BBA 75007

ELECTRICAL PROPERTIES OF BIMOLECULAR PHOSPHOLIPID MEMBRANES

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(Received June 21st, 1966)

SUMMARY

The electrical properties of bimolecular phospholipid membranes separating two aqueous electrolyte solutions were investigated. The membranes were formed from solutions of egg phosphatidyl choline or pure synthetic dioleyl phosphatidyl choline in *n*-decane. From measurements of the electrical capacity, the membrane thickness was calculated to be 70 ± 10 Å. In NaCl or KCl solutions the resistance of the membrane was very high (up to $10^8\ \Omega \cdot cm^2$). When Cl^- in the solutions was exchanged with I^-, the membrane conductivity increased by a factor of about 10^3; it returned to the original value when I^- was again replaced by Cl^-. With I^- solutions of different concentrations on both sides, a steady membrane potential of nearly the limiting value was observed. This membrane potential was almost unchanged even in the presence of a 100- to 1000-fold excess of Cl^-. A definite interpretation of these results is difficult before the mechanism of charge transport within the membrane (ionic or electronic) is established.

INTRODUCTION

The ability of certain lipid molecules to build up two-dimensional arrays plays an important role in the structural organisation of living cells. Lamellar arrangements of lipids are found not only in cell membranes, but also in such highly specialized structures as mitochondria, chloroplasts, and the photoreceptors of the vertebrate retina. Both electron microscopy[1-3] and X-ray diffraction techniques[4,5] have led to the conclusion that the thickness of the lipid layer in these lamellar systems is about 50–100 Å. This finding is consistent with the concept that the basic structure of the cell membrane includes a bimolecular sheet of lipid molecules, arranged so that the hydrocarbon tails constitute the interior of the membrane, whereas the polar end-groups point toward the outer medium[6,7]. It appears that in many cases protein molecules are embedded in the bilayer or attached to its polar surfaces. In this way spatially ordered arrays of enzymes and other functional molecules can be built up. There is now considerable evidence that the fundamental energy conversion processes

like oxidative phosphorylation in mitochondria[8,9] or photosynthesis in chloroplasts[10] take place in ordered systems of enzymes arranged on a lipid matrix.

At the present time the processes occurring in natural lipoprotein membranes are only very poorly understood on a molecular level, because any attempt to make direct physical measurements on structures like mitochondria or photoreceptors meets with great difficulties due to the complexity and smallness of the object. In view of these difficulties experiments with artificial bimolecular lipid membranes of known composition offer many advantages. The first attempts to build up thin lipoprotein membranes of macroscopic area were made by LANGMUIR AND WAUGH[11] who obtained films of lecithin (the major phospholipid component of many cell membranes) at the interface between benzene and an aqueous protein solution. These films were not very stable and no further experiments were reported. In 1962, MÜLLER *et al.*[12-15] described a simple method by which very thin lipid membranes with an area of a few square millimeters could be formed in an aqueous salt solution. The optical appearance of these membranes is similar to the so-called black soap films in air, whose thickness is in the order of 100 Å. Compared with natural lipid membranes the artificial films show a very high electric resistance, but a capacitance lying well within the range of values found with cell membranes. Since the brain extract used by MÜLLER and his co-workers was a mixture of several lipids and proteolipids, the composition of these membranes may have been rather complex. Recently it has been shown by HANAI, HAYDON AND TAYLOR[16] that „black" membranes could be obtained with pure preparations of lecithin (= phosphatidyl choline) dissolved in a hydrocarbon such as *n*-decane. However, the structural requirements for the formation of stable membranes seem to be rather critical. HUANG[6], WHEELDON AND THOMPSON[17] found that membranes could be formed with a lecithin containing a high proportion of unsaturated fatty acid chains, whereas a completely saturated lecithin (such as synthetic L-α-dipalmitoyl phosphatidyl choline) failed to give stable membranes. By measuring the intensity of reflected light, HUANG AND THOMPSON[18] obtained a value of 72 ± 10 Å for the thickness of the black membrane. This value agrees fairly well with the thickness determined from measurements of the electrical capacitance of the membrane[16,19,20]. As the length of a fully extended lecithin molecule (with C_{18} fatty acid chains) is about 30–40 Å, it seems very probable that the artificial lipid membranes consist in fact of a bimolecular sheet of oriented lecithin molecules.

