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PHOSPHOLIPID ORGANISATION : HYDROPHOBIC CHANNELS IN BIOLOGICAL MEMBRANES

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The structure of the biological membrane has been discussed. The existence of two types of organisation of lipids namely regular organisation and defective organisation has been postulated. The nature of the defect structures and their biological significance is discussed. The concept of hydrophobic channels has been introduced and their importance is discussed with special reference to the permeability of potassium ion.

INTRODUCTION

IN recent years biological membranes have assumed a great importance because of the realisation that they play significant roles in most of the cellular processes. The cytoplasmic membranes act as selective gateways for transport of substances and thus control the osmotic balance between the interior of the cell (cytoplasm) and the exterior. For example, the concentration of amino acids inside the cell is almost ten times larger than that outside. Many of the chemically modified amino acids are selectively left out during this transport. Within the cell one finds a high K^+ ion concentration and a low Na^+ ion concentration while the extracellular medium has a much higher Na^+ ion concentration. This ionic gradient plays a crucial role in nerve membranes in the generation and conduction of impulses (Hodgkin & Huxley, 1952). The mitochondrial membrane is the site of oxidative phosphorylation. Photosynthesis in plants takes place at the chloroplast membranes. In this paper the structure of the biological membrane has been analysed in greater detail and its biological significance has been discussed. The presence of some hydrophobic channels in the membrane for the transfer of various substrate molecules across the membrane has been postulated.

STRUCTURE OF BIOMEMBRANES

It is now known that all biomembranes, no matter what the source is, no matter what the functions they perform, have similar composition, in the sense that lipid and protein are their major constituents. The relative proportions, however, vary depending upon the nature of the function the membrane performs. In addition to lipids and proteins, the membrane contains various small molecules which form a very small fraction of the membrane. Proteins in the membranes have not been well characterised. However, it is believed that proteins are of two types viz., peripheral proteins which lie at the surface of the membrane being loosely bound to it and structural proteins which span the entire membrane and are tightly bound to it. On the other hand it is known that lipids are essentially of three types viz, phospholipids like various lecithins, phosphatidyl ethanolamine phosphadidyl serine etc., neutral lipids like cholesterol and glycolipids like cerebrosides. Phospholipids constitute large proportion of the total lipid content. Most of the lipids have two long non-polar hydrocarbon chains and a short polar chain. The hydrocarbon chains may be saturated or unsaturated.

Some of the lipids are charged (phosphadidyl serine) while many others are neutral. Some others are Zwitterionic. Thus the biological membranes have lipids of diverse physical properties. It is, therefore, reasonable to expect that they may not mix with each other and may form pools of lipids, each pool containing lipids having similar properties. The various degrees of freedom in these pools may be expected to be different. The organisation of the lipid molecules in different pools may be different.

The various lipids owing to their amplipatic character have a tendency to form different types of structures when they come in contact with water. The structures they form are determined by the relative proportions of the lipid and water. The most common structure they form is the bilayer structure which contains a central core of fatty acids surrounded by two planes of hydrated polar groups in the aqueous environment. X-ray studies in a number of biological membranes have shown that the lipids exist mainly in the bilayer form (Blaurock, 1971; Wilkins *et al.*, 1971; Blaurock & Wilkins, 1972). Thus as a working model for the biological membrane, the bulk of the lipid is assumed to be in a bilayer form and the proteins are assumed to be embedded in the lipid matrix. Some proteins are assumed to span the membrane while others are not. This constitutes the "fluid mosaic model" of Singer and Nicolson (1972). However, as yet there is no conclusive evidence for the presence or absence of proteins spanning the entire membrane.

Physical State of Lipids

There is strong evidence to believe that the lipids in the vicinity of the protein molecules have completely different properties than the lipids in the bulk. The lipids within a certain region around the protein are immobilised. On the other hand, the physical state of the lipid in the bulk is temperature-dependent and can be best understood by the study of phase diagrams. All the lipids exhibit two phases *viz,* gel phase and liquid crystalline phase. In the gel phase, the motions in the lipids are quite restricted while in the liquid crystalline phase, the lipid molecules are randomly distributed and possess greater freedom. The temperature at which the transition occurs from the gel phase to the liquid crystalline phase is dependent on the nature of the lipid concerned. The unsaturation in the hydrocarbon chains, the charge density in the polar groups are known to have significant effects on the transition temperatures. This implies that in the biological membrane where a wide variety of lipids are present, it is probable that regions of gel phase and liquid crystalline phases coexist, the two phases consisting of different types of lipid molecules. Such an inhomogeneous distribution of lipid molecules has been demonstrated in bacterial systems. For example, pulse labelled radioautographic studies of exponentially growing cells of *Bacillus megatesium* using radioactive palmitic acid show a highly non-uniform distribution of radioactively labelled phospholipids (Morrison & Morowitz, 1970).

