Lipid-Protein Interactions and Cohesional Forces in the Lipoproteins Systems of Membranes[1]

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Abstract

The lipid-protein positional relationship described by the simple Danielli model leads to a concept of the basic structure which is consistent with recent findings. The stability of membranes is insured by internal cohesion forces and by the potential barrier of surrounding water molecules. The properties of cohesional forces are reviewed with reference to membrane stability and order. The existence of a higher degree of organization is discussed in the light of recent electron microscopic data. A "particulate" concept which is compatible with the simple model is discussed with regard to transport phenomena and component turnover in membranes.

The Basic Structure of Membranes

THE UBIQUITOUS OCCURRENCE of biological membranes in the cells of all known organisms, and their involvement in a multitude of patterns adapted to so many functions imply a considerable latitude of their basic design in its acceptance of qualitative and quantitative modifications in the supply of structurally essential component molecules. For indeed, this basic design has survived many mutation-induced changes in this supply along with fluctuations occurring during the life-cycle of all species, and to a large extent, those occurring in disease. This remarkable adaptability is evidenced by the wide variety of constituent molecules found in membranes. Among these, the ever present polar lipids and protein appear to be essential components of the basic structure. While it may be safe to consider the protein as a genetically determined, species-representative component, it is not possible to attribute the same character to the lipids without important reservations. Indeed, very significant alterations in chain length and unsaturation occur under mere dietary influences in the rabbit (1,2) and human (3) erythrocyte membrane lipids, and those of mitochondrial membranes of sea mammals and birds (4). While similar events probably arise in many other membranes, the present data on this phenomenon clearly indicates that a structural specificity of lipid chains is not an essential feature of the basic design. On the other hand, since no significant changes in species-distribution of lipid classes have been reported, it is still possible to assume that a correspondence of the genetically determined protein with this distribution exists and consequently, that a direct structural relationship between lipids and protein involves the polar moiety of lipids.

It should be evident that the Danielli model (5), Figure 1, which for over 25 years remained the uncomplicated expression of our views on the structure of biological membranes, does represent a lipid-protein positional relationship which is consistent with the above conclusion. It should be evident also that

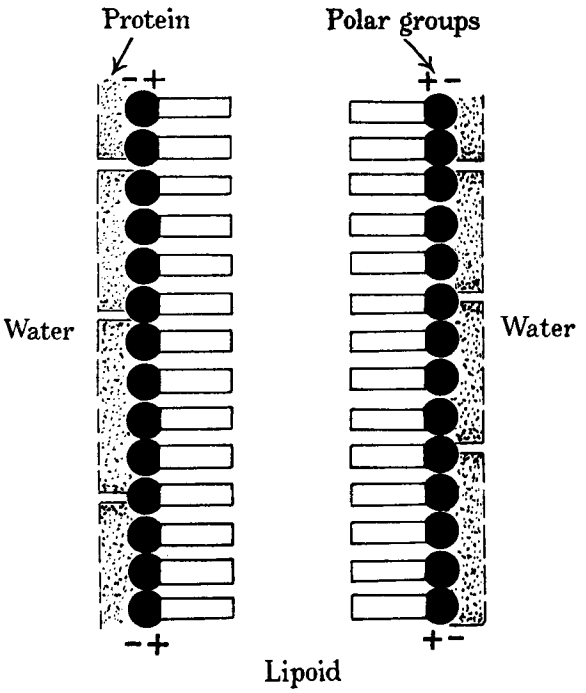

FIG. 1. Danielli Model, 1935 (5).

this model was intended to represent a general, undetailed concept of this relationship, and that the value of the concept itself is in no way decreased by the fact that the model does not describe a complete membrane. Indeed, a slightly more recent version (6), Figure 2, shows Davson and Danielli well aware of the fact that a complete description should account for superstructural components conferring specific functional properties (7). The remarkable adaptability of the basic design strongly suggests that the involvement of superstructural components with the primary layer of structural protein might be superficial only, these components being removed and replaced without important alteration of the membrane basic structure. Such arrangement would indeed explain, among others, the relationship between rough and smooth endoplasmic membranes. It would also permit the same basic membrane elements to fulfill several functions within the cell. If this is true, much of the protein assigned to specific functions of membranes, such as enzymes, may have no participation in the basic structure. Hence one could be completely misled by considering the protein to lipid ratio as an index of basic structure differentiation (7). Under this scheme, Robertson's "unit membrane" (8) would correspond to a basic unit preferably called "lipoprotein barrier" (7) to avoid any confusion with the complete membrane. This unit is not expected to be compositionally the same for all membranes or even for all membranes within the same cell. Aside from genetically-directed differentiation of the protein and lipids, it is all too evident that at least the chain composition can be affected by differences in

