

60 Lipid Bilayers as Models of Biological Membranes

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Real membranes

WHEN WE TALK about biological membranes, we must recognize, first, their unity in some general features and, second, their enormous diversity in many specific features.

The most obvious aspects of unity are their thickness, around 70 Å; their composition, protein and lipid; the barrier they present to permeation; and the property of fragments of membranes to round up into vesicles and of holes to seal.

The most obvious features of membrane diversity are variations in thickness; great variations in the protein-to-lipid ratio; great variations in the kinds of lipid and kinds of protein; great variations in permeability; and infinite variations in function.

SEVERAL KINDS OF NATURAL MEMBRANES Glancing briefly at the varieties of function, we have, first, a membrane enclosing every cell; this is the *cytomembrane* or *plasmalemma*, which exerts chemostatic controls on the interior and transmits numerous signals. It is characterized by the presence of glycolipids (Rouser et al., 1968). There

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is the *double membrane surrounding every nucleus*. This membrane has, in many cases, been shown to have "holes," seemingly violating the principle that the real membranes minimize their perimeter. The contradiction is, however, only apparent, because the perimeters of these holes are not perimeters of the membrane; they are places at which the nuclear membrane folds over (Figure 1).

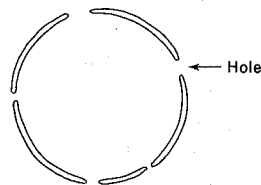


FIGURE 1 Nuclear double membrane with "holes." The perimeters of the holes are not perimeters of the membranes, but are places at which the membrane folds over.

There is the *myelin sheath*, an elaboration of the *cytomembrane* of the glial cells associated with nerve fibers. Perhaps this membrane is the one of least sophistication and the simplest function—that of electrical insulation. Its asymmetry is more conspicuous than that of most other mem-

branes. Because it is multilayered in a very regular way, it has been a favorite subject for physical studies by means of low-angle X-ray diffraction.

There is the *mitochondrion*, with its interesting construction involving two totally different kinds of membrane, the inner one and the outer one. The *inner one* forms the cristae and is perhaps the most complex of all membranes. It is the carrier of the complicated machinery coupling the energy derived from respiration to the synthesis of adenosine triphosphate (ATP). It is, perhaps, unfortunate that such a disproportionately large share of the efforts of the biochemical and biophysical fraternities is spent on this superelaborate structure (and on the analogous chloroplasts), rather than on those membranes that show simpler specializations. The mitochondrion as a whole, as Lehninger explains in his chapter in this volume, is thought to have been derived in a very distant way from originally free-living bacteria, which, in the course of evolving, have become stripped of a great number of functions. The principal argument in favor of this hypothesis derives from some similarities of this inner membrane with the cytomembrane of bacteria. These similarities consist of the following: (1) both are the locus of oxidative phosphorylation; (2) both contain a specific phospholipid, diphosphatidyl glycerol (DPG); and (3) both lack sterols. As to DPG, it is an interesting lipid, which carries two negative charges in close proximity and, therefore, is prone to conformational changes when binding doubly-charged positive ions, such as Ca^{++} (Shah and Schulman, 1965). The absence of sterols is a characteristic that appears to be limited to the inner membrane of mitochondria and, possibly, to the lamellar membrane of chloroplasts (Mercer and Treharne, 1965). All other membranes of plants and animals contain ergosterol or cholesterol, or some closely related compounds.

The *outer membrane of the mitochondrion* is perhaps a close relative and an elaboration of the *endoplasmic reticulum*, the locus of many synthetic activities in the cell. Both the outer membrane of the mitochondrion and the endoplasmic reticulum are characterized by the absence of glycolipids.

In a different direction of sophistication, we should mention the excitable membranes of the *nerve axon*, of the *muscle fiber*, and of their *synaptic specializations*. Here the most conspicuous aspect is the high degree of modifiability, in an all-or-none fashion, suggesting a very high degree of cooperativity between the elements of the membrane. It seems probable to me that the changes in state observable in these types of membrane are analogous to phase transitions of thermodynamics. These phase transitions, here occurring in two-dimensional objects, would be governed by somewhat different rules from those of ordinary thermodynamics. The rules have, as yet, been incompletely worked out. An important specific model for the functioning of nerve excitation has been presented (Adam, 1970).

