THOUGHTS ABOUT THE MECHANISM OF MEMBRANE MOVEMENTS[1,2]

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INTRODUCTION

The number of properties ascribed to various biological membranes is so high as to throw some doubt on the concept of the "unit membrane" [33]. The original lipid theory derived by Overton [31] from his experiments on the penetration of many substances into plant and animal cells received, some thirty years later, a more detailed description by Gorter and Grendel [20]. They pictured the cytoplasmic membrane as a double layer of lipid.

It was soon realized that this hypothesis explained only one aspect of membrane behavior, namely, the role of the cytoplasmic membrane as a barrier separating the cell from its environment. However, these membranes have other properties which, to all appearances, present contradictory conditions as regards the membrane's structure. To mention but a few: (1) Although many experiments have shown that the cytoplasmic membrane is hardly permeable to ions and hydrophilic molecules, the substances needed for the cell's metabolism all belong to these classes. Thus, one is apt to say the membrane cannot be a continuous lipid structure. At least it must possess specific loci to permit the passage of these ions and molecules. (2) In some cases the membrane exerts considerable movements (pinocytosis and phagocytosis) suggesting a supple and fluid nature. On the other hand, the cells in many tissues form a strong and integrated structure suggesting a robust and tough membrane adhering to the membranes of neighboring cells. This tough membrane must also be postulated when pores

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of a precise and constant diameter are assumed in order to explain the difference in permeability of sodium and potassium ions. (3) Many years ago Danielli and Harvey [17] came to the conclusion that the lipid double layer must be covered with proteins. Later several other types of macromolecules (polysaccharides, nucleic acids) have been supposed to be present at the surfaces of the double layer. It may be surmised that these macromolecules give additional stability to the membrane structure. On the other hand, their presence at the outside of cells poses the question as to their origin and their passage from the inside to the outside of the cell.

Moreover, electron microscopy has shown membranes in many other parts of the cell: mitochondria, endoplasmic reticulum, Golgi complex, etc. As these membranes will have completely different functions it is to be expected that the composition of biological membranes will be characteristic from case to case. The expression "unit membrane" should be seen as a purely morphological term (see also Elbers [19]).

The basic pattern—double lipid layer covered with macromolecular layers—will exhibit very many elaborations in relation to the biological requirements. This fact complicates our problem considerably; in the future we shall seek the specific role of each of the membrane components. The way to this goal will be long and difficult as at this moment we do not even know exactly by what forces the membrane components are held together. When discussing membrane movements this lack of knowledge hampers the progress of our ideas. In the following sections the problem will be approached from the colloid chemical angle. Of course, many scientists have thought about the background of membrane movements. The lack of conclusive experiments has led to a number of flourishing theories in which practically all conceivable mechanisms have been indicated as the source of the movements. Thus it is very difficult to tell a new story; the best we can do is to reshuffle the old cards and play a new game of patience.

THE LIPID DOUBLE LAYER

Let us start by focusing our attention solely on the lipid double layer. Three different approaches have shown that phosphatid double layers may be stable while separating two phases consisting predominantly of water. Bungenberg de Jong and Bonner [6] studied the behavior of phosphatid coacervates. By shaking these coacervates with

the equilibrium liquid very thin but stable membranes will form at the sides of the coacervate drops. The well-known experiments by Mueller *et al.* [30] and by Thompson [38] in which phosphatid double layers were formed from a solution in tetradecane led to membranes which demonstrated a remarkable resemblance to biological membranes. Hoogeveen [24] studied membrane formation of phosphatids in water/tertiary butylalcohol mixtures and found that the tendency to form membranes depends strongly on the properties of the phosphatids (e.g., on the degree of saturation of the carbon chains).

Many years ago Bungenberg de Jong came to the conclusion that soaps and other charged detergents might be regarded as simple models of phosphatids. On the basis of this assumption an extensive program for the investigation of soap coacervates was started (Booij and Bungenberg de Jong [4]). The shape of soap micelles depends on an equilibrium between two opposing forces: the attractive hydrophobic forces between the carbon chains and the repulsive forces between the charged heads. In a normal soap solution the resulting shape is the spherical micelle. Large flat micelles (double layers) will be formed when the charged groups of the soap ions are "neutralized" by microions having a strong affinity for these groups. As in phosphatids the positive and negative groups are practically in equilibrium, the normal shape of a micelle is in this case a double layer (provided the medium is water). In *in vitro* experiments these double layers will agglomerate to myelin tubes. The formal analogy between soaps and phosphatids is stressed by the possibility to change the smectic phase of a phosphatid into a coacervate by deequilibration of the charges [8].

