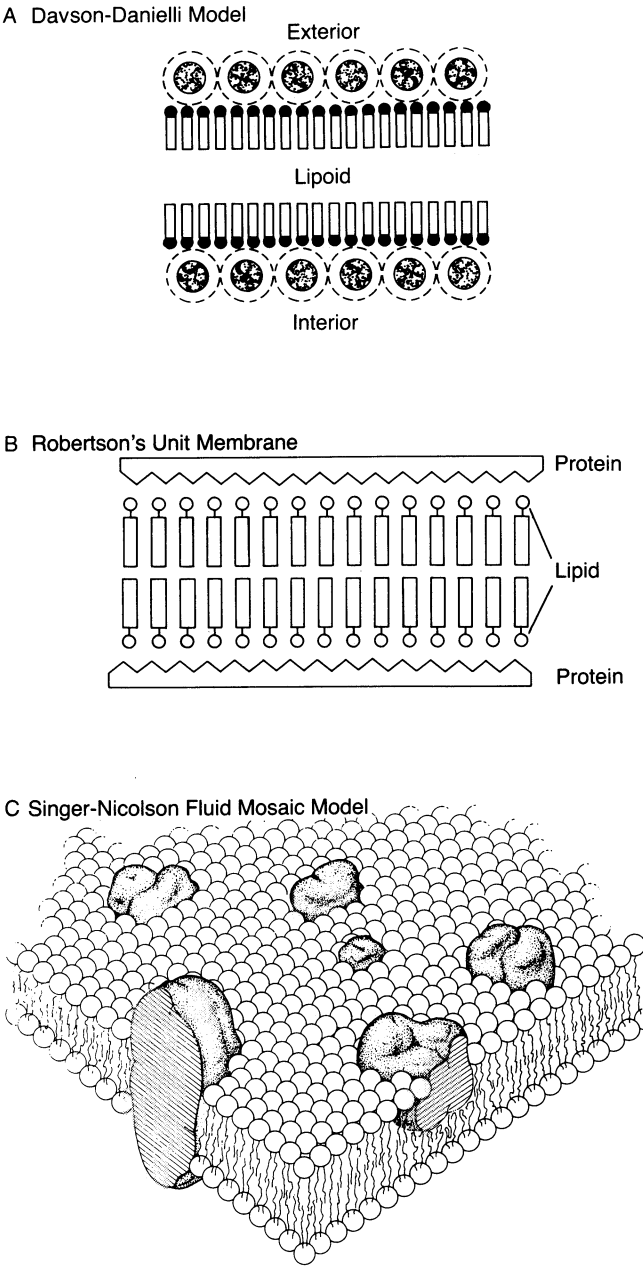


Figure 1.3. Three influential models proposed for biomembrane structure. (A) Davson-Danielli, (B) Robertson's unit membrane, and (C) Singer and Nicolson's fluid mosaic model. See text for details. Adapted from references 279 (Part A), 1230 (Part B) and 1349 (Part C). Drawing for part C provided by Dr. Singer.

1.3 Membrane Morphology

Two methods which have been of historic importance in defining membrane morphology have been X-ray diffraction analysis and electron microscopy. Both techniques have been used to confirm the bilayer model, but both methods are very limited in revealing molecular detail.

1.31 X-Ray Diffraction

X-ray diffraction techniques are capable of yielding high resolution structural information when applied to highly ordered crystalline samples. For less ordered samples, the power of this method is considerably limited. Some specialized membrane systems are naturally stacked in regular arrays and provide useful systems for applying X-ray techniques. Most notable is the myelin sheath of peripheral nerve, which is a membranous system which wraps around the axon many times, providing a regular concentric array of membranes. X-ray diffraction studies on myelin dating back to the 1930s are consistent with the bilayer model (1299, 427). Similar results have been obtained with the rod outer segment of vertebrate retinal cells (108), which also have a naturally stacked membrane system (disks), as well as with artificially stacked membranes formed by centrifuging (and collapsing) vesicular membrane preparations from sources such as the mitochondrion (1449) and the erythrocyte (426). In all cases, the data yield a similar electron density profile across the membrane, pictured in Figure 1.4.

In order to interpret the X-ray data, it is necessary to obtain the phases of the X-ray reflections in addition to the measured amplitudes. This is greatly simpli-

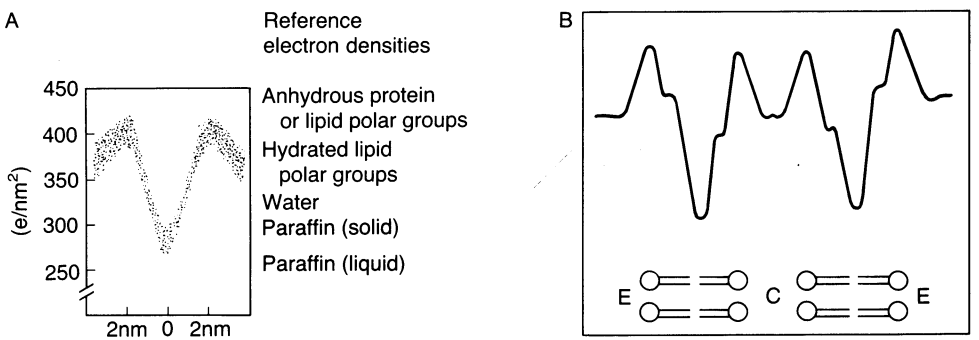


Figure 1.4. Electron density profiles of membranes from X-ray diffraction analysis. (A) Schematic of an electron density profile with calibration standards indicated. (B) The electron density profile of myelin. This represents a centrosymmetric pair of apposed membranes, with "C" indicating the apposition of the pair of cytoplasmic surfaces and "E" the extracytoplasmic surfaces. A schematic of the pair of membranes corresponding to the electron density profile is also indicated. Adapted from references 425a and 1057b.

fied for the stacked membrane systems because they all have repeating units with centrosymmetric symmetry (see 109). The data indicate that all the membranes have a similar structure, with a hydrocarbon interior (low electron density) and polar groups on either side (high electron density). There are only relatively subtle variations between different membranes seen in the X-ray data despite large differences in the protein composition of the various membranes examined, ranging from 20% to almost 80%. It is possible to obtain some information about the relative position of the bulk of the protein mass with respect to the lipid bilayer (e.g., embedded vs peripheral), but basically this X-ray technique does not yield molecular detail.

Wilkins et al. (1586) pointed out in 1971 that X-ray diffraction analysis can also be applied to dispersions of membranes and phospholipids. Reflections arise from the two polar layers on either side of the bilayer, yielding the thickness between polar headgroups (about 36 Å for pure phospholipid) and, from the ordered hydrocarbon chains, yielding the spacing between paraffin chains (about 4.2 Å when highly ordered). Again, the membrane samples from different sources yielded a similar pattern, confirming the universality of the bilayer model.

The lack of molecular detail obtainable from this technique has limited its application to biomembranes. However, these methods have been particularly useful for analyzing the ordered structures of lipid-water systems (1334).

1.32 Electron Microscopy

Transmission electron microscopy of thin sections of myelin and, in fact, virtually all membranes, shows a "trilamellar image," with two electron-dense bands separated by about 80 Å. This image is, in large part, dependent on the commonly used treatment of the samples by osmium tetroxide (956). Although the image, termed the "unit membrane image" by Robertson (1231, 1230), to stress its universality, has been viewed as confirmation of the bilayer model, the molecular basis of the osmium staining pattern is not known. It is clear, however, that the techniques used to prepare membrane samples for transmission electron microscopy can be damaging. In particular, osmium tetroxide treatment is known to cause extensive loss of protein (956) from the erythrocyte membrane. The trilamellar image in some way reflects the basic bilayer structure of membranes, but further molecular details of the location of the proteins are not obtainable.

