Model of Interaction of Polar Lipids, Cholesterol, and Proteins in Biological Membranes

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ABSTRACT

Membranes are proposed to consist of a hydrophobic core, two hydrogen belts, and two polar zones. The hydrogen belts consist of hydrogen bond acceptors, i.e. the carbonyl groups of phospholipids and sphingolipids, and hydrogen bond donors, i.e. the labile hydrogens of cholesterol, sphingosine, proteins, and water. The density of anhydrous hydrogen bonding and the impermeability of the membrane increase with increasing concentrations of cholesterol, sphingolipids, α-hydroxy acyl residues, plasmalogens, and ether phospholipids. Cholesterol owes its membrane-closing properties to its rigid longitudinal orientation in the membrane combined with the latitudinal orientation of the O-H bond. It is suggested that the intrinsic proteins of membranes are held in position by hydrogen bonding, as well as by hydrophobic and electrostatic forces, and that hydrogen bonding also mediates the penetration of membranes by proteins.

INTRODUCTION

Cholesterol is a major component of many biological membranes and obviously fulfills an important function in them, but it is not known what this function is. The sterol appears to form a complex with phospholipids, as shown by calorimetric scanning (1,2) and by the contraction of mixed phospholipid-cholesterol layers (3-5). Cholesterol suppresses the permeability of phospholipid membranes for water (6), cations, glycerol, and glucose (7,8) and the penetrability of monolayers by proteins (9). The effect upon permeability is shown only by cholesterol and related sterols having a β-OH group, a planar ring system, and an aliphatic side chain (8). Spin-label (10) and NMR (2) studies show that cholesterol reduces the flexibility of the carboxyl half of the aliphatic phospholipid chains in liquid-crystalline films. The spatial fit between the rigid ring structure of cholesterol and the paraffinic part of the common unsaturated fatty acids has been noted (11). It seems to be the present consensus of opinion that cholesterol condenses and rigidifies membranes without solidifying (gelling) them (2,7,12,13).

In the model presented in this article, condensation and rigidification by cholesterol play no role. This does not mean that these effects are negated, but I propose that they are not the only, and probably not the most important, functions of cholesterol in membranes. I suggest that the capacity of cholesterol to donate a hydrogen bond is of greater importance for the functioning of artificial, as well as natural, membranes. This hypothesis will lead to further consequences concerning the function of various phospholipids and sphingolipids and the structure of membrane proteins.

PREMISES

Molecular Models

All following statements concerning distances, bond angles, and other steric properties of the molecules have been obtained from spacefilling Corey-Pauling-Koltun models or in some cases from Dreiding models.

The following assumptions have been made. The conformation of ester groups C-C(=O)-O-C, and amide groups, C-C(=O)-N(H)-C, is *trans*. The conformations between the methylene groups, $-CH_2-CH_2-$, near the polar ends of the chain are also *trans*, and in phosphoglycerides, the first methyglene group of one of the chains must be gauche to the C=O if the chains are to be parallel (14). The further conformations of the chains or of the side chain of cholesterol are immaterial for our discussion. The A-ring of cholesterol has the chair conformation.

The hydrogen bond has a distance between the centers of the oxygen atoms of 2.6-2.8 Å and must be straight, i.e. the bonds in O-H···O form a straight line (15,16). These conditions are built into the commercial molecular models.

Hydrophobic Bonding and Hydration

The transfer of paraffinic chains from aqueous to nonpolar surroundings is accompanied by a loss in free energy of ca. 0.7 kcal/mole for each CH_2 group (17). The maximization of such "hydrophobic bonding" requires the total immersion of fatty acid chains and cholesterol into the nonaqueous phase and the closest possible packing of molecules in this phase.