In a very interesting study, Lucy [28] has tried to reconcile the ideas of a continuous lipid layer and of the presence of pores permitting the passage of small cations. He suggests that the membrane consists of small globular micelles of phospholipid, about 40 to 50 Å in diameter (Fig. 1). The spaces between the globular micelles (Fig. 1C) provide aqueous pores with effective radii of approximately 4 Å. This value tallies well with the various pore radii found in experiments in Solomon's group [35]. According to these experiments, however, the amount of pores in living membranes is in general much less than would be expected from the scheme given in Fig. 1. According to Lucy, this might be explained by the assumption that the lipids of the membranes can adopt either the configuration of the bimolecular leaflet or that of micelles. The differences between one cell type

and another and the variation in properties of different areas of plasma membrane surface within the same cell may depend upon the differences between the properties of the micellar membrane and the bimolecular leaflet. Rapid transitions to and from a micellar type

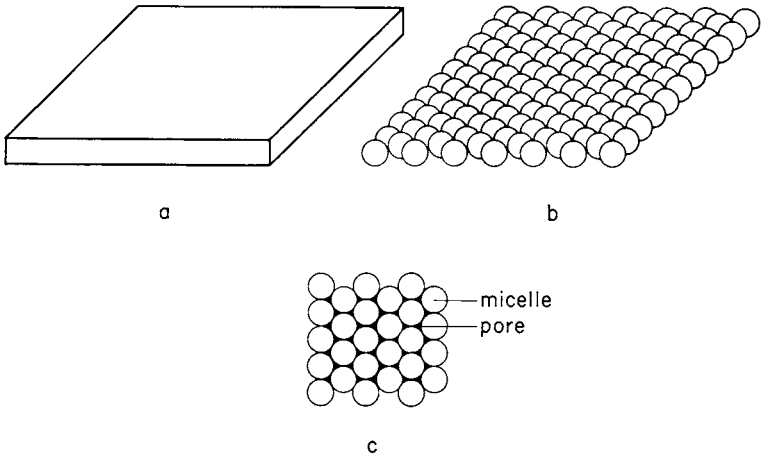

FIG. 1. According to Lucy, the biological membrane might consist of small globular micelles of phospholipids (B) instead of being a continuous double layer of phosphatid molecules (A). Scheme B will show pores with effective radii of approximately 4 Å (C).

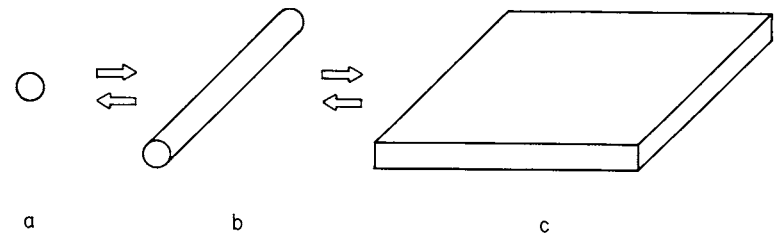

FIG. 2. Depending on the situation, detergent micelles may be globules (A), cylinders (B), or bimolecular leaflets (C).

of membrane would be of great biological value in relation to transportation of macromolecules, permeability, cell adhesion, and mobility.

Going back to our simple model, the detergent micelles, we see that there the transition from double layer to globular micelle proceeds

in stages (Fig. 2). Colloid chemical considerations lead to the expectation that the "micellar membrane" of Fig. 1 is an extreme change in the structure of the bimolecular leaflet, reached via the same kind of stages. In fact, it is likely that we will have to consider at least one stage more, judging from the presence of P- and O-coacervates

