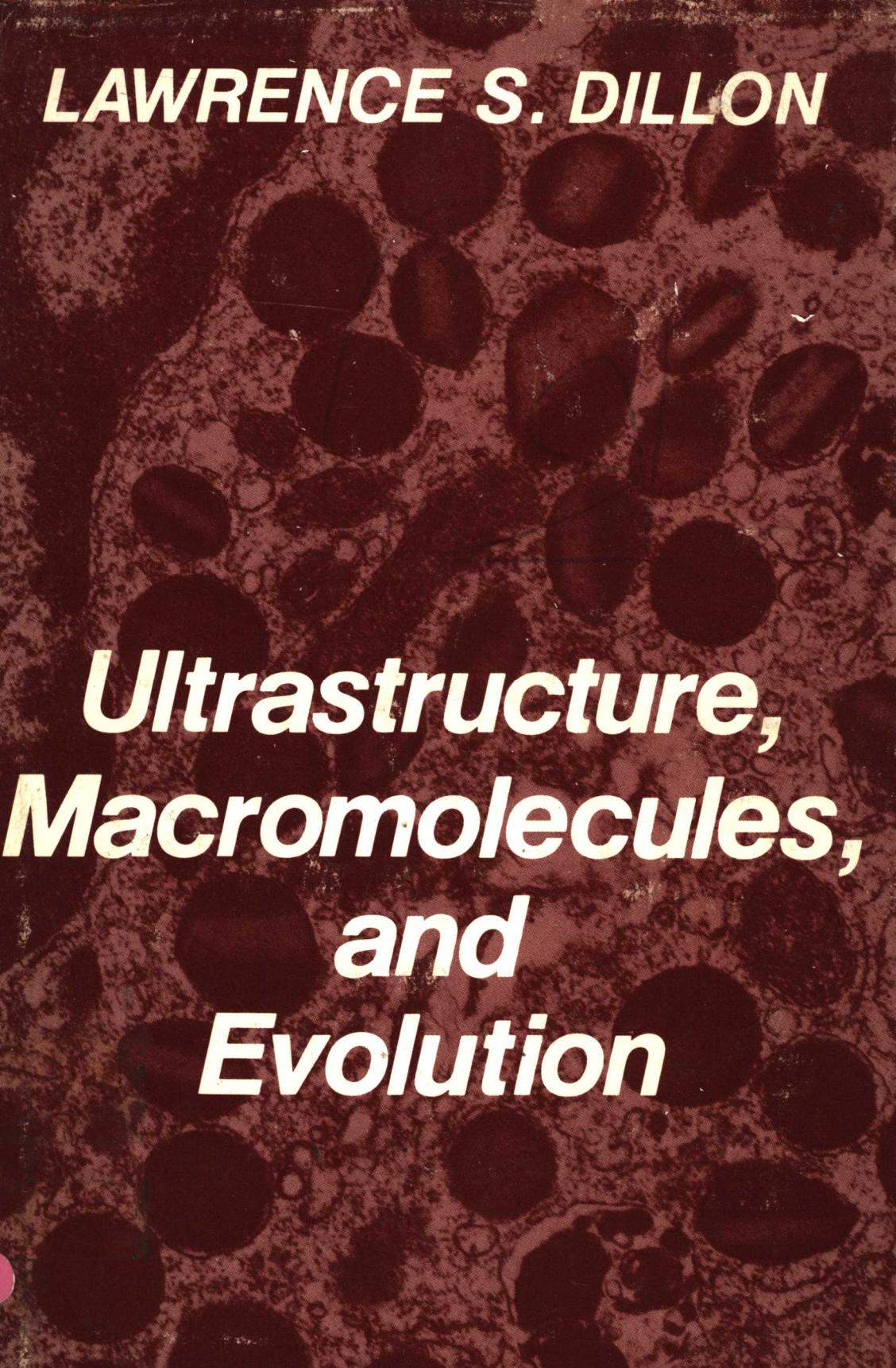
LAWRENCE S. DILLON



Ultrastructure, Macromolecules, and Evolution

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Preface

Thus far in the history of biology, two, and only two, fundamental principles have come to light that pervade and unify the entire science—the cell theory and the concept of evolution. While it is true that recently opened fields of investigation have given rise to several generalizations of wide impact, such as the universality of DNA and the energetic dynamics of ecology, closer inspection reveals them to be part and parcel of either of the first two mentioned. Because in the final analysis energy can act upon an organism solely at the cellular level, its effects may be perceived basically to represent one facet of cell metabolism. Similarly, because the DNA theory centers upon the means by which cells build proteins and reproduce themselves, it too proves to be only one more, even though an exciting, aspect of the cell theory.

