On the Orientation of Lipids in Chloroplast and Cell Membranes

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Abstract

The widespread recognition of the corpuscular nature of membrane ultrastructure demands re-evaluation of established concepts of their molecular organization. Many aspects of membrane physiology, composition, and metabolism provide support for the proposal that most membranes consist of two-dimensional polymers of lipoprotein subunits. Such a model allows the activity, specificity, and adaptability attributed to biological membranes. Evidence which supports this corpuscular model for membranes and some inadequacies of the bimolecular lipid leaflet model are pointed out.

The lamellae of plant chloroplasts are membranes which clearly consist of subunits (quantasomes). Their four surfactant lipids and pigments comprise 50% of the lipoprotein subunits. In each of these surfactant lipids there is found a limited and specific group of fatty ester components. This phenomenon suggests that the hydrocarbon chain of the fatty esters may specifically complement certain hydrophobic amino acid sequences in the membrane protein. The protein, then, would determine the sites where the lipid will be most firmly bound. It is proposed that the lipids of membrane subunits are bound by hydrophobic association of the hydrocarbon chains of the lipids with complementary hydrophobic regions within the interior of the protein. The resulting two-dimensional lipoprotein aggregate would possess the strongly anionic charged groups of the phospholipids on its surface. Metabolically-driven alterations in conformation of such a flexible lipoprotein ion exchange membrane allows a consistent interpretation of biological membrane transport phenomena.

Introduction

FUNCTION OF THE LIVING CELL requires remarkable specificity, efficiency and adaptability of interfaces known as membranes. The lipid-protein character of cell membrane has long been recognized. The structural relationships of the lipid molecules and their protein matrix have not been open to direct observation. As a result it has not been possible to interpret physiological function of membranes in terms of molecular structure.

An assembly of circumstantial evidence for the hydrophobic association of lipids and protein in chloroplast (1,2) and mitochondrial membranes (3) engenders the suggestion that most, if not all, membranes may be two-dimensional aggregates of lipoprotein subunits. This proposal is at variance with the classical bimolecular lipid membrane concept reviewed by Brady and Trams (4), but possesses several common attributes. Some of the limitations of the lipid bilayer model for membrane structure are described in the following paragraphs.

The Lipid Bilayer Model

The lipid bilayer model for the structure of membranes of cells was suggested by Davson and Danielli (5) on the basis of observed lamellar micellar aggregation (6) of natural surfactant lipids. Dramatic observations of lipid lamellar aggregation in myelin figures have been made by Stoeckenius (7,8). Lipid bilayer membrane stability and physical properties have been studied extensively (9,10). In order that these structures be involved in performance of membrane functions a very special matrix of superficial protein would be required (11). Its charge distribution must be roughly complementary to that of the adjacent anionic lipid layer and its reaction to molecular stimuli (ATP, cations, substrate approach, etc.) must result in specific alterations of the lipid layer such as production of holes, channels of appropriate charge gradients, and variations of the hydrophobic barrier. Such proteins would be essentially cationic in order to control lipid orientation most directly. The specific associations of cytochrome *c* with phospholipids provides strong evidence for this type of interaction (12) with basic proteins. Membrane proteins, however, are not basic proteins, and if anything, contain major amounts of the acidic amino acids (13–15).

Lipid monolayer studies have shown that branching, unsaturation, and short chains reduce film stability (16). Myelin is an example of a very stable natural lipid bilayer membrane structure. O'Brien (17) observed that when the Schwann cell is incapable of elongation of the C_{18} fatty acids, the myelin it produces is poorly organized. C_{16} and C_{18} acids are probably characteristic of the Schwann cell plasmalemma or cell membrane and therefore differ from the saturated C_{24} chains of lipids in normal myelin. Even though the "cell membrane" of the Schwann cell and its myelin layers are contiguous, one cannot conclude that they are identical. There must be a gradual transition from cell membrane lipoprotein to the lipid bilayer myelin as the lipid components of myelin are synthesized in the cell. The active functional membranes of most cells contain large amounts of unsaturated or branched chain acids. These are not the types which one would expect to find in stable lipid bilayer membranes.

The fatty acid components of lipids of the bilayer membrane form a relatively liquid phase like those in a soap micelle or crystal. The nature of the hydrocarbon chain of each class of complex lipid, therefore, could have little specific interaction with other lipids or with the adjacent protein. It appears that the lipid bilayer model could demand no specificity in the nature of the hydrocarbon chains of the lipids. In natural cell membranes, as we shall see, this is not the case, and hydrocarbon chains of each type of lipid are rather specifically selected.