In this paper we present some further investigations on the electrical properties of black lecithin membranes. The electrical capacitances of the membranes were determined by two different methods; the observed values confirm the earlier results of HANAI, HAYDON AND TAYLOR[16]. In order to obtain information on the ionic permeabilities, we measured the electrical conductivity in different electrolyte solutions. It was found that the I^- ion has a very specific influence on the electrical properties of the membrane. In I^- solutions the membrane conductivity is increased by a factor of about 10^3 compared with Cl^- solutions. Besides this, if the adjacent I^- solutions have different concentrations, membrane potentials of nearly the theoretical value are observed.

EXPERIMENTAL

Materials

Egg lecithin (= egg phosphatidyl choline) was prepared according to PANGBORN[21] and stored at —18° as a 2% solution in absolute ethanol. By means of glass beads the air space above the solution was kept as small as possible. The solution could be used for at least 8 months.

L-α-Dipalmitoyl phosphatidyl choline (synthetic puriss.) obtained from Fluka A.G., Buchs, was used without further purification.

L-α-Dioleyl phosphatidyl choline was synthesized using the well-known method of acylating L-α-glyceryl phosphoryl choline. L-α-Glyceryl phosphoryl choline was prepared according to the method of TATTRIE AND MCARTHUR[22] with commercial lecithin (Merck) as starting material. The cadmium chloride complex of L-α-glyceryl phosphoryl choline was allowed to react with oleyl chloride in the presence of pyridine. Oleyl chloride was prepared from oleic acid (from Fluka, $\geqslant 98\%$) and thionyl chloride[24]. The reaction mixture was extracted with aq. $NaHCO_3$ solution and further purified on an ion exchanger (Amberlite IR-45 and IRC-50 in equal amounts) and two silicic acid columns[25,26]. The infrared spectrum of the synthesized phosphatidyl choline agreed with the spectrum reported in the literature[27]. On the thin-layer chromatogram the product proved to be free of lysolecithin and fatty acids (Kieselgel G, Merck, with chloroform–methanol–water (65:25:4, v/v) as solvent; the spray reagent was alcoholic rhodamin B and Dragendorff reagent). The gas–liquid chromatographic analysis of the fatty acids obtained by hydrolysis of the synthetic lecithin showed the main peak of oleic acid ($\sim 96\%$) and some minor peaks (presumably 16:0, 18:0 and 20:1 fatty acids). The specific rotation of the dioleyl phosphatidyl choline was $[\alpha]_D^{20} = +4.8$.

Sphingomyelin (from beef brain, purified) was obtained from Sigma. In the thin-layer chromatogram the substance appeared as a single spot.

Membrane formation

If not otherwise indicated, a 1% solution of egg phosphatidyl choline in *n*-decane was used. This solution was freshly prepared every day from the stock solution. The membrane was formed at 35° on a small circular aperture in the wall of a teflon cell (see below) which was immersed in an aqueous solution. By means of a short piece of teflon tube mounted on a 1-ml injection syringe, a small portion of the phosphatidyl choline solution was transferred under the water surface and spread over the aperture as a thin liquid lamella. Under the combined influence of gravity and capillary forces most of the material was displaced toward the rim of the hole. When the aperture was observed with a low-power microscope in reflected light, intense interference colours were visible during the thinning of the lamella. About 1 min later, „black" fields appeared in the coloured area, indicating an abrupt decrease in the thickness of the film. These fields slowly expanded until almost the total area of the hole was covered with a uniform membrane, showing only a feeble light reflection. Membranes formed in this way were usually stable for several hours. In most experiments the diameter of the hole in the teflon cell was 3 mm, but membranes with a diameter of 5 mm ($F \simeq 20$ mm^2) could be formed without difficulties. In each experiment

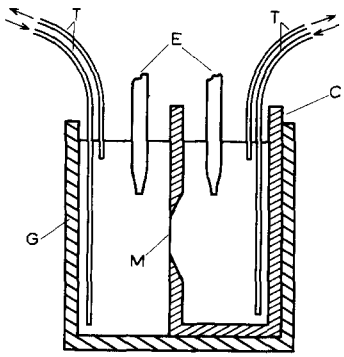

Fig. 1. Cell for electrical measurements. C, teflon cell; E, calomel electrodes; G, glass cell; M, membrane; T, teflon tubes.

the area of the black film (which usually was about 10% smaller than the total area of the hole) was determined with the aid of a measuring eyepiece.