In fact, if the matter is given closer scrutiny, evolution itself can be viewed as being a fundamental portion of the cell concept, for its effects arise only as a consequence of changes in the cell's genetic apparatus accumulating over geological time. Or, if one wishes, the diametrically opposite standpoint may be taken. For, if current concepts of the origin of life hold any validity, the evolution of precellular organisms from the primordial biochemicals must have proceeded over many eons of time prior to the advent of even the most primitive cell. Hence, evolution is the single basic principle, and the cell, in this light, is secondary, merely serving as the instrument that carries out the processes.

No matter which point of view is found more acceptable, it is clear that these two fundamentals of biology are so closely interrelated that in any treatise that transcends narrow taxonomic boundaries, discussion of either to the exclusion of the other makes meaningless that which holds great significance and tedious much that is exciting.

Nor can the macromolecules always be sorted into neat, discrete units and kept separated from descriptions of the cell parts. For, as the building blocks of protoplasm, they can no more be segregated from consideration of cellular

morphology than can bricks, lumber, and concrete be eliminated from a discussion of building construction. Hence, this topic joins those two that form the warp and woof of biological fabric, much like the pile in a Persian rug of intricate design.

The exposition of these three intertwined subjects from the broadest possible comparative approach, then, is the purpose of this book. Factual matters necessarily comprise the greater part of the text. But the facts indeed would be empty if from them no questions arose concerning their significance or if no problems for future investigation were revealed. For at this level and with this approach the newer and more classical aspects of biology can be seen to be but adjacent cells in a complex organism of a single science, and, as in those cells, the interfaces are found to interdigitate in a most intricate fashion.

Like its predecessor in the trilogy, this second member is not merely a review of the literature but more properly is to be considered an analytical synthesis from a diversity of viewpoints. As a consequence of its broad, in-depth approach, a number of novel aspects have come to light, so that new terms for several organelles have been introduced, the lysosomes and centriolar derivatives in particular having received such treatment. Moreover, an occasional regrouping of certain biochemicals has proven essential, and, where present information was insufficient, the need for such revision or redefinition has been indicated for other classes of macromolecules or cell structures. Similarly in those few instances that are supported by adequate researches, hypotheses concerning the functioning of such organelles as the flagellum have received analysis, and alternative concepts have been advocated. That phylogenetic theories and those of the origin of the cell parts have also received comparable treatment should, of course, be a foregone conclusion, for classification and phylogenesis are a direct consequence of evolutionary considerations.

Far too many persons have contributed to the completion of this volume than could possibly be mentioned here, but fortunately those who have made available electron and light micrographs or other illustrative material can be acknowledged appropriately with the figures. Among those who have been especially generous in this manner are Drs. Hilton H. Mollenhauer, of the U.S. Department of Agriculture, College Station, F. S. Sjöstrand, University of California at Los Angeles, E. M. Mandelkow and M. Mandelkow, Max Planck Institute, Heidelberg, and T. Eda, Teikyo University, so that especially warm thanks are expressed to them. Finally, my wife, as always, has collaborated in the preparation of the manuscript and artwork and in researching the literature, interpreting data, and polishing the phraseology.

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Biological Membranes

Although none is completely satisfactory, the very best cell part with which to begin a discussion of the structure and evolutionary history of the cell probably is the membrane (Frey-Wyssling, 1955). In the first place, it is the only organelle aside from the genetic mechanism that is universally present in living things—even the viruses are provided with a membranous capsid that covers the virion. Second, the presence of a membranous envelope endows cellular organisms with one of their most characteristic features. Because of it, living creatures have the ability to absorb materials from their surroundings, even against a gradient, that is, when the concentration of the given ion or compound in the organism is manyfold greater than that in the medium. In contrast, in nonliving systems, even when a membrane is present, the movement of chemicals is always from the greater concentration to the lesser. Although certain colloidal particles, such as coacervates and proteinoid microspheres, also possess a limited capacity for concentrating material against a gradient in biological fashion, the ability varies with dilution, composition of the milieu, and other factors without influence in living systems. Moreover, many organelles other than the plasmalemma of the eukaryote and prokaryote cells alike are constructed of membranes.