Finally, we should mention the most "clever" membranes, which can be found in the *specific portions of sensory cells*, where they are attuned to the transduction of various kinds of stimuli. These systems can be triggered to change their state and thus give a macroscopic response by absolutely minimal inputs. The input may be a quantized one—a single molecule or a photochemical change in a single molecule, in olfaction and vision, respectively—or it may involve minimal changes in some continuous parameter, as in mechanical, electrical, or thermal stimuli.

PERMEATION Let us stick to the simpler aspects and concentrate on permeation. Here we owe to the work of physiologists (Stein, 1967) a general classification:

Simple permeation, largely determined by the partition coefficients of the solutes between aqueous and lipid phases, and by diffusion within the lipid phase.

Facilitated permeation, involving carriers or pores or, perhaps, as we shall see, in some cases "carried pores." This kind of permeation is characterized by the phenomena of saturation and competition.

Next in sophistication we have *coupled carrier transport*, involving cotransport or countertransport of two species owing to the fact that the mobility of the carrier depends on more than one ligand. This kind of transport can cause motion of one component against its electrochemical gradient, by coupling it to the transport of another component that moves with its gradient.

We must and do have, in addition, *primary active transport* coupled to energy consumption in a manner that is not completely understood in any one case. Perhaps the closest to a complete understanding has been achieved in sugar transport in bacteria, in which it turns out that the transported species is a transiently phosphorylated one (Kaback, 1968).

The approach through the study of lipid bilayers

NAKED MEMBRANES It will take many years and many lines of attack for us to understand the structure and mode of function of all these membranes in their molecular details. The approach that I discuss here is that which starts out with lipid bilayers between aqueous phases and advances by modifying these bilayers in various ways, leading to simulation of some of the functions mentioned. This approach was pioneered by Mueller and Rudin, and to them we owe also the majority of the very interesting modifications of the properties of simple bilayers that can be obtained with the help of a variety of additives.

The simple bilayer is a miraculous structure in itself. It can be formed by smearing a lipid mixture across a hole in a partition separating two aqueous solutions. Under appropriate conditions, the nature of which is not clearly

understood, these lipid mixtures thin out to "black membranes," truly bimolecular structures in which the polar heads of the phospholipids face the water phases, and the hydrocarbon chains form a somewhat disordered and liquid layer about 50 Å thick. The disorderliness is an essential feature. It is generally assured, at body temperature, by there being a sufficient number of kinks, cis-double bonds, in the hydrocarbon chains. Also necessary for good membrane formation is the presence of cholesterol or tocopherol or tetradecane or some such neutral compound, for reasons not clearly understood. The membrane part is surrounded by a rim of lipid in the "bulk phase." The structure of this bulk phase has never been characterized. To which of the several possible lipid-water phases (Luzati, 1968) does it correspond? Or does it retain enough solvent to remain a disordered liquid?

This form of lipid bilayer is under tension, and is mechanically in a metastable state. When the membrane tears, the lipid is swallowed up by the bulk material along the rim of the hole across which the membrane is formed. It does not easily break because a relatively large activation energy is needed to start a tear. The liquidity of the hydrocarbon middle part is essential for giving the membrane plasticity. If the membrane is too stiff, it becomes mechanically fragile. One should appreciate the astonishing disproportions of such a membrane: a membrane 1 mm in diameter and 70 Å in thickness, if scaled up, would correspond to a thin piece of paper 30 feet in diameter.

The so-called surface tension (a few dyne cm^{-1}) of such double layers, measured by bowing them out with overpressuring of the liquid on one side, is not an ordinary interfacial tension. It is a measure of the relative energy of the double-layer versus the bulk phase.