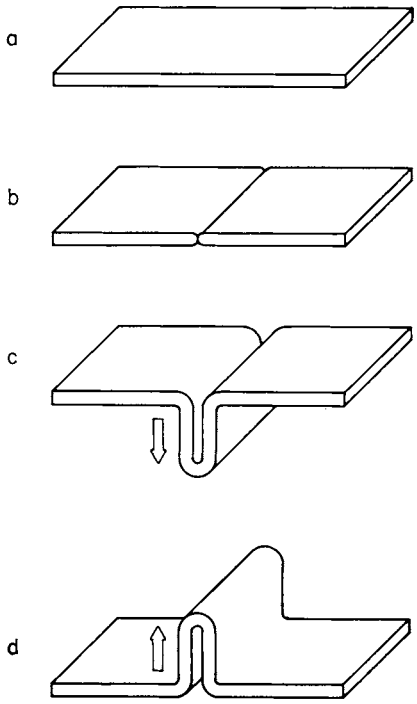

FIG. 3. Colloid chemical considerations lead to the view that the drastic change A → B in Fig. 1 may proceed via various stages. If a loosening effect takes place at both sides of the membrane, a slit may appear (A → B) at the same time. In the case of a one-sided influence, folding of the membrane may be the result (C or D).

in detergent and phosphatid systems [4, 15]. When starting from a densely packed and relatively impenetrable double layer in stage P (see next section) we may expect a number of stages which may be characterized by a reduction of the forces holding the lipid part of the membrane together, thus leading to an increased penetrability and flexibility of the membrane. Though subscribing to the general idea presented by Lucy, we feel that we must look for less drastic changes in the

membrane. We suspect that a change to a completely micellar stage (Fig. 1B) will result in the destruction of the cell. This change might perhaps occur strictly localized and during a very short time.

If we try to apply the findings of the colloid chemistry of detergents to the membrane problem we may expect that a "slit" will arise if a loosening effect takes place on both sides of the membrane (Fig. 3). In general, however, this influence will take place on one side only, presumably resulting in a folding of the membrane (Fig. 3C and D).

In passing it may be said that the phases described by Luzatti and Reiss-Husson [29] are at the other extreme of the scale. These occur only if the water content of the system is very low. Thus they are not very important for the normal biological membrane, but they may be present in the human skin, in insect cuticula [27], etc.

THE ROLE OF MACROMOLECULES AND MICROIONS

It would be extremely helpful for our problem to study the interaction of phosphatids and proteins *in vitro*. The obvious experimental difficulty is that these two kinds of substances are soluble in different solvents. That is why there have been various attempts to simplify the problem by choosing molecules showing some physicochemical resemblance to the membrane constituents. Thus, Bungenberg de Jong *et al.* [7, 10, 11, 12, 13, 14, 16] and Pankhurst [32] studied the interaction between gelatin and detergents, while Booij [3] pursued some relevant detail of Scott's investigations [34] on the interaction between positive detergents and negative macromolecules.

If we add a positive detergent (e.g., cetylpyridinium chloride) to a polysaccharide with a low density of charge (arabinate) a coacervate or viscous precipitate results. The interesting point is that the amount of positive detergent may, depending on the relative concentrations, be much larger than one would expect from the number of negative charges of the macromolecule. The ratio increases with increasing concentration of the detergent until a definite level is obtained [as the electroneutrality must hold, this means that an excess of microanions will be present as gegenions (counterions) in the coacervate]. As the interaction depends strongly on the length of the carbon chain of the detergent we must conclude that some detergent micelle is covered by the macromolecules. In all probability the packing of the carbon chains in the detergent micelle will not show extreme variation under the influence of the bound macromolecules and micro-

anions. Thus we may say—as the ratio detergent/macromolecule can vary strongly—that the amount of macromolecule bound per unit of surface of the detergent micelle (the model of the phosphatid double layer) will show strong variations under different conditions.

Though the degree of packing in the micelle will not vary considerably we may suppose that slight variations in the biological membrane will be of great importance for the penetrability of small molecules (e.g., water) and ions. La Mer [26] showed that a compressed monolayer of saturated long-chain alcohols or fatty acids strikingly retards the evaporation of water. Introduction of a double bond or a methyl group decreases the resistance factor considerably. In the biological membrane we are certainly not dealing with layers of saturated carbon chains only; the layers consist of carbon chains of various lengths and the number of double bonds is considerable. Thus, from La Mer's experiments, we would expect a practically negligible resistance to the penetration of water. It must be stressed that this rapid penetration of water will take place even if the biological membrane is a continuous double layer of lipid.

We know, however, that in many cases the permeability of the biological membrane to water is rather low. To quote a recent example [21]: Under the influence of digitonin the permeability to water of amphibian cells increases strongly, demonstrating that the original permeability to water was low. The conclusion might be that the presence of macromolecules at the surface of the lipid double layer imparts an additional force of the membrane, resulting in a tight packing of the carbon chains. Conversely, any change in the mutual attraction between the macromolecules and the lipid layer will result in a change in permeability, even without changing the general configuration of the bimolecular leaflet. Thus we may expect that under the influence of a factor progressively lessening the macromolecule/phosphatid interaction, the passive passage of water, of the hydronium ions, and finally even of the potassium and sodium ions will set in.