The more recent (by now "classical") techniques of freeze-cleaving and freeze-etching yield some information about membrane proteins. The samples are rapidly frozen and not subjected to the deleterious procedures used for preparing thin sections. As pictured in Figure 1.5, the procedure involves the following steps (see 432):

1. After freezing, the sample, which may consist of a suspension of cells or membranes, is fractured with a knife at low temperatures (-100°C) and under high vacuum. This creates shear forces which result in a shear plane passing

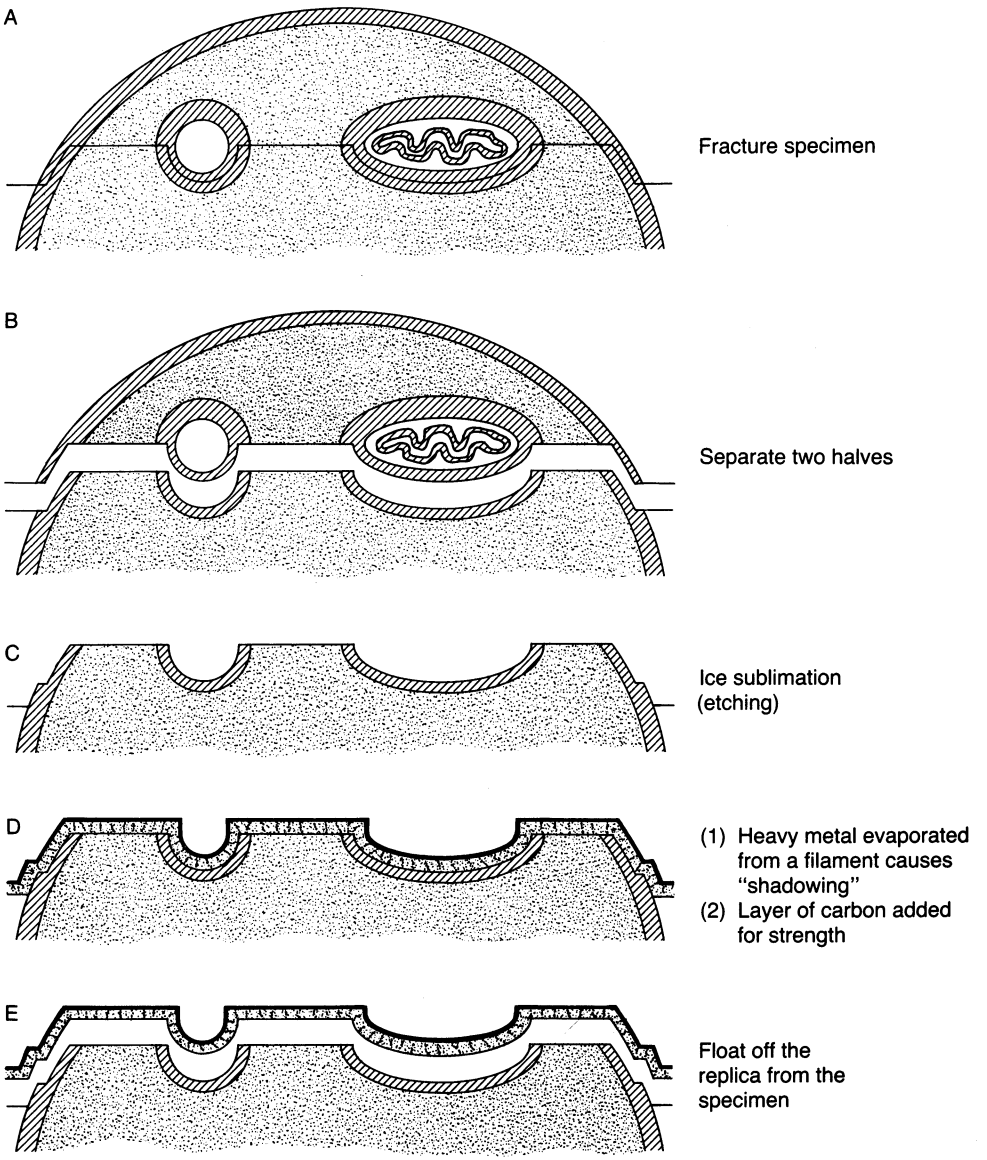


Figure 1.5. Schematic illustrating the freeze-cleavage technique. (A) A frozen cell is split by a cleavage plane which traverses, in part, through the middle of various membranes. (B) The two halves are separated. (C) The sample may be etched, exposing surface features. (D) A platinum layer is shadowed onto the sample, followed by a layer of carbon. This creates a replica of the surface of the sample. (E) The replica is removed and then examined by electron microscopy. Adapted from ref. 698. Introduction to Biological Membranes, by M. K. Jain and R. C. Wagner, Copyright © 1980. Reprinted by permission of John Wiley & Sons, Inc.

through the sample. Experience has shown that when the shear plane passes through a membrane it preferentially passes through the middle, thus dividing the membrane in half. The interior of the membrane is now exposed in these places.

2. If desired, the sample can be freeze-etched by simply allowing ice to sublime under vacuum. This results in exposing the surface structures of the cellular membranes.
3. A replica of the surface is now constructed. It is this replica which is actually observed in the electron microscope. The replica is produced by first shadowing the sample with platinum at about a 45° angle to highlight topological features. The platinum replica is given mechanical strength by depositing a layer of carbon on the sample. The sample is now thawed and the replica is floated off and picked up on an appropriate grid for examination.

The most significant features visualized by the freeze-cleavage studies are numerous intramembranous particles, about 80 Å to 100 Å in diameter, in the plane of the cleaved membranes. They are usually randomly dispersed but may also be aggregated in groups. Considerable study has shown that these particles correlate with membrane proteins. There are no corresponding features found in thin section electron microscopy. The topological features seen in the two halves of the cleaved membrane are not necessarily complementary, indicating that some of the particles are associated primarily with one or the other half of the bilayer. Freeze-fracture studies were prominently cited by Singer and Nicolson in their formulation of the fluid mosaic model, since these studies strongly implied that globular proteins reside in the interior of the bilayer and are not constrained to the bilayer surface (1349).

Box 1.1 An Example of Particles Visualized by Freeze-Cleavage

Figure 1.6 shows the results of freeze-cleavage of liposomes composed of egg yolk lecithin reconstituted with a crude preparation of Band 3, a membrane protein from the human erythrocyte (1625, 1626). Band 3 is a major protein component of the red blood cell membrane and it is known to function as an anion transporter (see Section 8.33). In the absence of this protein, the phospholipid vesicles present a smooth surface in freeze-cleaved preparations. Reconstitution of Band 3 in the phospholipid results in the appearance of particles which are indistinguishable from those observed in the red blood cell membrane (1625, 1626). Furthermore, it is known that at pH 5.5, the particles in the red cell aggregate within the plane of the membrane and that this aggregation is dependent on an interaction between the Band 3 protein with two other proteins, spectrin and actin. These latter two proteins are part of the cytoskeletal network found on the inner surface of the erythrocyte membrane (see Chapter 4). The reconstituted Band 3-lecithin system manifests similar behavior, and clumping of the particles is observed in the presence of added spectrin and actin at pH 5.5, but not at pH 7.6 (1625, 1626).

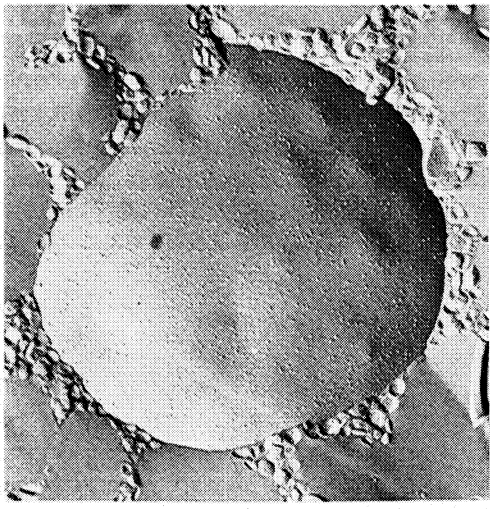


Figure 1.6. Freeze-fracture electron micrograph of reconstituted vesicles composed of egg phosphatidylcholine and a crude preparation of Band 3 from the human erythrocyte. The particles observed here resemble those seen in similar preparations of erythrocyte membrane. From ref. 1625.

These kinds of data helped to strengthen the concept that membrane proteins are globular units free to diffuse laterally in the plane of the membrane. Ironically, the static electron micrographs from freeze-cleave studies contributed to the appreciation of the dynamic potential of the membrane. We shall see later in Chapter 5 that there are many proteins which are not freely diffusing in the "sea of lipid," including Band 3.

1.4 Membrane Isolation

There has been a growing appreciation over the past 3 decades of the enormous number of cellular functions which are membrane-dependent. Both plant and animal cells are highly compartmentalized, and many of the cytoplasmic organelles are membranous, as outlined in Section 1.1. In addition to the organelles indicated for the generalized case, there are specialized membranous systems such as the sarcoplasmic reticulum of muscle cells, the myelin sheath around peripheral nerve axons, the chloroplast thylakoid membrane, and the disk membranes of the rod cells in the retina. Prokaryotic organisms also have membranes, although the internal elaborations found in the eukaryotic systems are generally not present. Gram-positive bacteria such as *Bacillus subtilis* have a single cytoplasmic membrane, whereas gram-negative bacteria such as *Escherichia coli* have, in addition, an unusual outer membrane external to a thin peptidoglycan cell wall (see Section 4.42). Some specialized organelles are also found in prokaryotic organisms, notably the chromatophore containing the photosynthetic apparatus in purple non-sulfur bacteria such as *Rhodobacter sphaeroides*. Some animal cell viruses, known as enveloped viruses, also are surrounded by true membranes, and these have been quite useful systems for study.