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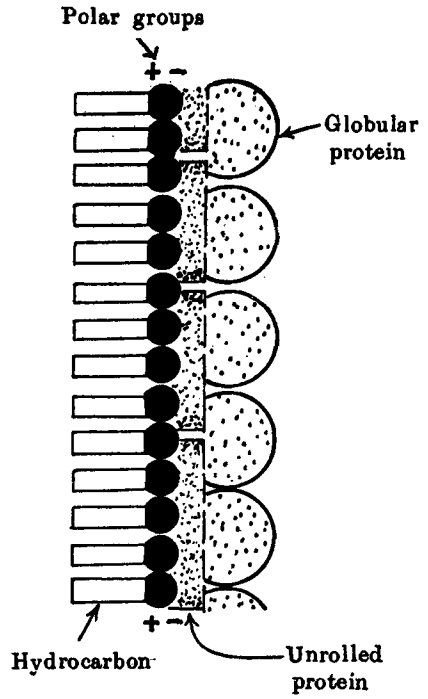


FIG. 2. Arrangement of superstructural components, Danielli, 1938. Cf Ref. 6.

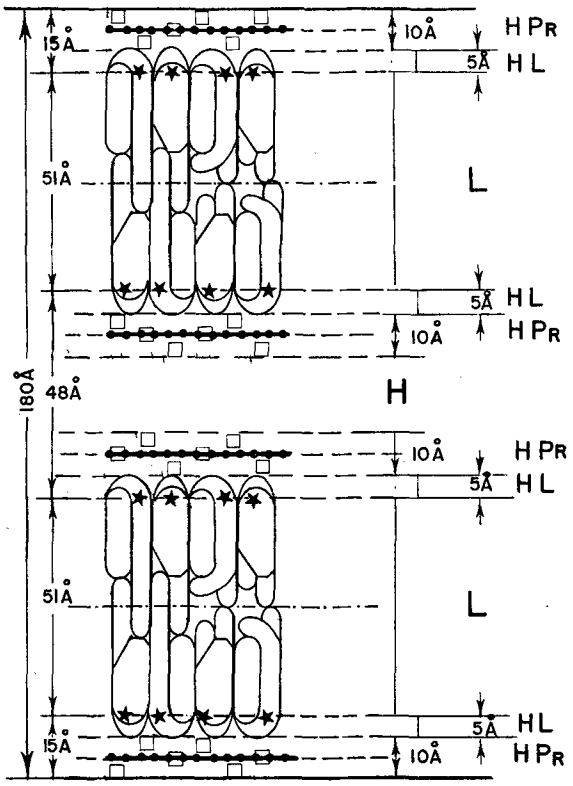
the supply of lipids, such as could exist in different cellular compartments. If in addition, the "lipoprotein particle" adaptation of the basic design proposed by the author (7) and further developed in the present article is operative, then a population of lipoprotein particles differing both in protein and lipid components would represent the "lipoprotein barrier" material within a cell. Cellular membranes would differ partly according to what section of this population is included in their "lipoprotein barrier."

Adding to the versatility inherent to this basic structure, the bimolecular lipid leaflet which it features is expected to possess, at least potentially, the remarkable adaptability to changes in shapes and forms observed with the related lipid bilayer in micellar systems.

Although the sum of data accumulated over 2 decades in support of the Danielli model is considerable, a more detailed picture could only emerge recently, thanks to improvements in sample preparation, analytical methods, X-ray and electron microscopic techniques discussed elsewhere (7,9,10). Much of the new and convincing data was obtained from studies on myelin. Derived from the multilayering of the glial cell membrane, this structure constitutes a bulky, easily isolated material. Because it is amenable to polarized light and X-ray diffraction studies in the fresh, unfixed state, artifactual effects on the structure could be eluded. The key structural parameters that were obtained, unequivocally showed the arrangement of lipids and protein to be that of the Danielli model (11). My own studies of myelin, by combining these and many other data, successively provided a detailed, tridimensional arrangement of lipids (9) and protein (10) based on exact molecular dimensions. The complete model (Fig. 3) which accounts for a large number of unrelated observations on the properties of myelin (10) adds considerable strength to the findings of the X-ray studies. On the other hand, the direct connection of the myelin layer with the glial cell membrane (8,12) together with the absence of obvious morphological discontinuity between the two structures, suggests that the arrangement of lipids and protein is probably the same in both. There is no evidence authorizing the view (13) that a complete change of configuration occurs in the course of myelinization, even in the likely event, as yet unproved, that some constituents are eliminated in the process (7). It has been suggested that myelin is derived from the "lipoprotein barrier" material of the glial cell membrane (7), and that this structure may therefore express the basic design of all membranes in the typical arrangement of a bimolecular lipid leaflet sandwiched between thin layers of hydrated structural protein. A complete membrane would include additional superstructural elements.

Among other arrangements which have been proposed for the basic structural relationship in membranes, that described by Benson (13) places the hydrocarbon chains of lipids within the frame of globular protein molecules, and the polar groups of lipids and protein outside. Molecular models clearly show that many lipid chains will not fit in this fashion unless the convolutions of the protein chain corresponded to a very open structure. With only 15% lipid by weight, and assuming that lipid chains occupy all spaces in which they can fit, the structure still offers many channels open to smaller molecules. Yet the absence of oxidation of polyunsaturated chains in membranes, and the lack of osmiophilic reaction in the region which they occupy suggest the existence of a tight system.