Electron microscopy supported the lipid bilayer membrane model as long as stained sections were the primary subjects for examination. The "three layer"

pattern of two adjacent dark lines bordering an unstained central line has come to be recognized as "the unit membrane" (18,19). It is the result of osmium or manganese accumulation at the interface between the charged groups of the phospholipids and the adjacent proteins. Stoeckenius (8) has reviewed the evidence in support of this deduction. Coordination complexes of the heavy atom with lipid phosphate ionic groups and protein nitrogen sp^3 orbital ligands may well account for the observed strict localization of bound metal in the stained membrane. The distance between these stained regions in a lipid bilayer membrane being a liquid phase, would be much less than twice the length of a C_{18} fatty acid or 30–35Å. The spacing in phospholipid myelin figures has been established by Stoeckenius (8) as 38 to 40Å for the lipid bilayer repeating unit. The observed separation is not this but up to 140Å in some cell membranes. Such a disparity could hardly result from a mistaken presumption of location of the metal in the stained membrane. It seems reasonable to suspect that some membranes may have structures different from that of myelin.

The Corpuscular or Lipoprotein Subunit Membrane Model

Appearance of Membrane Subunits

Shadowed surfaces of many cell membranes reveal a mosaic-like array of repeating units (20). In *Halobacterium halobium* (21,22) these units are 140Å in diameter (Fig. 1 and approximately equal to the thickness of the cell membrane as estimated from electron micrographs of osmium-stained sections. In *Mycoplasma laidlawii* the membrane structures have been estimated to be 80Å in thickness by Razin et al. (23). In all cases the sections of these obviously corpuscular membranes stain with osmic acid to give the typical "unit membrane" pattern of two parallel black lines. Chloroplast lamellae also possess subunits. The freeze-etch electron microscopic technique of Mühlethaler (24) gives the most striking picture of membrane surfaces. This method is based upon the fracture of frozen cells along membrane surfaces. Water is sublimed from the freshly cleaved surface to reveal the lipoprotein subunits in bold relief. These are shadowed by metal evaporation and the replica is reproduced by the electron microscope. The image suggests that chloroplast membrane surfaces are a mosaic of identical subunits. Many intracellular membranes appear to possess structure suggesting that

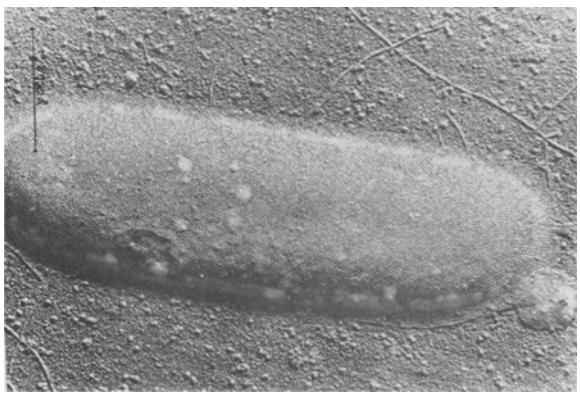

FIG. 1. External appearance of *Halobacterium halobium* showing 130 Å diameter membrane subunits. Bacterial surface was shadowed with carbon and the replica magnified 42,500×. (Reproduced by courtesy of A. L. Houwink, Mohr and B. J. Spit.)

FIG. 2. Electron micrograph of OsO_4-stained section of bean leaf showing chloroplast with lamellae and two mitochondria. Cell wall at top of figure surrounds darkly-stained cell membrane (actually a double black line). Chloroplast and central vacuole, white area, lower left, are surrounded by their own membranes. Reproduced by courtesy of T. E. Weier.

they are formed by aggregation of identical subunits. By entirely independent analytical method, low-angle X-ray scattering analysis, Kreutz (25) revealed a periodicity of electron density in the plane of chloroplast lamellae. These 38Å repeating units are, surprisingly, revealed in electron micrographs of stained sections as shown in Figures 2 and 3 (2). Whether these represent discrete lipoprotein subunits or not is debatable but highly suggestive. On such bases, Sjöstrand has proposed a globular structure for the plasmalemma (26).

