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Role of Charge-Fluctuation Forces in Adlineation of Similar Molecules

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Role of Charge-Fluctuation Forces in Adlineation of Similar Molecules‡

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Abstract—The biological cell membranes are birefringent. They melt at relatively low temperature and yield molecules several of which form liquid-crystalline melts and elastic crystals. This paper considers the possible modes of packing of the major constituents of the membranes, namely, phospholipids, glycolipids and cholesterol, based on three governing factors: (i) London–van der Waals or the charge-fluctuation interaction (ii) self-recognition and pairing of similar molecules in a heterogeneous mixture and (iii) the requirement that the packing must prohibit abrupt changes in force-fields. A few representative arrangements out of a very large number of possibilities which are otherwise consistent with optical and crystallographic data are presented. Three of the major implications of this packing are discussed: (i) generating of a large number of specific sites, (ii) possibility of elucidating the contact aminoacids in proteins covering the top or the sides of lipid aggregates and (iii) modulation of the state of the molecular collection, by non-covalent solvent-type interaction by molecules and ions. Neurotransmitter hormone, psychoactive protein from schizophrenics, anaesthetic gases and ions of biological significance are chosen to serve as examples. The significance of the state of the aggregates and its modulation in regard to gross physiological phenomena like ion-transport or ion-permeation through membranes, bioelectric potentials, semi-conduction and possibility of conformational changes in membrane proteins are considered.

It is somewhat remarkable that in homeotherms and even in poikilotherms a somewhat narrow zone of environmental tempera-

‡ Substance of this communication was presented at the International Liquid Crystal Conference, Kent, Ohio in August 196.

ture is compatible with the living state. If this temperature goes beyond permissible limits then in addition to the failure of a variety of complex compensatory mechanisms in which a number of systems and organs participate there is also a change in the state of various structural systems. In the case of the cell membranes, which are comprised of molecules, several of which can exist in liquid crystalline state, melting may be observed at temperature below the boiling point of water, the actual temperature range varying according to composition of the membrane at the time of measurement. This signifies that quite weak forces, weaker than hydrogen bonds, are involved in keeping the molecules together in the membranes.

The biologic cell membranes are comprised of phospholipids, cerebrosides, cholesterol, polypeptides and numerous ions. The water that is present may be in part organized by structures, and in part liquid water. The lipids constitute a significant proportion of the dry mass of the membranes; values up to 80% have been assayed in case of myelin and 35 to 40% in preliminary analyses of surface or plasma membrane fractions.[19] The lipid molecules present in the membranes have the following structural plan: the glycerol or the base sphingosine bears on one end acylgroups, containing 12 to 26 methylene groups and on the other a sequence of atoms namely oxygen, phosphorus or carbon (the latter in cerebrosides), oxygen, carbon, carbon and a polar group. This polar group may be N^+H_3, or $N^+(CH_3)_3$. In case of cerebrosides there is the hydroxyl of a sugar residue, in inositides hydroxyls of inositol. Cardiolipins bear a hydroxyl with possibly charged oxygens. Serine, threonine or other amino acid are attached at polar ends in some cases. The polymethylenic chains may have a variable length being comprised of 12 to 26 carbon atoms and bearing 0 to 3 double bonds. Thus essential plan is a polar head group on one end and polymethlene chains on the other. Because of a large number of essentially equivalent and single bonded non-polar methylenic groups large charge-fluctuation forces of the London–van der Waals type may contribute significantly to the structure formation and its stability. Each CH_2–CH_2 interaction

being much weaker than a hydrogen bond (0.1 kcal/mole at 5 Å interchain distance) it is necessary to direct one's attention to them while looking for weaker interactions as pointed out above and to estimate these forces and to investigate if they could be affected by physiological or pharmacological stimuli.

London–Eisenschitz–Wang or the charge fluctuation forces may further determine the gathering of similar molecules in a mixture, a proposition considered relevant in several other type of molecules in other situations [23,53] and may thus lead to an ordered region of similar lipid molecules. Orderly arrangement of lipid molecules is an essential aspect of all detailed models of the biologic membranes which are compatible with experimental data from optical and X-ray studies. In this matter the model proposed by Finean[16] for myelin has dominated the concept, with later refinements suggested by Vandenheuvel[50] and others including Finean.[18] He proposed in case of myelin two bimolecular leaflets of lipids adlineated lengthwise in each leaflet with polar ends pointing vertically on the inner and outer surface and covered on free surface by protein (Fig. 1). Vandenheuvel[50] suggested that cholesterol contacts by its α-surface the fatty acid chain while its hydroxyl is near the polar end-group of the glycero- or sphingo-lipid. If the lipid phase may be considered as comprised of continuous aggregate of lipid molecules with capacity to migrate or at least to rotate as suggested by thermal and dielectric data then it is reasonable to assume that the principle of least abrupt change of force-field, applied hitherto to interfaces[25,26,32,33] may also be applicable here. Analysis presented here shows that a large interaction energy and numerous specifically ordered regions can possibly occur in the membranes.

Lastly, variation in the magnitude of dispersion forces can be brought about by various molecules considered as solvents. These may cause changes in the state of the membrane and its physiological function. Needless to say that molecules forming covalent or electrostatic interactions in membrane molecules can cause changes but are excluded from consideration here.

Methods

Using the oscillator model of Drude one can study the charge fluctuation interaction in the " classical " limit when the classical oscillator frequencies are so low that the energy difference between successive quantum levels is small comparing with thermal energy, measured by KT. In this case the electrons would perform Brownian motion in various quantum states available to thermal energy. On the other hand in the " quantum " limit the oscillator frequencies are such that excited states are out of thermal reach and fluctuation of unexcited quantum states are taken into account. A general treatment for dealing with two similar isotropic oscillators between these two limits is available.[53,54] However, in several situations in biological processes one often deals with molecules which have oscillators of diverse frequencies and orientations. There are several ways of dealing with this.[39,41,53,54] It can, for instance, be shown that the energy of the London interaction, $= \Delta A$ can be represented by

$$-\Delta A = (\hbar/4\pi i R^6) \int_{-\infty}^{+\infty} \alpha_I(\omega)\alpha_{II}(\omega)d\omega$$

where α_I and α_{II} represent the spur of the product of the dynamic polarizability interaction tensors of the molecules I and II taken on the imaginary axis of the complex ω plane. Experimental difficulties, however, preclude the current availability of experimental data that can be used. One has then to take recourse to the approach of Salem[44–46] who has, by using the second-order perturbation theory and the closure approximation, obtained a simple closed formula for the energy of interaction. This is applicable to molecules with axial symmetry or to spherical systems. The merit of the method lies in the inclusion of an explicit two electron correlation term which brings the calculation in nearer conformity with experimental data. In this method of calculation the inaccuracy induced by the closure approximation is removed considerably by approximations for both polarisability and dispersion energy and choosing as common average energy

that which corresponds to the observed polarizabilities. Salem[44,45] has estimated the nine interactions between CH_2 units in two linear parallel opposed polymethylenic chains, using the quantum mechanical averages from ordinary molecular orbitals and neglecting the orientation effect and found that the London energy denoted by him as W is given by

$$W = - A \frac{3\pi}{8\lambda} \cdot \frac{N}{D^5}$$

where the chain is comprised of N identical units of length λ and at distance DÅ from the interacting unit in another chain.