Attempts to reconcile this open structure with known properties of membranes are not facilitated by Benson's suggestion that this arrangement could



★ P, IN PHOSPHOL. ⊷ AMIDE GROUPS. □ AMINO AC. RES.

FIG. 3. Radial section through unit layer of myelin of rat sciatic nerve showing arrangement of cholesterol-lipid complexes L, hydrated protein layer HPr, and hydrated, polar layer of the lipids HL. Intraperiod region H is occupied by water molecules.

provide holes specifically adapted to house specific lipid chains. The staggering complexity of configurational requirements for a number of chain-specific holes large enough to accommodate 50% lipid only makes the model more improbable. As a general feature of membranes, the chain-specific hole hypothesis could not explain the relative chain-unspecificity previously discussed. Because this model was inspired by a study of chloroplasts, it may be pertinent to mention the recent evidence obtained by Park (15) for the lipids being outside the protein units. Furthermore, although quantitative and qualitative species-specific differences have been observed in both the lipid classes and unsaturated chains of chloroplasts, a high average level of unsaturation of from 2 to 2.5 double bonds per chain is common to all species examined. How could this feature, perhaps one of the most significant lipid factors in relation to chloroplast function, manifest any functional expression if the lipid chains were buried in the unreactive "hydrophobic" interior of membrane protein molecules? It would seem that in this model designed to emphasize the lipid-specific individuality of membranes there is a gain in structural individuality which is deprived of functional expression.

If instead, the lipids formed the bimolecular leaflet of the Danielli model, at least over-all characteristics such as unsaturation could find a functional expression by affecting the permeability of the membrane, for instance. Also, by decreasing the gap between charged external layers, chains of short effective length could affect the capacitance of the system. Such effective functional participation as a group is not inconsistent with the limited specificity for lipid chains observed in several membranes.

It can be concluded from the foregoing discussion that the Danielli model is still very useful in interpreting the basic structure of membranes. The task of filling in details is not an easy one although the amount of reliable analytical data available is considerable. The difficulty lies not in the lack of structural data but in the unreliability of this data below a certain level of molecular aggregation. The probability and extent of artifactual alteration in fixed, embedded electron microscopic specimens increases rather quickly for details of decreasing size. In spite of much effort, the level of uncertainty in these observations remains well above the limiting definition.

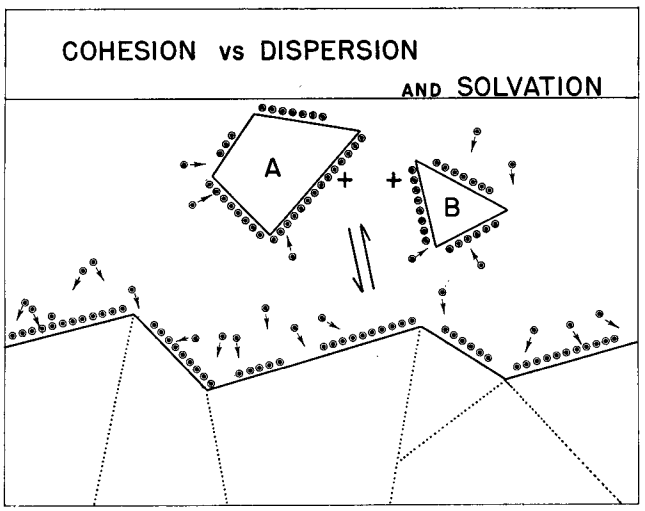


FIG. 4. Molecular aggregate (bottom) in equilibrium with solvated molecules (A, B). Small circles symbolize solvent molecules.

Hence there is much room for interpretation of observed details and for speculation as to their significance in relation to the natural state.

Molecular Models

A study of molecular models would seem to provide the only means of correcting gross errors of judgement by permitting the selection of concepts which are at least physically possible. It is to be hoped that this simple requirement will be more generally regarded as necessary in the present field as it is in many others where the workability of a design is of primary importance.

Whether improvements in technique will or will not lower the level of uncertainty of electron microscopic observations, the study of molecular models will remain important as a complementary, independent approach to the problem at all stages of development. A physical expression of concepts provides means to discover not only errors of interpretation but also possible solutions. Eventually, accurate models constitute the only means to estimate cohesional forces between constituent molecules and accounting thereby for the stability and functional ability of the proposed system.

While it is easy to build models of single molecules from available atomic models (9,15), the specific configuration that component molecules adopt in the polymolecular complex of membranes very much depends on the anistrophic interplay of molecular forces of cohesion. Hence a knowledge of these forces, and the use of this knowledge in conceiving models are perquisite. Studies which do not involve the use of molecular models also fail to account for the role of classical cohesive forces.

Stability

The role of cohesive forces in biological structures is not basically different from what it is in any stable molecular aggregate. The question is why do component molecules remain united, i.e., why is the probability for them leaving the aggregate very low?