In addition, a major generalization has prevailed in the literature that has attempted to ascribe a single pattern of molecular structure to all membranes regardless of their site within the cell. Because specialized aspects of these ubiquitous structures are more profitably discussed with the organelles of which they are a part, present considerations are confined first to general features and then to two all-important organelles of the cell, the plasma and nuclear membranes.

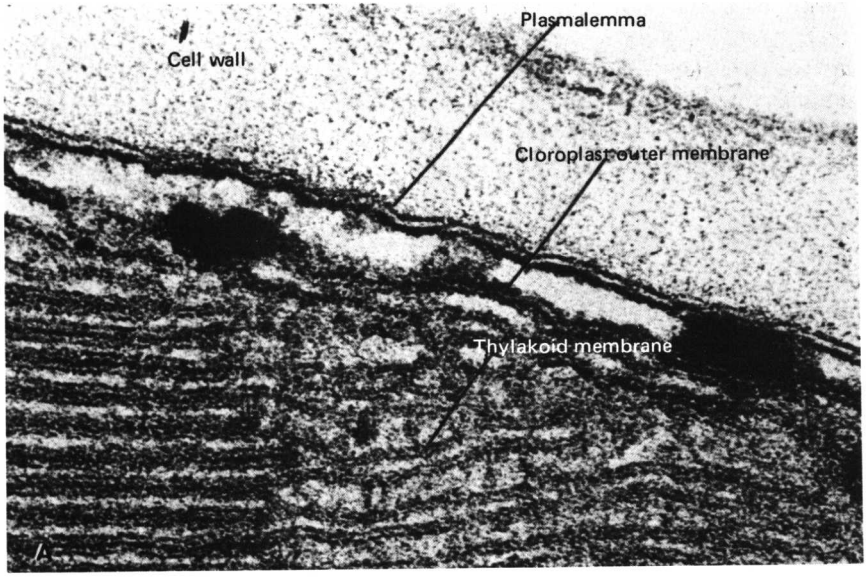
1.1. MEMBRANES IN GENERAL

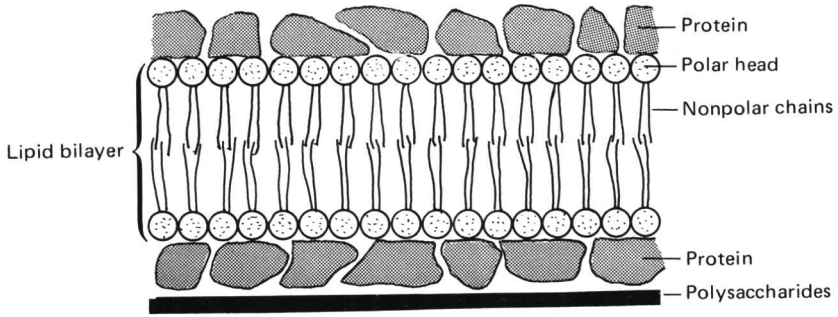
As a group, membranes of cells average 75 Å in thickness but range from as little as 50 Å to as much as 200 Å, but in light of recent findings, the lower limits and average cited are probably subject to correction (Sjöstrand, 1978). In comparing single-cell sections of mouse kidney and pancreas by electron microscopy, Sjöstrand (1963a) reported two width classes of membranes. The thinner one, measuring from 50 to 60 Å, was confined to mitochondria and endoplasmic reticula, and the thicker one, ranging between 90 and 100 Å, included the plasma membrane and that surrounding zymogen granules. In cross section under the electron microscope, natural membranes typically have the appearance of what has become known as the "unit membrane."

1.1.1. The Unit-Membrane Concept

In essence, the original unit-membrane concept advanced by J. D. Robertson (1959, 1960) was a modification, based on electron microscopic and X-ray diffraction data, of an older theory of biological membrane structure (Danielli and Davson, 1935). It proposed that all such membranes are constructed over a single molecular pattern, regardless of the species or site within the cell from which they are derived. Among the most convincing data that support the theory is the consistency of their appearance in electron micrographs (Figure 1.1A,B). In the plasma membrane, as well as in those from the endoplasmic reticulum, nucleus, Golgi apparatus, and other organelles, three layers appear to be present when suitably stained. The two outside layers are uniformly dark (electron dense or opaque), while the intermediate one is consistently pale (electron transparent). To explain this configuration, the unit-membrane concept proposed that biological membranes consist of two parallel layers of phospholipid molecules, held together by van der Walls' forces. The constituents of the respective layers face in opposite directions, so that the electron-transparent nonpolar carbon chains lie adjacent to form the central clear region. Thus the two electron-dense faces consist of the polar moieties that become electron opaque with osmium staining (Figure 1.1B). In addition, each face was conceived as being covered with a monomolecular layer, the thinner external (distal, or E) face consisting of proteins and the thicker internal (proximal, plasmal, or P) one being constructed variously of proteins or carbohydrates. While the general pattern was believed to be universal, the actual chemical nature of the phospholipids and proteins was considered to vary both from species to species and from organelle to organelle in the same cell.

