Some Further Experiments
on Bimolecular Lipid Membranes

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The authors of this paper have previously reported some electrical properties of simple lipoidal bimolecular leaflets in aqueous solutions (1). Recently, further investigations along similar lines have been carried out, the results of which are summarized in the following paragraphs.

The Frequency Dependence of Capacitance and Conductance

In the earlier experiments it was established that a bilayer of pure egg phosphatidyl choline containing less than 50 per cent by volume of n-decane had a capacitance of 0.38 $\mu f/cm^2$, this value being independent of applied voltage up to at least 50 mv and independent of frequency between 200 and 5×10^6 cps. It was also unaffected by the nature and concentration of the surrounding electrolyte solution for NaCl, KCl, and $CaCl_2$.

In an attempt to discover more about the electrical properties of the film at frequencies below 200 cps the time dependence of the response to a sudden change in the d.c. potential across the film was examined. The transients were characterized by a single time constant and, using the measured value of the d.c. resistance, a value of the capacitance of the film was calculated. The capacitance was thus found to be not significantly different from 0.38 $\mu f/cm^2$ at frequencies of from 0.16 to 0.001 cps and applied voltages of up to 50 mv.

The consideration of these results in terms of the molecular structure of the bilayer raises the question of the relative contributions to the membrane capacitance and conductance of the hydrocarbon and phosphatidyl choline layers respectively. It has already been inferred from experimental results (1) that both the capacitance and conductance of the polar group layer are completely masked by the hydrocarbon region, and that the measurements therefore relate only to the hydrocarbon region. As the aqueous phase, polar group layer, and hydrocarbon constitute a three layer dielectric (see for instance Schwan (2)), the detailed equations for this structure were derived. By inserting reasonable values for the capacitance and conductance of the polar group layer it has been shown that although the relaxation frequency introduced by the polar groups may be either high or low, depending on their

conductance, the magnitude of the associated dispersions should be negligibly small.

The Orientation of the Phosphatidyl Choline Groups in the Bilayer

From an inspection of a molecular model it can be seen that the quaternary ammonium groups can be either coplanar and up to 5 or 6 A below the phosphate groups, or, at the other extreme, both these groups can be in one and the same plane parallel to the leaflet. For the former orientation, when in an electrolyte solution, a complex diffuse double layer would build up around the fixed charges of the lecithin. A somewhat simplified theoretical analysis of this system has been carried out, and the average potential in the plane of the quaternary ammonium groups has been calculated. This potential is given experimentally, to a first approximation, by the electrokinetic potential, which has been measured for the appropriate interfaces in several NaCl solutions of various concentrations. The experimental potentials are close to zero and differ considerably from the theoretical values. On the other hand, if the quaternary ammonium and phosphate groups are coplanar, a zero potential would be expected. From this evidence, and from qualitative electrostatic free energy considerations, there seems little doubt that the quaternary ammonium and phosphate groups in the lecithin bilayer are effectively coplanar.

The conductance of the phosphatidyl choline layer is difficult to estimate. However, the conductivity data of most polar non-ionized liquids suggest that the conductance of this region must be at least 10^{-3} to 10 mho/cm^2. This may be compared with the conductances found for the whole membrane (see following section) of ca. 10^{-9} mho/cm^2. The maximum potential that can be applied to the membrane is approximately 200 mv and therefore the maximum average potential gradient which can be set up in the polar group region is approximately 2v/cm.

The D.C. Conductance

The membrane conductances were usually very low and were below the range of accurate measurement of our a.c. equipment. By d.c. methods, however, the conductances were readily measurable although at first sight rather irreproducible. Membranes were then bulged under a hydrostatic pressure so as to vary their area, and it was found that some (with low conductances) showed a direct proportionality between conductance and area which was reproducible, while others (with high conductances) did not and gave erratic results. This strongly suggests that the high conductances and the irreproducibility were caused by a variable border leakage. The true membrane conductances were ohmic, but depended on the concentration of NaCl in the adjacent solutions (2.5×10^{-9}, 1.3×10^{-9}, and 0.50×10^{-9}

mho/cm² for 1.0, 0.1, and 0.01 N NaCl respectively). One interpretation of this data is that the current is carried not by Na⁺ or Cl⁻ but by a combination of one or both of these ions with the phospholipid.