Most studies on membranes require as a prerequisite the purification of the particular membrane to be examined. Each system presents unique preparative problems. For example, if one is interested in studying the plasma membrane of a particular cell population (e.g., hepatocytes of the liver) it is obviously advantageous to first isolate these cells from the whole tissue. Then, one must consider the optimal procedures for cell disruption and for physically separating the membrane of interest from the other cellular components. An important consideration is the criterion used to assess the purity of the membranes obtained.

1.41 Cell Disruption

It is desirable to select a procedure which will effectively disrupt the cell without destroying the membrane structure to be isolated. In the case of many animal cells, relatively gentle procedures can be used, such as a Dounce or Potter–Elvehjem glass–Teflon type of tissue homogenizer (e.g., 436). This disrupts the cells by shear forces by forcing the suspension through a narrow gap between a Teflon plunger and the glass wall of the apparatus. This should strip off the plasma membrane and sever the connections between the various organelles but still maintain the integrity of the individual organelles. Specialized regions of the plasma membrane, such as the basolateral and apical membranes of epithelial cells, can also be severed by these procedures. It is usually desirable to work under conditions in which the organelles remain intact to minimize release of hydrolytic enzymes (e.g., from lysosomes) and to optimize subsequent separation procedures.

Harsher procedures are required to disrupt cells which have walls, such as bacteria, fungi, and plant cells. Sometimes the cells are pre-treated with degradative enzymes prior to physical disruption to assist in breaking the cell wall. For example, Tris-EDTA and lysozyme treatments can be used when disrupting *E. coli* (1102). The more harsh disruption techniques rely on grinding, sonication, and extrusion. Grinding is usually done in the presence of an abrasive such as sand, alumina, or glass beads. Small-scale work can be done with a mortar and pestle, but mechanical devices can also be used. Sonication is often used for breaking bacterial cells. Presumably, this technique works by creating shear forces in solution produced by cavitation. Shear forces are also produced by extruding the cell suspension through a small orifice, e.g., as occurs with the French press. There are many variations on these techniques and the choice will depend on the particular system being examined.

It should be noted that disrupted and fragmented membranes will usually spontaneously form vesicles. Examples (see 435) include (1) microsomes derived from plasma membrane, endoplasmic reticulum, or specialized systems such as the sarcoplasmic membrane of muscle cells; (2) submitochondrial particles, from inner mitochondrial membrane; (3) synaptosomes, derived from pinched-off nerve ends at synaptic junctions; and (4) bacterial membrane vesicles (Kaback vesicles) from the cytoplasmic membrane of *E. coli*. Other membrane systems such as

Golgi also vesiculate. In most cases, the size of the vesicles is critically dependent on the method used to disrupt the cells. Since the vesicle size in large part determines the sedimentation rate (see next section) and behavior in subsequent purification steps, the disruption step is of obvious importance. Some membranes do not form vesicles, notably the lateral or contiguous membranes of animal cells (see Figure 1.2), which are stripped off as pairs of adjacent membranes derived from neighboring cells, held together by junctions. The presence of these junctions prevents vesicle formation, and these membranes are isolated as sheets or ribbon-like structures (402).

The choice of medium used for cell disruption can also be important. For example, in order to maintain the structure of sealed membranous organelles, it is important to use a breakage medium which is iso-osmotic with the organelle interior. Sucrose (0.25–0.30 M) is most commonly used for this purpose, but sorbitol and mannitol are also utilized and in some cases favored (e.g., 52). It should be noted that the subsequent preparative steps for intact organelles are usually critically dependent on the maintenance of isotonic conditions.

1.42 Membrane Separations

By far the most widely used technique for membrane separations is centrifugation. (See Box 1.2; refs. 1209, 1459.) Particles can be separated from each other on the basis of their sedimentation rate or on the basis of differences in their buoyant density. The former is called S-value or zonal centrifugation, whereas the latter is equilibrium density or isopycnic centrifugation. In practice, separations are actually based on a hybrid of these two methods. Figure 1.7 shows the location of a number of subcellular particles in "S-ρ space." On the axis are plotted the sedimentation coefficients of the "particles," and on the ordinate is the density. Separations based on sedimentation rate are clearly pictured by comparing S-values along the axis. For example, nuclei have a relatively high S-value, indicating that the sedimentation rate is substantially higher than those of most other subcellular organelles. Nuclei can be pelleted by differential sedimentation of cellular homogenates leaving the other organelles in the supernatant. On the other hand, smooth and rough endoplasmic reticulum cannot be separated by zonal sedimentation.

In order to separate different membrane fractions from a cellular homogenate, it is often necessary to take advantage of the differences in density. This is done by centrifuging in a density gradient of a centrifugation medium (1178). Most often, sucrose density gradients are utilized. There are, however, serious drawbacks to the use of sucrose. In order to attain the density required to separate various membrane fractions, it is necessary to use high concentrations of sucrose which are both highly viscous and hypertonic. Exposure of subcellular organelles to hypertonic sucrose solution results in dehydration and, often, readjustment of the solution afterwards to isotonic conditions results in lysis and damage of the organelle (e.g., 1020). Another problem is that many membrane organelles are

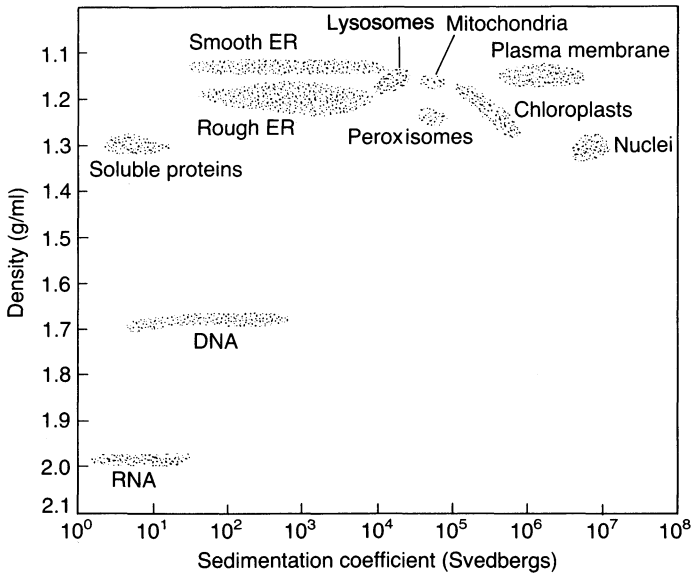


Figure 1.7. Subcellular particles displayed in S-ρ space. The ordinate is equilibrium density and the abscissa is the apparent sedimentation coefficient on a logarithmic scale. Note that the actual values will vary in different gradient media. The values for RNA and DNA apply to CsCl gradients. Adapted from ref. 1178.

permeable to sucrose. This can also result in osmotic disruption of the organelle. Penetration by sucrose can also alter the effective density of the particles being separated (1140).

To overcome some of these drawbacks, other density gradient media are becoming increasingly utilized. Some are listed in Table 1.1.

(l) *Ficoll*: A high molecular weight (ca. 400,000) hydrophilic polymer of sucrose which can be used to cover a density range up to about 1.2 g/ml. A major advantage is low osmotic pressure compared to the equivalent concentration of sucrose (w/v %). Density gradients can, thus, be constructed which are isotonic throughout by including sucrose (0.25 *M*) or physiological salts in the medium. Disadvantages include high viscosity and very non-linear dependence of the viscosity and osmolarity on concentration (1039).

Table 1.1. Physical properties of gradient media

	Concentration (% w/v)	Density (g/ml)	Viscosity (cP)	Osmolality (mOs/kg H_2O)
Sucrose	20	1.06	30	700
Metrizamide	30	1.16	2	260
Ficoll	30	1.10	49	130
Percoll	26	1.13	10	10

Data from ref. 1140.

(2) *Metrizamide*: A triiodinated benzamide derivative of glucose (mol. wt. 789) (1039). At equivalent concentrations, metrizamide solutions are more dense than Ficoll solutions. A major advantage is very low viscosity to facilitate rapid separation. At a concentration of 35% metrizamide, the solution has approximate physiological osmolarity, so most separations can be performed without exposing the membranes to hypertonic solutions. *Sodium metrizoate* is a related compound with similar properties but with the difference that it is isotonic at about 20% (w/v). This is used primarily for isolating intact cells. *Nycodenz* is also a derivative of triiodobenzoic acid with three hydrophilic side chains. Upon centrifugation, it will form its own density gradient rapidly, and is used for the preparation of subcellular organelles (e.g., 403) (Accurate Chemical and Scientific Corp., Westbury, N.Y.)

(3) *Percoll*: A colloidal suspension of polyvinylpyrrolidine (PVP)-coated silica (1140). The coating mitigates many of the toxic properties of the silica gel. Major advantages are that Percoll does not penetrate biological membranes, and solutions have low viscosity and low osmolarity. Because of the large particle size, centrifugation at moderate speeds results in self-generation of the Percoll density gradient (e.g., 30,000 × g for 30 minutes). Hence, separations are usually very rapid. Centrifugation medium can be made isotonic throughout by inclusion of salts or sucrose. Shallow gradients are easily generated which result in very high resolution of membrane fractions based on buoyant density (e.g., 38, 914, 680).