The portion of an anonymous aggregate shown at the bottom of Figure 4 is assumed to be in contact with solvent; only a few solvent molecules, represented by small circles, indicate specific roles such molecules may have in the process of aggregation. It is assumed that molecules involved in this process are much larger than solvent molecules and represent a variety of shapes, sizes, and properties.

In general it is found that a dynamic equilibrium exists between the aggregate and molecules, such as A and B, located in the surrounding medium. This is, for instance, the case of organic molecular crystals in contact with their saturated solution under constant thermodynamic conditions. In the case of biological structures a steady state (homeostatic equilibrium) must be postulated since participant molecules are continuously removed from, and replaced in the surrounding medium through biochemical processes independent of the process of aggregation.

Assuming constant temperature, concentration, and pressure, a comprehensive discussion of the problem should take into account the properties of the large molecules within the aggregate, the properties of solvated molecules, and those of solvent molecules per se.

Even a cursory examination of the problem suggests that cohesive forces between aggregate molecules should play an important role in resisting the dis-

persive effect induced by thermal agitation. It should be evident that this role should grow in importance with increasing number and strength of cohesive bonds per molecule since this will decrease the probability for all bonds to be broken simultaneously for any molecule. Hence the size of molecules, in increasing the number of bonds per molecule, and the degree of fit, by permitting a large number of stronger bonds per molecule, are both very important factors favoring aggregation. A total of from −10 kcal per mole or from 10 to 15 times −kT, the energy associated with thermal agitation, is deemed sufficient to insure the stability of a molecular aggregate (16,17).

The role of solvated molecules in stabilizing the aggregate is settled once it is assumed that the system represents a state of equilibrium (or steady state) largely in favor of aggregation, since this implies that the continuous exchange between aggregated and free molecules does not induce any change in the system. Component turnover, evidently related to this process, is discussed further in the present article.

The forces arising from the solvent per se are of two kinds: those resulting in the transfer of energy by continuously pelting solvent molecules and those resulting from interactions between surface elements and solvent molecules. In addition, the space filling solvent molecules constitute a potential barrier to aggregate dissociation.

Intermolecular Cohesive Forces

Effects of the *short range London-van der Waals interactions* between nonbonded atoms have been known for a long time and were the first to be measured with considerable accuracy. The forces induced become appreciable when the distance r between centers is shorter than about 6 Å. Both repulsive (positive) and attractive (negative) components occur simultaneously and the resultant energy V is often described by the following general expression known as the Lennard Jones "6–12 potential."

$$V = A\ r^{-12} - B\ r^{-6}$$

Substitution for constants A and B of appropriate numerical values leads to expressions corresponding to specific atom pairs. Thus

$$V_{C,C} = 2.994 \times 10^5\ r^{-12} - 3.253 \times 10^2\ r^{-6}$$

was proposed by Bartell (18) in the case of two carbon atoms. V vs. r plots of such expressions show curves reaching a minimum on the negative side of V values, i.e., corresponding to a distance r where maximal attraction occurs. The value V = o is obtained very soon when r decreases beyond this point, and the curve rises sharply toward high positive values. This means that considerable energy is required to bring atoms not much closer than the distance for maximal attraction. The latter is the sum of van der Waals radii. These have been determined from observations on a large number of crystals (19). Maximal attraction occurs for two hydrogen atoms when the distance is 2.4 Å from center to center. How close nonbonded hydrogen atoms will approach before repulsing each other is indicated by the potential barrier (about 3 kcal) to the rotation of the C−C bond in ethane and substituted ethanes (20–24) where the closest possible distance between hydrogen atoms across the C−C bond is 2 Å. A measure of the forces of repulsion between adjacent atoms across CC bond is given by the fact that the length of this bond increases with the number of adjacent hydrogen or other atoms (25,26).

These observations show that the considerable energy derived from covalent bonds is needed to bring hydrogen atoms to a distance of 2 Å. Because such energy is available in cyclic compounds (9) it is possible for hydrogen atoms attached to nonadjacent carbon atoms to lie close to each other. This could not happen, either to hydrogen atoms similarly located in aliphatic chains when the chains are folded or twisted, or to peripheral hydrogen atoms when molecules are brought in contact.

The calculation of London-van der Waals forces can be made from models. The total energy is obtained as a summation including interactions between all possible pairs of nonbonded atoms. This simple but very tedious process is best accomplished with the use of computers.

The forces between individual atom pairs are small. However, a great number of them simultaneously arise when relatively large molecules are brought together. This is easily demonstrated by using the simple expression proposed by Salem (27) for the specific case of two parallel hydrocarbon chains, or the curve derived from this expression (9). With −1 kcal per CH_2 for chains at their closest possible approach, 4.17 Å, the molal energy corresponding to C_{20} chains is −20 kcal, for example.