Torn membrane joins the bulk phase, which is distinct from natural membranes, which do not ball up on being torn but retain their thickness. They reduce their contour length to zero by forming vesicles. Natural membranes have a *contour tension* rather than a surface tension. By contour tension I mean an analogue to the surface tension of liquids. Liquid droplets minimize their surface area, at constant volume, by rounding up to form a sphere. Liquids do so because each molecule tries to maximize its interaction with neighboring molecules of the liquid, and a molecule in the interior of the liquid has more neighbors, and thus more interaction, than one on the surface. Therefore, the liquid tries to have as few molecules on the surface as possible, i.e., it minimizes its surface area and forms a sphere. Analogously, any molecule in a natural membrane has more neighbors if it is in the interior of the membrane *area* than if it is at the *perimeter*. Therefore, a natural membrane, if it is constrained to be a membrane of constant thickness, will try to minimize its perimeter. It can do so in three-dimensional space by forming vesicles, thus re-

ducing the perimeter to zero. This is a general feature of fragments of real cellular membranes.

Besides the Mueller and Rudin technique, there are several other methods for forming lipid bilayers. Pagano and Thompson (1967) have described a procedure for forming bilayers in a manner analogous to the formation of soap bubbles: the lipid solution is put into the tip of a pipette, overlaid with an aqueous phase, and blown out under water. Large bubbles can thus be formed that have a bilayer around most of their surface and a cap of bulk material at the top (Figure 2). Such bubbles can be kept in suspension

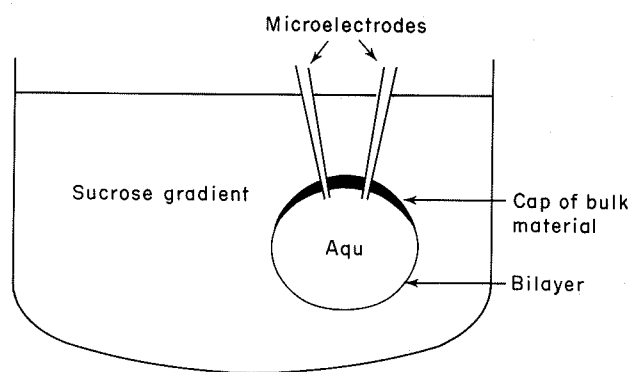


FIGURE 2 Lipid bilayer bubbles (Pagano and Thompson, 1968). The bubble is suspended in a sucrose gradient and carries a cap of bulk lipid solution. The cap can be penetrated by microelectrodes, and the bubble as a whole can be transferred from one aqueous environment to another.

by being blown into a sucrose gradient. The bubbles can be picked up by being sucked into a pipette and transferred to a different environment, and they can be impaled with microelectrodes. In this way, the bubbles can be used for measuring the permeation of tagged ions (^{36}Cl or ^{22}Na), and they can be used for measuring the transference of ions during the passage of electrical current (Pagano and Thompson, 1968; Price and Thompson, 1969).

Another interesting procedure has been described by Tsofina et al. (1966). These authors found it difficult to incorporate proteins into lipid bilayers of the type produced by Mueller and Rudin. On the other hand, it has been known from earlier work that *monolayers* of lipids formed at the interface of air and water or at the interface of an organic solvent and water can be strongly modified by the presence of proteins in the aqueous phase. It has been widely believed that this modification is due to a true incorporation of protein into the monolayer. Therefore, Tsofina et al. proceeded to construct bilayers from two lipid-protein monolayers in the arrangement sketched in Figure 3. Bilayers were indeed formed, but it was not actually