He later suggested[46] that interaction energy between two methylenic groups is given by

$$W_{CH_2 \leftrightarrow CH_2} = - \frac{A}{D^6(\text{Å})}$$

in kilocalories per mole.

In the present study Salem's techniques have been used for estimating charge-fluctuation interaction energies between the parallel and apposed hydrocarbon chains of relevance.

The dispersion forces are reduced when the molecules are immersed in liquid. A complete quantum mechanical theory of the reduction of these forces in dense media has been recently developed by Kestner and Sinanŏglu.[31] In the present study using, this point of view, the solvent is considered in two alternative situations (i) as a third body influencing the interaction between two methylenic groups and (ii) as a continuum. Data utilized for calculation were: Hinze and Jaffé's on ionization potentials of carbon in valence state te te te te,[27] Hoffman's on orbital energies,[28] of Dalgarno,[9] Fajans[15] and Fajans and Joos[16] on polarizabilities, and American Institute of Physics Handbook[3] and Handbook of Physics and Chemistry[24] for densities and ionization potentials of free ions.

Results and Discussion

The results of calculation on London interaction energies are outlined in Table 1.

TABLE 1 Order of London–van der Waals Interaction Energies in Polymethylenic Chains in Fatty Acids and in Polymethylenic Elements of Sphingolipids.

	Interchain distance 4.17Å
1. Myristic Acid	= – 13.83 k cal/mole
2. Palmitic Acid	= – 15.80 k cal/mole
3. Stearic Acid	= – 17.79 k cal/mole
4. Sphingolipids:	
Sphingomyelins—	
(i) Lignoceric acid residue + nonamidelinked fatty acid	= – 35.56 k cal/mole
(ii) Hydroxylignoceric acid residue beyond CHOH + non-amidelinked fatty acid	= – 34.58 k cal/mole
Cerebrosides—	
(iii) Nervonic acid residue + nonamidelinked fatty acid	= – 33.59 k cal/mole
(iv) Oxynervonic acid residue + nonamidelinked fatty acid	= – 32.60 k cal/mole
5. Nine carbon interaction between side chains	= – 8.89 k cal/mole

Note: (a) In (i), (ii), (iii), (iv) 13 carbon interaction in the non-amidelinked fatty acid chain is calculated.

(b) In (iii) 8 carbon interaction beyond the double bond and 13 carbon interaction before the double bond towards the amide linkage, while in (iv) 8 carbon interaction beyond the double bond and 12 carbon interaction before it are calculated.

In these estimates of the dispersion interaction, the saturated portions of the chains have been considered. A nine carbon interaction is mentioned since the usual site of the first double bond is the 9th carbon. The double bond may introduce a " kink " as pronounced as a V in the chain[2] and the interchain

distance at a level farther than the ninth carbon may be greatly increased thereby reducing the interaction energy, which is " distance specific "[45]; when chains that are 5Å apart come closer by 1 Å the interaction energy is tripled. Divergence of the two hydrocarbons residues beyond the 9th carbon may markedly weaken the interaction and lead to liquid state of the aggregate. It may be remarked that the double bond occurs early in the non-amidelinked chain in sphingomyelins and here it is feasible to consider adlineation and interaction because such a chain can be readily accomodated in the aggregate. The strength of the interaction, bending of polar groups, rotation of molecules, and the temperature at which the aggregate exists are relevant considerations in this.

It must be mentioned here that several of the assumptions in calculations proposed by Salem[44–46] are subject to revision later. For example, the deviation from spherical character of CH_2 groups, problem of the pair-wise additivity of interaction in parallel linear arrays of dispersion oscillators[55] and the nonideality of interaction as a dipole–dipole interaction may induce modifications. The orientation effects have also to be considered. The high frequency part of the microscopic dielectric constant due to medium has to be accounted for by solvent interactions. However, the method does seem to lead to accord between theoretical and several experimental data and explains a number of properties like liquidity of unsaturated fattyacid monolayers. It seems to provide a guide, although crude, to actual interaction energies. Whatever be the precise magnitude of interaction energy connected with the charge fluctuation forces the results are interesting because of the concept that a molecule must seek out a similar molecule in a mixture made by Hamaker[23] and later stressed and analysed by Jehle, Bade and Yos[30,53,54] stating that a very significant preference of response is obtained in case of the second order interaction discussed above. For example if a sequence of molecules I, I, II, I, II, III, I, I, I, is considered, II and III being two different single molecules of similar shape and I the medium molecules, then neglecting the second nearest neighbour inter-

action it can be seen that interaction energy ($\Delta A_{I,II}$ between molecules I and II and so on) in the arrangement in this sequence is

$$3\Delta A_{I,I} + 3\Delta A_{I,II} + \Delta A_{I,III} + \Delta A_{II,III}$$

In case of the sequence I, I, II, II, I, III, I, I, I, the energy is

$$3\Delta A_{I,I} + 2\Delta A_{I,II} + 2\Delta A_{I,III} + \Delta A_{II,II}$$

The difference in the interaction energy between the two sequences is

$$\begin{aligned}\Delta A_{II,II} - \Delta A_{I,II} + \Delta A_{I,III} - \Delta A_{II,III} = \\ - \tfrac{1}{2}(\Delta A_{II,II} + \Delta A_{I,I} - 2\Delta A_{I,II}) \\ + \tfrac{1}{2}(\Delta A_{II,II} + \Delta A_{III,III} - 2\Delta A_{II,III}) \\ - \tfrac{1}{2}(\Delta A_{I,I} + \Delta A_{III,III} - 2\Delta A_{I,III}).\end{aligned}$$

The first two terms on the right side are negative definite. Their sum usually exceeds the last term which is a positive definite.

After the strength and stability of interaction in the two chains in given molecules are evaluated and the perference of similar molecules to come together is seen, then the next question to answer is whether assymetric molecules will have (i) the necessary geometry for a close lengthwise fit and (ii) the ability to rotate or migrate in a dense crowded aggregate. As regards the consideration of various molecules a close fit is, indeed, possible as is shown in Figs. 5–15 which are a few representative examples. Indeed, unsaturation in fatty acids attached at 1 or 2 ester position is no bar to a sufficient although less strong interaction as can be seen in Fig. 8. In the arrangement depicted by Vandenheuvel[50] on the basis of orthogonal projections for models of glycero- and sphingolipids the fatty acid chains bearing even three double bonds projects only about 1 Å lateral to the cholesterol molecule which is adlineated on the side and can still pair with similar molecules. In case of cis-monounsaturated chains the V-shaped chain[2] may still adlineate with another similar molecule. The

thermal rotation may offer the necessary opportunity of effective contact. In this matter it is worthwhile to investigate if one of the functions of the body temperature is to provide the opportunity for optimal occurence of such an event. In other words temperatures much lower than the usual body tempetature may give infrequent adjustment of contacts while those above will randomise the molecules by excessive thermal motion competing with the dispersion interaction.