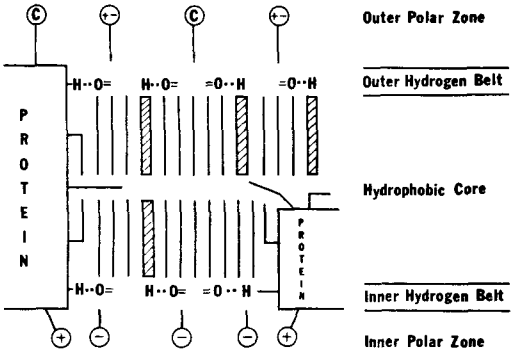

FIG. 1. Schematic model of a plasma membrane. Lines represent hydrophobic lipid chains or amino acids; shaded rectanges represent hydrogen bond donors (cholesterol, sphingolipids); the circles are charged head groups of phospholipids or cationic amino acids residues; C stands for carbohydrate.

Carbohydrate groups, on the other hand, and ionic groups, such as the phosphate, ammonium, and carboxyl groups of phospholipids, have an equally strong affinity for the aqueous environment (18). The head groups of the polar lipids in lipid bilayers must, therefore, be hydrated thoroughly, and any membrane model that implies dehydration of these groups in favor of contact or bonding to nonpolar residues must be discarded. This critique is directed in particular against those models (19-21) which call for phosphate-cholesterol hydrogen bonding with partial extraction of the cholesterol into the polar phase.

MEMBRANE MODEL

The preceding considerations are compatible with the generally accepted membrane model of a lipid bilayer with a hydrophobic core enveloped by two polar zones. The model presented here has, in addition, two interposed hydrogen belts, i.e. well defined planes of lipid-lipid and lipid-protein hydrogen bonding.

In Figure 1, the fluid mosaic membrane model, which envisions the intrinsic proteins floating in a continuous, viscous-liquid lipid bilayers, has been accepted (18,22). Bilayer assymmetry -with acidic lipids on the inner side of the membrane (23,24)–also has been assumed. It should be noted, however, that the concept of the hydrogen belt does not depend upon such conditions. The picture of the membrane (Fig. 1), which might represent a fragment of a plasma or erythrocyte membrane, has been purposely left abstract to suppress any intimations of specific lipid-lipid complexing, steric fit of nonpolar residues, influence of unsaturation, or stoichiometric proportioning of membrane components, all of which are probably of importance but are not essential for the discussion of the basic model.

The =O symbols of Figure 1 represent the carbonyl oxygen atoms of the ester groups of phosphoglycerides and of the amide groups of sphingolipids. These groups accept hydrogen bonds from the OH groups of cholesterol and sphingosine and from labile hydrogens of the amino acids of membrane proteins; if such donors are lacking, hydrogen bonds are accepted from water. The floating proteins are held in position by hydrophobic bonding in the core of the membrane and by electrostatic bonding to the phosphate groups in the polar zone. They are prevented from bouncing and swaying by being buckled into the hydrogen belts.

The permeability of the lipid bilayer in the model is regulated by the density of hydrogen bonding in the hydrogen belts. In layers of phospholipids without cholesterol, the C=O groups bind to water or, as the case may be, to cations or hydrogen bond donors, such as glycerol or glucose, perhaps with the mediation of water. These solutes can, thus, pass into and through the membrane. Hydrogen bonding to cholesterol dehydrates and blocks the C=O groups. Only 50% of them have to be bonded to close the membrane almost completely. A possible explanation for this ratio is given later.

The passage of ions and other molecules through biological membranes generally is believed to be mediated by membrane proteins. Therefore, the C=O groups of the phospholipids in the model do not accept the solutes themselves but the enzymic or carrier proteins that transport the solutes. The surplus of C=O groups is likely to be bonded to cholesterol (or sphingolipid), but there may well be some excess of water-bonded C=O groups. These could cause various degrees of porosity of the lipid matrix in various membranes.