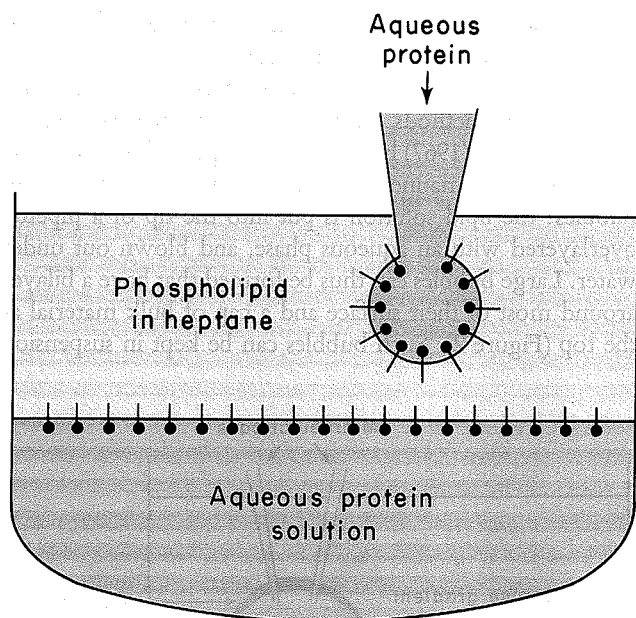


FIGURE 3 Bilayer constructed from two lipid-protein monolayers (Tsofina et al., 1966). One monolayer is formed at the interface of the lower aqueous protein solution (Aqueous) and the benzene layer containing the phospholipid. A second monolayer is formed at the interface of the benzene phase, and the drop of aqueous protein solution is extruded from the pipette by reaching in from the top. These two monolayers are moved toward each other by appropriate mechanical procedures and, at the place of contact, form a true bilayer.

established whether the bilayer did contain protein. The method is a very interesting one, because it offers the hope of constructing bilayers containing *different proteins* in the two monolayers.

Finally, I wish to mention a procedure invented by Träuble and Grell (1970) in Eigen's laboratory (Figure 4). Here, too, the bilayers are formed stepwise. In the first step, an emulsion of water droplets is formed in a bulk phase of benzene to which a small amount of phosphatidyl choline has been added. The emulsion is formed by intense ultrasonic irradiation. The droplets are of fairly uniform size, about 400 Å in diameter, and are coated with a *monolayer* of the phospholipid. This emulsion is then layered on top of a different aqueous phase. Some more phospholipid is added to the benzene to form an interfacial layer between the bulk aqueous phase and the benzene, and the droplets of the emulsion (the prevesicles) are forced from the benzene phase into the water phase by high-speed centrifugation. In their passage through the interface, the prevesicles cover themselves with a second layer of phospholipid and now constitute vesicles surrounded by a true lipid bilayer. Here the possibility exists of making the bilayer asymmetric, with respect to the polar lipid, and making the inside aqueous phase different from the outside one. Moreover, that these vesicles can be prepared with a total surface area per milliliter vastly exceeding that obtainable by any other method opens the way to a great number of physical studies not accessible with the other procedures. Vesicles of phospholipids can also be formed in a single step, by sonication of a suspension of lipid in water (Huang, 1969).

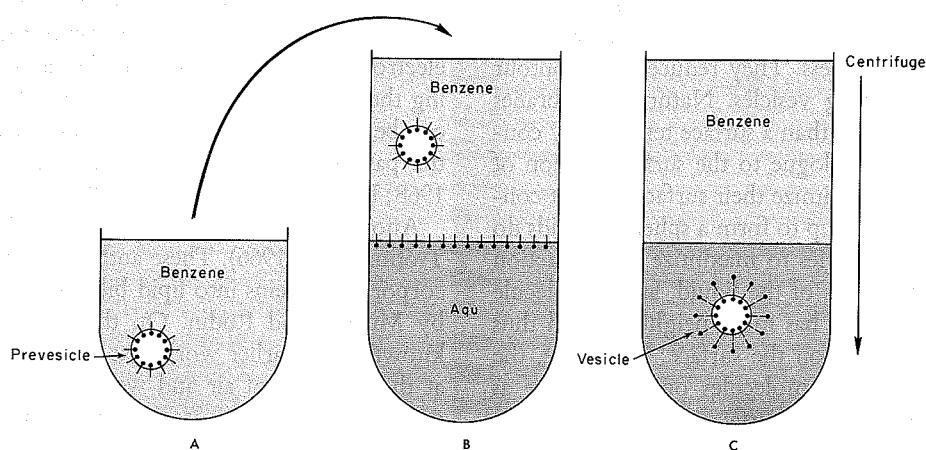


FIGURE 4 Production of masses of microvesicles by a two-step procedure (Träuble and Grell, 1970). Step 1. Formation of a water emulsion in a benzene phase containing small amounts of phospholipid. Prevesicles are formed on intense sonication. Step 2. Another aqueous phase is overlaid with

the emulsion, and an interfacial monolayer of phospholipid is formed. Step 3. The prevesicles of the emulsion are forced through the interfacial monolayer (high-speed centrifugation). Vesicles bounded by a lipid bilayer are formed.