So far as the opportunity of rotation and migration is concerned our studies on the dielectric dispersion on cell membranes as a function of temperature have revealed that enthalpies of rotation of micelles, which vary with physiological and pharmacological state and ion concentration, can be as low as 1.7 kilocal/mole. Some Treatment by taraxine, a psychotoxic serum protein obtainable from schizophrenic patients in phases of depression, reduces this to a value $\frac{2}{3}$ or $\frac{1}{4}$ of normal. The change seems to be particular since the lysergic acid diethylamide (LSD) did not reduce this, but rather elevated the enthalpy, a result also induced by acetylcholine which is a neurohumoural transmitter. This indicates that rotation of groups of molecules can occur with ease. The capacity for migration of acyl groups or of whole molecules to take new positions are to be expected from the solvent interaction discussed below. In summary it can be said that dispersion forces can cause strong interaction and bring together similar molecules.

The Molecular Aggregates and the Principle of "Least Abrupt Change of Force-Fields."

If individually-stable molecules, having sought each other out, have adlineated, what kind of assembly of molecule can one have in the aggregate? For answering this the above principle is invoked, first proposed by Hardy,[25] Langmuir[32,33] and Harkins[26] for a superficially different situation, the interfacial phenomena.

Before we do this it is necessary to state that there are several impediments at this stage in elucidating the packing of the concerned molecules in a model for cell membranes in general. The

first difficulty is the paucity of data on membranes. The X-ray and other data of the type available for myelin[17] are not available for other cell membranes. Therefore in contradistinction to myelin (Fig. 1) the questions that are open for membranes in general are the following:

Cholesterol is not nearly equimolar with the combined amount of sphingo- and glycerolipids in various membranes and it is to be ascertained if it adheres to the same or other sides of these lipids. It is also not certain if the phosphorus-phosphorus distance between the opposite members of a bimolecular leaflet is in all cases $\simeq 50$ Å. The protein may not cover the entire surface. It is also to be investigated if under dynamic alterations of the state of the membrane associated with the migration and rotation of molecules leading to localised contraction and relaxation of the membranes the restrictions of space imposed by local rigidity of protein coverings might cause helical conformation in the long fatty acid chains. Under some conditions formation of a globular micelle may result. The second difficulty is that whatever is known leads to the expectation of a large number of structures to be analysed. If one were to take the lipid core of the model of bimolecular leaflet of myelin,[17] and grant a P–P distance of about 50 Å between the opposite polar ends in the leaflet the variety in molecular constitution predicts a variety of associations. The variables are the constituent units of the lipid molecules. The head groups of glycero- and sphingolipids may bear ethanolamine choline or sugar groups and in some additionally serine, threonine[29] or other amino acids. Indeed this anionic polar group may be a more complicated one as in gangliosides. The non-polar regions comprising mostly of the polymethylenic fatty acid chain may vary in length and in number and site of double bonds, as well as the site of bonding on to the glycerol or sphingosine base. Although the α surface of cholesterol adheres to the sphingomyline or glycero-lipids in some situation it could be that it does so on the other side of the lipid. Thus a very large number of associations have to be conceived differing in stability, geometry and interaction energy. Needless to say covalent bonding or Kirkwood–

Shumaker charge cluctuation forces in hydrotactoids and the charge-exchange are excluded because they require a close approach. Even then an early complete solution is precluded.

However, application of the principle to the heterogeneous aggregates in membranes capable of rotation and acyl or whole molecule migration leads to some important and useful results. It appears that attainment of equilibrium would involve partner choosing and orientations such that interaction energies undergo smooth decrement from a central region of strong interaction, such as two molecules of sphingolipids with lignoceric acid chains. Since the cerebrosides have large hydrocarbon chains and bear a sugar moiety, instead of a highly polar one and further because this group does not exceed the projected cross section of the hydrocarbons attached, it is reasonable to anticipate that the cerebrosides would make strong organisational centers. Cardiolipins found in the pellets of cell membranes from the brain would also provide stable points in whatever membranes they occur. Out of the very large number of molecular associations possible a few are illustrated in Figs. 5–15. As an example, one can see that in Figs. 5 and 6 there are two units of sphingolipid–cholesterol pairs apposed back to back, each unit has a pair of chains. Thus, there are six interchain interactions along vertical direction in the plane of the paper and four in the horizontal. There would thus be interaction corresponding to 1.25 unit chain length/chain, if all chains are equal. In case they are unequal, the energy would still correspond to 1.25 unit but the unit length would be that of the smaller chain, since the chains are in antiparallel juxtaposition, and taking the case that the chains are all similar in respect of the residues attached in 1 and 2 ester positions on sphingosine backbone. In case the units are so arranged that the lipid and cholesterol in a pair alternate as in Fig. 11, the interchain interaction will be only six for the 4 pair units. This gives 0.75 unit chain length interaction per chain even in case of molecule possessing saturated side chains. Depending upon the chain dimension one could suggest the following sequence in decreasing order of the interaction energies for adlineating with similar molecules: (1)

cerebrosides, (2) phosphotidylcholines, (3) sphingomyelins, (4) phosphatidyletheanolamines, (5) phosphatidyl serines. In each of (2), (4), (5) the molecules will have the following sub-sequence: saturated 18, 16 carbon, monounsaturated 18 carbon, saturated 14 carbon, monounsaturated 16, 14 carbon. Monounsaturated *cis* will be stronger than monounsaturated *trans* and polyunsaturated will be considerably weak.

It should be noted that even when the chains are in close contact with a pair in a neighbouring row the positively charged nitrogen atoms remain at considerable distance from each other. Nonetheless, the order of repulsion energies at this end alone would be expected to increase in the order: (1) cerebrosides, (2) phosphatidylcholines and sphingomyelins, (3) phosphatidyl ethanolamines and (4) phosphatidyl serines and others bearing charged aminoacids. This repulsion is bound to affect to some extent London interaction in neighbouring methylenic units and may influence the order of their adlineation energies mentioned before. Because of the restriction of P–P distance of $\simeq 50$ Å in opposite polar ends across the bimolecular leaflet one uses the molecules in one layer of bimolecular leaflet to determine what molecules they would contact and precisely how the contact would be made. If the molecules are suitable then this could arise by translation in the plane of the layer, or by crosswise overlapping as examplified in Fig. 7.