In judging the possibilities of membrane movements as a result of changes in macromolecule/bimolecular leaflet interaction the concept of localized versus nonlocalized adsorption may be useful [23]. Almost all theoretical work on adsorbed monolayers has been on two mutually exclusive models. According to one of these the surface of the adsorbents provides a set of sites for the attachment of the molecules. The term "immobile adsorption," which is sometimes used, is not wholly adequate, because—depending on the circumstances—the

adsorbed molecules may hop from one particular site to another. In the case of nonlocalized adsorption the translational motion of the adsorbed molecules in directions parallel to the surface is practically continuous; here one may assume a free translational motion.

We may picture the two extreme cases with the bimolecular leaflet as the adsorbing surface (Fig. 4) and ask ourselves what will happen if by some influence the ratio macromolecule/phosphatid (the amount of macromolecule bound per surface unit of the phosphatid micelle) changes. In the case of a decrease the simplest result is a liberation of the excess macromolecule from the surface. Here we are reminded of the experiments by Ishihara [25]. He found that fertilization of sea urchin eggs is followed, in addition to the well-known change in permeability, by a release of acid polysaccharides. According to the view described above these two facts might easily be correlated.

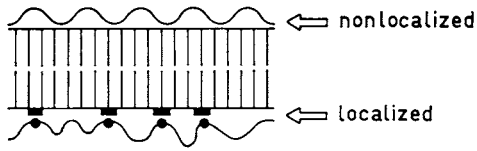

FIG. 4. Bimolecular leaflet covered with macromolecules, adsorbed either nonlocalized or localized.

If, however, the macromolecules form a more or less integrated structure, the result of a change in the ratio macromolecule/phosphatid may be completely different. In the case of nonlocalized adsorption the two layers now must shift in respect to each other in order to reach a new equilibrium. This shift of the layers of the membrane parallel to each other has been called "sliding" of the membrane [2]. Of course, if this sliding is only partially possible one of the layers must fold with respect to the other. The events taking place in the pinocytotic vesicle described by Dr. Marshall in this symposium might be recalled. We saw that 90% of the water in the primary vesicle disappears in a short time; the membrane remains smooth, however, but the slime material originally adsorbed to the membrane is folded considerably.

In the case of strong localized adsorption the situation may be completely different. Here the membrane will tend to retain its original structure. However, as the surface of one layer increases with respect to that of the other, forces will be exerted on the membrane that will lead to a folding of the complete membrane.

In practice, adsorption will nearly always be a combination of localized and nonlocalized adsorption. It stands to reason that in the case of the biological membrane the preponderance of one of these aspects will depend very much on the composition of the membrane. Even for the lipid double layer itself (Fig. 5), there exist many possibilities for localized or nonlocalized adsorption. Thus, on a given phosphatid double layer one macromolecule may be preferentially bound in one way, another in a different way. Parenthetically it may be noted that the two layers of the bimolecular leaflet may slide with respect to each other, as they are bound by nonlocalized adsorption.

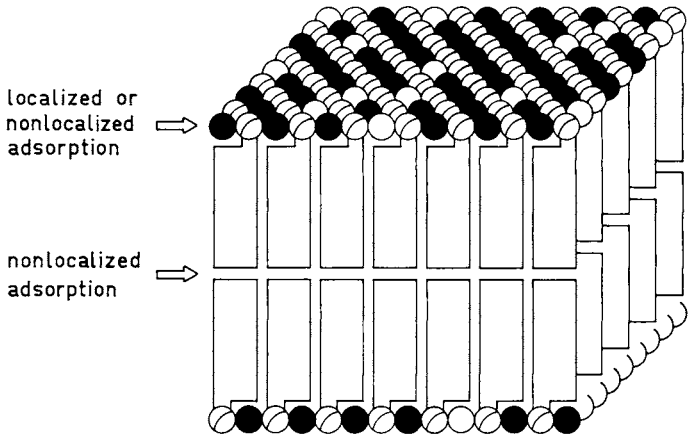

FIG. 5. The phosphatide double layer has a large number of possibilities for localized or nonlocalized adsorption.
(Positive ●; negative ⊖; uncharged ○.)