ARGUMENTS

Steric Arguments

The direction vertical to the surfaces of the membrane (Fig. 1) I define as longitudinal. This orientation probably is held, on the average, by the fatty acid chains and by the long axis of cholesterol. The direction parallel to the surfaces and the belts I call latitudinal. A latitudinal angle describes the deviation from this direction; the angle is positive toward the core of the membrane, negative toward the outside. For example, hydrogen bonds lying completely in the plane of a hydrogen belt would have a latitudinal angle of 0°, a bond pointing verti-

cally away into the aqueous phase an angle of -90°.

In the membrane model (Fig. 1), the =O groups function as acceptors of hydrogen bonds from either water or cholesterol; they should, therefore, be approachable from both longitudinal and latitudinal directions. If a phospholipid model is arranged in its most probable conformation, with both aliphatic chains in close contact and the ionic groups longitudinally extended, the C=O bonds can assume an almost perfect latitudinal orientation with an angle around 0°. Such an orientation is obviously ideal for latitudinal lipid-lipid bonding, but it also allows bonding from water molecules. With large negative C=O angles, water bonding will become favored, but such angles are improbable: space filling models show that they would loosen the packing of the aliphatic carboxyl end chains and thus cause the hydration of CH_2 groups; such hydration is energetically unfavorable.

Cholesterol, if arranged longitudinally in the membrane, can have latitudinal O-H angles between ca. +10 and -50°. In the extreme position of +10° (pointing slightly inward toward the membrane core), the hydrogen extends at a right angle from the side of the angular methyl groups, the β-side, of the molecule. Carbonyl···HO hydrogen bonding in this configuration is compatible with tight, parallel packing of fatty acid and cholesterol. The +10° configuration of the cholesterol O-H bond also yields the maximal exposure of the "back" of the oxygen to water, with the possibility of the oxygen accepting one or two hydrogen bonds (Fig. 2). The energetic advantage of such additional bonding is discussed below.

In the alignment shown in Figure 2, the C-3 of cholesterol is situated at a latitude between that of C=O and the first CH_2 group of the fatty acid. Such an alignment is thermodynamically highly probably, because it yields the maximal separation of hydrophilic (polar) and hydrophobic phases. With this alignment, the end methyl group carbons of the cholesterol side chain are ca. equidistant, in fully extended models, with carbon 14-15 of the fatty acid.

Energy of Hydrogen Bond

Hydrogen bond energies usually range from 4-8 kcal/mole (15,16). Dihedral oxygen -O-, forms relatively weak bonds, e.g. the water-water bond energy is ca. 4 kcal/mole. Carbonyl oxygen, C=O, is a stronger hydrogen bond acceptor; to cite an especially relevant example, cholesterol-triglyceride hydrogen bonding is favored strongly over cholesterol-cholesterol bonding (25). It must be assumed that the C=O

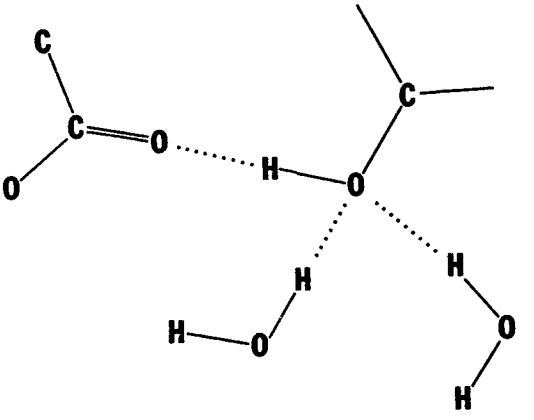

FIG. 2. Hypothetical alignment of phospholipid carbonyl, cholesterol hydroxyl, and water in the hydrogen belt.

group of pure phosphoglyceride bilayers, since they are accessible to water and no other hydrogen donors are available, form hydrogen bonds with water.