(4) *Sorbitol and mannitol*: Sometimes used in place of sucrose because they reportedly do not penetrate some biological membranes as readily as does sucrose (1179).

Note that glycerol is not used as a density gradient medium because sufficiently high densities cannot be attained. Alkali salts such as CsCl are used only when high densities are required. The salt concentrations required for equilibrium density are often deleterious.

Other techniques are also used for separating membranes from cell homogenates, though much less frequently than centrifugation.

(1) *Phase partitioning* (14, 15): Separates membranous particles according to their surface properties. Two (or three) immiscible aqueous layers are formed by mixing different water-soluble polymers with water. Examples are polyethylene glycol–dextran and dextran–Ficoll. The particles separate according to their relative affinities for the phases. The phases can be designed to separate species based on such properties as surface charge or hydrophobicity.

(2) *Continuous free-flow electrophoresis* (583, 584, 403): Separates on the basis of electrical charge. The sample is added continuously to a thin film of buffer flowing vertically. An electric field is applied perpendicular to the direction of flow. Particles are separated by electrophoresis across the flowing sheet of buffer, which is collected in a series of fractions at the bottom of the separation chamber.

(3) *Affinity adsorption*: Separation is based on a biospecific interaction between a membrane component and a solid phase. The advent of monoclonal antibodies, in particular, has made it possible to design preparative protocols based on the presence of a particular antigenic component in the membrane of interest. Once a monoclonal antibody preparation is available, the antibodies can

be covalently attached to a solid support and used to specifically bind to the membrane of interest. More frequently, this method is used to isolate a particular membrane protein (e.g., 1613). Problems encountered include the necessity of eluting the membranes without denaturation.

(4) *Silica microbeads* (553): Plasma membranes can represent as little as 1% of the total membrane mass of a eukaryotic cell. Consequently, the isolation of plasma membrane free of contaminating membranes can be a major problem. One approach specifically utilized for plasma membranes is to coat the intact cell (or protoplast) with a layer of cationic microbeads. These bind tightly to the outer surface of the plasma membrane and the plasma membrane sheets bound to the beads are easily separated on a sucrose gradient from all other contaminants by virtue of the high density of the beads. An additional feature is that the cytosolic face of the plasma membrane is exposed to solution in the final preparation.

Box 1.2 Sedimentation Velocity and Sedimentation Equilibrium

The rate at which a particle sediments in a centrifugal field is given by its sedimentation coefficient or S-value. This essentially is a measure of the steady-state velocity of the particle per unit of applied force:

$$S = \frac{dr/dt}{\omega^2 r} \qquad [1.1]$$

where S = sedimentation coefficient, usually given in Svedberg units (1 Svedberg = 10^{-13} sec)
r = distance from the center of the rotor
ω = circular velocity of the rotor, rotor rpm $\times$ $(2\pi/60)$ sec^{-1}

The S-value is determined by the size, shape, and density of the sedimenting particle and by the density and viscosity of the medium.

$$S = \frac{M(1 - \bar{V}\rho)}{Nf} \approx \frac{M(1 - \rho_{solution}/\rho_{particle})}{Nf} \qquad [1.2]$$

where M is the molecular weight of the particle
$\bar{V}$ is the partial specific volume of the particle or approximately the inverse of the particle density
$\rho_{particle}$ is the density of the particle
$\rho_{solution}$ is the solution density
N is Avogadro's Number
f is the frictional coefficient, which measures the amount of friction due to the particle moving through the solution

Note that the tabulated S-values, $S_{20,w}$, are all given for standard solution conditions: water at 20°C. This equation says that the rate of sedimentation is directly proportional to the mass of the particle which has been corrected for buoyancy by subtracting the mass of displaced solution, and is inversely proportional to the frictional resistance encountered. This frictional coefficient contains information

about size and shape of the particle. For spherical particles, the frictional coefficient $f = 6\pi\eta R$, where η is the solvent viscosity and R is the radius of the particle. Everything else being equal, any shape other than a sphere will have a higher frictional coefficient and, therefore, sediment more slowly.

Differential sedimentation takes advantage of the differences in *S*-values of particles to be separated. If these differences are large, one class of particles will pellet at the bottom of the centrifuge tube, and the other particles will remain in the supernatant. The differences in S-values may result from differences in particle mass, shape, or density or, as is usual, a combination of all three. For example, whole cells and nuclei are removed from disrupted membranes by pelleting. Mitochondria can similarly be separated from microsomes (see Figure 1.7).

Density gradient centrifugation is required when simple pelleting will not suffice. In this case, the density of the medium is varied from most dense at the bottom of the tube to least dense at the top. The gradient may be in any form, and the most commonly used gradients are either linear or step gradients. The gradient serves several purposes. It stabilizes the solution in the centrifuge tube against convective disturbances. This allows one to separate stable zones of material which have sedimented at different rates by stopping the centrifuge before pelleting has occurred or equilibrium has been reached. Importantly, density gradient centrifugation also allows one to do isopycnic centrifugation. In this case the particles sediment to a point in the tube where $\rho_{particle} = \rho_{solution}$, at which the sedimentation velocity is zero (see Equation 1.2).

Note that if the particle is more dense than the surrounding medium, it will sink ($S > 0$) in the centrifugal field. If the particle, however, is less dense, then $S < 0$, and the particle will rise. This phenomenon is called *flotation* and is frequently used to isolate membranes. When membranes are separated by isopycnic centrifugation, one is exploiting the differences in membrane density, which are usually determined by the protein/lipid ratio. For example, rough endoplasmic reticulum with attached polysomes is more dense than the smooth endoplasmic reticulum. Bacterial inner and outer membranes from gram-negative organisms also are clearly separated on the basis of density due to compositional differences.

Often membrane purification protocols are empirically optimized and represent a hybrid separation due to S-value differences and density differences. In a single density gradient some particles (or bands) may be at equilibrium (isopycnic) whereas others may not be equilibrated at the time the centrifugation is stopped.

1.43 Criteria of Membrane Purification

By far the most critical assessment of membrane purity is to assay for particular components known to be present uniquely or predominantly in the membrane being purified. Usually, these are enzymes, called marker enzymes. Examples of marker enzymes which have been used to monitor membrane purification are shown in Table 1.2. In performing these assays one must be aware of the potential problem of enzyme latency, which can result if an enzyme is inside of a vesicular membrane and unable to interact with its substrate. Other potential problems are discussed in ref. 436, but in many cases the procedures have been standardized.

Table 1.2. Markers used to monitor membrane purification from mammalian cells.[1]

Cell fraction	Marker enzyme activity
Plasma membranes	5'-Nucleotidase
	Alkaline phosphodiesterase
	Na^+/K^+-ATPase (basolateral in epithelial cells)
	Adenylate cyclase (basal in hepatocytes)
	Aminopeptidase (brush border in epithelial cells)
Mitochondria (inner)	Cytochrome *c* oxidase
	Succinate-cytochrome *c* oxidoreductase
Mitochondria (outer)	Monoamine oxidase
Lysosomes	Acid phosphatase
	ß-Galactosidase
Peroxisomes	Catalase
	Urate oxidase
	D-Amino acid oxidase
Golgi	Galactosyltransferase (see Figure 10.4)
Endoplasmic reticulum	Glucose-6-phosphatase
	Choline phosphotransferase
	NADPH-cytochrome *c* oxidoreductase
Cytosol	Lactate dehydrogenase

[1]From refs. 436, 698, and 404.

In some cases, the most convenient membrane markers are not enzymes but receptors for specific lectins, hormones, toxins, or antibodies. For well characterized systems, the protein profile on sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis can be used to indicate purity. For example, the outer membrane of gram-negative bacteria has a distinctive set of polypeptides not found in the cytoplasmic membrane (885). Other features used to evaluate a membrane preparation include morphology, as determined by electron microscopy, and gross compositional characteristics. For example, subcellular membrane preparations containing plasma membrane, Golgi, or mitochondria can be distinguished based on morphology. Cholesterol content can, in some cases, be used to evaluate a preparation. For example, mitochondria have a much lower cholesterol content than does Golgi or plasma membrane.