In our simple detergent/macromolecule model we have seen that a relatively large number of microions will be found in the resulting coacervate. Our experiments demonstrated that the number of detergent ions bound to a given macromolecule depend strongly on the accompanying gegenions (Fig. 6). This influence increases in the sequence (in the case of cetylpyridinium/arabinate interaction):

$$\text{Acetate} < \text{chloride} < \text{sulfate} < \text{bromide} < \text{nitrate} < \text{iodide} < \text{thiocyanate}$$

These ions show the same sequence when the influence on the charge of positive proteins is taken as criterion [5, 37]. Thus the following conclusion seems to be warranted: The stronger the affinity between

positive detergent and gegenion, the more detergent ions will be bound to an oppositely charged macromolecule.

As regards the "binding" of gegenions in the detergent/macromolecule system we may distinguish two types which show some relation to the types of adsorption described in the preceding pages: (*a*) "ionic atmosphere binding," and (*b*) "site binding." The first is the effect of the large electrostatic field of the macroion (or detergent micelle) on the dissociated ions in its vicinity; the second is the specific association between counterions and the "fixed" ionic groups. Strauss and

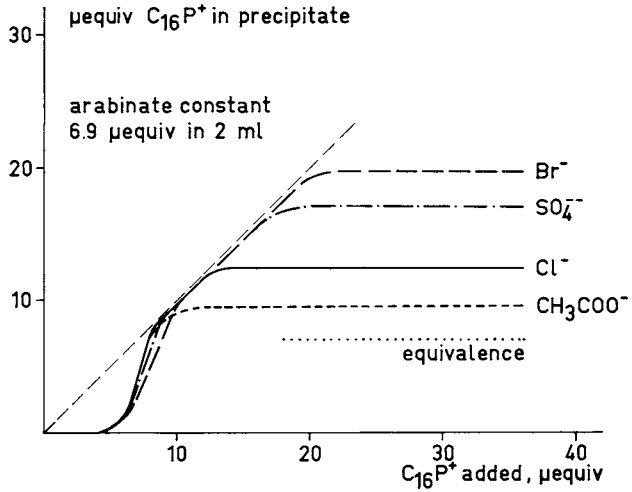

FIG. 6. The maximal amount of cetylpyridinium ions bound to arabinate depends very much on the accompanying anions.

Yuen [36] have shown by dilatometry experiments that site binding is accompanied by the release of water molecules from the solvation shells of the participating species. This phenomenon may serve as a useful criterion for characterizing site binding, as ionic atmosphere binding can be expected to leave the solvation shells intact.

The characteristic difference between P- and O-coacervates has never been elucidated. The experiments clearly show that the surfaces of the P- and O-micelles have different properties—only P-coacervates give rise to the occurrence of membranes on shaking—though in both cases bimolecular leaflets seem to be present. The fact that an O-system may be transformed into a P-system (via a phase transition)

by the addition of a not too short alcohol leads to the idea that the ionic groups in the O-system are bound by ionic atmosphere binding, while in the P-system site binding (with loss of solvation shells) occurs. The surface of the latter system will be less hydrophilic, and it is interesting to note that only this system forms very thin membranes on shaking.

In the biological membrane (Fig. 7) the core is formed by phosphatids, sterols, and other lipids. It is unlikely that this double layer has in itself a strong resistance against penetration of water. The macromolecules covering the double layer together with the surface of the lipid micelle provide a specific possibility for cation binding. Depending on the type of cation and the texture of the surface, this

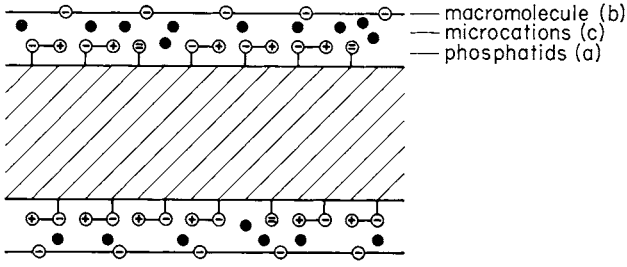

FIG. 7. The three kinds of components of a biological membrane are (a) the core formed by phosphatides, sterols and other lipids, (b) macromolecules covering this double layer, (c) cations, bound either by ionic atmosphere binding or by site binding.

binding will in many cases be site binding. Then the carbon chains in the micelle will be packed very closely together, thus strongly decreasing the water permeability. Antagonistic actions of calcium and potassium ions on the permeability to water might find their explanation along the lines developed above, as calcium is bound much more strongly to phosphate groups than potassium. In this volume Dr. Robertson gives an interesting example of his experiments on swelling of mitochondria.