The Unitary Concept. In turn, the unit-membrane concept leads into another generalization, termed the unitary theory of cell structure (Robertson, 1967). According to this view, the eukaryotic cell consists of three "phases." Phase 1, the greatest portion of the cell, is the nucleocytoplasmic matrix, which





B. UNIT-MEMBRANE STRUCTURE

Figure 1.1. The unit-membrane concept. (A) Transverse section of a corn-leaf cell, 1 month old, 200,000×. Each of the three kinds of membrane shown appears to consist of two dense layers enclosing a transparent one. Careful comparisons, however, show that no two are exactly alike. (B) The transparent inner layer is accepted as being comprised of a lipid bilayer, whereas the dense ones are made up of protein. (A, courtesy of Crane and Hall, 1972.)

is made into an integrated whole by the pores of the nuclear membrane. Phase 2, the membranous portion, is made continuous from the plasma to the nuclear membrane by means of the endoplasmic reticulum, Golgi apparatus, and mitochondrial membranes (Daniels, 1964). Phase 3 is an external fraction brought into the interior of the cell by invagination, as when solid matter is taken in during phagocytosis or liquid by pinocytosis (Section 1.2.1). This concept thus

makes clear that the original internal surface of the plasma membrane, even in its derivatives, is always adjacent to the nucleocytoplasmic matrix and that the E face consistently contacts substances from the milieu, even during phagocytotic and secretory activities and also during translocation to another region or organelle. This polarity of the membrane phase is viewed as constant in this concept, not the physical continuity of the several organelles.

1.1.2. Present Status of the Concept

As further investigations into the nature of biological membranes reached fruition, the validity of certain aspects of the original unit-membrane concept began to be questioned. Possibly the first paper to raise some doubts was that cited earlier (Sjöstrand, 1963a), which pointed out the absence of uniformity in thickness of the membranes from different types of organelles (Figure 1.1). Some other data that in one way or another can be reconciled with the concept only with difficulty are briefly summarized below

Protein-to-Lipid Ratios. In reevaluating the theory in light of biochemical composition, Korn (1966) disclosed serious problems that were encountered in correlating his data with the theoretical requirements of the unit-membrane concept. He pointed out that if all membranes are similar, their protein-to-lipid ratios should approximate one another closely. Instead he reported extensive variation in the ratios in the instances investigated. Among bacteria, for example, he showed that in *Streptococcus faecalis* the molar ratio of amino acid to phospholipid was 14.2:1.0 (Shockman *et al.*, 1963), in *Bacillus licheniformis*, 19.6:1.0 (Salton and Freer, 1965), and in *B. megaterium*, 22.6:1.0 (Weibull and Bergström, 1958). Furthermore, cholesterol was absent in all these bacteria, while it was consistently present in vertebrate membranes. Among the vertebrate tissues for which membrane analyses were available, he found similar wide divergence in the proportions of the constituents. In erythrocytes the molar ratio of amino acid to phospholipid to cholesterol approximated 16:1:1 (Maddy and Malcolm, 1965), whereas in myelin a ratio of only 2.4:1:0.67 had been reported (O'Brien and Sampson, 1965).

Another aspect analyzed by Korn presents similar difficulties. If, as the unit-membrane concept proposes, two monomolecular layers of protein cover the surfaces of a bimolecular layer of lipids, the ratio of surface area of the lipids to that of the proteins should be unity. In calculating the area ratios, Korn assumed amino acids on the average to occupy 17 $Å^2$, phospholipid molecules 70 $Å^2$, and cholesterol molecules 38 $Å^2$. Among bacteria, the results indicated that the protein-to-lipid surface area ratio varied from 3:1 to more than 5:1, and in erythrocytes 2.5:1. Hence, as Korn pointed out, there is an excess of protein simply to provide a covering layer, if the lipids are arranged as hypothesized in a bimolecular fashion.