Lipid-Protein Association

Both natural surfactant lipids and proteins may be considered as being aggregated by hydrophobic interactions [1] (27). These result from the very large net entropy increase as the hydrophobic side chains of the polypeptide or the hydrocarbon chains of the surfactant lipids are transferred from the water phase where of necessity they direct orientation of surrounding water molecules. Kauzmann (28) pointed out that as a hydrophobic structure is removed from the water phase, the organization of the surrounding water is diminished with a concomitant increase of entropy. Aggregation of phospholipids and proteins involve related equilibria of vertical equilibria (Fig. 4) and are driven by the very large $T\Delta S$ factor involved. It was clear from the myoglobin structure

[1] The term "hydrophobic association" is preferred to the widely used "hydrophobic bonding" since no covalent or hydrogen bonding is implied.

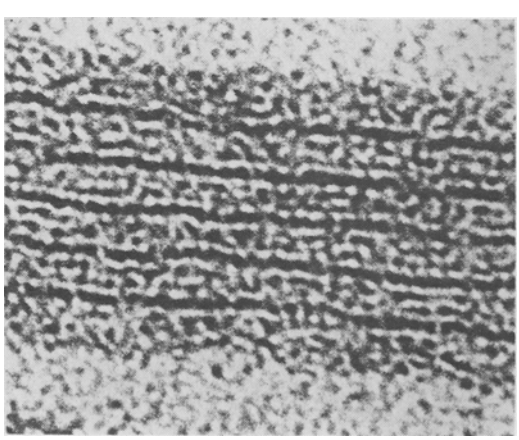

FIG. 3. Enlarged section of electron micrograph of stained chloroplast lamellae showing repeating lipoprotein subunits, white-centered regions. Reproduced courtesy of T. E. Weier.

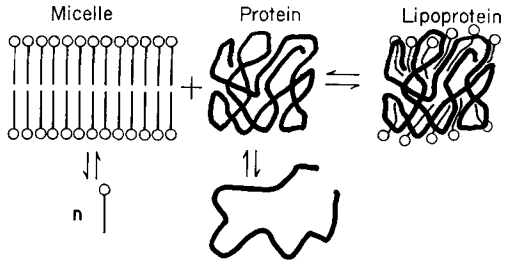

FIG. 4. Equilibria involved in hydrophobic association of surfactant lipids and membrane protein. Vertical equilibria strongly favor aggregation of lipid into micellar structure and hydrated protein into globular protein. Horizontal equilibrium describes association of lipid with hydrophobic interior of membrane lipoprotein subunit (presented at Symposium on Chemical and Biological Functions of Cell Membranes, 150th National Meeting of ACS, Sept. 9, 1965 by A. A. Benson and S. J. Singer).

(29) that the interior of this protein is tightly packed and largely hydrophobic while the hydrophilic and charged amino acids predominate on the molecule's exterior.

It is not hard to extrapolate these relationships to suggest that membrane proteins possess amino acid sequences such that they can maintain a hydrophobic interior when certain surfactant lipids are available for hydrophobic association. This relationship is suggested by the equilibrium expressed horizontally in Figure 4. The resultant lipoprotein represents our conception of a simplified membrane subunit. Successive aggregation of such lipoprotein subunits by hydrophobic interaction of adjacent molecular surfaces was pointed out by Green and collaborators (3,30,31) and occurs in many proteins and in biological membranes. Reconstitution of membranes from subunits derived from membranes of *Mycoplasma laidlawii* has been accomplished recently by Razin et al. (23).

Such a model suggests that the genetically-controlled amino acid sequence of membrane proteins may permit rather specific hydrophobic association with lipid chains. The protein sequence, then, would determine the lipids most stably bound and thereby the distribution of charged and hydrophilic moieties on the membrane surface. As we know, the membrane surfaces possess active sites for ion binding, enzymatic function, substrate binding and transfer and even for lipid haptene binding (4). In order that such a model exhibit specificity, one might anticipate that the various complex lipids of a given membrane possess a limited group of fatty acid or hydrocarbon chains. That this is actually the case has become strikingly clear.

Hydrocarbon Chain Specificity in Membrane Lipids

Chloroplast lamellae (32,33) are made up of four major surfactant lipids. They serve as important reservoirs of carbohydrate and partially reduced sulfur in photosynthetic metabolism and appear to be metabolized without simultaneous removal of their diglycerides from the membrane (34). The galactolipids are, in most plants, galactosyl dilinolenins

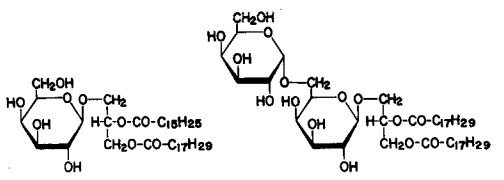

FIG. 5. The galactolipids of chloroplasts.