MEMBRANE MOVEMENTS

Many years ago Bungenberg de Jong and Dekker [9] observed characteristic movements in a gelatin/arabinate coacervate placed in a dc electric field. The same kind of movements come to the fore in a phosphatid coacervate. An analysis of the driving force of these

movements showed that the interfacial tension between coacervate droplet and medium plays a decisive role. This interfacial tension in a "neutral" gelatin/arabinate coacervate is surprisingly low [18]. When an excess of gelatin or arabinate is present the interfacial tension drops considerably. The addition of a neutral salt (e.g., KCl) also decreases the interfacial tension. In an electric field the equilibrium between the positive and negative macromolecules will be disturbed and the resulting decrease in interfacial tension will cause movement of the surface. The same phenomenon will be found in a KCl diffusion field.

If the idea of site binding may be applied to the biological membrane one might suggest that this type of binding is different on the inside and the outside of the membrane. This results in two different phase boundary potentials at both sides of the membrane. It would not be surprising if the total potential, which is practically always called a membrane potential, is in reality the sum of two phase boundary potentials. Let us now suppose that in such a membrane a one-sided disturbance of the equilibrium of charge takes place. This leads to a decrease of the interfacial tension and a movement of the membrane (or a part of the membrane in respect to another part) will be the result. Moreover, as in general the site binding will also decrease, the phase boundary potential changes. Thus, movement of the membrane will in many cases be associated with potential differences along the membrane (see also Bingley and Thompson, [1], who studied bioelectric potentials in relation to movement in amoebae).

In the case of nonlocalized adsorption of the double layer to a more or less tough outer structure (e.g., composed of proteins and polysaccharides) movements of these two layers in respect to each other seem possible. Cyclosis in plant cells might be cited as a likely example of this kind of mechanism, as in this case the velocity of streaming is highest near to the cell wall. In general the study of flow profiles will be more rewarding than the study of flow velocity.

It might even be possible that the propagation of an impulse along a nerve or muscle can be seen in the light of these ideas. Suppose we start with a membrane where the layers cannot move easily with respect to each other. The cations are bound by site binding (the situation will be the P-stage). An applied potential will, if high enough, pull these ions from their sites, provoking a strong local disturbance in the membrane, perhaps even leading to an area of globular

micelles. Then passive passage of potassium and sodium ions will take place. The neighboring region will quickly change from the P-stage to the O-stage, which will result in a rapid motion of cations along the membrane (compare Aris's findings on interfacial conduction). One might also say that site binding changes into ionic atmosphere binding. The process is, of course, accompanied by rapid changes in phase boundary potential.

In yeast cells Van Steveninck found that the outside can bind a relatively large number of metal ions (e.g., Ni, Co., etc.; see Van Steveninck and Booij [39]). An analysis of the process shows that the metal ions are bound to polyphosphates. The interesting point is that these polyphosphates are directly related to active glucose uptake; each glucose molecule is taken up together with a phosphate monomer. Under the influence of glucose metabolism polyphosphate reappears at the outside of the yeast cell. This means that either the polyphosphate is synthesized in the interior of the cell and then passes to the outside, or that polyphosphate is synthesized *in situ* at the outside of the cell. In the latter case adenosine triphosphate (ATP) must pass to the outside [and adenosine diphosphate (ADP) will return to the interior]. In both cases rather large and strongly hydrophilic substances pass from the inside to the outside and vice versa. It would be extremely unlikely for these molecules to pass the double layer of lipids. On the other hand, a passage through pores seems equally impossible, as these specific pores should have a large diameter. This poses a general problem, as macromolecular material in all types of cells is deposited on the outside (antigens, enzymes, structural proteins, and polysaccharides).