The cell

The arrangement for the electrical measurements is shown in Fig. 1. It consisted essentially of an outer glass cell (G) (2 cm × 2 cm × 2 cm) and an inner cell (C) made of teflon. Both compartments were filled with an aqueous salt solution. The membrane was formed on the hole in the wall of the teflon cell. The glass cell was surrounded by a thermostated metal block with a window for observation of the membrane. For the measurement of membrane resistance or concentration potentials, saturated calomel electrodes (E) were inserted in the cell. The whole assembly (cell + electrodes) was carefully screened, the electrode in the outer compartment being at the same potential as the screen.

In some experiments, after the membrane had been formed in a solution A, the liquid in one or both compartments had to be replaced by a second solution B. This could be done with the aid of four motor-driven injection syringes which were mechanically coupled, forming a push-pull arrangement. Each syringe had a capacity of 20 ml and was connected with the cell by a teflon tube (T). Small differences in the liquid levels during the exchange could be compensated by means of an additional syringe (not shown in the figure). With this arrangement the solution in one or both compartments could be completely exchanged within about 5 min without destroying the membrane.

Electrical measurements

The electric resistance of the membrane was measured with a variable voltage source and a Keithley 150 A microvolt-ammeter with a sensitivity of 10^{-10} A full scale. For the observation of membrane potentials the membrane was formed in an electrolyte solution of concentration c_1; thereafter the electrolyte at one side of the membrane was replaced by a solution of concentration c_2. After the exchange, the potential difference between the two solutions was measured using calomel electrodes and a Knick millivoltmeter with an internal resistance of $>5 \cdot 10^{11}\ \Omega$.

The electric capacitance of the membrane was measured by two different methods. In the first method the membrane was made part of a *RC*-network, in which

an external resistance R of known value lies parallel to the membrane capacitance C. By means of a square-wave generator and a series resistance $R_s \gg R$ rectangular current pulses were applied to the network. From the rise time $\tau = RC$ of the voltage across the resistance R, measured by a Tectronix 533 cathode-ray oscilloscope, the membrane capacitance C could be determined. The maximum voltage across R was 50 mV peak to peak. The cell was filled with 0.1 M KCl, and platinum electrodes coated with platinum black were used. Under these circumstances, the resistance of electrodes + solution was much smaller than R, and the resistance of the membrane very high compared with R, so that the equivalent circuit of the cell was given by a single capacitance C.

In a second series of experiments, the capacitance of the membrane was measured with an alternating current bridge method[16]. A Wayne Kerr B 221 bridge was used in combination with an external source (General Radio 1210-C oscillator) and detector (General Radio 1232-A tuned amplifier and null detector). Platinum electrodes as before were inserted in the cell. With the high sensitivity of the detector, a voltage of about 20 mV peak to peak was sufficient for balancing the bridge. With this arrangement, the impedance of the cell + membrane could be measured in terms of the equivalent parallel components of resistance and capacitance over a frequency range from 200 cycles/sec to $2 \cdot 10^4$ cycles/sec. Since the membrane resistance was very high compared with the resistance R_0 of the electrodes + solution, and the capacitance of the teflon cell very low compared with the membrane capacitance C, the equivalent circuit could be approximated with sufficient accuracy by a series combination of R_0 and C. The values of R_0 and C could then be obtained from the measured values of the equivalent parallel components in the usual way. It was also possible to measure the membrane capacitance while a d.c. voltage was applied across the membrane in addition to the small a.c. bridge signal.

