Structure and Function of the Plasma Membrane

A biochemical perspective

EDWARD D. KORN

From the Section on Cellular Physiology, Laboratory of Biochemistry, National Heart Institute, National Institutes of Health, Bethesda, Maryland 20014

ABSTRACT The paucimolecular unit membrane model of the structure of the plasma membrane is critically reviewed in relation to current knowledge of the chemical and enzymatic composition of isolated plasma membranes, the properties of phospholipids, the chemistry of fixation for electron microscopy, the conformation of membrane proteins, the nature of the lipid-protein bonds in membranes, and possible mechanisms of transmembrane transport and membrane biosynthesis. It is concluded that the classical models, although not disproven, are not well supported by, and are difficult to reconcile with, the data now available. On the other hand, although a model based on lipoprotein subunits is, from a biochemical perspective, an attractive alternative, it too is far from proven. Many of the questions may be resolved by studies of membrane function and membrane biosynthesis rather than by a direct attack on membrane structure.

My role today is to set the stage for the speakers to follow, who will discuss some recent developments in studies of the mechanisms of transport of solutes into cells. At the center of the problem of cellular transport lies the fact that there exists a selective barrier between the cytoplasm of the cell and the external environment through which a solute molecule can move often by the expenditure of cellular energy and by the mediation of a number of distinct components which together make up the particular transport system. This selective barrier to free diffusion is the plasma membrane. Now, it is apparent that although the kinetics of transport may depend on many other cell parameters, a complete understanding of the mechanisms of transport must include a description of the molecular composition and organization as well as the function of the plasma membrane. I shall present one man’s assessment of where we now stand.

THE PROBLEM IS POSED

For many years cell biologists have been in general agreement that the problem of membrane structure was solved, at least to a first approximation,
TRANSPORT ACROSS CELL MEMBRANES

Protein Polar groups

FIGURE 1. The Davson-Danielli diagram of the plasma membrane. Figure reprinted by permission from Circulation, 1962, 26:1022.

Lipoid

To the biochemist concerned with the metabolic roles and dynamic aspects of membranes, the bimolecular leaflet model seems inadequate, however, for a variety of reasons that I shall not explore in depth at this time. It is at least esthetically displeasing to have the functional components of the membrane, the enzymes, restricted to the surfaces of phospholipid structures in arrangements dictated by the lipid patterns. The classical model emphasizes the role of the membrane as a passive barrier to free diffusion and an effective electrical insulator rather than as the site of dynamic metabolic and biosynthetic events. Recently, however, an alternative view has developed that

FIGURE 2. A lipoprotein subunit diagram of cell membranes according to Benson (5).
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considers membranes to be aggregates of lipoprotein subunits, which are in themselves the fundamental units of both structure and function (4, 5) (Fig. 2). This approach has the appeal of shifting the emphasis from the lipids to the proteins, which are the usual, but not exclusive, biological mechanism for specificity and versatility. It has the additional attraction of regarding the functional and structural components of the membrane as different properties of the same lipoprotein molecules.

But appeal and proof are quite different. Can one decide between the two models? It is my opinion that we do not now know the structure of any biological membrane. I believe that much of the evidence for the bimolecular leaflet model is indirect and circumstantial, that many of the interpretations of the data are neither rigorous nor unique, and that the more recent data point in other directions. We are free, therefore, to consider other possibilities, but there is still insufficient evidence on which to base a reasonable alternative model of membrane structure, certainly for the plasma membrane.

I shall now explore some of the reasons for these conclusions. Elsewhere I have discussed certain aspects of this problem in more detail (6). This discussion is restricted to the plasma membrane. Necessarily it involves a critical appraisal of the experimental basis for the traditional view of membrane structure. If a less than complete presentation of the data in support of the classical theory is given, it is because that position has been so well argued by its proponents.

PAUCIMOLECULAR MODEL

The original paucimolecular model for biological membranes was based largely on the following observations. The relative permeability of certain cells to nonpolar and polar molecules indicated the presence of a lipoidal barrier at the cell surface. The electrical properties of the few membranes that had been studied supported that conclusion. The lipid content of the red cell was thought to be exactly that amount which would cover the cell surface twice—hence the double layer; and the bimolecular leaflet was regarded as a uniquely stable arrangement of phospholipids. Since the predicted surface tension of the lipid leaflet was much higher than the measured values for cell surfaces, a layer of protein was postulated.