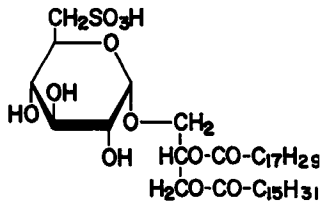

FIG. 6. The plant sulfolipid (positions of the linolenic and saturated acid are not yet established).

where up to 96% of the fatty acids are α-linolenic residues. Allen (35) has reported that the mono- and digalactolipids differ in that the monogalactolipid may have up to 25% of 16:3 acid (Fig. 5). Even though digalactosyl diglyceride appeared to be formed by galactosylation of the monogalactolipid (34) their diglyceride components differ markedly. The sulfolipid, 6-sulfo-6-deoxy-α-D-glucopyranosyl diglyceride, contained equal amounts of saturated (16:0) and unsaturated (18:3) lipid (36) as shown in Fig. 6. The only major phosphatide, phosphatidyl glycerol, in chloroplasts possesses up to 35% of Δ^3tr 16:1 acid (Fig. 7). Haverkate and van Deenen (37,38) observed that this acid occurs in no other lipid components of these membranes. The other major surfactant substance in chloroplasts is chlorophyll. Its C_{20} phytol chain is a further variation in hydrophobic structure. The phytylether phosphatides of halophilic bacteria (39) have selected an identical hydrophobic structure. The four methyl side chains of phytol are arranged with spacing like that in a polyalanine segment of a protein. It is not unreasonable to presume a close physical association of substances with such similar structure. Coenzymes Q possess polyisoprenoid chains characteristic of their sources. That from green plants, plastoquinone, possesses a $C_{45}H_{73}$ polyisoprenoid chain.

The widespread specificity of fatty acids components in membrane lipids has become increasingly apparent. Fleischer et al. (40) has noted specific fatty acid components associated with mitochondrial lipoprotein enzymes. The phospholipids of mammalian tissues possess diglycerides with remarkably specific fatty acid components (41). The work of Lands (42,43) in demonstrating the fatty acid specificity of reacylation of lysophosphatides has defined a mechanism whereby the unique fatty acid composition of phosphatides may be derived. The specificity in fatty acid components of reactions such as blood clotting or galactolipid cleavage, for example, is well recognized. To recognize this specificity it is necessary, of course, that the lipids be derived from homogeneous preparations of membranes from cell organelles. As fractionation procedures for membrane subunits become more sophisticated and selective, analyses of the lipid components may become more meaningful and the specificity for the nature of their hydrocarbon chains more exactly defined.

Location of the hydrophilic and ionic moieties of the surfactant lipids in membrane lipoproteins is

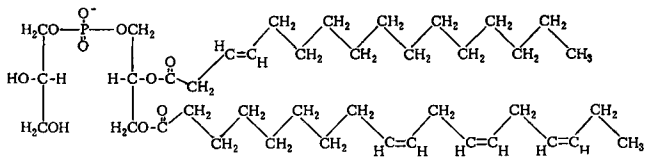

FIG. 7. Phosphatidyl glycerol. The structure shown comprises 25% of the phosphatidyl glycerol molecules in chloroplasts (36).

suggested by much independent but as yet circumstantial evidence. Rapport et al. (44,4) has shown that the lactosyl group of Cytolipin H is the haptene (N-lignoceryl-1-0-lactosyl-sphingosine) to which antibodies to its lipoprotein are specific. Therefore, the specific hydrocarbon chains of the ceramide may be expected to be buried within the lipoprotein interior. In another approach, Heemskerk and van Deenen (45) showed that erythrocyte membranes are not subject to exogenous phospholipase A, an observation consistent with strong association between diglyceride and protein.

Chloroplast Lamellar Membranes

In all photosynthetic plants, the chlorophylls are associated with a lamellar organization of lipoprotein capable of the electron transport and primary enzymatic activities involved in photosynthesis. The gross composition of these membranes is about 50% protein and 50% lipids (dry wt basis) (46,32,33). The information relating to molecular composition and ultrastructure has engendered speculation upon the molecular organization of lamellar components. Weier and Benson (2) have depicted the arrangement of surfactant lipids, pigments and protein shown in Figure 9.