RESULTS

Chemical composition and stability of bimolecular membranes

The first experiments were made with natural egg phosphatidyl choline which is known to be a complex mixture of molecular species differing in the nature of the fatty acid residues. It seemed therefore desirable to use a phosphatidyl choline of chemically uniform composition. From the experiments of HUANG, WHEELDON AND THOMPSON[17] it was already known that pure dipalmitoyl phosphatidyl choline with completely saturated fatty chains failed to give stable bimolecular membranes. It appears therefore that a certain degree of unsaturation is essential for membrane stability. In fact, we found that with a *n*-decane solution of pure synthetic dioleyl phosphatidyl choline (one double bond per fatty acid residue) one could obtain black membranes which were stable for several hours. With respect to their electrical properties (see below) these membranes were not very different from the egg lecithin membranes. Likewise, a 1:1 mixture of dipalmitoyl phosphatidyl choline and dioleyl phosphatidyl choline also gave stable bimolecular membranes. However, as the bimolecular film did not necessarily have the same composition as the bulk solution, the molecular proportion of saturated and unsaturated phosphatidyl choline in these membranes was unknown.

In this connexion an interesting question arises, namely: what structural pre-

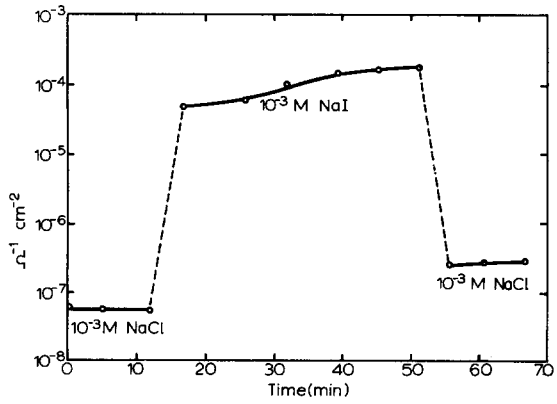

Fig. 2. Conductivity change of an egg phosphatidyl choline membrane during replacement of Cl^- by I^- in the outer medium.

requisites must a molecule possess in order to form bimolecular films in an aqueous solution? This question is complicated by the fact that solvent molecules (*n*-decane in our case) may constitute a part of the membrane structure. A few attempts were made to build up black membranes with lipid molecules other than phosphatidyl choline, but with limited success: only sphingomyelin which has the same polar group as phosphatidyl choline was found to give bimolecular membranes. These membranes were formed in the usual way from a 1% solution of beef-brain sphingomyelin in chloroform–decane (1:1, v/v). They were stable for only a few minutes.

Electrical resistance of the membrane in different electrolyte solutions

In NaCl or KCl solutions the electrical resistance of egg phosphatidyl choline membranes was very high but of poor reproducibility. Although the resistance of a given membrane was in most cases fairly constant over a period of about 2 h, the measured resistance values ranged between 10^6 and 10^8 $\Omega \cdot cm^2$ for different membranes. One may therefore suspect that the observed resistance values did not give the true resistance of the phosphatidyl choline films, but rather depended on the presence of trace impurities.

However, a very pronounced and reproducible effect on the membrane conductivity was observed when in the outer solutions the Cl^- ion was replaced by the I^- ion. A typical experiment is represented in Fig. 2. The membrane was formed in a 10^{-3} M NaCl solution and showed a constant conductivity of about $5 \cdot 10^{-8}$ $\Omega^{-1} \cdot cm^{-2}$ after the whole area was in the "black" state. When the medium on both sides of the membrane was then replaced by 10^{-3} M NaI, the conductivity increased by a factor of about 10^3. This change in the electric resistance of the membrane was reversible: if the original state (10^{-3} M NaCl on both sides) was reconstituted, the conductivity returned to a very low value. (As not all I^- ions could be removed in the second exchange, the conductivities at the end and at the start of the experiment were not exactly equal.) The strong increase in the membrane conductivity was observed also with other I^- solutions regardless of the cation. In this case it is evident that the conductivity was a real property of the black film an not caused by border leakage: when the membrane was formed in a I^- solution, the resistance was very high in the