These arguments are not as impressive as they once appeared to be, quite apart from the fact that they were based on studies of a very few, often atypical, cells. We now realize that the physiological parameters of transport are not restricted to the physicochemical properties of the membrane. The lipid content of the red cell ghost was underestimated in the original studies and is, in fact, sufficient to cover the cell surface only 1.3 times under the same assumption of close packing as was made previously (6). Similar data are not available for any other membrane. X-ray diffraction studies have
demonstrated that phospholipid-water systems can assume stable structures other than the bimolecular leaflet (7), and alternative structures for phospholipid-cholesterol complexes have been suggested by electron micrographs (8). Finally, recent studies of synthetic bimolecular leaflets (9) have demonstrated that the phospholipid bilayer has a surface tension as low as that of the cell surface, so that the original reason for postulating a protein surface layer no longer exists.

**UNIT MEMBRANE MODEL.**

We now consider the unit membrane version of the bimolecular leaflet model, which was arrived at by another, although not totally independent, route. Early X-ray data led to the suggestion (10) that myelin was a lamellar structure in which the repeating unit consisted of two bilayers of phospholipid and several layers of proteins which might be arranged in any one of many possible configurations. Four possibilities are shown in Fig. 3. When later X-ray data (11) indicated that the size of the repeating unit should be halved, attention was focused on the two alternatives represented by the two halves of Fig. 3 D. There is still probably no unique interpretation of the X-ray pattern.

By this time electron micrographs of myelin (Fig. 4) had revealed a pattern of dense bands whose spacings could be related to the X-ray diffraction data (13): the major dense lines corresponded to the large X-ray repeat, and the intermediate dense lines reduced the distance by half. When it was determined that the dense bands in somewhat similar micrographs of artificial mixtures of phospholipids and water corresponded to the hydrophilic surfaces, the extrapolation was made that osmium and permanganate are specific markers of the polar groups of phospholipids in natural membranes (2). This led to the interpretation of the micrographs of myelin shown schematically in Fig. 5.
The dense bands are said to represent polar groups of repeating phospholipid bilayers, and the transparent bands are thought to represent the location of the fatty acyl chains. Protein (or nonlipid) was placed between the polar surfaces of adjacent bilayers. This protein is usually indicated in the β-
conformation because of space considerations and to provide sufficient surface area to cover the surface of the lipid bilayer (14).

What does the structure of myelin have to do with the plasma membrane? In the electron micrographs (Fig. 4) myelin can be seen to be continuous with the plasma membrane of the Schwann cell in such a way that the major dense bands of myelin appear to be derived from the apposition of adjacent inner surfaces of the plasma membrane while the intermediate dense bands might be similarly formed from the outer surfaces of the plasma membrane.

![Figure 5. Schematic representation of an electron micrograph of myelin and its interpretation in molecular terms based on Robertson (2).](image)

By extrapolation of the structure postulated for myelin, therefore, the trilaminar image of the Schwann cell plasma membrane was interpreted as a bimolecular leaflet of phospholipid covered on both surfaces by protein (or more strictly, nonlipid) (Fig. 5). Finally, since at the time most cellular membranes appeared as trilaminar images in electron micrographs, the structure was extended to all membranes. It is important to note that the only connecting link between myelin and the plasma membrane of the Schwann cell, other plasma membranes, and membranes in general is the apparent similarity of their electron microscopic images. We shall return to the problem of interpreting the microscopic image, but for the moment let us consider the composition of membranes.
Plasma membranes have very different concentrations of protein, cholesterol, and phospholipids (Tables I and II). Myelin is particularly unusual in its very low protein content, in its high cholesterol content (which it shares with erythrocytes), and in its unique content of cerebrosides. The differences among surface membranes are most obvious when we compare animal membranes with bacterial membranes. The latter contain no cholesterol and have a very different spectrum of phospholipids. Cholesterol is emphasized because detailed models of myelin stress the importance of specific interactions between cholesterol and phospholipids (14, 23). The fatty acids of membranes are also widely varied. Mammalian membranes contain polyunsaturated fatty acids (17), Gram-negative bacteria contain mainly C_{16} and C_{18} saturated and monounsaturated fatty acids (24), Gram-positive bacterial content.