We have seen that disturbance of the equilibrium of charges at the outside of the membrane may lead to a sliding of the membrane layers with respect to each other. In many cases folding of the lipid double layer will be the result. If the inner layer is able to bind several substances by localized adsorption these substances pass to the "interior" of the cell on folding of the inner layers (Fig. 8). The folds may be pinched off, and the substances bound to the phosphatid double layer eventually reach the cytoplasm. The reverse process may bring material (macromolecules included) to the cell's surface. The proposed mechanism greatly resembles pinocytosis, but the important difference is that in pinocytosis the membrane behaves as a unit, while in the "sliding membrane hypothesis" the layers in certain cases may move in respect to each other. In this view the moving

part may contain carriers for active transport. In the customary carrier hypotheses of active transport loaded carriers pass either through the lipid double layer or through specific pores. In the first hypothesis it is very difficult to see how the rather large hydrophilic part of the carrier passes the hydrophobic layer (as a large number of hydrogen bonds must be broken); in the latter hypothesis pores of incredible diameter must be assumed.

From the preceding pages it will be clear that membrane movements in general require energy. That this energy will be supplied as energy-rich phosphate is obvious. On might suppose that a myosin-like contractile protein plays a role, but the difficulty is that such

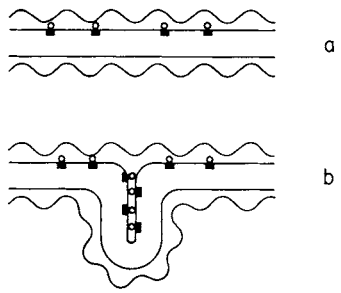

FIG. 8. Substances bound by localized adsorption to the lipid double layer (a) may be brought to the "inside" of the cell by a process of sliding of the membrane layers with respect to each other (b).

a protein must be anchored to other proteins in a very definite structure. This condition seems to be contradicted by the noted flexibility of the membrane. As we have seen before, changes in the charge pattern may lead to membrane movements. The easiest way to change the charge pattern is, of course, by phosphorylation of lipid components of the membrane. This reminds us of the experiments by Hokin and Hokin [22]. Though refuting their hypothesis that the phosphorylated product (phosphatidic acid) is the specific carrier of sodium ions, we have the feeling that these experiments are of great general importance. The generation of phosphatidate and other negatively charged phosphatids in the membrane means that the charge pattern is changed; this reaction must be seen as one of the motors for membrane movements. Of course, it must be expected that this reaction and other phosphorylations of membrane constituents will be characteristic for all kinds of active membrane movements and transport.

As phosphorylation reactions call for enzymes we must suppose that these reactions take place only under the influence of specific "trigger substances," which start the membrane movements (see Dr. Crane's chapter). The type of movement will be determined by the composition of the membrane, the ratio between localized and nonlocalized adsorption, and the amount of site binding. From the preceding pages we may gather that a colloid chemical study of membrane substances and their interactions will be particularly rewarding in trying to unravel this biological problem.

CONCLUSIONS

These thoughts on membrane movements are based on few definite experiments, but they try to harmonize several seemingly conflicting ideas. The aim has been to show that specific functions of biological membranes may be understood without forsaking the general idea of a double lipid layer covered on both sides with macromolecules. This general idea leaves a wealth of possibilities as regards the details of composition and these structural details define the reaction of the membrane to a certain stimulus. It is suggested that the layers of the membrane are more or less independent, though executing a profound influence on each other. In the case of nonlocalized adsorption the layers may slide in respect to each other, and this eventually may lead to folding of one of the parts of the membrane.

Another useful concept is that of site binding—specific binding of ions accompanied by the release of water molecules from the solvation shells of the participating ions and ionic groups. It is supposed that most biological membranes show a large amount of site binding, which results in a tight packing of the carbon chains and consequently a low permeability, even to water. The *in vitro* model for this situation would be the P-coacervate of association colloids. In the O-coacervate ionic atmosphere binding would be predominant. A change from the P-stage to the O-stage (e.g., provoked by ions) would lead to an increase in permeability.

Experiments on coacervate drops led to the conclusion that disturbance of the charge equilibrium causes a decrease of the interfacial tension and consequently to movement of the surface. There are several ways in which the charge equilibrium in biological membranes may be disturbed, for example, exchange of ions (calcium/potassium antagonism), phosphorylation of membrane constituents, etc. Then movements of the total membrane or parts of the membrane or ions

in the membrane would result. The type of movement depends on the structure of the membrane and the type of stimulus.

Thus, according to this concept, a biological membrane is not only a selective barrier. It must be seen as a living organelle of high complexity and mobility, varying from cell to cell, possessing its own enzymes, some of which (permeases) are engaged in active uptake of substances, and having numerous different functions.

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