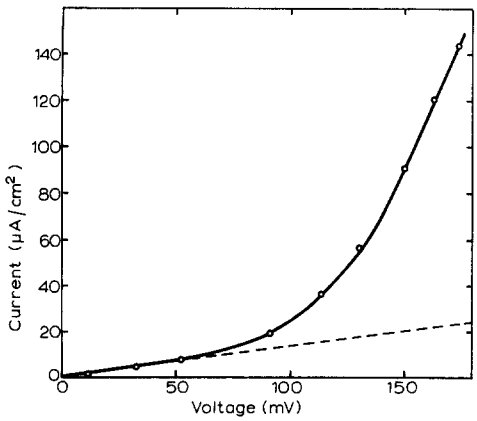

Fig. 3. Current–voltage curve of a dioleyl phosphatidyl choline membrane in 10^{-2} M KI.

early stage as long as only a thick coloured lamella was present, but decreased by several orders of magnitude during the expanding of the black area.

A strictly linear relation between the applied voltage V and the current I through the membrane was observed only at voltages $V \leqslant 30$ mV. This was true with Cl^- as well with I^- solutions. The resistance values given above refer to this linear region. A complete current–voltage curve of a membrane in 10^{-2} M KI is represented in Fig. 3. The current increased more rapidly than the voltage; at $V = 100$ mV the deviation from linearity was more than 100%. If voltages $V \geqslant 200$ mV were applied, dielectric breakdown occurred with most membranes after a few seconds. At this voltage the electric field strength in a 100 Å thick membrane is $2 \cdot 10^5$ V/cm, corresponding in order of magnitude to the breakdown field strength in hydrocarbons.

Membrane potentials

If a membrane separates two solutions of the same electrolyte but with different concentrations c_1 and c_2, an electrical potential difference $\Delta\psi \equiv \psi_2 - \psi_1$ is built up in general. The value of $\Delta\psi$ depends on the transference numbers n_+, n_- of the permeating ions in the membrane. If the membrane is much more permeable to one ion than to the other, the potential difference assumes its limiting value

$$\Delta\psi = \pm \frac{RT}{F} \ln \frac{c_2}{c_1} \qquad (1)$$

(R = gas constant, F = Faraday constant, T = absolute temperature; the activity coefficients are both assumed to be unity). With $c_2/c_1 = 10$ the limiting value of $\Delta\psi$ is 61.1 mV at 35°.

Membrane potentials with a fixed concentration ratio of $c_2/c_1 = 10$ were measured with several electrolytes. In the case of NaCl, KCl and KIO_3 inconstant and non-reproducible potentials in the order of 30 mV were obtained. A well-defined membrane potential was observed, however, with iodide solutions. Fig. 4 represents the potential difference $\Delta\psi$ between the outer solutions of an egg phosphatidyl choline membrane as a function of time (for pure dioleyl phosphatidyl choline membranes the result was almost the same). At the start of the experiment both solutions contained 10^{-2} M KI, and $\Delta\psi$ was zero (apart from a small asymmetry potential). When solution 1 was ex-

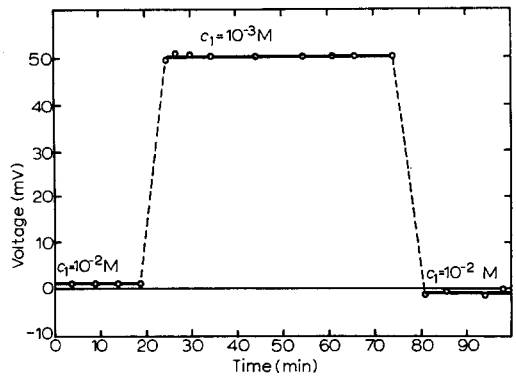

Fig. 4. Potential difference $\Delta\psi = \psi_2 - \psi_1$ across an egg phosphatidyl choline membrane. At start and end of the experiment both solutions contain 10^{-2} M KI; in the intermediate part solution 1 is replaced by 10^{-3} M KI.

changed with 10^{-3} M KI, a steady potential difference of 50 mV was observed, which remained constant over at least 1 h. If then solution 1 was again replaced by 10^{-2} M KI, the potential returned to nearly zero.