### TABLE I

**PROTEIN AND LIPID COMPOSITIONS OF ISOLATED PLASMA MEMBRANES**

<table>
<thead>
<tr>
<th></th>
<th>Protein:lipid</th>
<th>Cholesterol</th>
<th>Cholesterol : phospholipid</th>
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</thead>
<tbody>
<tr>
<td>Myelin (15)</td>
<td>0.25 w/w</td>
<td>25 %</td>
<td>0.9 mole/mole</td>
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<tr>
<td>Erythrocyte (16)</td>
<td>2.5 w/w</td>
<td>25 %</td>
<td>0.9 mole/mole</td>
</tr>
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<td>Liver cell (17)</td>
<td>1.5 w/w</td>
<td>14 %</td>
<td>0.4 mole/mole</td>
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<tr>
<td>Gram-positive bacteria (18)</td>
<td>3 w/w</td>
<td>0 %</td>
<td>0 mole/mole</td>
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<tr>
<td>Halophil bacteria (19)</td>
<td>2 w/w</td>
<td>0 %</td>
<td>0 mole/mole</td>
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### TABLE II

**MAJOR PHOSPHOLIPIDS OF PLASMA MEMBRANES**

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<th></th>
<th>Myelin (15)</th>
<th>Erythrocyte (20)</th>
<th>Liver cell (17)</th>
<th>Gram-positive bacteria (21)</th>
<th>Gram-negative bacteria</th>
<th>Halophil bacteria (22)</th>
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<td>Sphingomyelin</td>
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<td>Phosphatidyserine</td>
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<td>15</td>
<td>8</td>
<td>2</td>
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<tr>
<td>Phosphatidylcholine</td>
<td>21</td>
<td>30</td>
<td>37</td>
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<tr>
<td>Phosphatidylglycerol</td>
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<tr>
<td>Diphosphatidylglycerol</td>
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<td>7</td>
<td></td>
<td>13</td>
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<td>Ether analogue of phosphatidylglycerol phosphate</td>
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<td>85</td>
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* J. L. Dittmer. Personal communication.
mainly C₁₄ and C₁₇ branched chain fatty acids (24), and in halophil bacteria fatty acids are totally replaced by a highly branched alcohol (22). Myelin uniquely contains a high proportion of long chain α-hydroxy fatty acids (25).

Differences among membranes are also revealed by their enzymatic composition. Plasma membranes of animal cells (26) probably contain more than 20 enzymes, including a number of esterases, phosphatases, and peptidases; bacterial membranes contain hydrolytic enzymes and a group of dehydrogenases (27); the membrane of the red cell is rich in enzymes, probably including the glycolytic enzymes (28). Thus plasma membranes are a complex composite of catalytic proteins, not even considering those additional proteins that are involved in the multitude of specific transport processes. The enzymatic activities of other cell membranes are, of course, even more pronounced. Myelin, alone among membranes, is probably without significant metabolic activity (29), as befits its biological function. Myelin is essentially an inert lipid shield, whereas plasma membranes are highly metabolic lipoprotein complexes.

Why then assume that the structure of plasma membranes is the same as the structure of myelin? The only reason is the apparent similarity of their electron microscopic images. It is very important, therefore, to assess critically the basis for interpreting electron microscopic images in molecular terms.

THE CHEMISTRY OF ELECTRON MICROSCOPY

In a typical protocol for electron microscopy, the tissue is fixed with 1–5% glutaraldehyde, postfixed with 1% OsO₄ (sometimes fixation is in 1% KMnO₄), dehydrated by extraction with a series of aqueous ethanol solutions, washed in propylene oxide, soaked in epoxy monomers, and polymerized at 60°C for 24–48 hr. Then sections about 500 Å thick are cut and “stained” with uranyl acetate and lead citrate prior to being placed in the electron beam.

To interpret the resultant photographic image, one must know precisely what is responsible for the absorbed and scattered electrons. One must know to what groups the Os, Mn, U, and Pb atoms are bound and be certain that the position of the “tagged” molecule, or at least the “tag,” is the same in the final material that it was in the living cell. Very little information on these points is available.