Information about the motional digrees of freedom of the lipid molecules in the bulk has been obtained by ^{13}C, ^{1}H NMR (Levine *et al.*, 1972) and spin label ESR (Devaux & McConnel, 1972) experiments. It has been shown that there are three kinds of motions possible: (1) Rotational motion around the various bonds; (2) lateral, diffusion of molecules; and (3) flip-flop or the diffusion of molecules from one side of the membrane to the other. Of these, the flip-flop motion has been found to be the slowest having half-life of the order of several hours. The lateral diffusion of

molecules is sufficiently fast, the diffusion constants being of the order of 10^{-8} cm^2 sec^{-1}. As regards rotational motion around the bonds, these experiments have shown that a motional gradient exists along the fatty acid chains and also in the polar chain. The ^{13}C data for lecithins in the liquid crystalline phase indicate that the motion increases from the glycerol backborne of the lipid both towards the terminal methyl of the fatty acid and towards the NMe_3^+ of the head group (Levine *et al.*, 1972). Measurement of three bond coupling constants of protons with protons or ^{13}C atoms or ^{31}P atoms in different solvents give exact informations about the conformationl flexibilities around various bonds and also about the population distributions in these conformational states. For example, in the polar group, the conformation around C—C—O—P bonds has been found to be trans (Birdsall *et al.*, 1972).

Organisation of Lipids

The organisation of the lipid molecules will be different in the gel phase and in the liquid crystalline phase. In the gel phase where the mobility of the molecules is less, they will be arranged in a definite order and there will be cooperativity among the lipid molecules. In the liquid crystalline phase the packing is slightly looser, but even here there is considerable order and co-operativity among the lipid molecules at the lipid-water interface and one can certainly think of definite organisation at the interface. Over the boundaries between the gel and liquid crystalline phases, there will be disorder in the organisation of the molecules. Organisation of the lipid molecules requires the knowledge of their conformation. Since there is a large amount of conformational flexibility in the lipid molecules, the organisation of the lipid molecules will also be dynamic, but the general features of the organisation will be same at any instant of time. Lateral diffusion of lipid molecules adds to the dynamic nature of organisation but is not expected to alter the general features of organisation.

The conformations of the phospholipid molecules have been determined both theoretically (Gupta & Govil, 1972; Gupta *et al.*, 1975; Pullman & Saran, 1975) by NMR (Seelig *et al.*, 1976) and X-ray (Hitchcock *et al.*, 1974) experiments. There is fairly good agreement between theoretical predictions and experimental observations. It is found that a large number of conformations are possible. These can be grouped into two families 'P' and 'S'. In family P the molecules have ($g^{\mp}$, g^+) conformation with respect to O—P—O bonds in the polar group, while in family 'S' the molecules have (g^-, g^-) conformation with respect to O—P—O bonds in the polar group. Phospholipid molecules in one of these conformations have been organised in the form of bilayer as shown in Fig. 1. This would correspond to the gel phase in the membrane. In the liquid crystalline phase such an organisation of the hydrocarbon chains and polar head groups is not maintained rigidly, but the organisation of the polar groups at the lipid-water interface may be expected to be similar. In Fig. 1, the molecules have been tilted by about 60° so as to maximise the hydrophilic interactions, i.e. no matter whether the conformation belongs to family 'P' or family 'S', the polar group is completely immersed in the aqueous phase. The area on the surface per polar group will be about 60 Å. The interplanar spacing between such layers is about 4.2 Å.