This result may be interpreted by the assumption that the membrane is much more permeable to the I^- ion than to the K^+ ion. The previous conductivity experiments suggested that a similar difference exists between the I^- and the Cl^- ion. This was confirmed by membrane-potential measurements in which on both sides a large excess of KCl was added to the KI solution. In the arrangement

$1 \cdot 10^{-3}$ M KI	membrane	$10 \cdot 10^{-3}$ M KI
$99 \cdot 10^{-3}$ M KCl		$90 \cdot 10^{-3}$ M KCl

the K^+ concentration on both sides was the same, so that only Cl^- and I^- could give rise to a membrane potential. If the membrane were equally permeable to Cl^- and I^- the potential difference should have been zero because the total anion concentration was the same in both solutions. If, on the other hand, the membrane was much more permeable to I^- than to Cl^-, a membrane potential near the limiting value $\Delta\psi = 61.1$ mV of the iodide concentration potential should have been observed (as the ionic strength was the same on both sides, the activity coefficients cancel each other out in Eqn. 1). In fact, a steady membrane potential of 61.0 mV was obtained under these conditions. Even with 1 M KCl on both sides in addition to 10^{-3} M and 10^{-2} M KI respectively, the observed potential difference still remained 55–58 mV. Thus, a phosphatidyl choline membrane is capable of detecting activity differences of iodide in a 100- to 1000-fold excess of chloride.

Capacitance measurements

Within the limits of error both types of capacitance measurements yielded the same results, but the most accurate values were obtained with the bridge method. The capacitance of egg phosphatidyl choline membranes in 0.1 M KCl was found to be independent of frequency in the range of 20–20 000 cycles/sec and had a value of 0.33 ± 0.02 μF/cm^2. Membranes formed from pure dioleyl phosphatidyl choline gave almost identical results. A somewhat higher, but equally frequency-independent

value of 0.38 ± 0.01 μF/cm^2 was obtained by HANAI, HAYDON AND TAYLOR[16,19]. In contrast to the poor reproducibility of conductivity measurements, the capacitance was a well-reproducible property of the phospholipid membrane. Variations in the capacitance values (per cm^2) from one membrane to another were within a few per cent and resulted probably from errors in the determination of the membrane area. Besides normal capacitance measurements in which the voltage across the membrane was always below 25 mV, the influence of a steady d.c. voltage on the membrane capacitance was also studied. It was found that the capacitance increased by about 10% when a d.c. voltage of 100 mV was maintained across the membrane in addition to the small a.c. bridge signal. As the capacitance is a measure of the membrane thickness, the result indicates that the membrane is compressed under the influence of the electric field.

DISCUSSION

It is interesting to examine whether the measured electrical properties of the phosphatidyl choline film support the proposed bimolecular structure. From the very low conductivity one can conclude that the greater part of the membrane is inaccessible to inorganic ions. In fact, with a membrane thickness of about 100 Å (see below), the observed resistance of $10^8\ \Omega \cdot \text{cm}^2$ corresponds to a specific resistance of $10^{14}\ \Omega \cdot \text{cm}$. This would agree with the assumption that the interior of the membrane is formed by the hydrocarbon chains of the phospholipid molecules.

The capacitance measurements may be interpreted by regarding the whole system as a parallel plate condenser in which the electrolyte solutions on both sides of the membrane are the conducting plates and the membrane is the dielectric. If C is the capacitance and ε_m the dielectric constant of the membrane, then the thickness d of the membrane is given by

$$d = \frac{1}{4\pi} \times \frac{\varepsilon_m}{C} \tag{2}$$

Since the dielectric constant of most aliphatic hydrocarbons ranges between 2.0 and 2.2 (for instance $\varepsilon = 2.06$ for *n*-hexadecane at 20°), we use $\varepsilon_m \simeq 2.1$ as a mean value. With the measured capacitance $C = 0.33 \cdot 10^{-6}$ F/cm^2 ($= 2.97 \cdot 10^5$ cm^{-1} in electrostatic units) one obtains $d = 56$ Å. Three possible sources of error have to be considered in this calculation:

1. The membrane is not a homogeneous phase but has rather to be treated as a multi-layer dielectric with a central apolar core and two polar surface regions. It can be shown[20], however, that the polar layers which have a much greater capacitance than the hydrocarbon core contribute very little to the capacitance of the membrane. Therefore d is the thickness of hydrocarbon layer, and one has to add 5–10 Å on either side in order to obtain the total thickness of the film.