There is inherent contrast in biological materials, but it is not known which molecules are responsible. It is not known with what groups the section “stains” uranyl acetate and lead citrate react. The reactions involved in fixation with KMnO₄ are unknown, although lipids do not seem to be involved (30). There are no definitive data, but it might be expected that both KMnO₄ and OsO₄ would react with proteins, especially the amino and sulfhydryl groups, and it would be surprising if the tertiary and secondary structures of proteins were unaffected by these reagents. Qualitative evidence
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Figure 6. The structures of oleic acid, an osmic acid ester of 9,10-dihydroxystearic acid, and 9,10-dihydroxystearic acid, or their methyl esters. The osmic acid ester shown is the expected product of reaction between oleic acid (or methyl oleate) and osmium tetroxide under anhydrous conditions according to Criegee (32). R = H or CH₃.

(31) suggests that OsO₄ cross-links proteins and that KMnO₄ degrades proteins. On the other hand, glutaraldehyde is a useful fixative specifically because it cross-links free amino groups of proteins. Obviously this will not leave the protein molecules in their original states. There is no experimental basis for assuming that fixation preserves the “molecular morphology” of proteins.

Reactions of OsO₄

Somewhat more can be said about the chemistry of fixation with OsO₄. As I have already discussed, the evidence for the orientation of phospholipids in myelin and, hence, the derivation of the unit membrane theory is based to a significant degree on the assumption that osmium specifically labels the polar groups of phospholipids. Since the work of Criegee (32), however, it has been known that under anhydrous conditions OsO₄ reacts with an olefin, such as an unsaturated fatty acid, to form an osmic acid ester of the glycol derived from the olefin by oxidation (Fig. 6). To rationalize the interpretations of the electron micrographs it was necessary, therefore, to assume that under the conditions of fixation the osmic acid ester is hydrolyzed and releases lower oxides of osmium that somehow migrate to, and are bound to, the polar groups of phospholipids (33). I have recently investigated the reaction of

\[
\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{C}==\text{O}\text{CH}_3 \\
\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{C}==\text{O}\text{CH}_3
\]

Figure 7. Bis(methyl 9,10-dihydroxystearate) osmate, the product of reaction between methyl oleate and osmium tetroxide under conditions resembling fixation (34).
OsO₄ with unsaturated lipids in an aqueous environment under conditions closely resembling those of fixation and have reached different conclusions (34–36).

The product of reaction between OsO₄ and either oleic acid or methyl oleate is a diester in which 2 molecules of the glycol are linked through 1 molecule of osmic acid (Fig. 7). The reaction product has been isolated in nearly 100% yield by silicic acid chromatography and identified by thin-layer and gas-liquid chromatography; visible, infrared, and nuclear magnetic resonance spectroscopy; molecular weight determination; and elemental analyses. The esterified osmium is stably bound, and there is no evidence for any other bound osmium. Free glycols are not formed. Similar reactions with phosphatidylcholine containing two monounsaturated fatty acids have indicated the probable presence of two reaction products. In one, the osmic acid diester links two fatty acids within the same phospholipid molecule (Fig. 8), and the other is probably a polymer in which the osmic acid forms a link between fatty acids of different phospholipid molecules (Fig. 9). Furthermore
when amebae are fixed with OsO₄, all of the unsaturated fatty acids disappear, no glycols are formed, and about 50% of the oleic acid can be recovered as the bis(dihydroxystearic acid) osmate shown in Fig. 7. The remainder of the oleic acid is probably esterified through osmic acid to derivatives of polyunsaturated fatty acids that we can neither isolate nor characterize. The conversion of unsaturated lipids into polymeric esters of osmic acid would be expected to alter significantly their arrangement in membranes.