Chemical Composition of Lipids. As summarized in that same study,

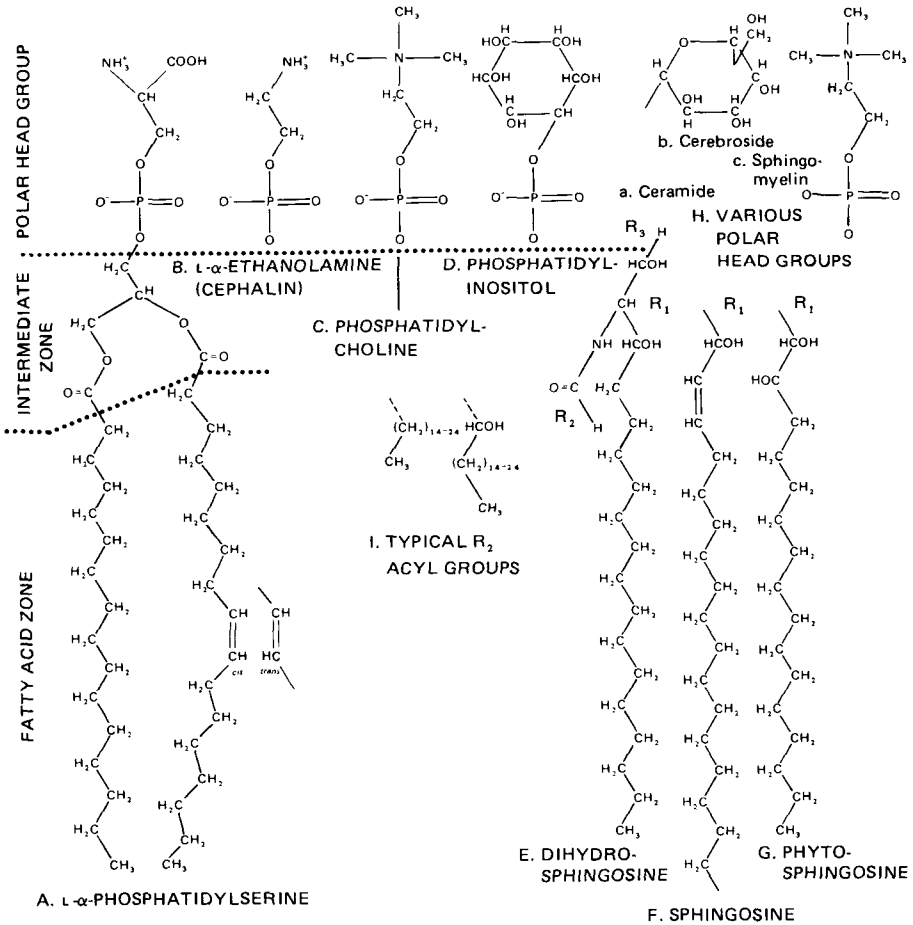


Figure 1.2. Molecular structure of some membrane lipids. Many lipids of membranes consist of a polar head group (A–D, H), to which two fatty acid chains typically are attached by structures located in the intermediate zone (A). The ceramide (a) is illustrated by E.

the chemical composition of the lipids also deviates widely from taxon to taxon. Among bacteria, no steroids occur in the membranes (Kaneshiro and Marr, 1965; Op den Kamp *et al.*, 1965), and in such species as *Azotobacter agilis* and *Escherichia coli*, the lipids are entirely represented by a single phospholipid, phosphatidylethanolamine (Figure 1.2B). On the other hand, that same phospholipid and two others are present in the membranes of *Bacillus megaterium*; the two additions include phosphatidylglycerol and lysylphosphatidylglycerol, the latter in small quantities. In contrast, most metazoan membranes contain cholesterol (Figure 1.3A), but the percentage present is subject to extensive fluctuation from tissue to tissue. Whereas in myelin (O'Brien and