London-van der Waals interactions occur between all nonbonded atoms independently of all other interactions such atoms promote. They therefore exist as components of all other type bonds and are not specific to hydrocarbon chains. On the other hand, because other bonds are absent they can be measured directly in saturated hydrocarbons. The latent heat of fusion and the latent heat of vaporization of saturated hydrocarbons represent energies of cohesion. The heat of fusion corresponds to the energy required to break London-van der Waals bonds existing in the crystalline state and so bring about the disorderly liquid state. The heat of vaporization is related to the complete rupture of all remaining bonds. Latent heats are not exactly equivalent to the reverse of cohesion potentials (28) but are close enough for the present demonstration. The latent heats of fusion and vaporization of saturated hydrocarbons listed in Table I are taken from the International Critical Tables and have been available for decades (29).

It should be noted that the L_v value per CH_2 which in the case of the lower hydrocarbon is 1.28

TABLE I

Latent Heats of Fusion (L_F) and of Vaporization (L_V) of Normal Hydrocarbons (kcal)

	M.W.	L_F			L_V			$L_F + L_V$	
		/g	/CH_2 (mole)	/mole	/g	/CH_2 (mole)	/mole	/CH_2 (mole)	/mole
C_4	58.12				*0.0915*	1.28	5.32		
C_{10}	142.28	0.035	0.5	5.0[a]	0.060	0.840	8.5	1.34	*13.5*[b]
C_{20}	282.5	0.035	0.5	10.0[a]	0.060	0.840	17.0[b]	1.34	27.0[b]
C_{60}	840	0.035	0.5	30.0[a]	0.060	0.840	51.0[b]	1.34	81.0[b]

[a] Value per gram as for paraffin wax; value/CH_2(mole) calculated for 14 g.
[b] Extrapolated from values for C_{10}.

kcal, falls to 0.84 kcal in the case of decane partly because the measurements were not made at the same temperature.[2] From the curve mentioned earlier (Fig. 4 in Ref. 9) it can be found that −0.84 kcal corresponds to straight chains 4.3 Å apart.[3] Thus chains are packed closely, and London-van der Waals interactions are still important in the liquid state. Molar latent heats of vaporization in the case of liquid molecules the size of phospholipids (mol wt 840) fall in the range of covalent energies. From the last column giving the sum of heats of fusion and vaporization, it is apparent that a 60% increase in cohesive energy will arise from crystallization. For the larger molecules, the absolute increase in cohesive energy brought about in this way represents a considerable amount of extra energy per mole. It is to be expected therefore, that order can be induced in a system of such molecules under the influence of London-van der Walls forces, since molecules will tend to adopt situations corresponding to the best possible fit. It has been shown also (30) that London-van der Waals forces could lead, not only to long range attraction between macromolecules, but also to a specific attraction for molecules of like polarizability.

Hydrogen bonding involves a different form of short range interactions which has been reviewed previously (9).

Water molcules contain both the required donor and acceptor type atoms ($-O-H^+$ and $^-:\ddot{O}<$), and are therefore highly dominated by hydrogen bonding. In aqueous solutions of compounds which can form hydrogen bonds in a water-free system, the competition of water molecules for hydrogen bonding is so strong that little of it occurs between solute molecules. The disorderly state of bulk water molecules also prevents hydrogen bonding from promoting a considerable degree of order. Very different is the situation where movements of water molecules are highly restricted, as for example, when water molecules are adsorbed, bound or crystallized. In an earlier communication (7) the tendencies for hydrogen bonding found in protein, in polar lipids, and in water, were shown to be mutually satisfied by the presence of a water monolayer between the protein and lipid components of membranes. This arrangement, which explains many important membrane properties, should make a large contribution to the stability and order in membranes. Whether the concept is correct or not, the presence of bound water molecules in membranes does indicate a contribution of hydrogen bonding which cannot be ignored.

Charge-induced interactions provide long range forces. Although both protein and lipid possess the necessary ions, it is doubtful that direct ionic interactions leading to salt formation occurs between them. Ionic interactions between these ions and small inorganic ions are numerous however, and among these, those leading to linkages between protein and lipids through divalent cations.

Charge induced forces arising from coulombic interactions have been discussed elsewhere (9,27). While it has been pointed out (27) that the dielectric constant of the membrane will reach a relatively low level, it is also possible that in the orderly array of water and other molecules surrounding the ions, the value of the dielectric constant will be direction-dependent. The resultant anistropy in ionic field strength should enhance the structural specificity of coulombic bonds. Clearly, an estimation of these forces requires the use of appropriate models. If however, an average of −5 kcal is assigned to these bonds (27), the total molal energy for protein molecules containing 100 amino acids of which only 10 are engaged in this type of bonding with lipids would be −50 kcal. This does not take into account the ionic bond energy derived from divalent cation bridges.

Forces Arising from the Solvent per se

As indicated by the rise of boiling points under increased pressure, the dissociation of a condensed phase is impeded by the surrounding free molecules. Under 760 mm Hg pressure, the boiling point of the C_{20} hydrocarbon (mol wt 282) is about 325C above room temperature. Taking 0.5 cal gm^{-1} as the heat capacity of this compound, it can be estimated that 44 kcal $mole^{-1}$, or 2.2 kcal $mole^{-1}$ CH_2 are required to raise the level of kinetic energy of molecules high enough for them to overcome the barrier and form a gas phase of their own. Energy requirements for larger molecules are evidently higher.