When unsaturated lipids react with OsO₄ in the test tube, in addition to the esterified osmium an approximately equivalent amount can be recovered as OsO₂. Its formation might be explained by the reaction scheme proposed in Fig. 10. In the model reaction the OsO₂ is not bound to the lipids; if formed during fixation, its location in the cell is not known. One possibility is that the

\[
\begin{align*}
4 \text{CH}_3(\text{CH}_2)_7\text{CH=CH}(\text{CH}_2)_7\text{C}-\text{OR} + 4\text{OsO}_4 & \rightarrow 4 \text{CH}_3(\text{CH}_2)_7\text{CH}-\text{CH}(\text{CH}_2)_7\text{C}-\text{OR} \\
2\text{OsO}_3 & \rightarrow \text{OsO}_2 + \text{OsO}_4
\end{align*}
\]

OsO₂ might orient at the interfaces between lipoprotein structures and water. The OsO₂, then, might be only a marker for the aqueous/organic interface and not a specific indicator of the presence of polar groups of phospholipids at that interface. These conclusions have recently been confirmed in studies of the reaction of OsO₄ with lipid monolayers (37).

There is yet another reason for doubting that the electron microscopic image necessarily tells one anything about the disposition of lipid molecules. The typical trilaminar image of the inner mitochondrial membrane is revealed by fixation in OsO₄ even when all of the lipids have been removed before fixation (38). In fact, all of the lipids can be extracted from myelin (39), after fixation in glutaraldehyde, with no effect on the image obtained with subsequent fixation in OsO₄. If lipid is not necessary to obtain the trilaminar image, then that image cannot be used to define the orientation of the lipid. In membranes other than myelin, there is no other experimental basis for the
bimolecular leaflet. It would be very interesting to know if the X-ray diffraction pattern of myelin, which has always been attributed to lipid bilayers, is also retained after the removal of the lipid.

I believe the foregoing discussion exposes many of the weaknesses of the traditional arguments for the bimolecular leaflet theory and indicates the desirability of considering alternative structures for cell membranes and, perhaps, even for myelin. Let me emphasize again, however, that the evidence in support of other models is inadequate and unconvincing, although provocative, at this time.

**The Proteins of Membranes**

Recent investigations have focused on the proteins rather than the lipids of membranes. Isolated membranes have been examined by the techniques of optical rotatory dispersion, circular dichroism, and infrared spectroscopy, which provide information concerning the configuration of the peptide backbone of the proteins. By these criteria the erythrocyte ghost (16, 40), the plasma membrane of the Ehrlich ascites carcinoma cell (41), the proto-

![Graph showing optical rotatory dispersion of mitochondrial structural protein.](image-url)

*FIGURE 11. The optical rotatory dispersion (42) of mitochondrial structural protein in the soluble form (pH 11) and aggregated state (pH 9). The decreased amplitude of the maximum and minimum and the red shift are characteristic of membrane proteins. Figure reprinted by permission from Proceedings of the National Academy of Sciences, 1967, 58:1292.*
plast membrane of *Bacillus subtilis* (42), and the plasma membrane of halophil bacteria (42) all seem to have between 20 and 50% of their protein in the \( \alpha \)-helix configuration. No protein in the \( \beta \)-conformation can be detected, the remainder being present presumably as random coil. Very similar data are obtained for the mitochondrial “structural” protein and even for the protein of myelin (42). These latter data are difficult to reconcile with the usual interpretations of the X-ray and microscopic studies of myelin, but they are quite compatible with studies of proteolipids (43)—the soluble products into which myelin is converted by dissolution with chloroform-methanol. If much of the protein of myelin is in the \( \alpha \)-helical conformation, there would be insufficient protein to cover phospholipid bilayers. Proposed models would have to be altered accordingly.

Precise interpretations of these protein spectra are difficult and uncertain, but the optical rotatory dispersion and circular dichroism measurements of all the membranes share an interesting feature. The maxima, minima, and transition points all show a red shift of several millimicrons. This red shift has been attributed by Wallach and Zahler (41) to binding between hydrophobic portions of phospholipids and the \( \alpha \)-helical regions of proteins. Lenard and Singer (40), on the other hand, attributed this red shift to strong interactions between \( \alpha \)-helical regions of adjacent proteins. This latter interpretation is consistent with the recent data of Steim and Fleischer (42), who have found an identical red shift in the circular dichroism spectrum of mitochondrial structural protein that contains no lipid (Fig. 11).