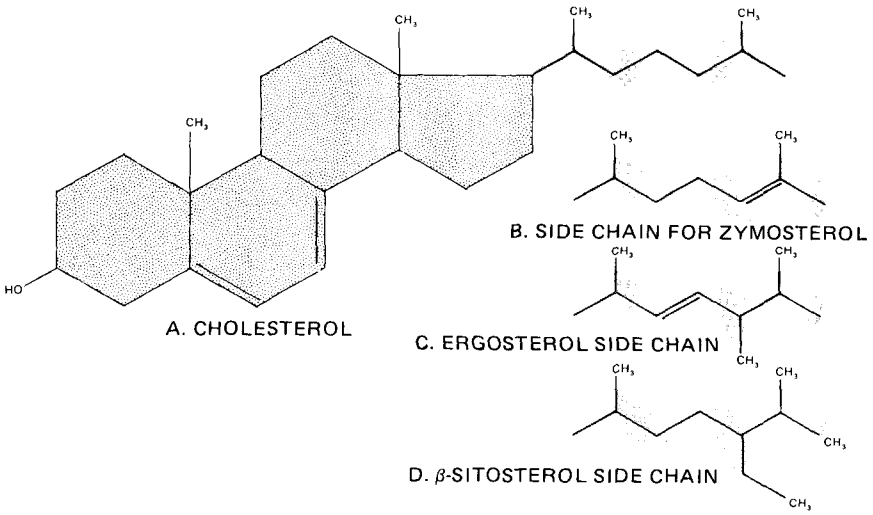


Figure 1.3. Sterols of membranes. Typically sterols have a complex molecular structure, similar to that shown in coarse stippling (A); various side chains, shown in dark stippling, are appended to the representative structure.

Sampson, 1965) and erythrocytes (Ways and Hanahan, 1964) this steroid is the most abundant lipid, providing 25% of the entire fraction, in mitochondria and microsomes (Dallner *et al.*, 1965) it is found only sparingly, with phosphatidylcholine (Figure 1.2C) providing approximately half. Nevertheless, in spite of the wide fluctuation in composition, reexamination of the above data from a different point of view exposes an evident trend in the lipids—from a simpler condition in bacteria to a more complex one among metazoans. Consequently, although too few analyses are as yet available to permit more than a suggestion of the possibility at this time, it is not unlikely that an evolutionary sequence in the lipid components will eventually be found to exist from the lower to higher taxa.

Water Relationships. Another source of doubt as to the validity of the unit-membrane concept derived from the relationship of water to the other components. Because the central layer was theorized to consist of lipids and therefore to be hydrophobic and the outer lamellae wettable and hydrophilic, the moisture content of membranes should be largely concentrated in the latter rather than in the former. Several studies intimated that water may actually be distributed in the opposite fashion. One such investigation on the amoeba, *Pelomyxa carolinensis* (Brandt and Freeman, 1967), used the electron microscope to examine some effects of various concentrations of $CaCl_2$ and NaCl upon the plasma membrane. If the concentration of $CaCl_2$ was maintained at a

uniform low level while that of NaCl was increased, the membrane's diameter increased from a mean of 94 Å to one of 140 Å; raising the $CaCl_2$ level without lowering that of NaCl then restored the membrane to its original thickness. Because the outer layers remained unaltered during these experiments, the investigators concluded that their observations resulted in part from the redistribution of water and electrolytes *within* the central lamella. Hence, water was evidenced to be present in the latter, at least under certain conditions.

Other Conflicting Data. A different approach supporting similar conclusions was employed by Branton and Moor (1964). By use of freeze-etching techniques on cells of the onion root tip, they found that the nuclear membrane did not conform to all theoretical implications of the unit-membrane concept. With these procedures, after the cell has been fractured with the ultramicrotome knife, the exposed surface is "etched" by permitting water to evaporate for a time before a carbon replica is made for the actual examination. If water is distributed as outlined above to accord to the theory's demands, shrinkage should be greater in the outer lamellae than in the inner; however, the opposite results were obtained. Because of these and earlier comparable data, several workers (e.g., Sjöstrand, 1963b; Lucy and Glauert, 1964) suggested that membranes may consist of a phospholipid matrix in which protein globules are embedded, or may be a combination of micelles and lamellae (see Kavanau, 1965, for a review of this concept). Nevertheless, the freeze-etch studies, it must be emphasized, did suggest a three-layered condition for the membranes examined; only the sequence of the constituent molecules was questioned.