The work of Irwin and Bloch (46) revealed the almost universal direct relationship between α-linolenic acid content and oxygen producing capability in chloroplasts. Although exceptions occur, the galactosyl dilinolenins are essential lamellar components and seem to occur nowhere else. The alteration in lipoprotein conformation which occurs when chloroplasts suffer degradation by galactolipase effectively uncouples their internal electron transport system (47). α-Linolenic acid possesses three isolated six-atom planar olefinic systems near the outer end of the C_{18} chain, as shown in Figure 7. These may associate with π orbitals of the aromatic systems of the membrane protein and further strengthen the hydrophobic affinity of the unsaturated chains for certain internal regions of the protein. Further, the three *cis* olefinic bonds can easily assume a helical orientation conducive to close association with a single protein chain.

Reconstitution of Membrane Lipoproteins

Fleischer et al. (48,49) succeeded in activating mitochondrial electron transport by addition of lecithin. It is generally accepted that many lipoprotein enzymes require lipid for their enzymatic activity. To restore biological activity of the delipidized protein by addition of lipid requires reorganization not possible with crude techniques. Shibuya and Maruo (50) succeeded in restoring much of the electron transport activity of delipidized chloroplast lipoprotein by an ingenious method. The dispersed lipid reassociated with an aqueous suspension of the dispersed protein when the mixture was frozen at −31C for five days. As may be inferred from the equilibria of Figure 4, reduction of water activity by freezing will allow partial relaxation of the forces driving the protein into its normal clenched globular conformation. The lipids may then diffuse into the proper interstices and restore the essential conformation of pigment and electron transport components. Considerable time was required because of the low diffusion rates in the solid phase. When lipoprotein membranes are formed in the developing cell, simultaneous synthesis of both lipid and protein may facilitate aggregation in a specific structural conformation.

TABLE I

Fatty Acid Composition of Chloroplast Lamellar [a] Lipids (C. F. Allen, 36)

Complex lipid	Fatty acid composition
Galactosyl diglyceride	25% 16:3, 72% α-18:3
Digalactosyl diglyceride	87% α-18:3, 5% 16:3
Phosphatidyl glycerol [b]	32% Δ^3tr-16:1, 47% α-18:3, 11% 16:0
Sulfoquinovosyl diglyceride [c]	39% 16:0, 53% α-18:3, 7% 18:2
Chlorophyll *a*	100% phytol ester

[a] Chloroplast lamellae contain most of the lipids and pigments of isolated chloroplasts and the major fraction of the lipids of most green leaves.

[b] Fifty percent of spinach leaf phosphatidyl glycerol is 1-phosphatidyl-2-Δ^3tr-hexadecanoyl-3-α-linolenyl-L-glycerol (Haverkate, 37).

[c] The position of the saturated, palmitic acid and of the linolenic acid is not yet established. By anology with the other diglycerides, however, the unsaturated linolenate ester is in the α-(3-) position of the glycerol.

Function of Lipoprotein Membrane

Contractile Phenomena

Structural alterations in chloroplast, mitochondrial and other membranes occur as a result of metabolic activity. These swelling and contractile phenomena (51–57) are the result of changes in membrane lipoprotein in conformation resulting from oxidative phosphorylation, electron transport, substrate binding, etc. These gross and readily observable structural changes certainly reflect even more dramatic contortions of the polypeptide chains involved in membrane metabolic processes.

Ion Transport

The presence of contractile proteins in the red cell membranes (58) and the observed enhancement of the ATPase activity of these proteins in the presence of phospholipids, Na^+, and K^+ led Onishi and Kawamura (56) to suggest that "a complex of contractile proteins in combination with phospholipids play an important role in the active transport phenomena." The model represented by Figure 8 may be envisaged as a section of a flexible two-dimensional ion exchange membrane. Its charged groups of the associated phospholipids and sulfolipids may bind cations with specificities determined by the proximity of the surrounding peptide chains. Whether the system could discriminate between sodium and potassium on the basis of the 50% difference in K^+ and Na^+ hydrated diameters is equivocal but plausible. Alteration of peptide configuration around the phosphorylcholine moiety of lecithin could well be a major mechanism