1.5 Composition of Membranes

The major components of membranes are proteins and lipid. Carbohydrate may comprise as much as 10% of the weight of some membranes, but the carbohydrate is invariably in the form of glycolipid or glycoprotein. The relative amounts of protein and lipid vary significantly, ranging from about 20% (dry weight) protein (myelin) to 80% protein (mitochondria). Tables 1.3 and 1.4 summarize the com-

Table 1.3. Composition of subcellular membranes from rat liver[1]

	Percentage of total phospholipid					
	Mitochondria	Microsomes	Lysosomes	Plasma membrane	Nuclear membrane	Golgi membrane
Cardiolipin	18	1	1	1	4	1
Phosphatidylethanolamine	35	22	14	23	13	20
Phosphatidylcholine	40	58	40	39	55	50
Phosphatidylinositol	5	10	5	8	10	12
Phosphatidylserine	1	2	2	9	3	6
Phosphatidic acid	—	1	1	1	2	<1
Lysophosphoglycerides[2]	1	11	7	2	3	3
Sphingomyelin	1	1	20	16	3	8
Phospholipids (mg/mg protein)	0.175	0.374	0.156	0.672	0.500	0.825
Cholesterol (mg/mg protein)	0.003	0.014	0.038	0.128	0.038	0.078

[1]Data from ref. 284. Additional tables of lipid compositions are found in ref. 1570. Endosomes are reported to have a composition similar to that of the plasma membrane (404).

[2]High values of lysophosphoglycerides should be viewed with caution, since this could result from breakdown during preparation.

Table 1.4. Protein and lipid composition of some animal cell and bacterial membranes. *L/P* is the ratio of lipid/protein (dry weight).[1]

Membranes	Major proteins	*L/P* (w/w)	Major lipids
Myelin (human)	Basic protein Lipophilin (proteolipid)	3–4	PC 10% PE 20% PS 8.5% SM 8.5% Ganglioside 26% Cholesterol 27%
Disk membranes (bovine)	Rhodopsin	1	PC 41% PE 39% PS 13% Trace of cholesterol
Erythrocytes (human)	Band 3 Glycophorin Spectrin Glyceraldehyde-3 phosphate dehydrogenase	0.75	PC 25% PE 22% PS 10% SM 18% Cholesterol 25%
Rectal gland plasma membrane (dogfish)	Na^+/K^+-ATPase (I)		PC 50.4% PE 35.5% PS 8.4% PI 0.5% SM 5.7% Cholesterol
Cholinergic receptor membranes (*Torpedo marmorata*)	Acetylcholine receptor	0.7–0.5	PC 24% PE 23% PS 9.6% Cholesterol 40%
Sarcoplasmic reticulum (rabbit)	Ca^{2+}-ATPase	0.66–0.7	PC 66% PE 12.6% PI 8.1% Cholesterol 10%
E. coli (inner membrane)		0.4	PE 74% PG 19% CL 3%
Purple membrane *(Halobacterium halobium)*	Bacteriorhodopsin	0.2	Phosphatidyl-glycerophosphate 52% Glycolipids 30% Neutral lipids 6%

[1] The major proteins in these membranes have been intensively studied and are discussed in other portions of the book. Abbreviations used: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; SM, sphingomyelin; PI, phosphatidylinositol; PG, phosphatidylglycerol; CL, cardiolipin. Data from ref. 330.

position of a number of membranes. The density of a membrane is directly proportional to the amount of protein in the membrane. Higher protein composition results in increased density as determined by isopycnic centrifugation.

To some extent, the protein components associated with a membrane will depend on the procedures used to isolate the membrane. A number of proteins are not strongly associated with membranes and can easily be removed by such procedures as washing at high or low ionic strength, washing at alkaline pH, or including a chelator such as EDTA in the buffer. In some cases, it is difficult to distinguish proteins which should properly be considered as membrane components from cytoplasmic proteins which may bind adventitiously to the membrane surface during the isolation procedures.

1.51 Membrane Lipids

The most striking feature of membrane lipids is their enormous diversity. The reason for the diversity is not at all clear, although there is an increasing awareness of the multiple roles of lipids in membranes (see Section 1.52). Certainly the major role of membrane lipids is to form the bilayer matrix with which the proteins interact. The major lipid classes are pictured in Figure 1.8 and are briefly discussed below.

Glycerophospholipids

These are the most commonly found membrane lipids. One of the glycerol hydroxyls is linked to a polar phosphate-containing group and the other two hydroxyls are linked to hydrophobic groups. Glyceride nomenclature is often in terms of the stereospecific numbering (*sn*) system. When the glycerol is drawn in a Fischer projection, with the hydroxyl in the middle drawn to the left, the positions are numbered as shown in Figure 1.9, and the prefix *sn-* is used before the name (e.g., *sn*-3 position). Several different stereochemical conventions are used: *sn*, D/L, and *R/S*. Figure 1.9 also illustrates the stereochemistry about carbon atom C-2 in the three conventions (see ref. 604). Natural phospholipids generally have the *R* (or D) configuration.

Most phosphoglycerides have the phosphate at the *sn*-3 position of glycerol. The phosphate is usually linked to one of the several groups as indicated in Figure 1.10, including choline, ethanolamine, *myo*-inositol, serine, and glycerol.

The long-chain hydrocarbons attached to *sn*-1 and *sn*-2 positive may be attached through ester or ether linkages. The chains themselves vary widely in terms of length, branching, and degree of unsaturation.

(a) *1,2-Diacylphosphoglycerides or phospholipids.* These fatty acid esters of glycerol are the predominant lipids in most eukaryotic and prokaryotic membranes, excluding archaebacteria (1296). Phosphatidylcholine is a major component in animal cell membranes, and phosphatidylethanolamine is often a major component in bacterial membranes. Table 1.5 lists a number of the more common

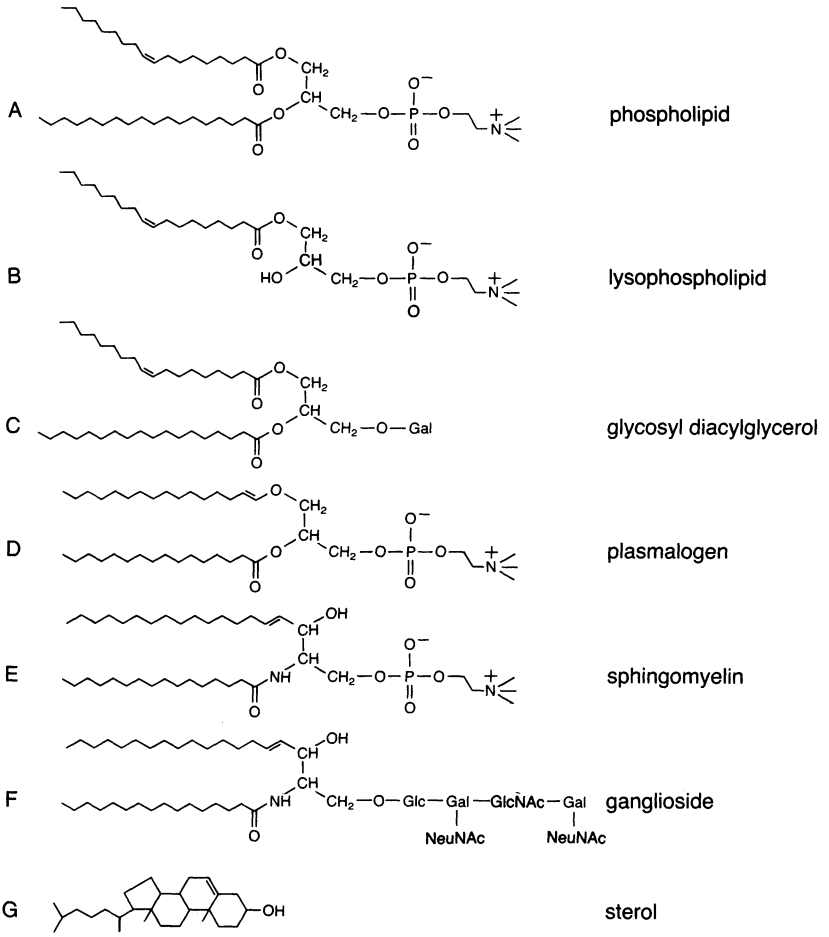


Figure 1.8. The structures of some classes of membrane lipids. The structures are drawn so as to emphasize the amphipathic nature of the lipids, with nonpolar groups on the left and polar moieties on the right. Gal, galactose; Glc, glucose; NeuNAc, *N*-acetylneuraminic acid (sialic acid); GlcNAc, *N*-acetylglucosamine. Adapted from ref. 658. Copyright 1982, Reprinted by permission of John Wiley & Sons, Ltd.

Figure 1.10. Structures of membrane lipids, illustrating the variety of polar head groups. At neutral pH, the amino group of the ethanolamine moiety will be protonated.