Geometry of these aggregates is being considered in detail elsewhere but two consequences of such an assembly are to be stressed: In the first place it can be seen that various distributions of polar groups and a polar side chains will generate a large number of interaction sites each with a characteristic charge distribution, strength of binding and available space at polar ends. This could restrict the choice of aminoacids and hence the kind of protein that would contact them at polar ends. It is reasonable to consider that the protein is greater in amount amongst the polar ends. Liquid transformation or microscopic " melting " (see below) may permit the formation of globular micelles and this may carry over the protein covering as lining to a pore created. In negatively

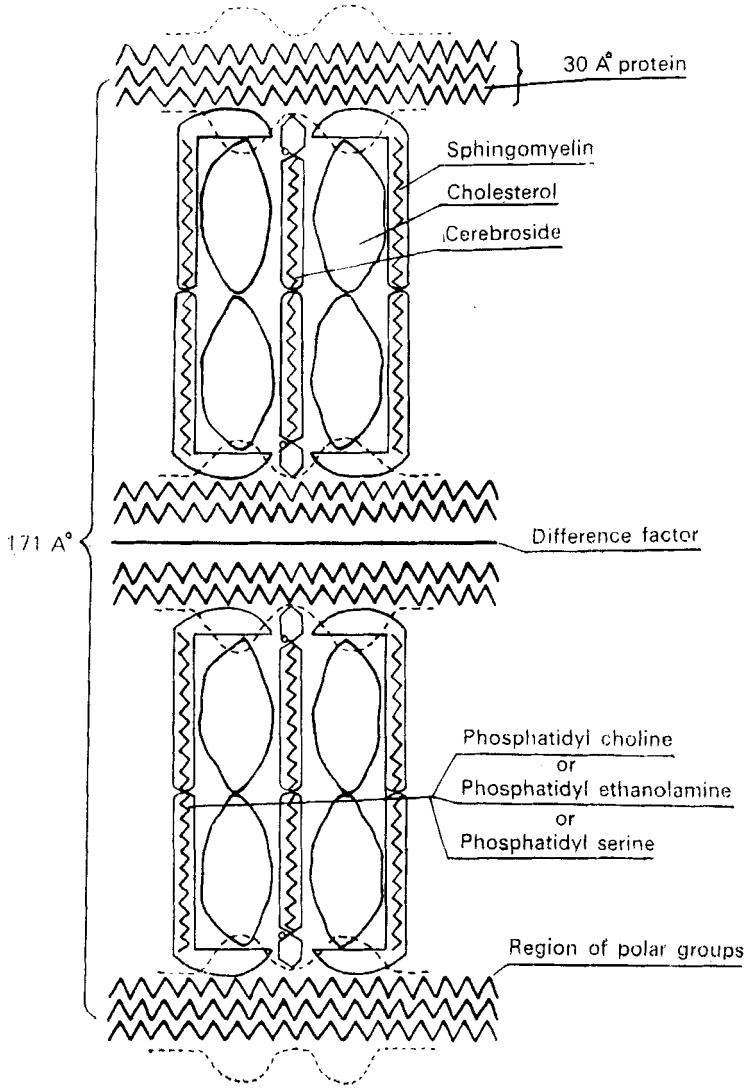
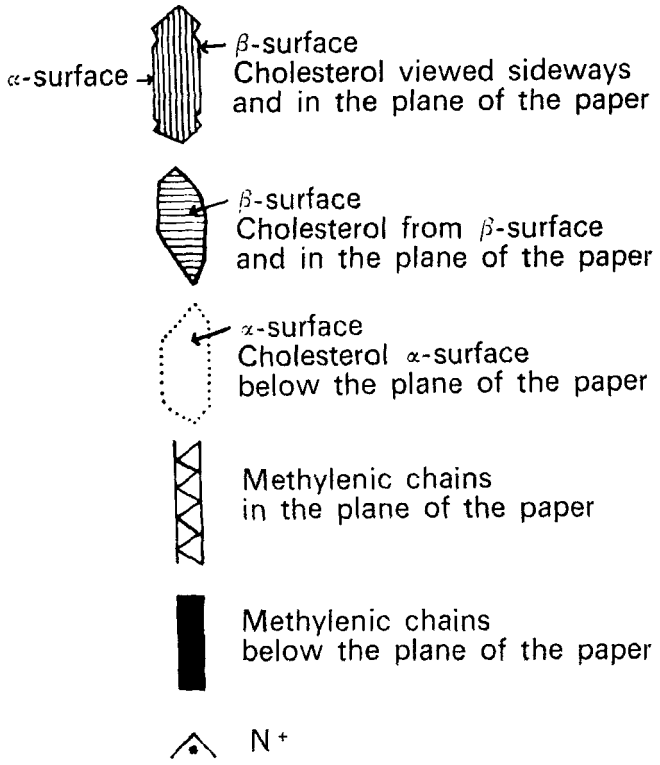


Figure 1. A diagram of a vertical cross section through the thickness of myelin as proposed originally by Finean.[17]

KEY FOR FIGURES 2–4



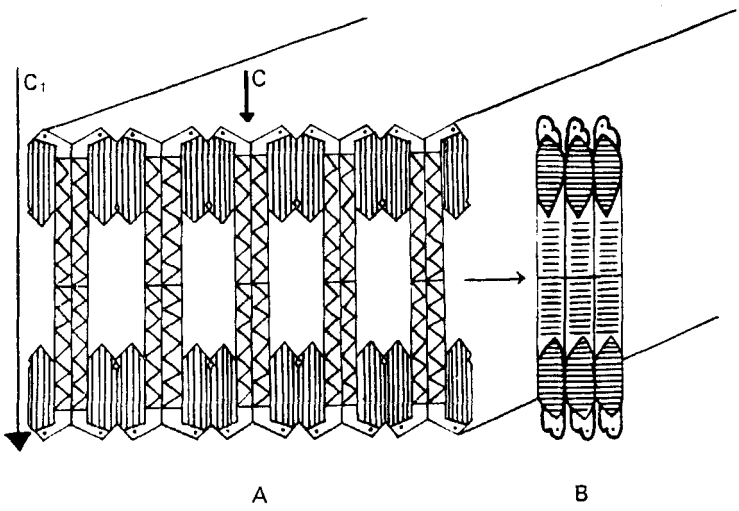


Figure 2. Diagram of the front (A) and lateral view (B) of molecules in a lipid bilayer, showing assembly of phospholipid molecules with two equal and saturated 16 carbon chains in 1 and 2 ester positions pairing with cholesterol molecules. End to end contact of fatty acid residues on opposite molecules result in $\simeq 50$ Å P–P distance between polar head groups. Dots in polar ends represent positively charged nitrogens.