2. It is questionable whether the dielectric behaviour of the hydrocarbon layer can be characterised by the dielectric constant of a bulk hydrocarbon. As water molecules are possibly incorporated between the fatty acid chains, this may be the most serious error.

3. Even if the membrane can be considered as a homogeneous film with thickness d and dielectric constant ε_m, which is on both sides in contact with an electrolyte

solution, the measured capacitance C is not identical with the geometrical capacitance $C_m = \varepsilon_m/4\pi d$ of the membrane. The reason is the following: When a voltage V is maintained between the two electrolyte solutions, the electric charge $C \cdot V$ is not concentrated in the surfaces of the film (as it would be in the case of an ideal condenser with metallic plates) but rather spread out in the solution over a distance of the order of the Debye–Hückel length. By solving the Poisson–Boltzmann equation the difference between C and C_m can be calculated (see APPENDIX). It turns out that under the conditions of our experiments the difference is negligible.

If the thickness of a single polar layer is assumed to be 7 Å, the capacitance measurements lead thus to an estimated value of 70 ± 10 Å for the total thickness of the membrane (hydrocarbon core + two polar layers). HANAI, HAYDON AND TAYLOR[16] obtained in a similar manner from their capacitance measurements a value of 48 ± 1 Å for the thickness of the hydrocarbon part of the lecithin film. These results are in agreement with recent calculations based on stereomodels[28] yielding a value of 34 Å for the length of a single phosphatidyl choline molecule (with C_{18} chains) in a bilayer. HUANG AND THOMPSON[18] determined the thickness of a black phosphatidyl choline film by measuring the intensity of reflected light and found 72 ± 10 Å. By low-angle X-ray diffraction measurements FINEAN[5] obtained values of 48–51 Å for the distance of opposite phosphate planes in fresh, unfixed myelin. A similar thickness (41.7–44.9 Å) of the lipid layer was found in a study of the lamellar phase in a concentrated phospholipid–water mixture[29].

The most remarkable result is the specific influence of iodide ions on the electrical properties of the membrane. Both the strong increase of the membrane conductivity in the presence of I^- and the occurrence of a concentration potential in I^- solutions can be explained by the assumption that the membrane is much more permeable to I^- than to other ions. This explanation, however, remains questionable, as long as the mechanism of charge transport in the membrane is not established. For the present it is still unknown whether the membrane conductivity is ionic (charge transport by migration of I^- ions) or electronic. The assumption of an electronic conductivity mechanism would imply that under the influence of the applied electric field the I^- ion is discharged on one side of the bilayer, giving off an electron which migrates through the membrane and combines on the other side with H_2O or H^+. A decision between the two conductivity mechanisms should be possible with the aid of isotopic tracer techniques. Experiments of this kind are now in progress.

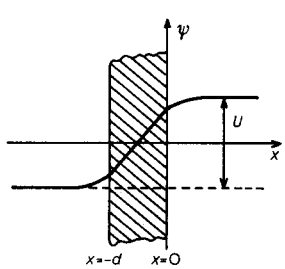

Fig. 5. Thin isolating film in contact with two electrolyte solutions. Electrical potential ψ as function of the distance x in the presence of an external potential difference U.

APPENDIX

Capacitance of a thin film between two electrolyte solutions

Consider a thin isolating film of thickness d and dielectric constant ε_m which is at $x = -d$ and $x = 0$ in contact with a 1:1 electrolyte solution of concentration c (Fig. 5). Within the film, the electrolyte concentration is assumed to be zero. When a potential difference $U = \psi_{x=\infty} - \psi_{x=-\infty}$ is maintained between the solutions, a space charge layer is built up in the vicinity of the planes $x = -d$ and $x = 0$. For $x \geqslant 0$ the concentrations c_+ and c_- of the cations and anions are given by the Boltzmann relations

$$c_+ = c\, e^{-\varphi(x)} \text{ and } \varphi(x) \equiv F[\psi(x) - \psi(\infty)]/RT \qquad (3)$$
$$c_- = c\, e^{\varphi(x)}$$