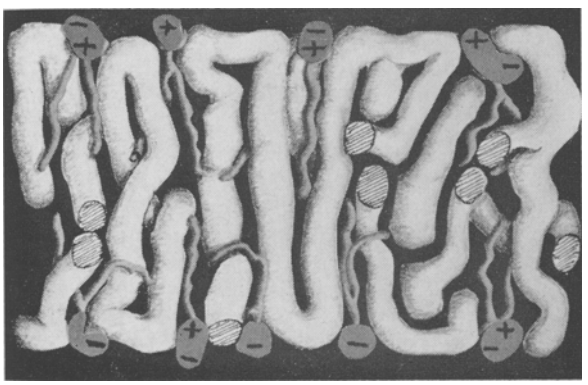

FIG. 8. Proposed model for association of membrane lipids and protein. Charged and hydrophilic exterior is capable of binding osmium and producing black line of electron micrographs. Central hydrophobic region is impervious to stain and gives white central line of electron micrographic image of membrane.

for control of anion binding or of binding and release of cations.

Ion transport is known to be accomplished in groups of up to three potassium ions as a result of hydrolysis of one molecule of ATP by membrane-bound ATPase. The classical "rotating tumbler" mechanism for ion transport could well function by inversion of an ion-binding lipoprotein subunit. A more plausible mechanism seems to involve an involution ("transviceration") of a segment of the lipoprotein, such as depicted in Figure 8, which could be driven by an energy-requiring alteration of some aspect of the protein structure which would render the new conformation with the concomitant ion transport, most stable. Whether the "energy input" might involve synthesis and insertion of a new phospholipid component of the lipoprotein carrier is not certain. Nikaido (59) has presented good evidence that galactoside transport, for instance, occurs with a simultaneous stimulation of phospholipid turnover as evidenced by enhanced ^{32}P incorporation into membrane phospholipids (60).

Sugar Transport

The blocking of ion and hexose transport in yeast (61) and in intestinal epithelium by uranyl ion UO_2^{++}, which has a strong affinity for coordination with phosphate groups is consistent with this model for active transport. In a system where the phosphate ionic groups of the associated phosphatides might be complexed by a large divalent cation like uranyl ion, the conformation of the lipoprotein hexose carrier could be effectively locked in place.

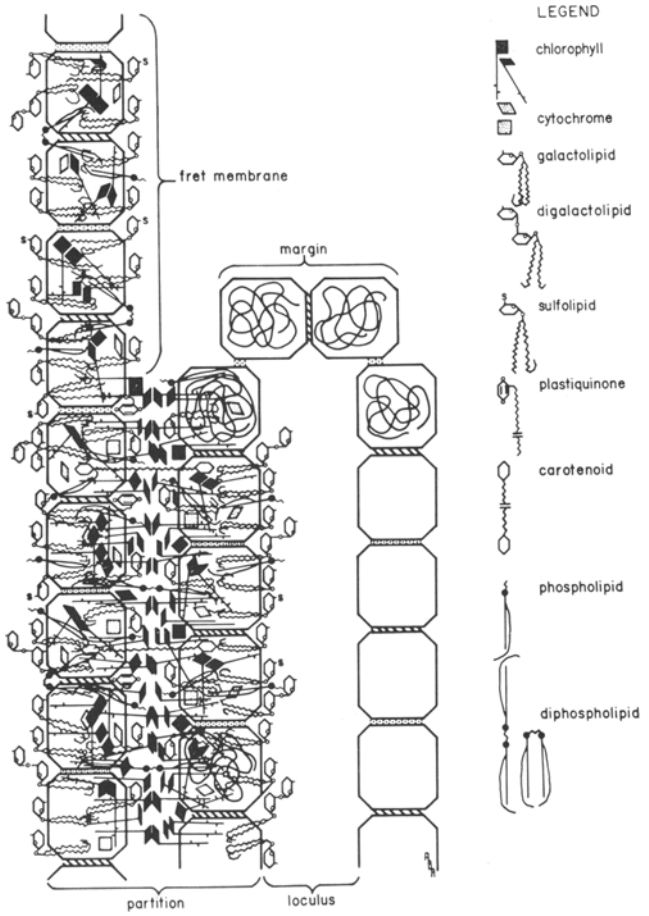

FIG. 9. Arrangement of lipoprotein subunits in lamellae of chloroplasts (2).