The negatively charged phosphate groups of phosphoglycerides increase the electronegativity of the neighboring carboxyl ester groups by induction, and thus make them better hydrogen bond acceptors (15,16). This inductive effect falls off with increasing distance; the C=O of the ester in position 2 of the glycerol can, therefore, be expected to be the better hydrogen bond acceptor. Once established, hydrogen bonding at position 2 will further reduce the acceptor capacity of the carbonyl in position 1 by reverse induction.

It must be understood that the possibility of a strong phosphoglyceride-cholesterol hydrogen bond does not guarantee its existence. The C=O···H-O bond in Figure 2 has to compete with C=O···H_2O hydrogen bonds; such bonds have to be broken, but the overall ΔG must still be negative. The reaction:

$$\text{OH (hydrated)} + \text{OC (hydrated)} \rightarrow \text{OH}\cdots\text{OC} + x\text{H}_2\text{O} \quad [1]$$

has, in fact, been estimated as having a ΔG of +1.4 kcal, regardless of whether the hydrogen bond is formed in polar or apolar environment (26). In our model (Fig. 2), two forces may drive reaction 1. First, because of the close packing of fatty acids and cholesterol and the slightly inward direction of the hydrogen bond, water is expelled from the hydrogen belt and can no longer compete. This means that a part of the hydrophobic bonding energy is expended to balance the positive ΔG of reaction 1. Second, and more important, the cholesterol oxygen, by turning its back towards the aqueous

phase and partly donating its proton to the C=O group, becomes a good acceptor for one or two protons from water. Through this mechanism, the C=O···H-O bond is reinforced heavily. Stated in other words, the positive free energy change reported for equation I (26) refers to the total dehydration of C=O and OH. In the arrangement of Figure 2, however, while there is dehydration of the hydrogen belt, i.e. the C=O, there is no net dehydration of the total system. There is also no reversal of the polarity of the water layer.

EVIDENCE

The preceding arguments lead to the conclusion that the carbonyl groups, as well as the hydroxyl group, must participate in some form of hydrogen bonding and that they are sterically, and also energetically, in a position to bind to each other. Bonding of the cholesterol to the phospholipid phosphate, which has been suggested (20,21), would require the dehydration of the anion, as well as the freezing of it in one position, both energetically improbably. Experiments which appear to show such bonding in anhydrous lecithin-cholesterol mixtures or hydrated multilayers (27,21) or below the liquid-crystalline transition point of the phospholipids (28,29) are irrelevant to the problems of membrane structure. The alternative, then, is a simple one: Are carbonyl and hydroxyl bonded to each other, or is each bonded to water only? We have no direct experimental answer: IR and NMR spectroscopies, which usually detect such bonds, cannot distinguish between the different kinds of hydrogen bonds in the presence of water (1,2). The evidence that can be offered at present for the hypothesis is, of necessity, less direct. It is, mainly, derived from studies originating in the laboratories of van Deenen and his colleagues (7,8,30,31).

Only the β-OH sterols with a flat structure and a side chain reduce the permeability of phospholipid membranes (8). Flat structure and side chain are probably necessary for close packing and hydrophobic bonding; the β-configuration of the hydroxyl is, in the light of our hypothesis, essential for latitudinal hydrogen bonding. Most significantly, epicholesterol, with an α-OH but otherwise identical in structure with cholesterol, does not reduce membrane permeability (8). In this sterol, the possible latitudinal angles of the labile hydrogen vary from $+60^\circ$ to -80°. In the more negative (longitudinal) orientations of the OH groups, both sterols could be expected to be hydrogen bond donors, $OH\cdots OH_2$, to water, and there is no reason to believe that the organization of the water would be much different in both cases, as has been suggested (7,8), but only cholesterol, I postulate, forms a latitudinal hydrogen bond. For epicholesterol to form latitudinal bonds, the hydrogen would have to be directed not vertically out of the β-plane as in cholesterol, but pointing to one of the edges of the molecular plane. This, we must assume, would put C=O and H-O too far apart, under the prevailing conditions of packing, to form a hydrogen bond. Similar steric considerations can explain why cholesterol cannot dose membranes of polyunsaturated phosphoglycerides.