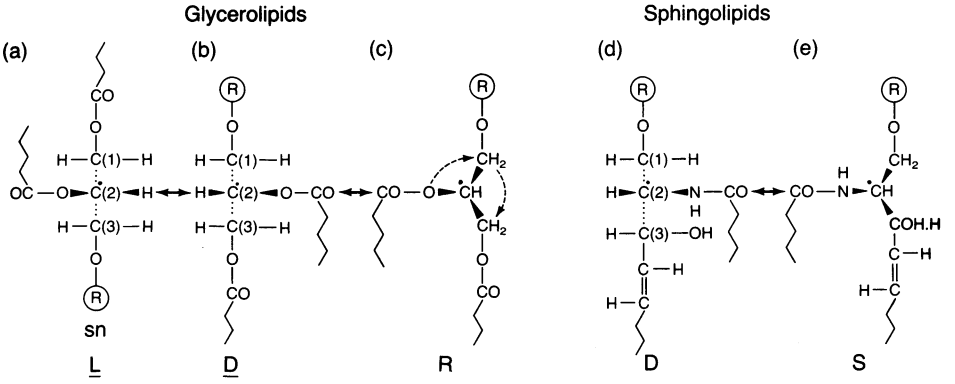
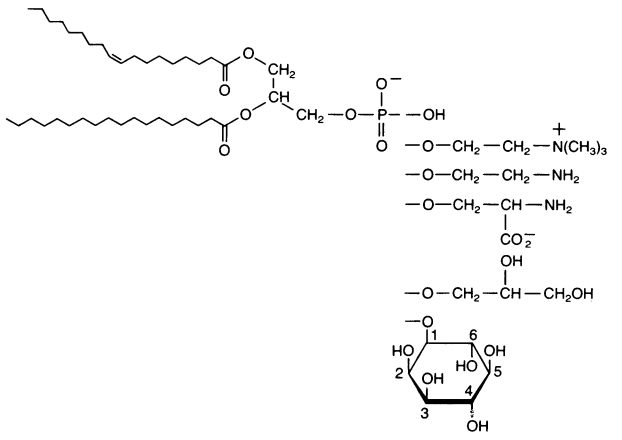
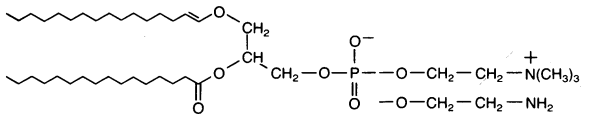


Figure 1.9. Stereochemical conventions for glycerolipids and sphingolipids. The *R/S*, D/L, and *sn* nomenclatures are illustrated for the C(2) positions. Adapted from ref. 604.





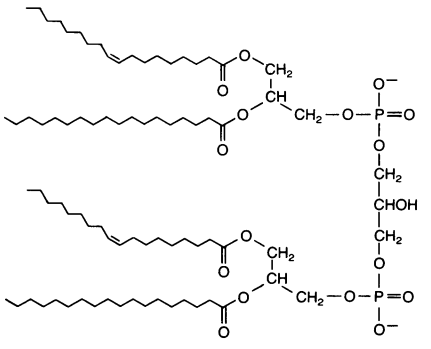


Table 1.5. Some fatty acids commonly found in membrane lipids. The common names are given.

Common name	Chain length: unsaturation
Lauric	12:0
Myristic	14:0
Palmitic	16:0
Palmitoleic	16:1 (9-*cis*)
Stearic	18:0
Oleic	18:1 (9-*cis*)
Vaccenic	18:1 (11-*cis*)
Linoleic	18:2 (9-*cis*, 12-*cis*)
γ-Linolenic	18:3 (6-*cis*, 9-*cis*, 12-*cis*)
α-Linolenic	18:3 (9-*cis*, 12-*cis*, 15-*cis*)
Arachidic	20:0
Behenic	22:0
Arachidonic	20:4 (5, 8, 11, 14-(all)*cis*)

fatty acids found in phospholipids, and Table 1.6 gives the fatty acid compositions of organelle membranes from rat liver mitochondria. The acyl chains nearly always have an even number of carbons, ranging from C14 to C24. Most common are C16, C18, and C20. The degree of unsaturation varies widely, but the most common unsaturated species are 18:1, 18:2, 18:3, and 20:4. In this notation, the first number indicates the chain length and the second figure is the number of double bonds. Nearly all naturally occurring double bonds are *cis* rather than *trans*. This places a kink in the molecule which is generally disruptive of ordered packing of the chains in the bilayer (see Chapter 2). Many phospholipid molecules have one saturated and one unsaturated chain. In animal cells, the unsaturated chain is usually found esterified to the *sn*-2 position of glycerol, and the same is true in *E. coli* (see Figure 10.16). Polyunsaturated chains are generally not conjugated. Branched chains, cyclic chains (e.g., cycloproprane-containing chains), and ß-OH groups are found in some bacterial membranes. Figure 1.11 gives the structures of some of these less common fatty acid structures (1262).

(b) In *archaebacteria* the stereoconfiguration of the glycerophospholipids is reversed, with the phosphoryl groups on the *sn*-1 position of the glycerol (1296). In many of these bacteria the hydrophobic constituents are isopranyl glycerol ethers rather than fatty acid esters (1296, 719, 1007, 307, 888) (see Figure 1.11).

(c) *Cardiolipids or diphosphatidylglycerols* (see Figure 1.10). These are essentially dimeric phospholipids. They are a significant component of the mitochondrial inner membrane, chloroplast membrane, and some bacterial membranes, but are rare in other membranes.

(d) *Plasmalogens*. These are phosphoglycerides where one of the hydrocarbon chains is linked via a vinyl ether linkage (see Figures 1.9 and 1.10). Ethanolamine plasmalogens are an important component of myelin and of the cardiac sarcoplasmic reticulum (554).

Table 1.6. Fatty acid compositions of some membranes from rat liver[1]

Membrane fraction	Fatty acids (as % total by weight)														
	14:	15:	16:	16:1	17:	18:	18:1	18:2	18:3	20:	20:1	20:2	20:3	20:4	22:6
Mitochondrial (outer)	0.4	27.0	4.1	21.0	13.5	13.5							1.1	15.7	3.5
Mitochondrial (inner)	0.3	27.1	3.6	18.0	16.2	15.8							1.0	18.5	3.8
Plasma membrane	0.9	36.9		31.2	6.4	12.9	tr	tr						11.1	
Smooth ER	0.4	28.6	3.1	26.5	10.6	14.9							1.4	14.0	0.7
Rough ER	0.5	22.7	3.6	22.0	11.1	16.1							1.8	19.7	2.9
Golgi	0.9	34.7		22.5	8.7	18.1	tr	tr						14.5	

[1]Data from ref. 1570. ER denotes endoplasmic reticulum.

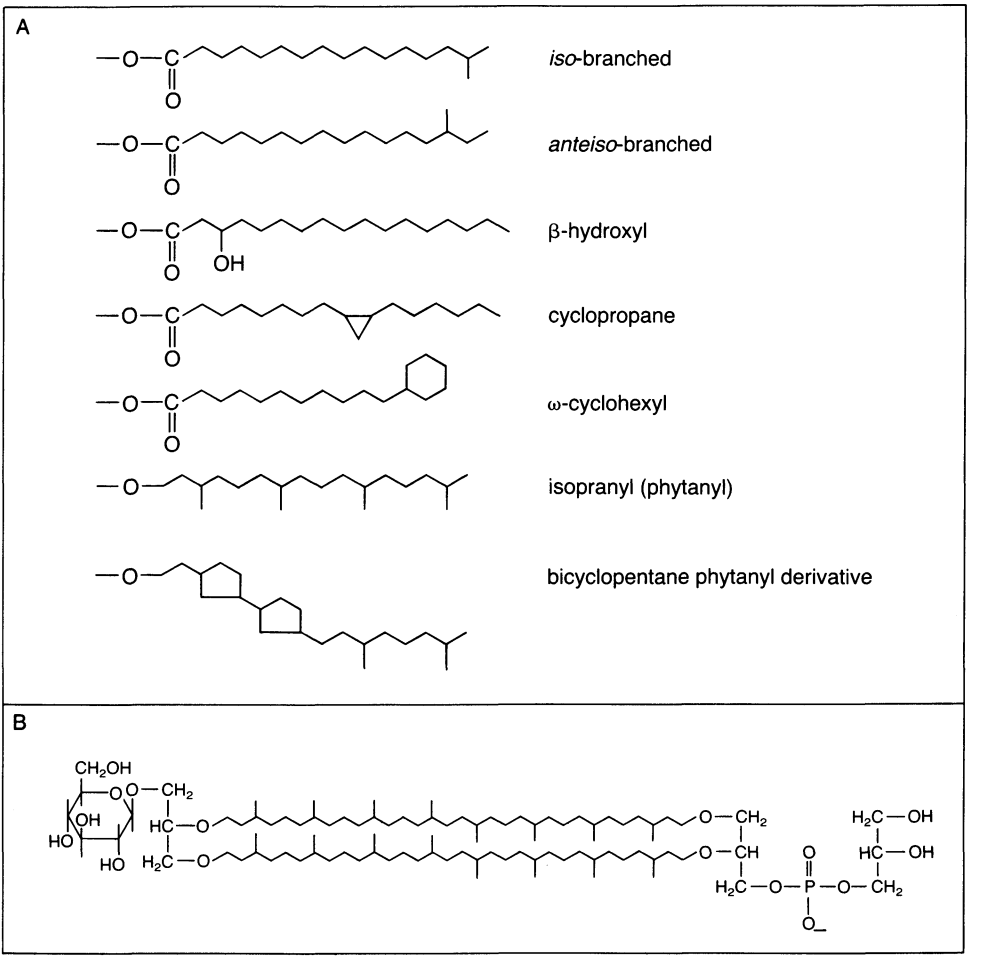


Figure 1.11. (A) Some less common acyl- and ether-linked hydrocarbon chains in the membrane lipids of bacteria. Many of the bacterial species containing these lipids are adapted for growth under extreme environmental conditions, e.g., thermophiles, acidophiles, and halophiles (see 1262, 307, 888 for reviews). Adapted from ref. 1262. (B) An example of an unusual 40-carbon tetraether that spans the bilayer of *Thermoplasma acidophilum* membranes. From ref. 111.