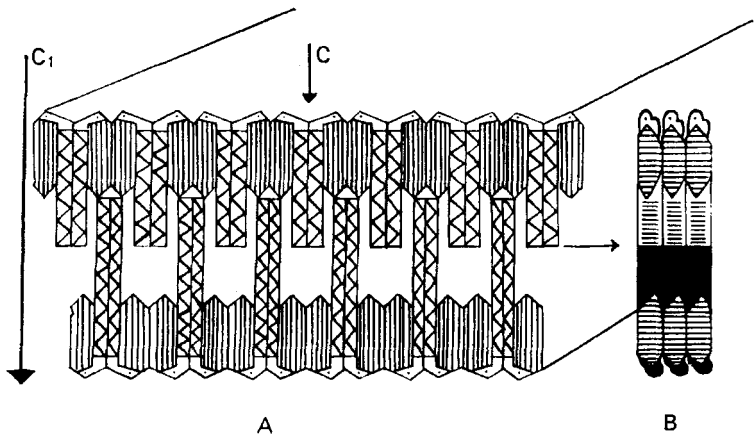


Figure 3. Diagram showing how phospholipids with chains longer than 16 carbons may still be accommodated by translation of one layer with respect to another and yet produce the same $\simeq 50$ Å P–P distance.

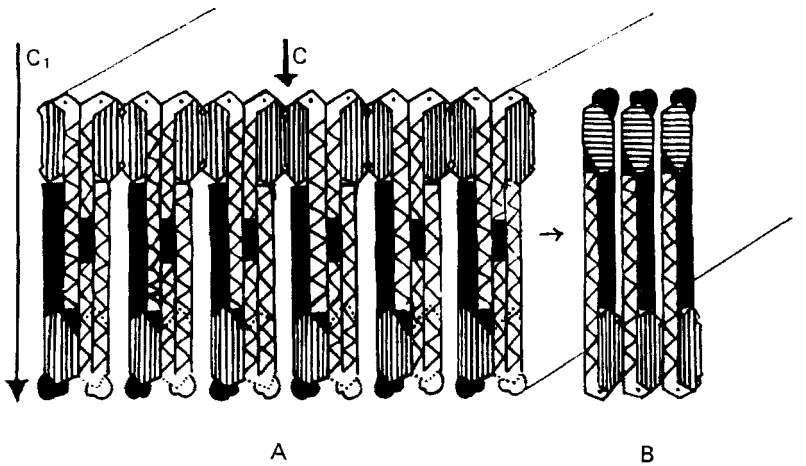
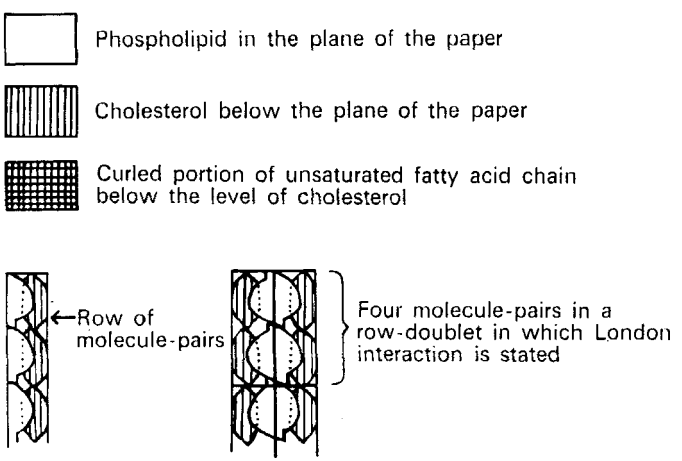


Figure 4. Diagram of sphingomyelin–cholesterol pair showing the consequences of the suggestion made by Vandenheuvel,[50] whereby the $\simeq 50$ Å vertical P–P distance is produced by molecules with one chain longer than 16 carbons by the rotation through 90° around the longitudinal axis of a phospholipid–cholesterol pair in the bottom molecular layer with respect to a similar molecule in the upper layer. A chain with double bond at 9C would curl up beyond the " tail " of cholesterol but would not project beyond the β-face of cholesterol.

KEY FOR FIGURES 5–15



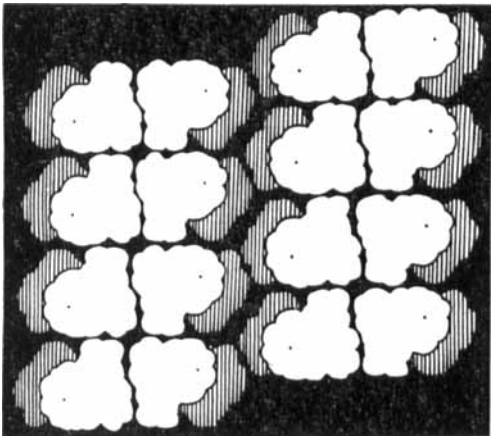


Figure 5. Superposed cross section of the molecule-pairs in one member of the bimolecular leaflet. Each pair comprises of sphingomyelin bearing cholesterol towards the concavity of its polar end. There is maximum contact between fatty acid chains in antiparallel arrangement of the rows of sphingomyelins. The translation of the row-doublet assures close fit and smallest intermolecular spaces. The charged nitrogens, however, between two adjacent row-doublets are closer than in Fig. 6. London interaction energy corresponds to 1.25 unit chain-length per chain in a collection of four molecule-pairs (eight fatty acid residues in two opposed molecule-pairs in a row-doublet). View from external surface. (Corresponding to direction in Fig. 2.)

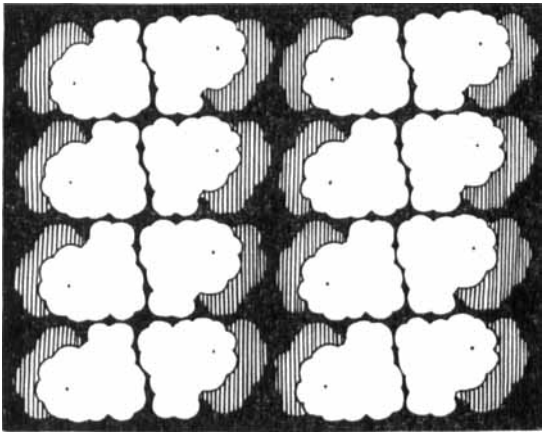


Figure 6. The elements in Fig. 5 now in register horizontally. The intermolecular spaces are larger. Also, the distance between the charged nitrogens between adjacent row-doublets is greater. London interaction energy per fatty acid chain same as in Fig. 5 but polar repulsions are greater.