Cholesterol reduces the average molecular area occupied by phospholipids in monolayers (3,4) and abolishes the energy jump at phospholipid phase transitions (2). A large number of sterols and ketosteroids has been tested in an effort to prove that these effects are correlated with the structures of the steroids and with their influence upon membrane permeability (30,8). Such a correlation might indicate that impermeability is the result of closer packing (condensation) of the membrane. The monolayer studies have yielded no support for such a proposal. The planar β-OH sterols all condense the membrane and reduce the permeability, but the (nonplanar, β-OH) coprostanol does not influence the permeability, although it causes considerable condensation. More striking, the keto analog of cholesterol, cholest-5-en-3-one, condenses the membrane as efficiently as cholesterol (30) but leaves it as permeable as before (8); other steroids have similar effects. Cholesterol acetate also condenses membranes (32); I predict that it will not significantly reduce their permeability.

In a recent study (31), it was concluded that there is no specific binding of the sterol-OH to any polar part of the phospholipids. This conclusion was based upon the condensing effect and the liquefying effect (reduction of the ΔE of phase transition) that cholesterol had on some phospholipids that lacked the C=O group in position 2, namely, 1-oleoyl-2-palmitylglycerylphosphorylcholine and 1-oleoyl-2-palmityl-2-deoxyglycerylphosphorylcholine. As pointed out above, membrane condensation is not identical with membrane closure, and the experiments (31) may, therefore, not have been relevant to the problem of membrane function. It is the more surprising that a closer examination of the results (Fig. 1, [31]) shows that the condensing effect of cholesterol upon these lipids amounted to only one-half of the effect that could be achieved upon diacyl phospholipids. This result seems to show that cholesterol does, indeed, establish a bond to car-

bonyls and preferentially to the ester group in position 2 of the glycerol.

SPHINGOLIPIDS, α-HYDROXY FATTY ACIDS, AND PLASMALOGENS

The 3-hydroxy group of sphingosine has no known biochemical function. In conventionally printed structural formulas, this group seems to be buried in the hydrophobic region of sphingolipids, but in a three dimensional model in which the *trans*-configuration of the amide group is taken into account and the chains are arranged parallel, the OH group moves to the same latitude as the amide C=O group. (The N-H group probably is buried between the heads of the chains.) Both C=O and OH now lie in the hydrogen belt. Obviously, the one can act as hydrogen bond acceptor, the other as donor. They cannot link to each other. The range of possible latitudinal sphingosine O-H angles may be much wider than for cholesterol, perhaps from +70° to -70°. The case for latitudinal bonding on the basis of steric arguments is, therefore, not as convincing as it is for cholesterol. Nevertheless, I suggest that sphingolipids are both hydrogen bond acceptors and donors serving as extenders and branches in the hydrogen belts.

The D-α-hydroxy acids found in some galactocerebrosides of the brain introduce an additional hydroxy group into the sphingolipid, and this must be a strong donor because of electronegative induction from the neighboring carbony. These cerebrosides could serve as cross-links in the hydrogen bond network. On the other hand, it is possible for the α-OH to link to the ring oxygen of the galactose while, at the same time, the C=O is linked by the 4-OH of the carbohydrate. This arrangement is possible only with galacto-, not with glucocerebrosides, and not with L but only with D-α-hydroxy acids. A new ring structure would be formed which would be stabilized by concerted electron shifts and which would totally immobilize the galactose in relation to the head groups of the sphingosine and the fatty acid, but would leave hydroxyls 2, 3, and 6 available for $OH\cdots OH_2$ hydrogen bonding. Galactose hydroxyl 3 would be the group farthest extended; it is interesting that this is the group that can carry a sulfate residue. A sphingolipid thus internally complexed would be a hydrogen donor only. It would resemble cholesterol in its largely planar structure (because of the rigidification of the chains by *trans*-methylene configurations), and it labile hydrogen would, at low latitudinal angles, extend out of the plane as in cholesterol. The main physicochemical difference of the sphingolipid would be a greater length and a large, rigid hydrophilic head group.