The recent isolation of the galactoside permease "carrier protein" of *Escherichia coli* as a small membrane-bound lipoprotein component by Fox and Kennedy (63) added strong support for the suggestion that active transport involves substrate-specific binding sites in a membrane. Such a site with its bound substrate could, as well, be transferred to the opposite side of the membrane by appropriate protein conformation alteration. The Na^+-dependence of active transport of sugars in the small intestine is well documented by the work of Crane (64). The "mobile carrier" system (65) is supported by the evidence that Na^+ interacts directly with the membrane and its carrier-bound sugar. The lipoprotein membrane model is consistent with this observation.

We are thus presenting a model for the cell membrane quite the opposite of the bimolecular lipid leaflet structure of Davson and Danielli (5). According to this model, globular lipoprotein subunits associate to form a two-dimensional membrane with a hydrophobic interior. The stability and effective function of such a membrane requires the association with specific surfactant lipids in order to attain proper protein conformation. The resulting membrane gives the same type of stained section electron micrographs as does a lipid bilayer and, further, shows the mosaic relief characteristic of a lipoprotein aggregate. The structural relationships of such a model are consistent with contemporary concepts of protein structure and membrane biosynthesis and function.

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REFERENCES

1. Benson, A. A., Ann. Rev. Plant Physiol. *15*, 1–16 (1964).
2. Weier, T. E., and A. A. Benson, in "Biochemistry of Chloroplasts," ed. T. W. Goodwin, Academic Press, Inc., London, 1966, in press.
3. Green, D. E., and O. Hechter, Proc. Nat. Acad. Sci. *53*, 318–325 (1965).
4. Brady, R. O., and E. G. Trams, Ann. Rev. Biochem. *33*, 75–100 (1964).
5. Davson, H. A., and J. F. Danielli, J. Cell. Comp. Physiol. *5*, 495–508 (1935).
6. Danielli, J. F., and E. N. Harvey, J. Cell. Comp. Physiol. *5*, 483–494 (1935).
7. Stoeckenius, W., J. Biophys. Biochem. Cytol. *5*, 491–500 (1959).
8. Stoeckenius, W., The Molecular Structure of Lipid-Water Systems and Cell Membrane Models Studies with the Electron Microscope, in "The Interpretation of Ultrastructure," ed. R. J. C. Harris, Vol. 1, Symposium Int. Soc. Cell Biol., Academic Press, Inc., New York, 1962, p. 349.
9. Mueller, P., D. O. Rudin, H. T. Tien and W. G. Wescott, in "Recent Progress in Surface Science," ed. J. F. Danielli, K. G. A. Parkhurst and A. C. Riddiford, Academic Press, New York, 1964, p. 379.
10. Thompson, T. E., in "Symposium Society Study Developmental Growth," Vol. 22, ed. M. Locke, Academic Press, Inc., New York, 1964, p. 83.
11. Kavenau, J. L., "Structure and Function of Biological Membranes," Vols. I and II, Holden-Day Press, San Francisco, 1965.
12. Das, M. L., E. D. Haak and F. L. Crane, Biochemistry *4*, 859 (1965).
13. Weber, P., Zeit. Naturforsch. *18b*, 1105–1110 (1963).
14. Menke, W., in "Biochemistry of Chloroplasts," ed. T. W. Goodwin, Academic Press, Inc., London, 1966, in press.
15. Criddle, R. S., R. M. Bock, D. E. Green and H. Tisdale, Biochemistry, *1*, 827–842 (1962).
16. Salem, L., Nature *133*, 476–477 (1962).
17. O'Brien, J. S., Science *147*, 1099–1107 (1965).
18. Robertson, J. D. Biochemical Soc. Symposia, No. 16, ed. E. M. Crook, Cambridge University Press, 1954, p. 1.
19. Robertson, J. D., Sci. Am. *206*, 64–72 (1962).
20. Benedetti, E. L., and P. Emmelot, J. Cell Biol. *26*, 299–305 (1965).
21. Larsen, H., in "The Bacteria," Vol. 4, Academic Press, Inc., New York, 1963, p. 297.
22. Kushner, D. J., S. T. Bayler, J. Boring, M. Kates and N. E. Gibbons, Can. J. Microbiol. *10*, 483–497 (1964).
23. Razin, S., H. J. Morowitz and T. M. Terry, Proc. Nat. Acad. Sci. *54*, 219–225 (1965).
24. Mühlethaler, K., in "Biochemistry of Chloroplasts," ed. T. W. Goodwin, Academic Press, Inc., London, 1966, in press.
25. Kreutz, W., Zeit. Naturforsch. *19b*, 441–446 (1964).
26. Sjöstrand, F. S., Nature *199*, 1262–1264 (1963).
27. Singer, S. J., Adv. Protein Chem. *17*, 1–68 (1962).
28. Kauzmann, W., Adv. Protein Chem. *14*, 1–63 (1959).
29. Kendrew, J. C., in "Nobel Lectures, Chemistry," Nobel Foundation, Elsevier Publ. Co., Amsterdam, 1964, p. 676.