To larger, insoluble molecules, such as the outer structural protein of membranes, the water-filled surrounding space probably constitutes a potential barrier only, since it is unlikely that independent movement of such molecules are permitted by the simultaneous rupture of a large number of bonds. On the other hand, the much smaller polar lipid molecules could be placed in situations where the strength of their binding to other membrane components is marginal. For these the water barrier should constitute an important factor in minimizing further dissociation.

Lipid turnover in membranes (31) is explained by the fact that some of these molecules actually escape from the membrane and are replaced, probably through a reversal of the escape process. The thermal motion of water molecules provides both the critical situations and forces leading to lipid expulsion. It is evident from the existence of Brownian motion that the random agitation of water molecules does result in local impacts involving considerable energy. Both the size of displacements and the scale of particles in motion far exceed molecular dimensions. A continuous pelting by water molecules should generate considerable turmoil at the molecular level of membrane surfaces. In spite of inertial damping by the water barrier, surface components should suffer momentary displacements, and many cohesional bonds should be broken in the process. In a few local disruptive upheavals lipid components must be frankly exposed. On the other hand, we know that the parting of surface components leading to the ejection of a lipid molecule is an event of relatively low frequency since lipid turnover is of the order of 1%/min (31). The number and strength of cohesive bonds are obviously large enough, not only to prevent complete breakdown of membranes, but also to permit a proportion of ordered area to remain functional at any one time.

The thermal energy of water molecules is not entirely directed to membrane disruption. A relatively large proportion represented by impact energy vectors normal to the surface should favor cohesion.

Water molecules associated with membrane elements in direct contact with the medium must pro-

[2] Temperature for butane was 20C, for decane, 160C.
[3] The chains are not straight nor parallel in the liquid state, the distance corresponding to −0.84 kcal should be somewhat smaller.

vide a cohesive contribution. This water coat, or crust, should result from the combined adsorption and hydrogen bonding of water molecules to polar groups, and from further hydrogen bonding of these molecules to each other. No more than one molecule deep, this coat is adjacent to counterions and associated water molecules of the Stern layer, which also provide a measure of protection.

The energetic contributions of "hydrophobic" interactions (32–34) involving water molecules and hydrocarbon appendages of surface components does not appear considerable in view of the total cohesive energy involved in maintaining membrane stability.

It should be evident that this contribution depends on the number of hydrocarbon groups involved in the formation of Frank-Evans (35) "icebergs" and also on the extent of this involvement at any one time. While both would be very limited in the outer region of the surface protein, they should undoubtedly become larger during the disruptive events leading to the exposure of lipids to water. The frequency and extent of these events, many of which do not result in lipid expulsion, cannot be properly assessed from turnover rates.

A "Particulate" Concept of Membranes

Recent electron microscopic studies have revealed many details in intracellular (36–39) and mitochondrial (40–45) membranes. The definition of structural details which only appear after specific treatments of the specimens, often leaves much to be desired; their significance in relation to the undisturbed tissue is still unsettled (46). Because the presence of subunits has been clearly established in membrane systems such as chloroplasts, for example (14), it is tempting to conclude that the observed details constitute evidence for a similar organization in the above, and perhaps in all membranes. Even if the observed features are artifactual, they nonetheless indicate the presence in membranes of a related structural arrangement which is manifested by their appearance under appropriate treatments. It is suggested that this is due to molecules in the thin layer of structural protein forming units interconnected by specific bonds. Should these bonds be broken under specific treatments, the free protein molecules, together with the lipids to which they are individually associated could undoubtedly form distinct micellar lipoprotein units of globular appearance. The general pattern of the artifactual arrangement would depend on several factors including the extent of the fragmentation process, the uniformity of protein molecules, the involvement of superstructural protein constituents, the fixation agent used, and many others.

Figure 5 diagrammatically represents an arrangement of the structural protein in hexagonal array. The hexagonal spiral is one of the possible configurations protein chains could adopt (47). Units of about 10,000 molecular weight constitute the protein of myelin (48). This represents a chain capable of forming a spiral of the type described with a diameter of about 60 Å (7). Bonds between protein units are assumed to differ significantly from those between chain elements of the same unit and from lipid-protein bonds. It is evident that this further definition of the structural protein does not alter the positional relationship of lipids to protein or any other basic feature of the Danielli model. Yet this arrangement provides more than a simple explanation for the formation of artifactual detail in electron microscopic preparations, which is but one manifestation of the postulated interunit/intraunit bond difference.

In a previous communication (7) the specificity of sites existing at the junction of adjacent units was assumed to lead to the preferential adsorption of particular solute molecules. Such sites which are symbolized by small triangles in Figure 5 would owe their specificity to a particular structural quality of the amino acid chains forming interunit bonds in their immediate vicinity. Specific molecular species would be preferentially adsorbed, not only because of structural correspondence, perhaps of the lock and key type, but also because of polar groups included in their molecule having an affinity for polar groups at the site.