**NONIONIC PROTEIN-LIPID BONDS**

There is other evidence for nonionic linkages between phospholipids and membrane proteins. Membranes are stable in solutions of high ionic strength. Phospholipids are bound to mitochondrial structural proteins only through hydrophobic bonds (44). Preliminary and still tentative interpretation of the nuclear magnetic resonance spectrum of erythrocyte ghosts (45) suggests that the fatty acyl moieties of phospholipids are strongly bound. Bonds between the lipid and protein constituents of myelin proteolipids seem to be nonionic (43). But perhaps the strongest evidence comes from titration studies of the isolated membranes of halophil bacteria (46). All of the polar lipids of these membranes are anions and, therefore, any ionic bonds must be to positive groups on the proteins. Yet it is possible to titrate all of the amino groups of the membrane proteins without loss of lipid. The amino groups can even be converted to anions by succinylation without loss of lipid.

**ALTERNATIVE MODELS**

Emphasis on the catalytic function of membrane proteins, on the probability of protein-protein interactions, and on the probable importance of hydrophobic bonding between protein and lipids leads to the consideration of mem-
branes as specific aggregates of lipoprotein subunits. One schematic model has been suggested (40) (Fig. 12). It differs in almost every significant way from the bimolecular leaflet, yet is compatible with all of the data that led to the paucimolecular model. The newer proposal places the hydrophilic regions of both the phospholipids and the proteins at the aqueous interface. It envisages appreciable sections of α-helical protein that penetrate through the membrane and are stabilized by the hydrophobic environment and by interaction with α-helical sections of other proteins. The fundamental continuity of the membrane depends on protein-protein interactions rather than lipid-lipid bonds. Lipids are involved in hydrophobic interactions with nonpolar groups on the protein, and perhaps with nonpolar lipids. In a three-dimensional view this model of the plasma membrane would resemble in many ways the explicit lipoprotein models proposed by others for mitochondrial (4) and chloroplast (5) membranes (Fig. 2).

**MEMBRANE FUNCTION AND BIOSYNTHESIS**

The biological implications of these newer models with regard to the plasma membrane are significantly different from those of the classical models. To penetrate a lipid bilayer, for example, a solute molecule must either pass through permanent or transient aqueous pores, or form complexes with specific lipid-soluble carrier molecules. In the lipoprotein subunit model, the membrane protein itself can serve in the catalytic role of the carrier model. It is only a distance of some 50–60 amino acids in the α-helical configuration from one side of the membrane to the other. One can imagine a conformational change of the protein as sufficient to move a solute molecule across the membrane. Admittedly these movements are greater than those normally involved in allosteric transformations of enzymes, but they are no greater than the distances through which protein molecules are thought to move during muscle contraction.

There is some evidence for the existence of membrane proteins that bind solute molecules. In mammalian cells, transmembrane transport of Na⁺ seems...
to be dependent on a membrane ATPase which is phosphorylated during this process (47, 48). The phosphorylation might be the molecular basis, and provide the energy, for the conformational change. Membrane as well as nonmembrane proteins are strongly implicated in the transport of galactosides (49), sugars (50), amino acids (51), and sulfate (52) into bacterial cells. Resolution of these transport systems may be a major step toward understanding membrane structure as well as membrane function.

The new and old membrane models also have quite different implications with regard to mechanisms of membrane biosynthesis (Fig. 13). The paucimolecular model is fundamentally based on the associative properties of phospholipids, and therefore implies that the first step in membrane biosynthesis is the formation of a bimolecular leaflet of phospholipid to which proteins are later added, probably in arrangements dictated by the phospholipid bilayer. The lipoprotein model implies a sequence of events in which the protein molecules are first synthesized. To these would be added the lipids as dictated by the primary, secondary, and perhaps tertiary structure.
of the protein. The lipoproteins would then aggregate to form membranes. Alternatively, the lipid might be added after the association of the protein into membranes. These alternatives should be experimentally distinguishable. If biosynthesis of membranes begins with formation of lipoproteins, then the behavior of phospholipids in model systems may have little relevance to biological membranes.

CONCLUSION

This discussion has covered considerable ground. I want to conclude by emphasizing that although in my view the paucimolecular model is not well documented, neither is it disproven. The lipoprotein subunit theory is very attractive and more easily reconciled with contemporary biochemical knowledge, but is equally unproven. It is entirely consistent with all available data that different membranes may have different structures, that different portions of the same membrane may have different structures, or that the same section of membrane may exist in different states at different times.