Let us return to the flat bilayers introduced by Mueller and Rudin. These are reasonably permeable to substances soluble in both water and lipid. One of these substances is water itself, whose solubility in hydrocarbon solvents is actually surprisingly high, around 10^{-3} molar. The nature of water dissolved in hydrocarbon has not been studied by modern methods. It is not known to what extent it is aggregated and, if it is, in what kinds of aggregates. Such a study would certainly be feasible and highly desirable.

These membranes are impermeable to substances of low solubility in hydrocarbon solvents or, more precisely, to substances for which the partition coefficient between water and the hydrocarbon phase is unfavorable for the latter. Foremost among these are anions and cations. As a result, the electrical conductance of such membranes is fantastically low, in most cases of the order of 10^{-8} mho cm^{-2} .

Läuger et al. (1967) made the interesting discovery that such membranes are relatively permeable to the iodide ion and much more so in the presence of the I_2 molecule. Probably this case is also a simple one of partitioning between water and lipid; the larger the volume over which the charge of the ion is distributed, the less energy is needed to transfer such an ion from its aqueous environment with a dielectric constant around 80 to the lipid phase with a dielectric constant around 2. The iodine molecule enhances the partition of the iodide ion in favor of the lipid phase. The mechanism of this enhancement has not yet been clarified unambiguously. It is possible that I_3^- or I_5^- ions are formed, thus spreading the charge over a larger volume and reducing the electrostatic energy needed for transfer to the lipid phase (Finkelstein and Cass, 1968). However, more indirect effects of I_2 on the lipid phase also can be conceived.

The unmodified lipid bilayer membrane imitates the thickness, electric capacitance ($\sim 1 \mu\text{f cm}^{-2}$), and the water permeability of real membranes. It fails in its electrical conductance in not being constrained to constant thickness when torn, and in all higher functions.

PROTON CARRIERS Next in sophistication we may consider the remarkable effects of *uncouplers*, i.e., substances that, in mitochondria, uncouple oxidation from phosphorylation. Dinitrophenol is the classic example, and related compounds have been introduced recently. When such compounds are added to mitochondria, the electron transport system of respiration continues to operate at a high rate, but the energy supplied by this transport is not used for the synthesis of ATP. The theories concerning the mechanism of this uncoupling naturally depend on our theoretical understanding of the coupling of oxidation and phosphorylation in the first place. One class of such theories derives from ideas of Peter Mitchell (1966; see also Lehninger, this volume). Mitchell noted that membranes, be-

cause they are invariably contour free, must necessarily divide space topologically into an inside and an outside. Thus, one can conceive of a biochemistry, situated in the membrane, which both creates and depends on the differences in the electrochemical potentials of solutes on the inside versus those on the outside. One can also conceive that the coupling between electron transport and phosphorylation might be mediated by such gradients. Specifically, Mitchell conceived that the electron transport creates a pH differential between inside and outside, and that the pH differential drives the synthesis of ATP. Uncoupling, from this point of view, would occur if the pH gradient fails to be established, for instance by making the membrane highly permeable to protons. It is still uncertain if this idea is correct with respect to the uncoupling of oxidative phosphorylation in mitochondria, but it has turned out that the uncouplers are indeed good proton carriers in lipid bilayers (Hopfer et al., 1968; Liberman and Topaly, 1968). At least that is what they seem to be at first sight. They increase the conductivity of the membrane in a manner that would be expected for a mechanism that allows both the neutral uncoupler and the anion to shuttle back and forth. The dependence of the conductance on the concentration of uncouplers and on the pH support the idea, but whether this is the whole story is still not clear. Certain experimental findings on the saturation of this mechanism point to complications. These have been pursued actively by L. J. Bruner (Bruner, 1970).

DEVIATION FROM OHMIC BEHAVIOR A striking feature of the conductance both of simple lipid bilayers and of those made more conductive by uncouplers is a symmetric nonlinearity in the I versus the V curve: with increasing applied voltage the current rises faster than proportionally to the voltage. The deviation from linearity becomes obvious around 30 mV and reaches a factor of about 5 near 100 mV. Läuger and his associates have made a thorough theoretical study of this nonlinearity (Walz et al., 1969; Neumcke and Läuger, 1969), and point out three possible causes, each of which is analogous to certain mechanisms well known in solid-state physics.