In addition to the regular arrangement of the polar groups as shown in Fig. 1, there can be defective organisation of the polar groups, wherein, the molecules are

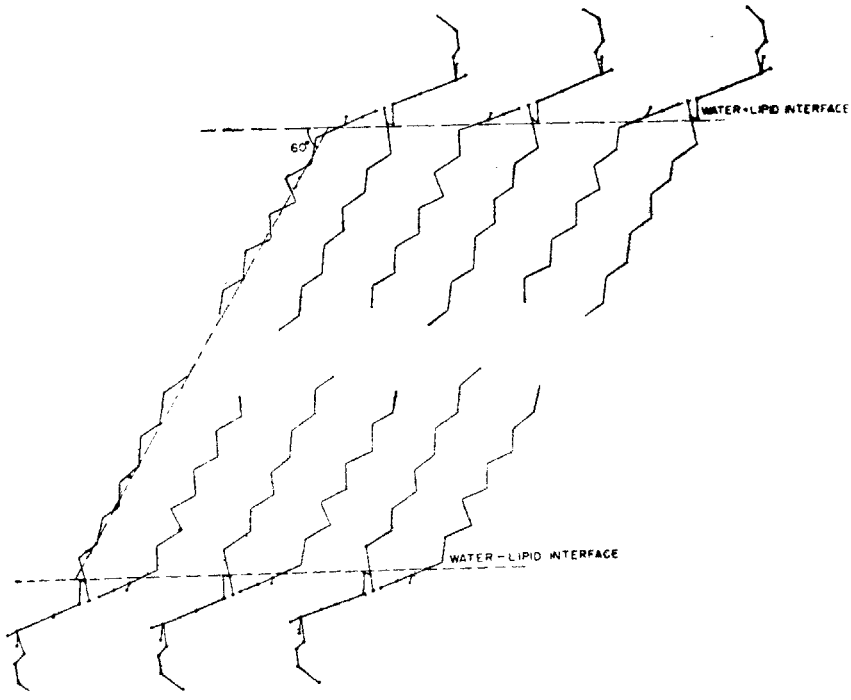

FIG. 1. Regular organisation of the phospholipid molecules in the form of a bilayer. This packing of hydrocarbon chains corresponds to the gel phase.

rotated with respect to one another by different angles. This leads to a greater separation between polar groups. The charge distribution at such places will be different from that in the regular organisation. These defects may play significant roles in many of the biological functions. They may act as vacancies and facilitate the lateral diffusion of molecules. They may act as active sites for the adsorption of various substrate molecules. The number of such defects will be determined by the free energy change that occurs in going from the regular organisation to defective organisation and is determined by Boltzmann statistics. Thus when the environmental conditions are constant, this number will be determined by temperature alone. Hence, the lateral diffusion of lipid molecules which will only change the location of the defect structure will not alter the total number of the defects.

The defects can be classified in two ways. (1) They can be homogenous or heterogeneous. If they are formed by the same phospholipid molecules, then they are homogeneous; otherwise they are heterogeneous. In the latter case, the lipid molecules must have similar physical properties so that they exist in the same island. (2) They can also be grouped on the basis of the number of lipid molecules involved in the formation of the defect. The area of the defect depends upon the type of the defect and also on the conformations of the molecules forming the defect. For a given type of defect the size of the defect will be larger if the molecules are in an extended conformation. As illustrations, Figs. 2 and 3 show the defects formed by three phospholipid molecules rotated with respect to one another by 120° and 240°. In these