REFERENCES


Discussion

Dr. Anne E. Kaplan: Dr. Korn's paper has given us much to consider concerning the structure of membranes. The question with which we have been concerned is that of the role of weak chemical bonds in reactions associated with physiological processes in membranes. The hydrogen bond is the first of such chemical bonds we have investigated with this question in mind. Our attention was drawn to it because of the work of Tanford (1961. *J. Am. Chem. Soc.* 83:1928), which showed that the energy of the hydrogen bond in a hydrophobic medium is of the order of −6 kcal/mole. This value is considerably higher than that of the hydrogen bond in an aqueous medium, where it is considered to be 0 to −1.5 kcal/mole. The increased strength in bond energy in transferring a group from an aqueous to a hydrophobic medium can act as a driving force through membrane structures.

In order to investigate this further, we synthesized a compound, N-palmitoyl-L-serine (Fig. 1, Discussion), which has several chemical groups useful in hydrogen bond formation: the 1-carboxyl group, the amide group at C-2, and the hydroxyl group at

1 These studies were carried out at The City University of New York, where the discussant was a member of the Doctoral Faculty for Biochemistry.
the third carbon atom of serine. Each of these groups is capable of forming hydrogen bonds. In addition, the bond between the amide carboxyl and the C-3 hydroxyl group can be seen in the model of this molecule. We have found that the tendency for hydrogen bonds to form is so strong in solvents such as chloroform that rigid gels result from interactions of the monomer. We have presented evidence for this in earlier work by measuring shifts in the infrared absorption maxima of these mixtures (Kaplan, A. E. 1967. J. Colloid Interface Sci. 25:63).

We have recently examined the capacity of N-palmitoyl-L-serine to interact with a variety of lipids in aqueous suspension. In these studies, we compared the interactions of the serine derivative with that of palmitic acid. The free acid has approximately the same molecular length as the serine derivative, but is considerably lower in hydrogen-bonding potential. The results are summarized qualitatively in Table I, Discussion. We have used membrane lipids such as cholesterol and phrenosine, a cerebroside found in the myelin sheath, in these studies because we are especially interested in membrane interactions. The four lipoidal substances, N-palmitoyl-L-serine, palmitic acid, cholesterol, and phrenosine, are all insoluble when homogenized with water at a concentration of 1 mm. If mixtures are made with N-palmitoyl-L-serine...
and cholesterol and/or phrenosine, a physical change is apparent immediately, for the lipid combinations form uniform suspensions in solution. In contrast, palmitic acid, a weaker hydrogen-bonding molecule, has no effect on the solubility of other lipids in water. The combination of cholesterol and phrenosine in water does not result in any solubility change, such as the addition of N-palmitoyl-L-serine brought about. Instead, these two membrane lipids act more in a manner comparable to the mixtures with palmitic acid.

We have examined the additional effect of protein on the lipid-water mixtures with defatted bovine serum albumin at a concentration of 400 µg/ml. The protein

<table>
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<tr>
<th>Table I</th>
<th>CHANGE IN SOLUBILITY OF CHOLESTEROL AND PHRENOSINE IN WATER UPON THE ADDITION OF N-PALMITOYL-L-SERINE IN COMPARISON WITH PALMITIC ACID</th>
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<tr>
<td>*NPS = N-palmitoyl-L-serine; PA = palmnitic acid; Chol = cholesterol; Phren = phrenosine, def BSA = defatted bovine serum albumin.</td>
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<tr>
<td>‡ + = opaque suspension; ++ = clear solution; - - = no change.</td>
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acts to alter the physical properties of the suspension of N-palmitoyl-L-serine and cholesterol, and N-palmitoyl-L-serine and phrenosine, and enables all components to dissolve in a single phase. At this protein concentration, however, the three lipids, N-palmitoyl-L-serine, cholesterol, and phrenosine, are not brought into solution by the albumin.

These results offer evidence that hydrogen-bonding groups can act to enhance the solubility of lipids with respect to water, with special emphasis on membrane lipids, which are extremely insoluble in water.

Dr. Kennedy: Thank you, Dr. Kaplan. The question is about the chemistry of the role of permanganate and fixation for electron microscopy.