The first possible cause is referred to as "ion injection." When an insulating layer is interpolated between two layers containing a large number of mobile charge carriers, an applied voltage will effectively be limited to the insulating layer, caused by the accumulation of charge carriers of opposite sign at the two interfaces. Because of the interplay of thermal diffusion and electrical fields, however, the charge layers at the interfaces will not be infinitely thin. They will extend some distance into the conducting phases. The extent of this spread of the space charge and of the voltage is the greater the lower the carrier concentration. The net effect of this space-charge distribution is a dependence on the applied

voltage of the concentration of the majority carrier at the lipid-water interface: the greater the applied voltage the higher the majority-carrier concentration and, therefore, the higher the ion injection. Walz et al. (1969) showed that for lipid bilayers this effect is appreciable only at ionic strengths of about 10^{-3} molar or lower, and that it should rapidly decrease with increasing ionic strength. Both these predictions are contrary to the actual findings, and therefore this cause cannot account for the bulk of the observed effect.

The second cause studied in detail (Neumcke and Lauser, 1969) considers the effects of the so-called image forces to which the ions in the lipid phase are subjected. These image forces are an expression of the polarization of the dielectric environments of the ions. The forces are situated very close to a lipid-water interface and lead to a strong attraction of any ion dissolved in the lipid phase toward the interface and thus to a lowering of its energy. In lipid bilayers, the effect of these forces is to increase the partition of the ions in favor of the lipid phase, and the increase is greater as the applied voltage is greater. In this case, quantitative analysis leads to results much closer to reality. One obtains an I curve versus a V curve, which is independent of the ionic strength and gives deviations from linearity of the right sign and the right order of magnitude, but they still are not quite large enough to account for the observations.

A third effect has, therefore, been considered and calculated in detail. This is the field dissociation effect first discovered by Wien many decades ago and accounted for quantitatively by Onsager in a classic paper (Onsager, 1934). Onsager showed that the applied field affects the association-dissociation equilibrium of dissolved salts, leading to a net increase in dissociation in the presence of high applied field strength and, therefore, in the number of charge carriers. The theory of Onsager has been modified (Neumcke et al., 1970) to the situation of thin bilayers and has been shown to account satisfactorily for the observed nonlinearity, when combined with the contribution of image forces mentioned above.

ION CARRIERS This nonlinearity does not seem to be present and is probably swamped out when lipid bilayers are modified by the addition of ion carriers, substances that increase the conductance of lipid bilayers still more than do the uncouplers. The history of this discovery is somewhat as follows. Among the vast number of antibiotics against bacteria discovered during the last decades are some that, when applied to mitochondria, lead to a strange accumulation of potassium ions. The mechanism of this K^+ accumulation is still controversial, but again the application of these agents to lipid bilayers has led to a beautifully simple result: the bilayers become highly permeable to K^+ and highly selectively so. Mueller and Rudin (1967b), who discovered this phenomenon, thought at first that these substances,

which are ring molecules, accommodate the hydrated potassium ion in the center of the ring and were tailor-made to fit this hydrated ion. They conceived of these ring molecules as inserting themselves in the membrane-like washers, forming pores through which the ions might move. The truth turned out to be even more startling. When the first X-ray diffraction structure of one of these molecules (nonactin) came out (Kilbourn et al., 1967), it showed that the carrier wraps itself completely around the *naked* ion, suggesting that this complex could act as a "carrier pore" for shuttling particular ions across the membrane. A similar structure has recently been established for another K^+ carrier, valinomycin (Pinkerton et al., 1969; Ivanov et al., 1969). Eigen, elsewhere in this volume, deals with the features that permit these molecules to carry out such a very specialized function.

HOLE PUNCHERS The next class of additives might be called "*hole punchers*." These are cyclic antibiotics containing a polyene chain as part of the molecule, but other portions of the ring are highly hydrophilic. Their effects on real membranes and on bilayers are contingent on the presence of cholesterol or some other sterol in the membrane. Nystatin and amphotericin B are the ones that have been studied most carefully (Finkelstein and Cass, 1968). These additives also increase the conductance of bilayers, but the effects that they produce are very different from those of ion carriers.

1. The additive must be present on both sides of the membrane to give reproducible results.

2. The conductance increase depends on a very high power of the drug concentration.

3. The conductance is based on an increased permeability to *anions* and is coupled with an increased permeability to water.