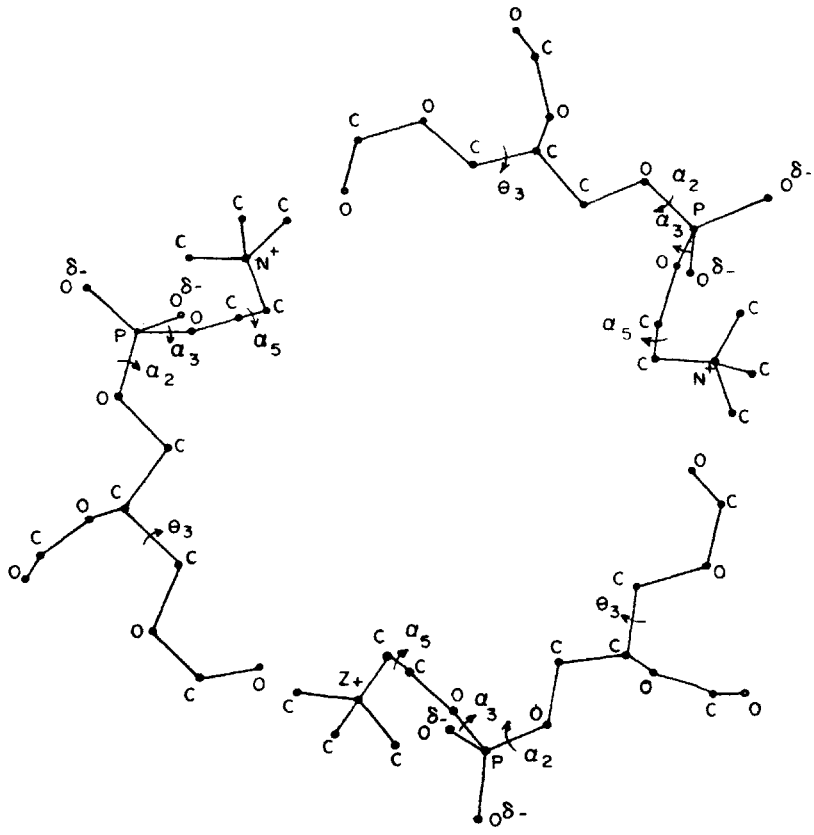

FIG. 2. Defective organisation of three phosphatidyl choline molecules.

figures, the change in the size that occurs due to a conformational change in all the three molecules is noticeable.

Thus we see that the different lipids and the proteins form a very heterogeneous structure in the membrane. The other small molecules like ATP and various carrier molecules which are also present in the membrane, or the adsorbed substrate molecules, do not see the same environment in the membrane and hence will not be randomly distributed. In other words they see definite but different potential profiles in different directions. A schematic diagram of the potential profile that would be seen by a diffusing molecule on the surface is shown in Fig. 4. The figure shows potential energy contours of the diffusing molecule. These contours are also the probability diagrams in the sense that along each contour the probability of finding a molecule at any point on the contour is the same. The magnitude of the probability will be determined by the magnitude of the potential energy. Lower the potential energy, higher will be the probability and vice versa. It is always customary to draw contours only up to a certain level of potential energy since higher energy contours would any way correspond to almost zero probabilities. There are certain regions in Fig. 4 containing clusters of contours and these are the regions where the probability of finding the molecule is very high. These may correspond to certain

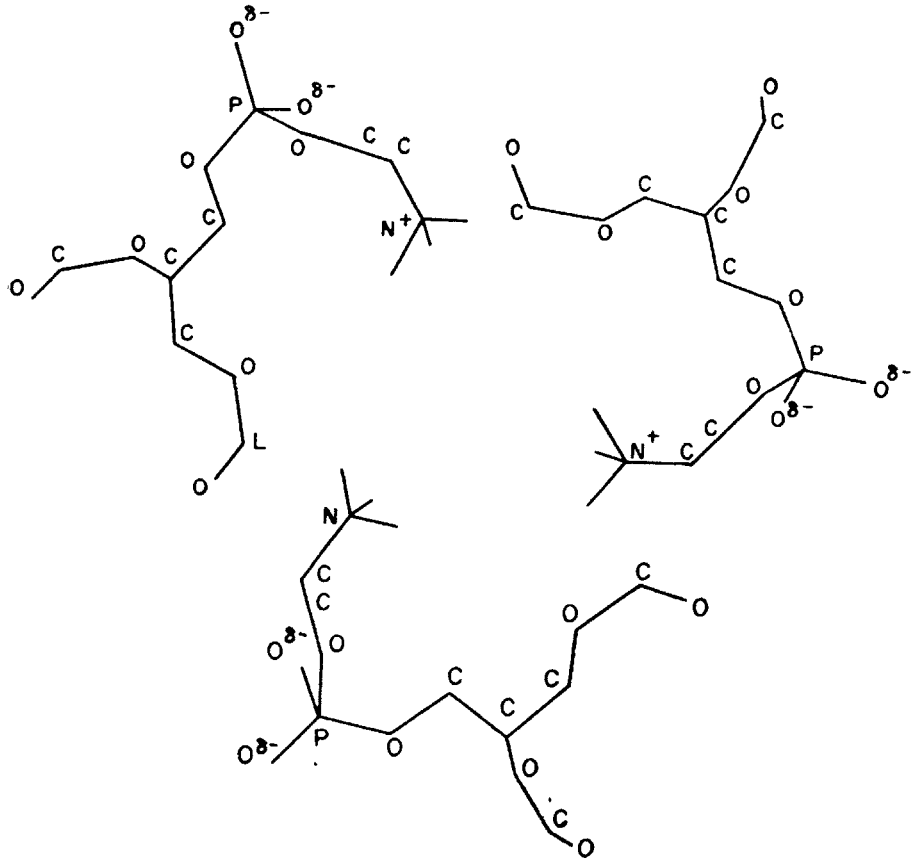

FIG. 3. Defective organisation of three phosphatidyl choline molecules in another conformation (*see text*).