The Current View of the Unit-Membrane Concept. More recently, a study of the trilamellar arrangement of membrane structure was made, in which the arrangement of the proteins was particularly stressed (Green, 1972; Green and Brucker, 1972). In part, these papers showed that the trilamellar appearance of sectioned membranes viewed under the electron microscope was the result of preparatory techniques. It is essential that objects prepared for electron microscopic study be thoroughly dehydrated before exposure to the extremely high vacuum employed in such instruments. One of the results of such dehydration, it was shown, was to induce the lipid fraction of the membrane to clump into a "water hose" configuration (Figure 1.8B), so that the bimolecular layer no longer remains (Figure 1.4). Today, although the unit membrane still exists as a working concept in a few quarters, especially in theoretical accounts or in those based on synthetic membranes (Fox and Keith, 1972), it has been replaced in biological researches on the subject by other models, such as those described in the following section.

1.1.3. Other Concepts of Membranes

Although innumerable models and varieties of membrane structure have been proposed since the unit-membrane concept first began to lose favor, the problem of the molecular organization of membranes is still not completely

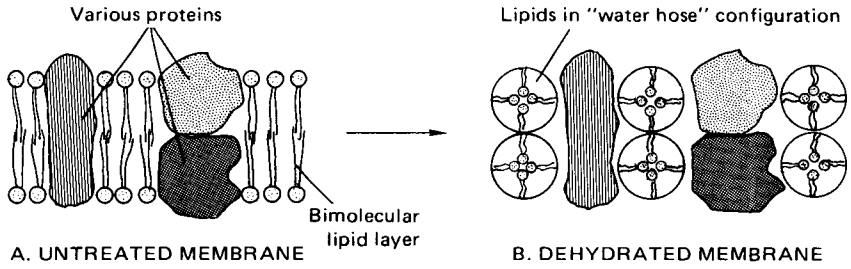


Figure 1.4. Effect of dehydration on membrane structure. According to Green (1972) dehydration during preparation for ultrastructural study induces lipids in membranes to assume a "water hose" configuration, with polar heads inward and the fatty acid chains outward.

resolved. To recount the details of all the models that have been advocated during the intervening years would require an unjustifiable amount of space, so only the more important and current proposals receive attention below. Out of the maze of data and ideas that have emerged from the numerous studies of the membrane, it is becoming apparent that all fall into a limited number of major categories.

Liquid Models of Membranes

The Fluid Mosaic Concept. Among the many recent models of membrane structure at the molecular level is the fluid mosaic concept (Singer, 1972, 1977; Singer and Nicolson, 1972). Actually this is a modification of the Wallach-Zahler (1966) concept, which proposed the arrangement of proteins and lipids but did not refer to fluidity. In this, as in most current models, the double layer (bilayer) of phospholipids remains, but here it is viewed as discontinuous and fluid—more specifically, it is considered to be a two-dimensional viscous solution whose constituents are highly oriented. The proteins, however, no longer are thought to cover the surfaces of the phospholipid bilayer but are treated as being embedded in them. That is to say, the proteins are considered to float within the liquid medium or sometimes to penetrate through it (Figure 1.5A). In addition, there may be extrinsic, or "soluble," proteins that float on the surface of the membranes; those that are contained in whole or part within the membranes are then referred to as intrinsic (Green, 1972). In some instances, certain of the phospholipid members of the matrix may interact with given proteins. This type of structure is dynamic and is believed to apply to most cellular membranes, including the plasma membrane and those of mitochondria, endoplasmic reticulum, Golgi material, and chloroplasts. Others such as myelin sheaths of neurones or the capsids of small viruses, nonetheless may be rigid, so all membranes are not perceived as being identical.

The thermodynamics of certain aspects of membrane structure are particu-

larly clearly phrased in Singer and Nicolson's (1972) explanation of the two types of noncovalent interactions that occur, hydrophobic and hydrophilic. The first variety of this class of interactions is defined as those thermodynamic factors that sequester hydrophobic and nonpolar groups away from water. Such sequestering is demonstrated by the immiscibility of such nonpolar substances as oils and other hydrocarbons with water. For example, at 25°C an expenditure of 2.6 kcal of free energy is required to transfer 1 mole of methane from a nonpolar medium, such as petroleum, to water. Similar requirements for free energy in comparable large amounts doubtlessly are involved in many other interactions of nonpolar bodies or parts, such as among proteins contained in aqueous solutions in which the nonpolar amino acid residues are considered to be largely confined to the interiors of the macromolecules, out of contact with the water.