The Effect of Some Proteins on Membrane Capacitance and Conductance

The solution of lecithin in decane used to form the membranes discussed above can be shown by electrokinetic measurements to adsorb some proteins strongly from aqueous solution. In fact the adsorption proceeds to at least a monolayer of closely packed protein. Experiments have been carried out with serum albumin, egg albumin, and insulin at various pH. In no case was the specific capacitance and conductance of the membrane significantly affected by the protein. Capacitances at very low frequency were determined by the decay curve method and again no frequency dependence was found between approximately 0.001 and 5 $\times$ 10⁶ cps. If the three layer dielectric equations are applied to this system, it is found that to account for the results the protein layer must have either a capacitance or a conductance which is high compared to that of the membrane as a whole, or both these properties. As the membrane conductance did not change on addition of protein, the latter evidently did have a high conductance.

The Effect of Lipid Composition on the Capacitance

So far two approaches to this question have been tried, neither of them being very satisfactory.

(a) Although the lipid composition of the oil phase from which the membranes are formed has been varied, and the corresponding membrane property measured, the results to date cannot yet be related to the final membrane composition. Cholesterol has been added to the original oil phase in mole ratios to egg phosphatidyl choline of 0 to 2.95. The membrane capacitance did not change over this range of composition. The measured conductances were of the same order of magnitude as they were for absence of cholesterol. The inclusion of isopropanol together with the cholesterol did, however, yield rather erratic capacitance values of up to 0.52 μf/cm², but still without a significant change in membrane conductance.

(b) The second approach has involved the estimation, from bulk dielectric constant measurements, of the dielectric constants for various ratios of mixed hydrocarbon residues saturated with water. It has been estimated that for mixtures of ring structures (such as in cholesterol) and normal saturated and unsaturated aliphatic chains, the dielectric constant may vary between 2.41 and 2.84 for 0.67 mole fraction of aliphatic chains. This makes the upper limit of capacitance of a hydrocarbon membrane, whose thickness is 45 A, approximately 0.56 μf/cm².

Capacitance and Conductance Measurements on Bulged Membranes

By bulging the membranes under a hydrostatic pressure, the area has been varied by a factor of 10. It has been shown that during this process, the capacitance varies, as expected, linearly with the area. The conductance measurements on the bulged membranes have been described in the section on d.c. conductance.

The Permeability to Water

Both osmotic flow and tritiated water diffusion have been used to determine the water permeability at 20°C. The reproducibility so far achieved in the former technique is not high and values of between 8.3 and 14.4 $\times$ 10^{-4} cm/sec. have been found for NaCl of various concentrations and concentration differences. By tritiated water diffusion, using the technique developed by Thompson (3), a value of 2.30 $\times$ 10^{-4} cm/sec. has been found which is not dependent on NaCl concentration between 0.1 and 1.0 N (result obtained by W. R. Redwood of this laboratory). This value compares with 3.75 $\times$ 10^{-4} cm/sec. quoted by Thompson (see reference 4) for somewhat similar membranes at 36°C. Both osmotic and tracer permeabilities are of the same order of magnitude as the corresponding data for many cell membranes and also resemble the biological data in that the osmotic result is the higher by a substantial factor. The reasons for this discrepancy have not yet been elucidated.

The Comparison with the Properties of Cell Membranes

Although the properties of cell membranes vary considerably, some general comparisons with the present model membranes are nevertheless possible.

It appears that the capacitances and conductances of the model membranes are lower than those of most cell membranes, although the capacitances of some cell membranes are probably not known sufficiently accurately for any significance to be attached to the discrepancies. Apart from the obvious possibility that some cell membranes may contain shorter chain lipids and therefore be a little thinner, variations in lipid composition and the presence of sandwiching protein structures do not seem likely to account for the capacitance discrepancies. It may be significant that both the capacitance and conductance of the model membranes could be greatly increased if a very small fraction of their area were made up of polar material in parallel with the lipid. Thus if 1 per cent of the membrane was made up of "polar pores" having an effective dielectric constant of 80, the capacitance / cm^2 would be increased by 0.16 μf and there would also be no difficulty in explaining, with this model, a conductance increase of $\times$ 10^6. It does not, therefore, seem

difficult to account for the more general electrical properties of cell membranes in terms of a slightly perforated bilayer.

The close similarity between the water permeabilities of the present membranes (in which, according to the electrical properties, there is no evidence of pores) and those of cells is again consistent with the concept of a cell membrane as predominantly bimolecular lipid leaflet.

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