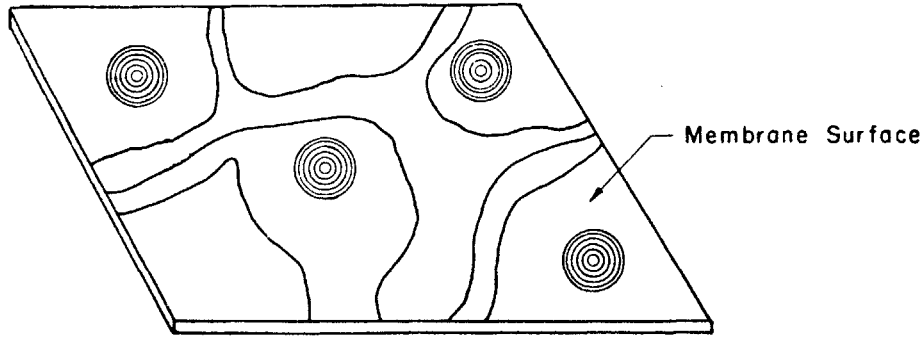

FIG. 4. Schematic diagrams of the potential profiles a molecule diffusing on the surface of the membrane would see.

binding sites or active sites of adsorption of the substrate molecules. It may be necessary to mention here that owing to the fluidity in the membrane these contours will be continually changing with time, but the gross features like the number of binding sites or active sites will remain unaltered. External forces like electric field across the membrane, *p*H etc., also influence these contour diagrams.

Transport of Substances across the Biological Membrane

The transport process of substances across the membrane involves in general three steps: (1) adsorption of the substance on the surface of the membrane; (2) lateral diffusion of the adsorbed molecule to a suitable site; and (3) translocation from one side of the membrane to the other.

Adsorption of the molecules occurs at certain active sites on the surface of the membrane. The nature of the active site for a given molecule will be determined by the charge distribution in the molecule. The active site can be in the proteins or in the lipid portions. In the latter case, the defects in the lipid organisation are one of the likely candidates for the active sites. Once the molecule is adsorbed on the surface, there are three competing processes that can occur: (*i*) the molecule may be desorbed; (*ii*) the molecule may be transported across the membrane at the same site; and (*iii*) the molecule may diffuse laterally to a different active site.

The fastest of these will dominate over the others. The rate of transfer across the membrane at any site will be determined by the potential profile the molecule would encounter while travelling across the membrane at that particular site. The rate of lateral diffusion will be determined by the rates of changes of potential profiles on the surface i. e., by the fluidity of the membrane. The rate of desorption will be determined by the strength of binding at the active site. All these phenomena are schematically shown in Fig. 5. Those active sites in the lipid portion of the membrane at which the substrates are transported across the membrane have been referred to as "hydrophobic channels" in this paper, in contrast to the "hydrophilic channels" that have been postulated to be existing in some of the proteins. These names are suggestive of the environment all along the channel, the molecule being transported would see. Thus

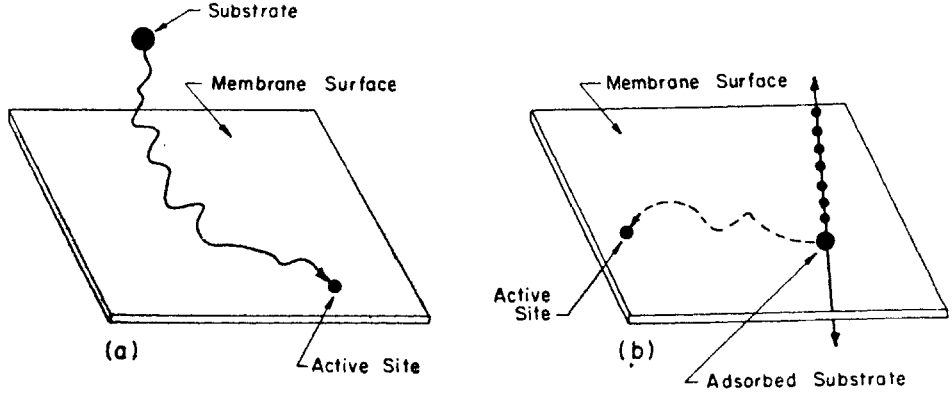

Fig. 5. Steps involved in the transport process *a*. Adsorption of the substrate molecule on the surface, *b*. Lateral diffusion of the adsorbed substrate (.....), transport across the membrane (———), and desorption (—.—.—.—.—).