Hydrophilic interactions, the second variety, are similar to the first except that they proceed in the opposite direction; they are defined as those thermody-

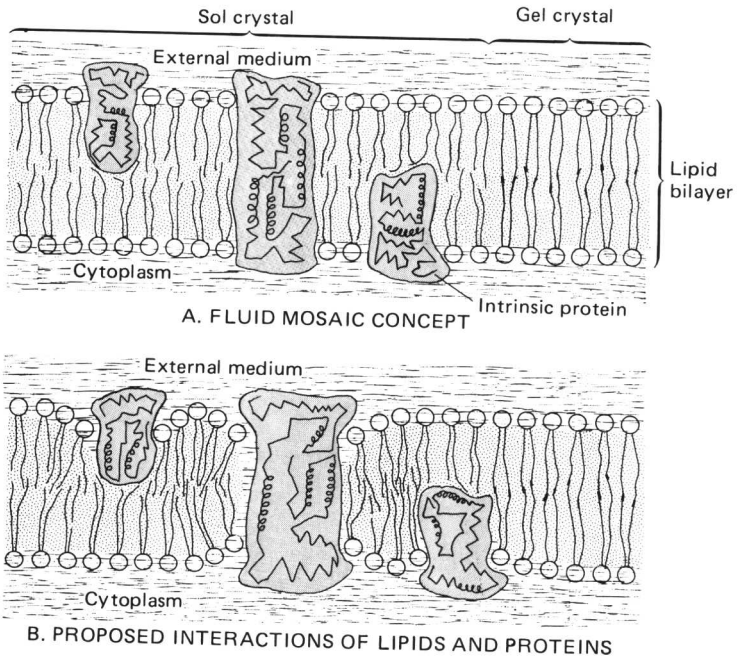
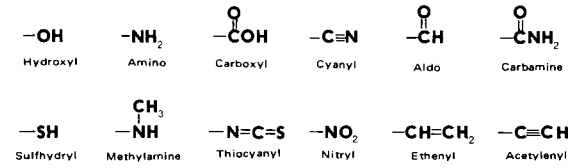
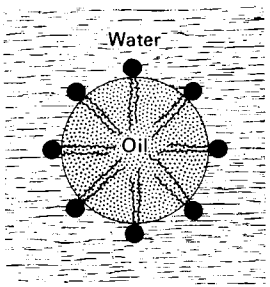


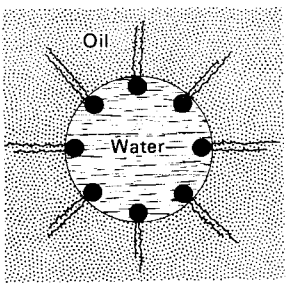
Figure 1.5. The fluid mosaic concept. (A) In this concept, the lipid bilayer is viewed as discontinuous, being interrupted by the presence of proteins that penetrate one or both layers. (B) A modification of the above, proposed here, suggests how the lipids may interact upon contact with proteins.



A. POLAR RADICALS



B. PHOSPHOLIPIDS IN OIL-IN-WATER EMULSION



C. PHOSPHOLIPIDS IN WATER-IN-OIL EMULSION

Figure 1.6. Polar groups and effects of polarity in various media.

namic factors responsible for the sequestering of ionic and polar groups from nonpolar environments. For instance, at 25°C the transfer of zwitterionic* glycine from water to the hydrocarbon acetone requires 6.0 kcal of free energy. Thus almost all of the ionic amino acid residues of proteins are considered to be in contact with water and usually are exposed on the exterior of the molecule, insofar as X-ray crystallographic studies can discern.

Polarity Aspects. Because polar radicals include OH^-, $COOH^-$, NH_2^-, and the others illustrated (Figure 1.6A), all amino acid molecules are polar at the end where the peptide bonds are formed. In addition, serine, cysteine, threonine, aspartic and glutamic acids, lysine, arginine, and tyrosine (Table 1.1) have polar radicals at their free ends. Thus a problem may be perceived to exist with the concept.

This problem involves the orientation of the polar and nonpolar amino acids in their respective environments. In their diagrams and discussions, Singer and Nicolson suggested that most protein molecules penetrate only halfway through the phospholipid bilayer, leaving one such layer undisturbed. However, the large number of amino acids with polar free ends usually present in protein molecules makes it extremely unlikely that only those protein strands that are almost exclusively nonpolar would be immersed in the phospholipid milieu, leaving the ionic and polar portions concentrated in the aqueous medium above the surface of the bilayer (Figure 1.5A). While perhaps a few pro-

*Bearing both a positive- and a negative-charged radical.