4. Anions are discriminated according to size.

5. The conductance has a very *high negative temperature coefficient* in contrast to that produced by the ion carriers. This permits the experimenter the neat trick of switching the conductance from one due to potassium to one due to anions by using bilayers to which both a K^+ carrier (valinomycin) and a hole puncher (nystatin) have been added. Simply by a change of a few degrees in temperature, the dominant conductance mechanism can be switched, and with it the membrane potential, if the solutions on the two sides are chosen appropriately.

GATEABLE ADDITIVES The last class of additives I wish to discuss are the gateable ones: EIM (excitation-inducing material, Mueller and Rudin, 1967a) and alamethicin (Mueller and Rudin, 1968). The first of these materials is a protein produced by a strain of *Enterobacter cloacae*; the second, a cyclic peptide containing 19 amino acids. Both substances

are still very imperfectly characterized chemically. Both substances confer on lipid bilayers a conductance which varies over several orders of magnitude, depending on the applied voltage. Conductance is cationic but can be changed to anionic by the addition of such polycations as protamine, polylysine, or spermine. By playing with the applied voltage, salt gradients, or the polycationic additives, one can simulate many striking electrical phenomena characteristic of nerve-axon membranes, including resting potentials, action potentials, and rhythmic discharges. The molecular mechanisms here involved are still obscure. Especially, the basic question still unanswered is whether the sudden transitions from one state of conductivity to another bespeak a cooperative phenomenon on a large scale, such as a phase transition of the whole membrane, or a microcooperativity involving local conformational changes of oligomers. Perhaps the strongest argument in favor of the latter view comes from the observations of Bean et al. (1969), which showed that, on the addition of EIM, quantized increases in conductance can be seen, each quantum amounting to a conductance change of about 4×10^{-10} mho. It seems plausible that these increases in conductance correspond to the opening of individual gates controlled by one or a few molecules of EIM. If such an interpretation is correct, then the excitations of the lipid bilayers produced with the help of these additives are not likely to be close relatives of true nerve excitations, because, for the latter, it seems probable to me that we are dealing with a true phase transition on a macroscopic scale (Adam, 1970).

NEW TECHNIQUES COMING OR HOPED FOR There is much need for more basic studies on lipid bilayers, naked or with additives. The lipids used should be chemically defined, as in the studies of the Lauger group; otherwise we cannot hope to obtain data interpretable in terms of specific mechanisms. Electrical measurements must be supplemented by other physical measurement, by the attachment of spin labels or fluorescent labels, or the development of technologies for absorption and reflection measurements adapted to these very thin layers.

A most promising approach is the study of the bulk phases of lipid-protein-water mixtures (Gulik-Krzywicki et al., 1969). Such mixtures give rise to a variety of phases which can be studied by X-ray diffraction techniques. The clarification of the structures possible in laminar phases, especially, should go a long way in helping us to assess the relative roles of lipid and protein in contributing to the basic structure of real membranes.

Another approach of great value for the understanding of the interaction of lipids and proteins in membranes is that pursued by Colacicco (1969). Here a monolayer of phospholipid is first formed at an air-water interphase. Various proteins are then introduced into the aqueous subphase, and

the penetration of the protein into the monolayer is assessed by its effect on the surface pressure of the monolayer.

Perspective

It would seem that Nature, when she had invented the principle of membranes, found that she had caught on to a good thing, and proceeded to exploit this principle with the passion of the true inventor. As yet we have as little understanding of this general principle as we had of chromosomes, say, 30 years ago. Studies of physiologists, biochemists, and electron-microscopists have yielded some guidance in the characterization of special membranes, but the gap between this characterization and the light shed by the study of model membranes is still enormous.

Considering the relevance of the study of membranes to our over-all progress in biology and psychobiology, I do not think that we shall learn from such studies how any central nervous system functions or even be led directly to the solution of the great puzzles of neuroembryology. I do have a strong feeling, however, that radical progress in any of these fields will not come until the gap has been bridged that now exists between our understanding of the simplest model membranes and, say, the transducer membranes of sensory physiology. Here I am sure discoveries are still to be made that will rank with the greatest we have had in molecular biology.

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