hydrophobic substrate molecules would prefer to cross the membrane through hydrophobic channels rather thanthrough hydrophilic channels and viceversa. Of particular importance among the various substrates are the K^+ and Na^+ ions which are known to play very important role in the generation of impulses in nerve membranes. It is believed that there are two mechanisms by which the ions can cross the membrane by passing through the hydrophilic channels. This is the simplest mechanism one can sepeculate. This suggestion is based on the observation that certain polypeptides like gramicidin A, alamethicin which increase the ionic conductivities of lipid bilayers are known to form channels across the membrane (Urry, 1971). Alternatively, the ions can form complexes with some neutral carrier molecules and these ion-carrier complexes which have hydrophobic outer surface might travel through the hydrophobic channels. These carrier molecules are believed to be shuttling across the membrane, continuously transferring the ions down the concentration gradient of the ions. This mechanism has been suggested on the basis of the observation that certain antibiotics like valinomycin, nonaction etc., which also increase the K^+ permeability of lipid bilayers behave as mobile carriers (Shemyakin *et al.*, 1967, 1969). The details of these mechanisms may be found in the review article by Hladky *et al.* (1974). In this paper, the carrier mediated transport mechanism has been analysed in greater detail.

The carrier molecules are an integral part of the biological membrane. The transport of the metal ions by these carrier molecules involves the following steps in sequence :—

(1) Formation of the carrier ion complex.
(2) Diffusion of the complex from one side of the membrane to the other.
(3) Dissociation of the complex.
(4) Diffusion of the carrier back to the other side of the membrane.

It is believed that the 2nd step is the slowest and hence the rate determining step in this process. It is possible to throw more light on the process by discussing it from the point of view of potential profiles. The potential profiles, the ion, the carrier, and the carrier-ion complex would see on the surface of the membrane will be completely different i.e., the probabilities of existence for these three species will be different at any place on the surface of the membrane. In other words the active sites for these three species will be different. The carrier molecule on the surface might itself act as an active site for the adsorption of the ion. Once the carrier-ion complex is formed, the complex could diffuse to a site where the probability of its existence is high, if this site happens to be a channel for the transport of the complex then the complex will be transported across the membrane. Since the channel can be considered as a deep potential well for the complex, the complex would always diffuse to a channel. All these processes have been schematically shown in Fig. 6. When the complex dissociates on the other side of the membrane, the free carrier molecule will diffuse back through a different region in the membrane. Thus the regions of diffusion of the carrier-ion complex and the free carrier molecule in the membrane will be spatially different. If by some external forces it is possible to alter the number of channels for the carrier-ion complexes, then the ionic permeability mediated by that particular carrier molecule will be dependent on those external forces. The electric field changes, *p*H changes etc., which cause conformational changes in the molecules, and thus cause changes in the organisation of the molecules in the membrane are likely to bring about such