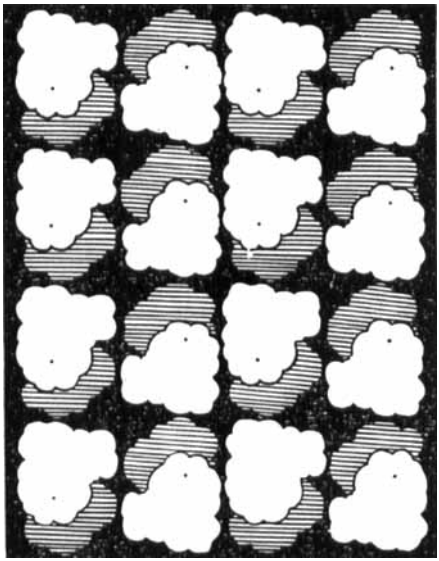


Figure 7. Effect of a Vandenheuval suggestion[50] for fitting the two layers in a bimolecular leaflet. One molecule-pair of Fig. 5 meets another one which is rotated by 90° around its longitudinal axis. Note poor contacts between the chains. In a row-doublet there are only partial contacts, elsewhere there is no immediate contact. London interaction energy is reduced to that for 0.75 unit chain length per chain. See text for further discussion, and Fig. 3 for fit by translation. View from top along C_t of Fig. 4 and ignoring the upper layer.

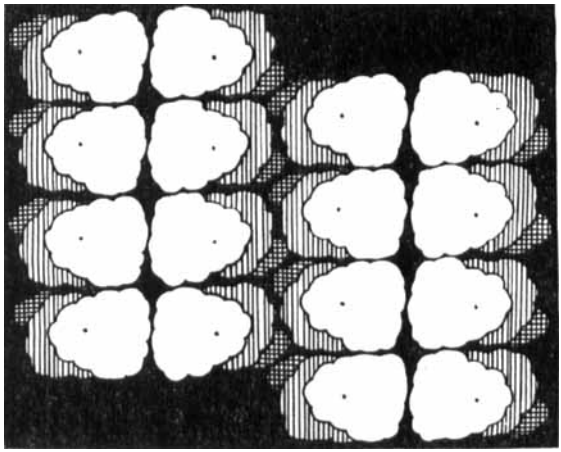


Figure 8. Molecule-pairs of β unsaturated phosphatidylcholine and cholesterol. Compare with Fig. 5. Distance between charged nitrogens between two row-doublets is greater. London interaction in four molecule-pairs in same situation as in Fig. 5 corresponds to 1.25 unit chain length. Note that the unit chain would be the initial straight portion of fatty acid at β position and therefore smaller of the two chains. The arrangement is less compact and rarefied in respect of adjacent row-doublets, leading to greater separability of row-doublets.

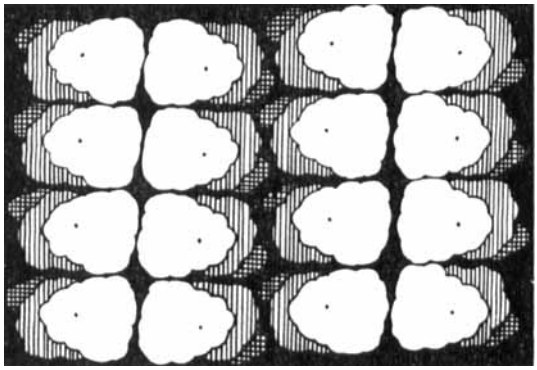


Figure 9. Molecules in Fig. 8, in horizontal register. Compare with Fig. 6.

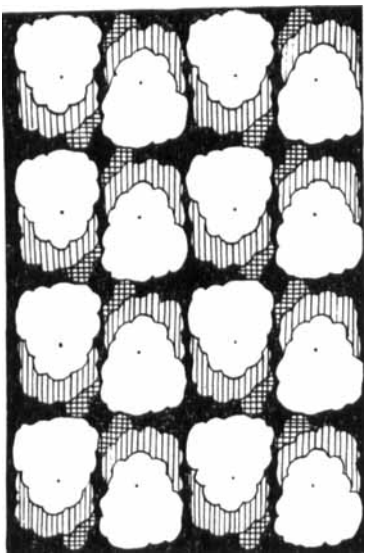


Figure 10. Molecule-pairs same as in Fig. 8 but belonging to the layer below arranged according to the Vandenheuvel suggestion.[50] London interaction energy for the four molecules collection in a row-doublet corresponds little over 0.5 unit chain length per chain. View from the external surface and ignoring the upper layer. It is to be noted that in Figs. 7 and 10, some overlap between acyl chains may cause some additional London interaction when the fit between upper and lower layers are considered.

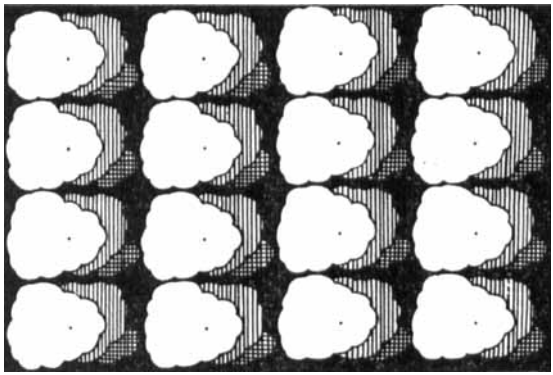


Figure 11. Molecules in Fig. 8 arranged in parallel arrangement. No row-doublets now and a loose-packed arrangement results with London interaction 0.75 unit chain length per chain in the four molecules.

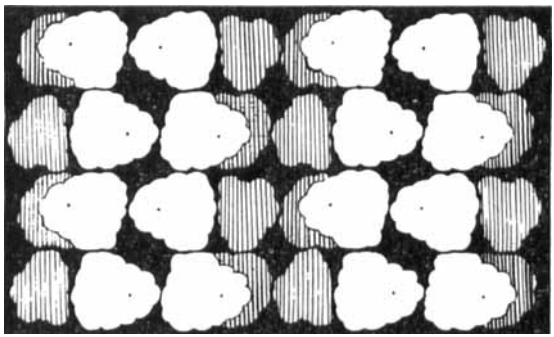


Figure 12. In this the α surface of cholesterol adlineates with sphingolipid or saturated glycerolipid on one side in half the molecules and on the other side in the other half. London interaction corresponds to somewhat more than 0.75 unit chain length per chain, since the bulges between adjacent glycerol or sphingosine bases reduce strongly the interaction between fatty acid residues in opposed molecules in row-doublets.

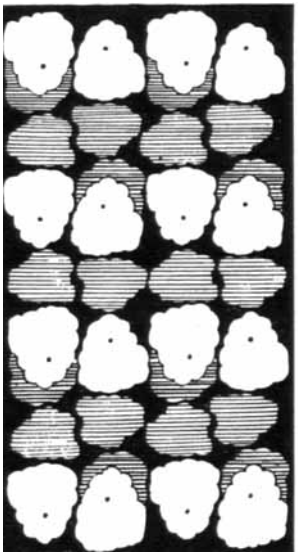


Figure 13. The molecules in the layer beneath those in Fig. 12, rotated as in 7 except that in order to achieve less strained contact with adjacent phospholipid fatty acid chains the molecules with cholesterol on the " back " surface are rotated in the same sense by 270°. Viewed from the external surface and ignoring the covering layer. London interaction relates to less than 0.75 unit chain length per chain. Note the interposition of cholesterol leads to easy separability of rows.