Plasmalogens, i.e. 1-(alk-1-enyl)-2-acyl phosphoglycerides, and ether lipids, i.e. 1-alkyl-2-acyl phosphoglycerides, have only one C=O group, in position 2. Since there cannot be more than one mole of cholesterol/mole of phosphoglyceride in natural membranes, because the membranes would crystallize, there is always an excess of hydrated CO groups in such membranes, probably mostly the less electronegative CO groups in position 1 of phosphoglycerides. In plasmalogens, even this group is cancelled, and the membrane, according to the hydrogen belt hypothesis, must be of minimal permeability. (Incidentally, removal of the electron-withdrawing CO in position 1 will increase the electron density, and, therefore, the hydrogen bond strength, in position 2). Thus, it becomes clear why plasmalogens abound in plasma membranes, but especially in the myelin membrane. The abundance of sphingolipids in these membranes is similarly explained.

MEMBRANE PROTEINS

The membrane has been treated here as a semipermeable lipid bilayer; biological membranes, however, contain proteins, and the permeation of membranes by solutes is thought to be accomplished mostly by these proteins. This might appear to invalidate all studies on pure lipid monolayers, films, or vesicles. However, lipid bilayers constitute almost certainly the continuous matrix of membranes, and our arguments do apply to this matrix. Furthermore, I believe that the membrane proteins themselves participate in the hydrogen belt. The arguments concerning the hydroxyl hydrogen of cholesterol must also apply to those labile hydrogens that are situated on the border between the hydrophobic and the hydrophilic part of membrane proteins. The required latitudinal orientation of the hydrogen must be a frequent possibility. Membrane proteins, then, can be viewed as being girdled by a hydrogen belt consisting of these protons and the C=O groups of phospholipids. Circumstantial evidence is supplied by the myelin sheath of nervous tissue. This membrane, which has a completely locked lipid-lipid hydrogen belt, also seems to be devoid of any intrinsic protein (33). It also has been shown that cholesterol inhibits the penetration of phospholipid monolayers by proteins (9).

The concept of the hydrogen belt illuminates many aspects of membrane permeability, and it is, as far as I have probed, compatible with all known facts. Further confirmation,

short of direct spectroscopic proof, may be expected from permeability studies with phospholipid analogues lacking C=O groups. More extensive studies on the correlation of membrane condensation and permeability may furnish additional evidence. The concept offers new views on membrane phenomena other than permeability. For example, the action of many hormones might involve the disruption of the hydrogen belt bonding of an acceptor protein and thus initiate the conformational changes that are believed to take place in such proteins on stimulation. Antibiotics, and lysing and fusing agents, such as lysolecithin or polylysine, may function by interrupting and disorganizing the hydrogen belts. Monoglycerides may be absorbed in the gut, because they are hydrogen bond donors. Alcohols, i.e. hydrogen bond donors, are required to solubilize lipids from tissues. The concentrations of cholesterol and protein in inner and outer mitochondrial membranes appear to be inversely related (34); this is understandable if both compete for phospholipid C=O groups. Many more such examples can probably be found.