30. Richardson, S. H., H. O. Hultin and D. E. Green, Proc. Nat. Acad. Sci. *50*, 821–827 (1963).
31. Criddle, R., in "Biochemitsry of Chloroplasts," ed. T. W. Goodwin, Academic Press, Inc., London, 1966, in press.
32. Park, R. B., and N. G. Pon, J. Molec. Biol. *3*, 1–10 (1961).
33. Lichtenthaler, H., and R. B. Park, Nature *198*, 1070–1072 (1963).
34. Ferrari, R. A., and A. A. Benson, Arch. Biochem. Biophys. *93*, 185–192 (1961).
35. Allen, C. F., JAOCS *43*, 233–331 (1966).
36. O'Brien, J. S., and A. A. Benson, J. Lipid Res. *5*, 432–436 (1964).
37. Haverkate, F., Dissertation, University Utrecht, 1965.
38. Haverkate, F., and L. L. M. van Deenen, Biochim. Biophys. Acta, *106*, 78–92 (1965).
39. Kates, M., L. S. Yengoyan and P. S. Sastry, Biochim. Biophys. Acta *98*, 252–268 (1965).
40. Fleischer, S., H. Klouwen and G. Brierly, J. Biol. Chem. *236*, 2936–2941 (1961).
41. Mangold, H., JAOCS *41*, 762–773 (1964).
42. Lands, W. E. M., JAOCS, this symposium (1965).
43. Lands, W. E. M., and P. Hart, J. Biol. Chem. *240*, 1905–1911 (1965).
44. Rapport, M. M., L. Graf and N. F. Alonzo, J. Lipid Res. *1*, 301–304 (1960).
45. Heemskerk, C. H. T., and L. L. M. van Deenen, Koninkl. Ned. Akad. Wetenschap. Proc. *67(B)*, 181–191 (1964).
46. Irwin, J., and K. Bloch, Biochem. Z. *338*, 496–511 (1963).
47. McCarty, R. E., and A. T. Jagendorf, Plant Physiol. *40*, 725–735 (1965).
48. Fleischer, S., G. Brierley, H. Klouwen and D. B. Slautterback, J. Biol. Chem. *237*, 3264–3272 (1962).
49. Fleischer, S., Sixth International Congress of Biochemistry, Abstracts p. 604–605 (1964).
50. Shibuya, I., and B. Maruo, personal communication and abstracts.
51. Lehninger, A. L., "The Mitochondrion," W. A. Benjamin, Inc., New York, 1964, Ch. 9.
52. Izawa, S., M. Itoh and K. Shibata, Biochim. Biophys. Acta *75*, 349–354 (1963).
53. Packer, L., P. A. Siegenthaler and P. S. Nobel, Biochem. Biophys. Res. Comm. *18*, 474 (1965).
54. Dilley, R. A., and L. P. Vernon, Biochem. Biophys. Res. Comm. *15*, 473–478 (1964).
55. Zurzycki, J., Protoplasma *58*, 458–482 (1964).
56. Ohnishi, T., and H. Kawamura, J. Phys. Soc. Japan *18*, 1559–1560 (1963).
57. Ohnishi, T., Seibutsubatsuri *4*, 18–31 (1964).
58. Ohnishi, T., J. Biochem. *52*, 307–308 (1962).
59. Nikaido, H., Biochem. Biophys. Res. Comm. *9*, 486–492 (1962).
60. Tarlov, A. R., and E. P. Kennedy, J. Biol. Chem. *240*, 49–53 (1965).
61. Rothstein, A., Circulation *26*, 1189–1200 (1962).
62. Newey, H., P. A. Sanford and D. H. Smyth, Nature *205*, 389–390 (1965).
63. Fox, C. F., and E. P. Kennedy, Proc. Nat. Acad. Sci. *54*, 891–899 (1965).
64. Crane, R. K., Biochem. Biophys. Res. Comm. *17*, 481–485 (1964).
65. Wilbrandt, W., and T. Rosenberg, Pharmacol. Rev. *13*, 109–183 (1961).