Dr. Korn: In the first place, potassium permanganate is a compound which is not
employed very much currently as a fixative because it is a destructive reagent. Although you get very clear images of membranes, the rest of the cell is fairly extensively damaged. But nothing is really known about the chemistry of permanganate fixation. It does not appear to react with the lipids at all. In the test tube in the cold, under the conditions employed for fixation, one cannot detect any reaction with lipids. In the cells that I have examined, the lipids are essentially unaffected after permanganate fixation. There are probably protein reactions, but other than that there is no evidence at all of which I am aware.

Dr. Mauro: Since you have been interested in the critique of the electron microscopic data associated with myelin, would you comment on the recent very interesting paper in The Journal of Cell Biology, by Dr. Napolitano and his colleagues, of The University of New Mexico, Albuquerque?

Dr. Korn: I am glad you asked. I forgot to comment on it and the earlier paper by Fleischer and Stoeckenius. That is rather an important point. In the case you are talking about, myelin was first fixed with glutaraldehyde, and in the other case, isolated mitochondria were not fixed until all of the lipids were extracted. At that point, the material was reacted with osmium and the resultant images were identical with the image obtained with the intact material. So it is very, very clear that you can get the identical image in these two instances, one of which is myelin, when the lipids are all gone. That being the case, it seems to me that one cannot employ the electron microscopic image to decide the orientation of lipids.

Question from the Floor: Could you comment on the earlier reports that micelles of saturated lecithin, etc., give electron microscopic pictures not distinguishable from that of unsaturated lecithin stained with osmium?

Dr. Korn: Insofar as I am aware, saturated phosphatides do not react with osmium, and pictures are not obtainable under those conditions. I don't know the papers you are referring to. If there is no olefinic group you cannot fix isolated lipids, as far as I am aware.

Question from the Floor: Would Dr. Korn care to comment on the results of Thompson in trying to fix black membranes for electron microscopy, where he couldn't fix them with osmium tetroxide, but he could fix them with lanthanum nitrate?

Dr. Korn: I don't have any comment. It is interesting, of course, because the phospholipids used do react with osmium. Apparently the resultant product is unstable.

Dr. Blank: I think it is fair to point out that the evidence for the bilayer theory is much stronger than just the electron microscopy. You mentioned this during your talk, but I think it should be emphasized.

I have two questions: (a) In connection with your criticism of the electron microscopy technique, would you make a similar evaluation of the optical rotatory dispersion technique? (b) With regard to the unit membrane concept, Robertson has spoken about the ubiquitous unit membrane, i.e. that it always has the same dimension. For those who have accepted the bilayer view of the membrane, the dimension seems to rise from the two layers of lipid. If you accept that the matrix is primarily protein, how do you get this same thickness all over?

Dr. Korn: There are at least two questions there, I think. I shall answer them in reverse order.

In the first place, in the image the membranes vary anywhere from 50 A to 150 A
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or 200 A. The inner membrane of the mitochondrion is very, very thin. And the argument of Robertson is that this kind of a change from 50 to 150 A is quite compatible, within the accuracy of electron microscopy, with the bimolecular leaflet, and perhaps this is the case.

Now for the initial question. First of all, obviously proponents of the unit membrane argument would be able to offer, and do offer, a stronger argument in support of it than I indicated. This has been done over quite a long period of time, and I didn’t feel obligated to repeat it here. I think it is fair to say, and I was hurried at the end so perhaps I didn’t emphasize it, that the interpretations of the optical rotatory dispersion spectra and the circular dichroism spectra are preliminary, and are difficult. The spectra are compared to the spectra of polypeptides usually in solution, and this may not be a fair thing to do. So I think that the quantitative aspects are certainly very hard to be certain of. Most of the authors of these papers say that any where from 20% to 50% of the protein is α-helices. I think the presence of α-helix is clear; the quantity of α-helix is uncertain. Another aspect of these data is the absence of β-conformation in all of these studies. Now, it is because there are a great many uncertainties about the interpretation of these very recent data that I have concluded that the recent developments are indicative of perhaps another structure, but by no means prove it. And I think it is very possible that the classical structure exists in parts of some membranes, or maybe in all of a few membranes, or perhaps membranes are transitory structures, and things flip around.