Since this form of adsorption implies a competition for polar groups engaged in interunit binding, it may lead to the rupture of interunit bonds at the site of interaction and to the local weakening and disruption of the membrane structure. As these events may result in a significant proportion of adsorbed molecules passing through the membrane, they describe a possible mechanism for molecular transport. Implications of this concept have been discussed to some extent in a preceding article (7). Among those not previously examined is a possible link between molecular transport and lipid turnover. Under the present scheme, both phenomena would be related to local disruptions involving the participation of

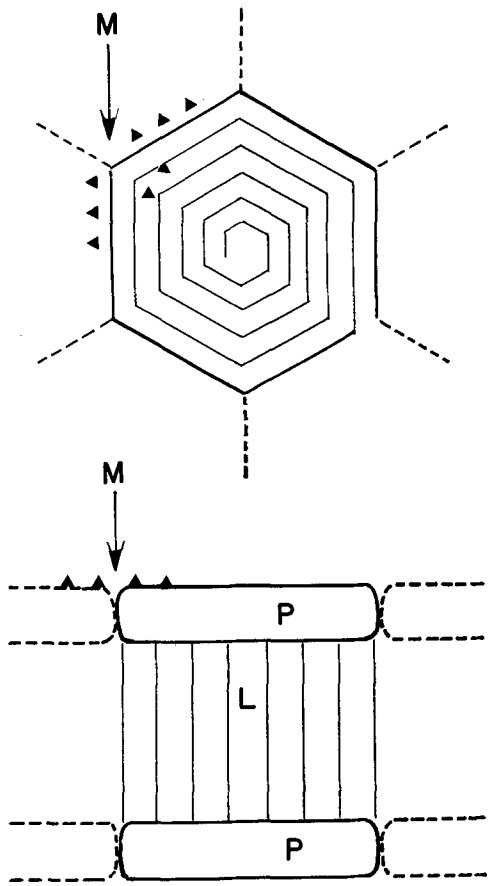


FIG. 5. Hexagonal spiral configuration of membrane protein unit. *Top.* Protein unit in the mozaic pattern of the membrane surface. *Bottom.* Cross section of membrane showing lipoprotein particle. P is protein unit on both sides of the membrane; L, lipids of bimolecular leaflet. In both views, triangles symbolize groups forming site specific for adsorption of solute molecule M.

thermal energy. If disruptions promoted by the adsorption of specific solutes may result in the ejection of lipid molecules, it is also possible that lipid ejection is the next obligatory step in the sequence leading to some forms of molecular transport.

Conclusions

For any given membrane, the problem of assigning to the various cohesive forces their share of the total effort is very much dependent on establishing a representative model, i.e., one embodying the typical structural features of the components. Among these should figure the amino acid sequence of the membrane protein for which there is at present no available information. Assuming this problem solved, one would have to take into account the dynamic equilibrium existing in membranes to assess the contributions of polar bonds (ionic, coulombic, hydrogen bonds, etc.). Since such bonds will be made and broken very frequently under the influence of thermal motion and of transient electric fields generated by small ions, a continuous reshuffling of forces must be expected (10). It is clear that statistical methods will have to be used in this analysis.

The proposed "particulate" concept of membrane structure embodies the simple features of the Danielli model. It is believed that the building elements of the "lipoprotein barrier" constituting the basic structural feature of membranes are lipoprotein units which abandon most of their structural individuality in the process of membrane formation. While the lipids of lipoprotein particles form the classic bimolecular lipid leaflet, structural protein units, now constituting the structural protein layers, conserve a vestigial expression of the former division. Additional superstructural elements confering distinctive functional properties complete the membrane. Alterations to the bond system linking the protein subunits is thought to permit partial or total manifestations of the former division. This concept thus implies a latent rather than on overt expression of membrane subdivision. Features observed in electron microscopic preparations are possible reflections of a propensity to reversion. On the other hand, many functional properties, including molecular transport and lipid turnover, could arise from a limited, localized and controlled expression of this tendency, of which the formation of temporary "pores" is a typical example. It would seem fitting that an important agent in causing the necessary disruptions be energy derived from thermal motion which is involved in all other phases of biological transport.

In looking for evidence of a general design in membrane structure, the possible incidence of artifactual effects in electron microscopic preparations is not the sole concern. In specialized evolutionary forms of membrane systems, a basic design is apt to be disguised, often beyond recognition. The distinctive property of superstructural elements may be directed to some particular function to such extent as to overshadow most of the familar ones. In gram positive bacteria, what looks like a "lipoprotein barrier" lies behind a wall which is many times thicker. This wall may be the evolutionary expression of superstructural elements whereby a structural function, initially assigned to the "lipoprotein barrier," has acquired considerable importance. Evolutionary modifications of this kind probably occur elsewhere to a lesser degree.