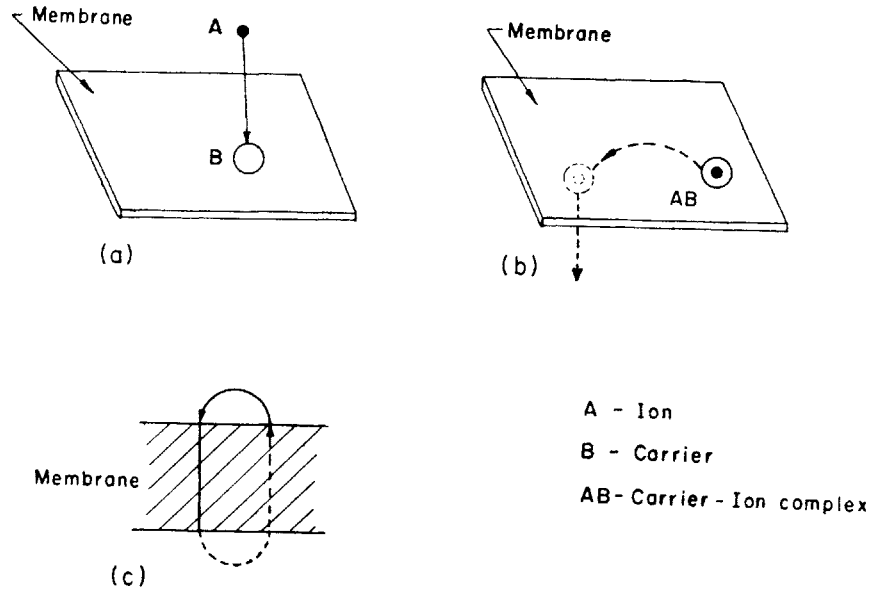

FIG. 6. Steps involved in carrier mediated transport of ions, *a*. Formation of carrier-ion complex. *b*. Lateral diffusion of the complex to a channe, *c*. Complete transport process indicating the spatial separation of regions of carrier-ion complex diffusion (—) and free carrier diffusion.....).

an effect. Such an effect of the electric field across the membrane has been demonstrated in one of our previous papers (Hosur & Govil, 1977). The main points will be briefed here.

The defect shown in Fig. 2 has been postulated to be a channel through which a carrier—K^+ ion complex would pass through, and has been called as a "hydrophobic potassium channel." This channel has been formed by three lecithin molecules. However, a similar channel can be formed by three phosphatidyl ethanolamine molecules as well, conformational changes in the phospholipid molecules forming the channel will lead to changes in the size of the channel or closing of the channel as shown in Fig. 3. Thus the number of open channels will be dependent on the factors which cause these conformational changes. Since phospholipid molecules are dipoles, the electric field across the membrane changes the energies of the phospholipid molecules through dipolelectric field interaction and thus causes a change in the conformation. Thus the fraction of the channels that are open and hence the permeability of K^+-carrier complex specific for this channel would be voltage dependent.

The effectiveness of the electric field changes in bringing about conformational changes is determined by the magnitudes of the dipole moments of the molecules and their orientation with respect to the electric field. For a given conformational change or transition, the effect will be more if the difference between the components of the dipole moments, perpendicular to the surface, of the two conformations involved, is large and vice versa e.g., if this difference, represented by $\triangle M$ (say) is 20 debyes, then for a 10 mV change in the potential across the membrane, the interaction

energy change is about 0.11 kcal/mole. This is a significant quantity compared with the thermal energy and hence the thermal forces will bring about a change in the populations of the two conformational states.

The dipole moments of the conformational families 'P' and 'S' of the phospholipid molecules have nearly opposite orientations and also the magnitudes of the dipole moments are of the order of 15–20 debyes. (Theoretical calculations on phosphatidyl ethanolamine, unpublished work). Thus the electric field changes across the membrane would be more effective in causing conformational transitions between these two families rather than within the same family itself. Two conformations represented as I and II have been chosen as representatives of families P and S respectively for our study. These have the following sets of torsional angles:—

I. $\theta_1=60°$, $\theta_3=60°$, $\beta_1=150°$, $\alpha_1=180°$, $\alpha_2=60°$, $\alpha_3=60°$, $\alpha_4=180°$, $\alpha_5=60°$.

II. $\theta_1=300°$, $\theta_3=180°$, $\beta_1=100°$, $\alpha_1=180°$, $\alpha_2=300°$, $\alpha_3=300°$, $\alpha_4=180°$, $\alpha_5=300°$.

The notation and the nomenclature of the torsional angles is the same as in our previous papers (Gupta *et al.*, 1975; Hosur & Govil, 1975).

Since the hydrophobic potassium channel shown in Fig. 2 is formed by three phospholipid molecules, the conformations I and II of them can give rise to four shapes of the channel. These correspond to the following four arrangements of the three molecules:—

(1) All the three molecules are in conformation I (Fig. 3).
(2) Two molecules have conformation I and one molecule has conformation II.
(3) Two molecules have conformation II and one molecule has conformation I.
(4) All the three molecules are in conformation II (Fig. 2).