where $\psi(x)$ is the electrical potential at distance x. Eqns. 3 are simplified in two respects. Firstly, the (very small) influence of pressure on the potential energy of an ion is neglected. Secondly, image forces resulting from the discontinuity of the dielectric constant in the plane $x = d/2$ are not taken into account. As the disturbance of the ionic distribution due to image forces is confined to a distance of 2–3 Å from the boundary[30,31], their influence on the capacitance is likewise small. When Eqns. 3 are inserted into the Poisson equation

$$\frac{d^2\psi}{dx^2} = -\frac{4\pi}{\varepsilon} F(c_+ - c_-) \qquad (4)$$

(ε = dielectric constant of water), one obtains

$$\frac{d^2\varphi}{dx^2} = \kappa^2 \sinh \varphi,\ \kappa^2 \equiv \frac{8\pi}{\varepsilon}\frac{F^2}{RT} c \qquad (5)$$

where κ is the reciprocal of the Debye–Hückel length. The solution satisfying the boundary conditions

$$\psi(\infty) = U/2 \text{ and } \left(\frac{d\psi}{dx}\right)_{x=\infty} = 0 \qquad (6)$$

is given by

$$\psi(x) = \frac{U}{2} + 2\frac{RT}{F} \ln \frac{1 - \tanh(\alpha/2)e^{-\kappa x}}{1 + \tanh(\alpha/2)e^{-\kappa x}} \qquad (7)$$
$$\alpha \equiv F[U/2 - \psi(0)]/2RT \qquad (x \geqslant 0)$$

The potential ψ_m within the membrane is a linear function of x; this follows from Eqn. 4 with $c_+ = c_- = 0$. Therefore

$$\psi_m = \frac{2x + d}{d}\psi(0) \qquad (-d \leqslant x \leqslant 0) \qquad (8)$$

With the boundary condition

$$\varepsilon_m \left(\frac{d\psi_m}{dx}\right)_{x=0} = \varepsilon \left(\frac{d\psi}{dx}\right)_{x=0} \qquad (9)$$

one obtains from Eqns. 7 and 8:

$$\kappa d \frac{\varepsilon}{\varepsilon_m} \sinh \alpha + 2\alpha = \frac{UF}{2RT} \qquad (10)$$

Now, the capacitance (per cm²) of the film is $C = \sigma/U$, where σ signifies the total charge per cm²:

$$\sigma = F \int_0^\infty (c_+ - c_-)\mathrm{d}x = \frac{4cF}{\kappa} \sinh \alpha \tag{11}$$

The integration was carried out using Eqns. 3 and 7. Finally we introduce U from Eqn. 10 and obtain

$$C \equiv C_m \frac{\sinh \alpha}{\frac{2\alpha}{\kappa d}\frac{\varepsilon_m}{\varepsilon} + \sinh \alpha} \tag{12}$$

where $C_m = \varepsilon_m/4\pi d$ is the geometrical capacitance of the membrane per cm². Hence, C_m is always greater than the measured total capacitance C. For a given potential difference U the value of α can be determined by graphical solution of the transcendental Eqn. 10. However, for small values of U, *i.e.* $U \ll RT/F \simeq 25$ mV, α is also a small quantity according to Eqn. 10. In this case the approximation $\sinh \alpha \approx \alpha$ holds, and Eqn. 12 becomes

$$C = \frac{C_m}{1 + \frac{2}{\kappa d}\frac{\varepsilon_m}{\varepsilon}} \tag{12a}$$

In a 0.1 M solution of a 1:1 electrolyte $1/\kappa$ is about 10 Å. Putting $d = 60$ Å, $\varepsilon_m = 2.1$, $\varepsilon = 81$, we obtain $C = 0.998 \cdot C_m$. As long as the electrolyte concentration is relatively high ($1/\kappa$ small), the difference between C and C_m can thus be neglected.

ACKNOWLEDGEMENTS

The authors wish to thank Professor Dr. K. BERNHARD for the gift of several lipids and Dr. P. LESCH and Dr. H. H. A. LINDE for helpful discussions.

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