Phosphosphingolipids

These contain the same kinds of polar substituents (e.g., phosphorylcholine) as do the glycerophospholipids, but the hydrophobic group is a ceramide. Sphingomyelin (ceramide 1-phosphorylcholine) is widely found in animal cell plasma membranes (Figure 1.8). In myelin, the predominant fatty acids are 24:1 and 24:0. Phosphosphingolipids are rarely found in plants or bacteria. Other phosphosphin-

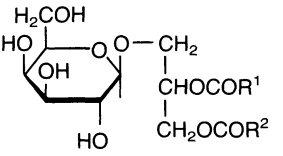
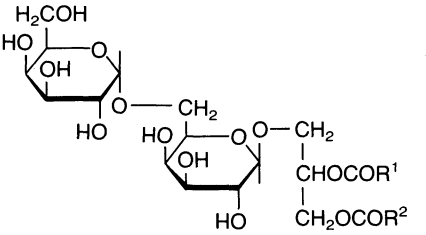
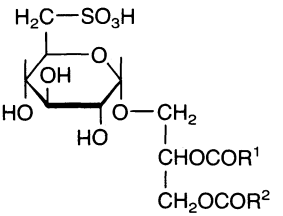
Common name	Structure
Monogalactosyl diglyceride (MGDG)	
Digalactosyl diglyceride (DGDG)	
Sulfolipid	

Figure 1.12. Representative structures of several glycerol-based glycolipids. Note that the sulfolipid has a carbon-sulfur bond. The R groups represent fatty acid hydrocarbon chains. These lipids are primarily found in plant leaves and in algae.

golipids are also widely distributed, such as ceramide l-phosphorylethanolamine, ceramide l-phosphorylinositol, and ceramide l-phosphorylglycerol (e.g. see 590).

Glycoglycerolipids (see 97 for review)

These are polar lipids in which the *sn*-3 position of glycerol forms a glycosidic link to a carbohydrate such as galactose. Glycoglycerolipids are predominant in the chloroplast membrane and are also found in substantial quantity in blue-green algae and bacteria. Monogalactosyldiacylglycerol (Figure 1.12) has been termed "the most abundant polar lipid in nature" since it comprises half of the lipid in the chloroplast thylakoid membrane (533). Gram-positive bacteria, in particular, have glycoglycerolipids with a variety of sugars. Archaebacteria also contain similar lipids, but as with the glycerophospholipids, the stereoconfigurations are reversed, with the glycosidic linkage at the *sn*-1 position (307, 888). Glycoglycerolipids are rare in animals.

A. Structures of Some Glycosphingolipids

Glucocerebroside	Cer—Glc
Ceramide lactoside	Cer—Glc—Gal
Globoside	Cer—Glc—Gal—Gal—GalNAc
Sulfatide	Cer—Gal(3)—OSO_3^-
Galactocerebroside	Cer—Gal
Gangliosides	
GM_3	Cer—Glc—Gal—NeuNAc
GM_2	Cer—Glc—Gal(NeuNAc)—GalNAc
GM_1	Cer—Glc—Gal(NeuNAc)—GalNAc—Gal

B. Detailed Structure of Ganglioside GM_1

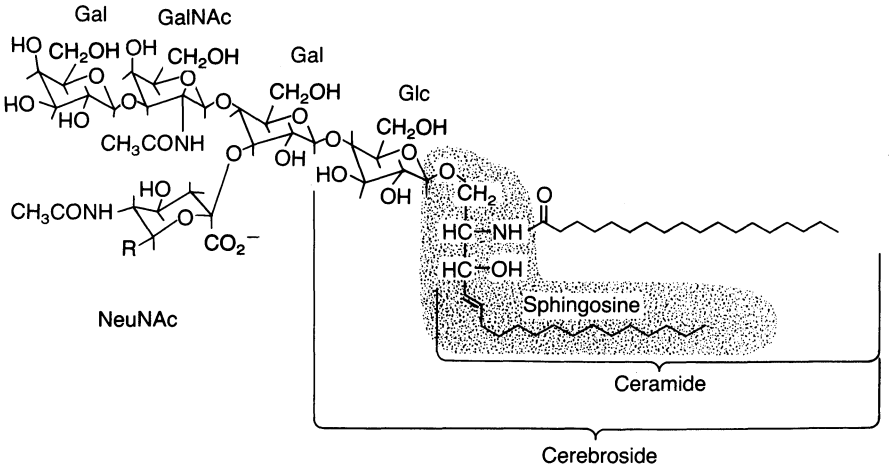


Figure 1.13. Structures of some glycosphingolipids. (A) Shorthand summary of several examples. Abbreviations used: Cer, ceramide; Glc, glucose; Gal, galactose; NeuNAc, sialic acid or N-acetylneuraminic acid; GalNAc, N-acetylgalactosamine. Note that GM_3 is also called hematoside and GM_2 is called Tay-Sachs ganglioside. (B) A more detailed structure of ganglioside GM_1, also showing the names applied to various parts of the structure.

Glycosphingolipids (see 271 for review)

These lipids have a glycosidic linkage to the terminal hydroxyl of ceramide. The classification scheme is according to the carbohydrate moiety, which can range from a single sugar to very complex polymers (see Figure 1.13). Monoglycosyl

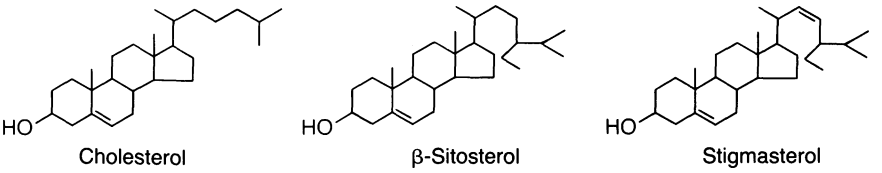


Figure 1.14. Structures of three sterols found in the membranes of eukaryotic cells.

ceramides are generally known as cerebrosides. Gangliosides are a class of anionic glycosphingolipids that contain one or more molecules of sialic acid (*N*-acetylneuraminic acid, NeuNAc) linked to the sugar residues of a ceramide oligosaccharide. Globosides refer to neutral glycosphingolipids which do not contain the negatively charged sialic acid residues.

The glycosphingolipids are found on the outer surface of animal cell plasma membranes, usually as minor components, but occasionally they can be a significant lipid class [e.g., epithelial plasma membrane (849)]. Monogalactosyl ceramide is the largest single component of myelin sheath of nerve. In some cases, the glycosphingolipids are present in intracellular membranes rather than in the plasma membrane (1416).

The glycosphingolipids in the erythrocyte membrane carry blood group antigens (494, 320). Human adenocarcinomas result in the accumulation of novel glycosphingolipids, which are fucosylated and which can be used to recognize these cells and to monitor the oncogenic progression (e.g., 573).

Sterols

These are found in many plant, animal, and microbial membranes. Cholesterol is, by far, the most commonly found sterol. This molecule is a compact, rigid hydrophobic entity with a polar hydroxyl group. Cholesterol is found in animal cell plasma membranes, lysosomes, endosomes (404), and Golgi. It constitutes about 30% of the mass of the membrane lipids of many animal cell plasma membranes. Whereas cholesterol is the major sterol found in animal cells, other sterols, notably sitosterol and stigmasterol, are found in higher plants. These plant sterols (phytosterols) frequently have an additional side chain at position C-24 and/or a double bond at position C-22 (see Figure 1.14). Ergosterol is often found in yeast and other eukaryotic microorganisms. Hopanoids are sterol-like lipids which are found in bacteria and some plants (see 1181 for review).

Minor Components

There are a number of other lipid components found in membranes which can be considered minor in terms of the amounts present. Free fatty acids and lysophospholipids are usually present, but at very low levels. One possible exception is the chromaffin granule membrane, where an extraordinarily high amount of free fatty acids has been reported (668). Monoacyl- and diacylglycerides are also minor components. Diacylglyceride serves an important function as a second

messenger in signal transduction in a mechanism by which a variety of biologically active substances can activate cellular responses. This signal response system will be discussed in more detail in Chapter 9.