REFERENCES

1. Van Deenen, L. L. M., J. De Grier, V. M. T. Houtsmuller, A. Montfoort and E. Mulder, "Biochemical Problems of Lipids," Ed. A. C. Frazer, Elsevier, Amsterdam, 1963, p. 404.
2. Mulder, E., J. DeGrier and L. L. M. Van Deenen, Biochim. Biophys. Acta *70*, 95 (1963).
3. Farquhar, J. W., and E. H. Ahrens, Jr., J. Clin. Invest. *42*, 675 (1963).
4. Richardson, T., A. L. Tappel, L. M. Smith and C. R. Houle, J. Lipid Res. *3*, 344 (1962).
5. Danielli, J. F., J. Cell. Comp. Physiol. *5*, 495 (1935).
6. Davson, H., and J. F. Danielli, "The Permeability of Natural Membranes," Cambridge University Press, Cambridge 1952, p. 67.
7. Vandenheuvel, F. A., JAOCS *42*, 490 (1965).
8. Robertson, J. D., Biochem. Soc. Symposia, Cambridge, England, *16*, 3 (1959).
9. Vandenheuvel, F. A., JAOCS *40*, 455 (1963).
10. Vandenheuvel, F. A., Ann. New York Acad. Sci. *122*, 57 (1965).
11. Finean, J. B., Circulation *26*, 1151 (1962).
12. Geren, B. B., Exp. Cell Res. *7*, 558 (1954).
13. Benson, A. A., this Symposium.
14. Park, R. B., J. Cell Biol. *27*, 151 (1965).
15. Jackson, H. R., and F. A. Vandenheuvel, J. Phot. Sci. *12*, 134 (1964).
16. Waugh, D. F., "Macromolecular Complexes," Ed. M. V. Edds, Jr., The Ronald Press Co., New York, 1961, p. 7.
17. Setlow, R. B., and E. C. Pollard, "Molecular Biophysics," Addison-Wesley Publishing Co. Inc., Reading, Mass., 1962, p. 157.
18. Bartell, L. S., J. Chem. Soc. *32*, 827 (1960).
19. Pauling, L., "The Nature of the Chemical Bond," 3rd ed., Cornell University Press, 1960, p. 260.
20. Pitzer, K. S., Disc. Faraday Soc. 10, *66* (1951).
21. Herschbach, D.R., J. Chem. Phys. *25*, 358 (1956).
22. Lide, D. R., Jr., Ibid. *30*, 37 (1959).
23. Kasuya, T., J. Phys. Soc. Japan *15*, 1273 (1960).
24. Laurie, V. W., J. Chem. Phys. *31*, 1500 (1959).
25. Stoicheff, B. P., Tetrahedron *17*, 135 (1962).
26. Bartell, L. S., Ibid. *17*, 177 (1962).
27. Salem, L., Can. J. Biochem. Physiol. *40*, 1287 (1962).
28. Linder, B., J. Chem. Phys. *35*, 371 (1961).
29. International Critical Tables, Vol. V, Ed. E. W. Washburn, McGraw-Hill Book Co. Inc., New York, 1929, pp. 137–138.
30. Yos, J. M., W. L. Bade, and H. Jehle, Proc. Nat. Acad. Sci. *341* (1957).
31. Hagerman, J. S., and R. G. Gould, Proc. Soc. Exptl. Biol. Med. *78*, 329 (1951).
32. Kauzmann, W., "Advances in Protein Chemistry," Vol. XIV, 1–63.
33. Nemethy, G., and H. A. Sheraga, J. Chem. Phys. *36*, 3399 (1962).
34. Nemethy, G., and H. A. Sheraga, J. Biol. Chem. *237*, 2506 (1962).
35. Frank, H. S., and M. J. Evans, J. Chem. Phys. *13*, 507 (1945).
36. Sjostrand, F. S., Nature *199*, 1262 (1963).
37. Sjostrand, F. S., J. Ultrastructure Res. *9*, 340 (1963).
38. Robertson, J. D., Anat. Rec. *145*, 276 (1963).
39. Robertson, J. D., J. Cell. Biol. *19*, 201 (1963).
40. Pease, D. C., Ibid. *15*, 385 (1962).
41. Fernandez-Moran, H., Circulation *26*, 1039 (1962).
42. Doumashkin, R. R., R. M. Dougherty and R. J. Harris, Nature *194*, 1116 (1962).
43. Parsons, D. F., Science *140*, 985 (1963).
44. Smith, D. S., J. Cell. Biol. *19*, 115 (1963).
45. Stoeckenius, W., Ibid. *17*, 443 (1963).
46. Sjostrand, F. S., E. Anderson-Cedergren and U. Karllson, Nature *202*, 1075 (1964).
47. Vandenheuvel, F. A., Symposium on Biophysics and Physiology of Biological Transport, Frascati (Rome), June 15–18, 1965.
48. Thompson, E. B., and Marian W. Kies, Symposium on Demyelinating Diseases, Ann. New York Acad. Sci. *122*, 129 (1965).