REFERENCES

1. Chapman, D., and G.H. Dodd, in "Structure and Function of Biological Membranes," Edited by L.I. Rothfield, Academic Press, New York, N.Y., 1971, p. 13.
2. Oldfield, E., and D. Chapman, FEBS Lett. 23:285 (1972).
3. Van Deenen, L.L.M., in "Progress in the Chemistry of Fats and Other Lipids," Vol. VIII, Part I, Edited by R.T. Holman, Pergamon Press, Oxford, England, 1965.
4. Lecuyer, H., and D.G. Dervichian, J. Mol. Biol. 45:39 (1969).
5. Demel, R.A., L.L.M. Van Deenen, and B.A. Pethica, Biochim. Biophys. Acta 135:11 (1967).
6. Finkelstein, A., and A. Cass, Nature 216:717 (1967).
7. Van Deenen, L.L.M., J. De Gier, and R.A. Demel, in "Current Trends in the Biochemistry of Lipids," Edited by J. Ganguly and R.M.S. Smellie, Academic Press, London, England, 1972, p. 377.
8. Demel, R.A., K.R. Bruckdorfer, and L.L.M. Van Deenen, Biochim. Biophys. Acta 255:321 (1972).
9. Papahadjopoulos, D., M. Cowden, and H. Kimelberg, Ibid. 330:8 (1973).
10. Hubbel, W.L., and H.M. McConnel, J. Amer. Chem. Soc. 93:314 (1971).
11. Rothman, J.E., and D.M. Engelman, Nature 237:42 (1972).
12. Phillips, M.C., in "Progress in Surface and Membrane Science," Edited by J.F. Danielli, M.D. Rosenberg, and C.A. Cadenhead, Vol. 5., Academic Press, New York, N.Y., 1972.
13. Rottem, S., J. Yashouv, Z. Ne'Eman, and S. Razin, Biochim. Biophys. Acta 323:495 (1973).
14. Sundaralingam, M., Ann. N.Y. Acad. Sci. 195:324 (1972).
15. Pauling, L., "The Nature of the Chemical Bond," Third Edition, Cornell University Press, Ithaca, N.Y. 1960.
16. Vinogradov, S.N., and R.H. Linnell, "Hydrogen Bonding," Van Nostrand Reinhold, New York, N.Y., 1971.
17. Tanford, C., J. Amer. Chem. Soc. 84:4240 (1962).
18. Singer, S.J., in "Structure and Function of Biological Membranes," Edited by L.I. Rothfield, Academic Press, New York, N.Y., 1971, p. 146.
19. Vandenheuvel, A., JAOCS 40:457 (1963).
20. Darke, A., E.G. Finer, A.G. Flook, and M.C. Phillips, FEBS Lett. 18:326 (1971).
21. Verma, S.P., and D.F.H. Wallach, Biochim. Biophys. Acta 330:112 (1973).
22. Singer, S.J., and G.L. Nicolson, Science 175:720 (1972).
23. Zwaal, R.F.A., B. Roelofsen, and C.M. Colley, Biochim. Biophys. Acta 300:159 (1973).
24. Bretscher, M.S., Science 181:622 (1973).
25. Parker, F.S., and K.R. Bhaskar, Biochemistry 7:1286 (1968).
26. Klotz, I.M., and S.B. Farnham, Ibid. 7:3879 (1968).
27. Zull, J.E., S. Greanoff, and H.K. Adam, Ibid. 7:4172 (1968).
28. Rand, R.P., and V. Luzzati, Biophys. J. 8:125 (1968).
29. Bittman, R., and L. Blau, Biochemistry 11:4831 (1972).
30. Demel, R.A., KR. Bruckdorfer, and L.L.M. Van Deenen, Biochim. Biophys. Acta 255:311 (1972).
31. De Kruyff, B., R.A. Demel, A.J. Slotboom, L.L.M. Van Deenen, and A.F. Rosenthal, Ibid. 307:1 (1973).
32. Kwong, C.N., R.E. Heikkila, and D.G. Cornwell, J. Lipid Res. 12:31 (1971).
33. Branton, D., and D.W. Deamer, Protoplasmatologia 11:E1 (1972).
34. Fessenden-Raden, J.M., and E. Racker, in "Structure and Function of Biological Membranes," Edited by L.E. Rothfield, Academic Press, New York, N.Y. 1971, p. 401.

[Received March 4, 1974]