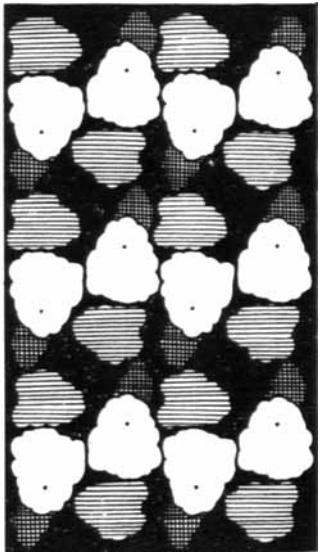


Figure 14. Cholesterol adlineated to the unsaturated glycerolipid on the surface opposite that shown in Fig. 8. The phospholipid is alternately rotated through 180° to bring full length of fatty acid residues in register in each row. It is assumed as suggested by Vandenheuvel[50] that β surface of cholesterol cannot strongly interact with phospholipid. If it does then compact arrangement would result with the β chain curling over the adjacent cholesterol in the horizontal row. Note extremely loose arrangement with large N^+N^+ distance in opposed rows. London interaction energy corresponds to about 0.75 unit chain length per chain in a collection of four adjacent molecule-pairs in parallel rows. Note the unit chain length is not smaller as in Fig. 8, but the full chain lengths of chains in both 1 or 2 ester positions are utilised. The area occupied by the four molecules is large. No row-doublets exist and easy separability of rows is to be expected.

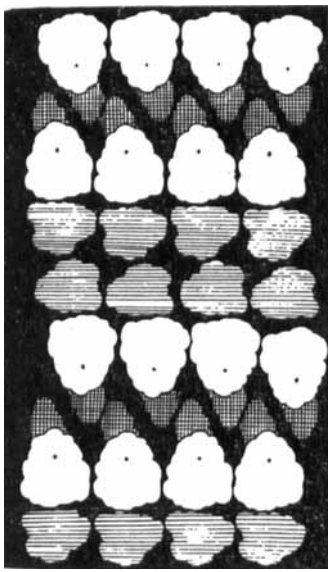


Figure 15. The assembly of molecules in the layer below molecules in Fig. 14, arranged in crosswise manner as in Fig. 10 and translated laterally to get closer fit amongst unsaturated chains. London interaction energy corresponds to little over 0.75 unit chain length per chain. View through the top covering layer.

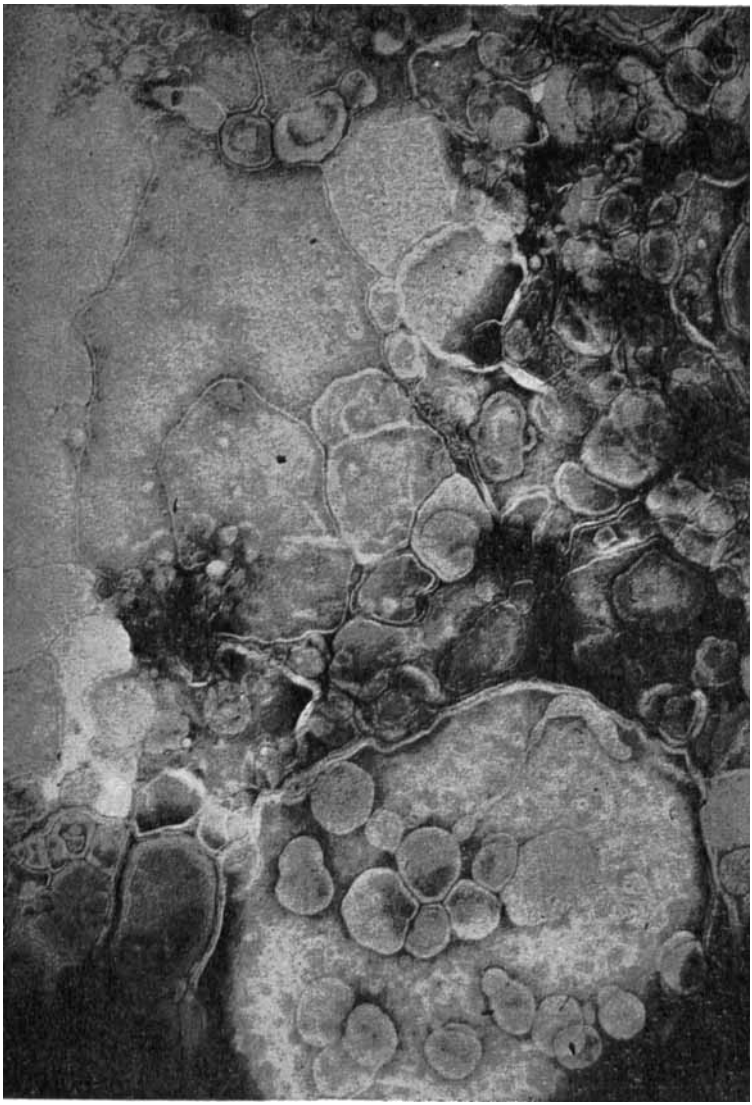


Figure 16. The neuronal membranes from rat cerebral cortex negatively stained with sodium phosphotungstate. See also Fig. 17. × 8,500.