Only the arrangement (4) has been postulated to be the conducting channel. These four arrangements will be in equilibrium as shown below :

$$(1) \underset{K'_1}{\overset{K_1}{\rightleftharpoons}} \cdot \qquad (2) \underset{K'_2}{\overset{K_2}{\rightleftharpoons}} \qquad (3) \underset{K'_3}{\overset{K_3}{\rightleftharpoons}} \qquad \ldots(4)$$

K_1, K_2, K_3 and K'_1, K'_2 K'_3 are the rate constants. One can write the following rate equations for the transitions between the four arrangements.

$$\frac{dN_1}{dt} = K_1'N_2 - K_1N_1$$

$$\frac{dN_2}{dt} = K_1N_1 + K_2'N_3 - (K_1 + K_2)N_2$$

$$\frac{dN_3}{dt} = K_2N_2 + K_3'N_4 - (K_3 + K_2')N_3$$

$$\frac{dN_4}{dt} = K_3N_3 - K_3'N_4$$

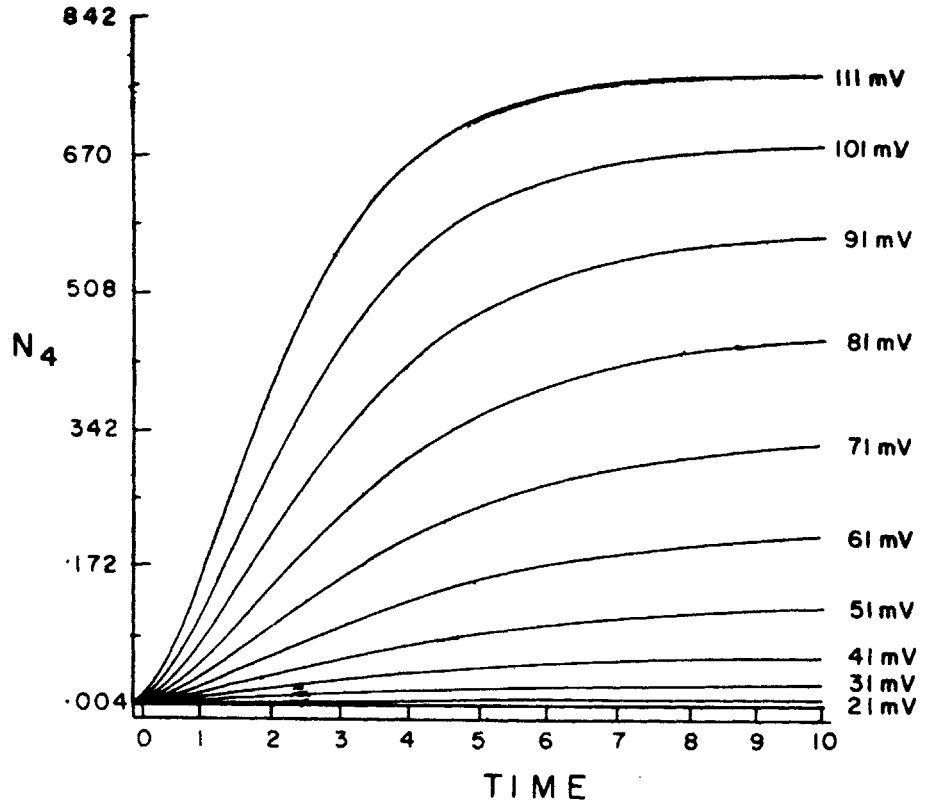

FIG. 7. Fraction of the conducting hydrophobic potassium channels as a function of time for various potential changes (depolarisation) across the membrane. (*see* Hosur *et al.*, 1977).

N_1, N_2, N_3 and N_4 are the relative populations of the arrangements (1), (2), (3) and (4) respectively. These equations have been solved to obtain the steady state and non-steady state solutions for the fraction of the conducting channels. The numerical evaluation of these quantities requires the knowledge of energy difference $\triangle G$, between the two conformations, activation energy $\triangle G^{\ddagger}$ for the conformational change I-II, in addition to the knowledge of the magnitudes and orientations of the dipole moments. Both $\triangle G$ and $\triangle G^{\ddagger}$ are dependent on the potential difference across the membrane since dipole-electric field interaction alters the energies of the two conformations. Numerical evaluations have been done for various values of these dipole moments and for various values of potential changes (depolarisation) across the membrane. Some of these curves are very similar to the Hodgkin-Huxley experimental curves of potassium conductance *vs.* time, in squid axons. These theoretical curves are shown in Fig. 7. The potential change indicated on each curve is the depolarisation of the membrane from a rest potential of 70 mV.

In conclusion one can say that the understanding of the structure of the membrane and its relevance to transport properties is still at a hypothetical stage. To the best of the authors' knowledge, none of the carrier molecules nor the channel forming

protein structures for the transport of metal ions have been identified in the biological membrane.

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