Polyisoprenoid lipids are also commonly found in membranes. These include ubiquinones and menaquinones, which function in membrane-bound electron transport chains. Other examples are undecaprenol and dolichol, which function respectively as lipid carriers for intermediates in prokaryotic cell wall biosynthesis and eukaryotic glycoprotein biosynthesis in the Golgi. These lipids can be stretched out to lengths considerably beyond the thickness of the bilayer and it is not known how they reside in the bilayer. It is also not known why the polyisoprenoid structure is apparently preferred for these various lipid carrier molecules.

1.52 Lipids Play Multiple Roles Within Membranes

Although the distribution of lipids in various membranes does not seem to be random (1257), there is no satisfying explanation for the observed patterns. Any single membrane can contain well over 100 unique lipid species. Why are there so many and why does each membrane have a unique distribution of lipids? The biosynthesis of membrane lipids and the mechanisms by which they are distributed to different membranes are discussed in Section 10.4. However, the reasons for the heterogeneity are not known, although lipids are increasingly being recognized as active participants in membrane-associated processes. Several factors can be considered.

1. Minimally the lipid mixture must form a stable bilayer in which the proteins can function. This is discussed in the next chapter.
2. Some lipids may be required because their shapes favor packing configurations that may be necessary to stabilize regions of high curvature, junctions between membranes, or optimal interactions with specific proteins (see 303, 267, 272). This polymorphic aspect of membrane lipids is discussed in the next chapter.
3. Some lipids are important as regulatory agents. Most notable are the derivatives of phosphatidylinositol in the plasma membranes of eukaryotic cells (see Section 9.73).
4. Some lipids participate in biosynthetic pathways. For example, in *E. coli* phosphatidylglycerol provides the glycerol phosphate moiety in the biosynthesis of periplasmic oligosaccharides (see Section 10.43).
5. Specific lipids may be required for optimal enzyme activity of particular enzymes. This topic is addressed in Chapter 6.
6. Gangliosides, in particular, have been implicated as playing a role in the regulation of cell growth (1368), in binding to specific receptors in the plasma membrane (210), and in adhesion (812).
7. Other lipid components are also known to play specialized roles. These include the polyisoprenoids, such as dolichol, ubiquinones, menaquinones, and carotenoids, and the platelet activating factor (576).

It must be remembered that it has been demonstrated that organisms can often tolerate quite drastic changes in their membrane lipid composition without deleterious effect. For example, by genetic manipulations, strains of *E. coli* can be made in which the membranes contain 34% phosphatidic acid, which is not normally found in the wild-type strains (1365; see Figure 10.16). Obviously, the exact lipid composition found in wild-type strains is not required for viability of the organism, at least under laboratory growth conditions.

1.53 Membrane Proteins

As shown in Tables 1.3 and 1.4, membranes contain between 20% and 80% (w/w) protein. It is the proteins, of course, which are the biochemically active components of the membrane and provide the diversity of enzymes, transporters, receptors, pores, etc. which distinguishes each particular membrane. Progress in our understanding of membrane proteins was initiated when biochemists learned to use detergents to solubilize these proteins from membranes in biochemically active forms. Initially, success was with the enzyme complexes of the mitochondrial inner membrane. The realization that membrane proteins were not predominantly ß-pleated sheet, as postulated to best fit the Davson–Danielli–Robertson "unit membrane" model, but contained significant amounts of α-helix, was a significant step forward. Also important was the insight that membrane proteins extended deeply into or completely through the lipid bilayer and were stabilized by hydrophobic interactions. This thermodynamic argument was essentially an extension of the principles of the "hydrophobic force" being developed to understand protein structure, i.e., nonpolar hydrophobic interior and polar hydrophilic exterior in contact with water.

As techniques for membrane protein purification were developed, more membrane proteins were obtained in homogeneous form. The insoluble characteristics of most membrane proteins and of the hydrophobic peptides derived from them made primary structure determination difficult. Progress on two membrane proteins, glycophorin and cytochrome b_5, helped to establish the structural themes which have been dominant since the mid-1970s. The amino acid sequence of glycophorin, a sialoglycoprotein from the erythrocyte membrane, indicated a short stretch of 23 nonpolar amino acids near the middle of the molecule (1461) (see Figure 3.17). Topological and other studies indicated that glycophorin extended completely through the erythrocyte membrane and that this hydrophobic stretch was in an α-helical form and was buried in the membrane (475). This work contributed to the now firmly entrenched concept of membrane-spanning α-helical domains in proteins. Electron microscopy image reconstruction studies on bacteriorhodopsin in the purple membrane of *Halobacterium halobium* (619) and X-ray diffraction studies on bacterial photosynthetic reaction centers (319) have provided the highest resolution data available for membrane-spanning proteins. These proteins consist of a series of α-helical segments traversing the bilayer (see Chapter 3).

Sequence studies on the intact form of microsomal cytochrome b_5 were also

very suggestive, showing a relatively short stretch of hydrophobic amino acids near the carboxy-terminus (1113, 437) (see Section 4.22). This "hydrophobic anchor" could be removed by proteolysis, releasing the heme-binding domain in a water-soluble form. The membrane-binding hydrophobic domain or "anchor" has been another dominant theme in the analyses of the structure of membrane proteins.

The major point is that membrane proteins are now generally viewed as being folded so as to present a nonpolar hydrophobic surface which can interact with the nonpolar portions of the lipid bilayer. Polar or charged regions of the protein can interact with the lipid headgroups at the surface of the bilayer. Many membrane proteins are transmembranous and extend through the bilayer. Other membrane proteins are probably bound to the membrane exclusively through interactions with other proteins.

Membrane proteins are generally bound to the membrane through noncovalent forces, such as the hydrophobic force or electrostatic interactions (see Chapter 3). There is, however, a small but growing number of examples of membrane proteins which are covalently bound to lipids (see Section 3.8). Many of the proteins in plant or animal plasma membranes are glycoproteins, such as glycophorin. The carbohydrate residues are always located on the extracytoplasmic side of the membrane.

Operationally, membrane proteins are classified as extrinsic (or peripheral) or intrinsic (or integral). This generally denotes the degree of harshness of the treatment required to release the protein from the membrane. Extrinsic proteins are dislodged by washing the membranes in low ionic strength buffer, in a buffer at low or high pH, and/or in the presence of a divalent cation chelator such as EDTA (1438). Such proteins are thought to be weakly bound to the membrane surface by electrostatic interactions either with the lipid headgroups or with other proteins. It is often hard to distinguish an extrinsic membrane protein from a cytoplasmic protein which has become adventitiously bound to the membrane during the isolation procedure. As much as 30% of the proteins associated with the erythrocyte membrane is solubilized by treatment at low ionic strength (922, 1348). Somewhat harsher treatments to release extrinsic proteins involve the use of chaotropic agents such as ClO_4^- or SCN^- (see 1438). These reagents are, in some cases, strong enough to disrupt some protein-protein interactions but are not sufficently strong to denature the individual polypeptides. Chaotropics or "water structure breakers" act by effectively reducing the magnitude of the hydrophobic force (278) (see Chapter 2).

Treatments involving detergents or, occasionally, organic solvents are required to release intrinsic membrane proteins. The detergents disrupt the lipid bilayer and presumably bind to the membrane proteins at the nonpolar binding sites normally in contact with the bilayer interior. Intrinsic membrane proteins require the continued presence of detergents to remain in a soluble, monodisperse form. Removal of the detergent invariably results in the formation of high-molecular-weight protein aggregates and, usually, precipitation. Further information on protein–detergent interactions and the structure and analysis of membrane proteins is in Chapter 3.

1.6 Chapter Summary

Historically, X-ray diffraction and electron microscopy have contributed substantially to our current view of biological membranes. It is now clear that the lipid bilayer forms the structural framework of virtually all biomembranes, and that structural similarities underlie the functional diversity one finds in comparing different membranes. There are a large number of chemically distinct lipids in any given membrane. The reasons for this diversity are unknown, although the unique biological functions of particular lipids are receiving increasing attention. Proteins comprise between 20% and 80% of biomembranes. Many of these proteins extend across the lipid bilayer and are usually solubilized by the use of detergents. Other proteins, called peripheral membrane proteins, are more easily dislodged from the membrane, such as by changing the pH, ionic strength, or chelating divalent cations.

Biochemical techniques have been developed to isolate and characterize distinct membrane populations from prokaryotic organisms, from animal cells, and, to some extent, from plant cells. These techniques most often take advantage of differences in the size and/or density of the different membrane populations in disrupted cell homogenates. Differences in surface properties and in electrophoretic behavior can also be exploited to separate different membranes from a cell homogenate. The isolation of pure membranes is an essential first step prior to biochemical characterization.