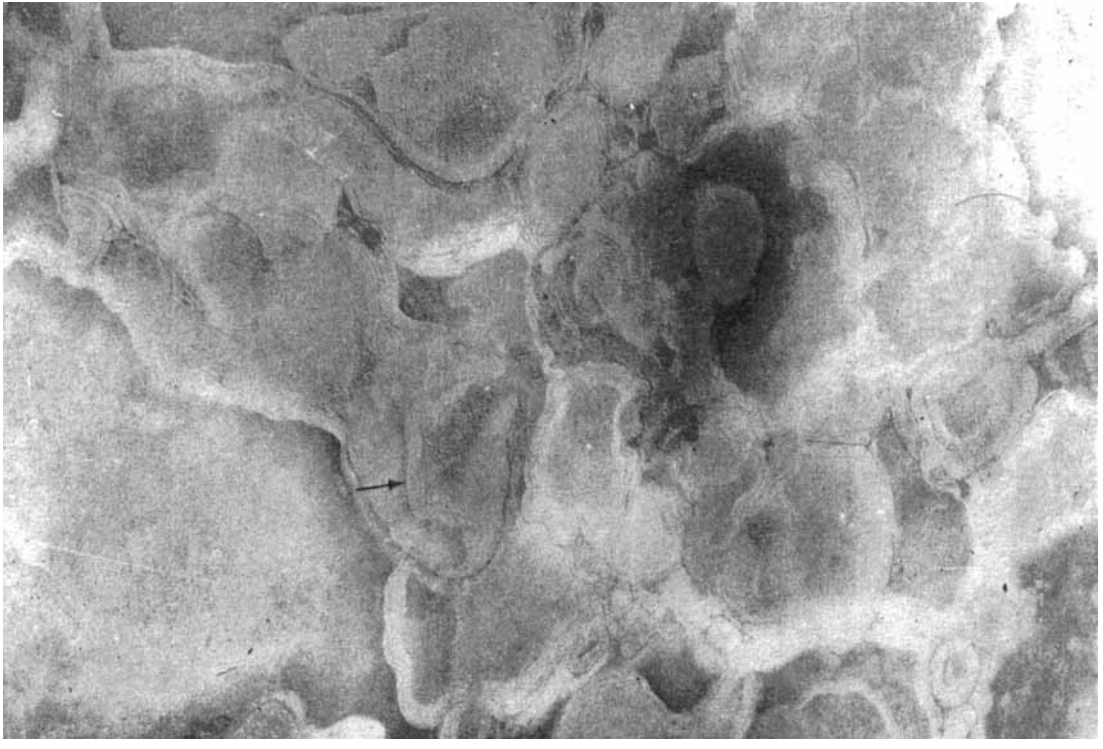


Figure 17. The neuronal membranes treated by acetylcholine chloride showing the "myelinic" lines at the borders of fragment which appears to extend into the fragments at several places for example at the arrowmark. × 63.000.

stained preparations " myelin figures " are often seen in the membrane near the borders of the fragments (Figs. 16 and 17). The area of these figures is increased after treatment by taraxine or lysergic acid diethylamide. This might indicate that protein is easily transformable by such agents and thus in certain states it may not completely cover lipid core.

The other interesting implications of the particular state and organisation of the aggregate is that it can be modulated and be rendered dynamically transformable. Quite clearly some transformations can be brought about by electrostatic or covalent interaction for example by saponin,[4,21,40] or by phosphalipase A (see Ref. 11). However, in general the " solvent " type interactions by various agents can be considered as follows:

The " Solvent " Interaction and Dynamic Transformations in the Molecular Aggregates

Tables 2 and 3 present an estimate of the reduction of dispersion interaction of energies by " solvents ".

Table 2 Reduction of the Dispersion Interaction by a Three-body Interaction due to the Presence of a Given Ion or Molecular Species

(i) Case when the ion is assumed to be at midpoint between the equivalent carbons in two opposed methylenic chains 4.17 Å apart.

Iodine ion	77.2 %	Calcium ion	5.5 %
Bromine ion	50.65%	Sodium ion	1.65%
Xenon atom	44.12%	Magnesium ion	0.77%
Potassium ion	9.92%		

(ii) Case when the ion is assumed to be at the apex of an equilateral triangle with the two carbons of opposed methylene groups

Iodine ion	9.93%	Magnesium ion	0.999%
Bromine ion	6.2 %	Lysergic acid	
Xenon atom	5.52%	diethylamide (LSD)	4.5%
Potassium ion	1.24%	Acetylcholine chloride	2.7%
Calcium ion	0.69%	Choline chloride	2.2%
Sodium ion	0.21%		

The reduction due to LSD, acetylcholine chloride and choline chloride treated as points in case (i) will be 36.4%, 21.6% and 17.6% respectively, but the molecules are too bulky to approach the sites without strain.

TABLE 3 Reduction of van der Waal's Coefficient‡ in Ionic " Solvent " Treated as Continuum

Potassium ion	11%	Sodium ion	3.6%
Calcium ion	11%	Magnesium ion	2.9%

‡ This coefficient indicates the figure to be multiplied by the geometric factor K[31] due to varying topography in different situations giving rise to the fraction that denotes the actual reduction of dispersion interaction.

Noticeable amongst the three body interaction is that of Xenon (44.12%) which is known to be an anaesthetic gas[8,34,35] and acetylcholine chloride which is neurotransmitter hormone and LSD, a psychotoxic drug. Better estimate of ionisation potential, orbital energies, the geometric factor K and integrations after detailed consideration of geometry of each individual " solvent " ion is liable to lead to a substantial refinement of values in Table 3. Nonetheless, the estimates show the capacity of these agents to act on the lipids in the cell membranes. They also show, because of similarity in action, that the interaction is not the whole explanation of the physiological or pharmacological activity in all cases, but that this effect must be taken into account in all theories proposed to account for the action of these agents. For instance, the drugs may open spaces for the transport of ions. The solvent effect of potassium, and of calcium may determine their presence as intracellular cations while that of sodium may explain its presence chiefly in the extracellular space. The influence of magnesium ion is very small. Thus, it is difficult to understand its sizable concentration in the cells. Possibly this may have to do with its small diameter (0.67 Å), comparing with others like K^+(1.33 Å)Ca^{++}(0.99 Å), Na^+(0.97 Å). The unidirectional flows must await further analysis of the role of intracellular structures which may determine the direction of the flow. In any case the point of view is compatible with various theories of cation permeability (see Refs. 10, 11, 12, 14, 22, 36, 42, 43, 47, 48, 51, 52).

The result of these solvent interactions can be very significant in as much as they can be (i) specific and (ii) physiologically

significant. The specificity can be understood from the following consideration. It is known that 6 to 9 k cal/mole is the energy required for the stability of acyl chains. The solvent interaction may therefore, not be effective at a cerebroside-cerebroside interaction of about 35 k cal/mole but in a nine carbon interaction (8.9 k cal/mole) which is on the border of stability the effect would be microscopic " melting " and raising up of " structure temperature " rendering transformations feasible. The transformation may involve lattice changes leading in some cases to net electric charge on micelles and alteration of spaces between molecules affecting transport properties. Sometimes the transport may be coupled, one ion bringing about a change so that another one can follow either from outside to within the cell or vice versa. Transport of macromolecules may also be understood in terms of the action of segment after segment of the molecule on the dispersion forces in the membrane. The state of water and conformation of covering protein, too, may change leading to physiological consequences. In this manner on the basis of the presence of quantum-mechanically coupled system the photovoltaic effect and photoconduction demonstrated by Tien in bilayer lipid membrane[49] may be easy to understand.

The question of approachability of interaction sites by bigger molecules must also be considered. This is easy to understand in some cases. For example one may ask if the site of interaction of acetylcholine is a choline-bearing lipid with small fatty acid chains. The longest distance in the choline end of acetylcholine is between H_{17}–H_{22} and measures 4.2434 Å.[6,37] This could well be posited between choline residues. The distance between choline when unencumbered by a bulky fatty acid chain can be quite small, as shown in case of glyceryl choline.[1]

In summary, above considerations indicate a fruitful line of attack on the proposition that lipids and their particular state in biological membranes which bears kinship to liquid crystalline state, may at least for analytic purposes and probably in fact, be the organiser of the membranes structure and function and be of important physiological significance.

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