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THE EFFECTS OF A DIRECT CURRENT POTENTIAL BIAS ON THE ELECTRICAL PROPERTIES OF BIMOLECULAR LIPID MEMBRANES

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SUMMARY

1. A study has been made of the capacitance and resistance of bimolecular membranes of pure lecithin in *n*-heptane in the presence of d.c. transmembrane potentials of up to ± 200 mV.

2. The applied d.c. potential had no effect on the d.c. or a.c. resistance of the membranes, but caused a capacitance increase which, in given circumstances, was proportional to the square of the potential[5].

3. For a given applied potential, the percentage change of capacitance depended on the electrolyte concentration in which the membrane was formed, reaching a minimum at concentrations of 0.1 M of 1–1 electrolytes (KCl, NaCl, KI) or of 0.025 M of a 2–2 electrolyte ($MgSO_4$).

4. Change of temperature of the system between 10° and 30° caused a negligible change in the electric parameters of the membranes, but caused a fall by a factor of 5 in the time constant with which the capacitance change appeared. A linear relationship was observed between the time constant and the ionic mobility.

5. It is suggested that the observed changes were due to ionic movement at the lipid–water interface.

INTRODUCTION

Optically black single membranes of phospholipids formed between electrolyte solutions were introduced by MUELLER *et al.*[1] as models for cell membranes. Studies by electrical[2] and optical[3] techniques have shown that these membranes are two molecules thick, the molecules lying with their hydrocarbon tails inside the membrane, oriented perpendicular to the membrane plane, and with their hydrophilic polar groups at the membrane–water interface. Studies have been made of the permeability and other properties of the membranes and of the effect on these of certain interesting compounds absorbed into the membrane structure[4]. However, only one paper[5], appearing after the present work was begun, has considered the effect of a particularly characteristic biological situation, namely, a transmembrane d.c. potential of up to 100 mV. We have studied the changes in electrical properties of lecithin membranes on application of such potentials in the hope of deriving structural information, specifically regarding the orientation of the dipolar head group of the phospholipid and its role in stabilizing the system. It is intended that the present series of experiments should serve as an introduction to a similar later series in which macromolecules will be adsorbed onto the membrane surfaces.

MATERIALS AND METHODS

Lecithin

Preliminary experiments were made using commercial lecithin, partially purified by chromatography on a silicic acid column. The results, however, were then variable, and the main body of experiments was made with pure lecithin, prepared from hens' eggs according to the method of VREEMAN[6] and giving a single spot when analyzed by thin-layer chromatography. The lecithin was prepared and used in a solution (usually 1 % w/w) in *n*-heptane and the solution was stored under nitrogen at room temperature. Despite the precautions taken in storing the lecithin solutions, the measured effects in, and the stability of, the membranes changed after 4–8 weeks, apparently as a result of oxidation of the lecithin (as indicated by the effect of bubbling air through an aliquot of a fresh solution); in such circumstances, a fresh batch of lecithin was prepared.

Formation of membranes

The membranes were formed in an arrangement similar to that described by HANAI, HAYDON AND TAYLOR[2]. The lecithin solution was painted across a smooth round hole, about 1 mm in diameter, in a teflon pot which was partially immersed in a larger cell containing an electrolyte solution. The membrane so formed could be observed by white light reflected from it into a microscope through which could be seen, as the membrane drained, first the appearance of coloured fringes and then black ("grey") areas. After the membrane had drained to complete blackness, most of its rim could be discerned by reflexions from the discontinuity, and the membrane area could be measured, when required, with the aid of a graticule in the microscope eyepiece. Although the membrane drained to blackness within about 5 min, electrical measurements showed that it required about 20 min to reach stability. No serious difficulty was found in forming membranes from pure lecithin in a heptane solution (*i.e.* without stabilizing agents), but there was some advantage in first painting the lecithin solution across the hole in air and letting it dry before immersing the pot; this "pre-treatment" seemed to improve membrane stability. In situations involving the manufacture of a succession of membranes, it was found necessary to clean frequently the area of teflon around the hole. The outer vessel was provided with a jacket through which was circulated water at constant temperature. Unless otherwise indicated, the experiments were run at 22°.

Electrical measurements

A block diagram of the electrical circuit is shown in Fig. 1. Pt foil electrodes, placed in the solutions on either side of the membrane were connected to a transformer ratio arm bridge for the measurement of a.c. electrical parameters. The two bridges used—a Wayne-Kerr type B221 in the frequency range 10^2–$2 \cdot 10^4$ Hz and a Hatfield type LE300/A1 in the range $1.5 \cdot 10^4$–$2 \cdot 10^{-6}$ Hz, each connected to an appropriate oscillator and detector—allowed a d.c. potential to be applied between the Pt electrodes during the a.c. measurement. The applied d.c. potentials were up to ± 200 mV. but the a.c. potential at the Pt electrodes was never more than 5 mV. An oscilloscope connected between the oscillator and detector showed a Lissajous figure in which the conductive and capacitative contributions to any lack of balance of the bridge could be separately seen. An *x*-*t* plotter, connected (through a filter) to the detector, could be used to record the total (*i.e.* conductive *plus* capacitative) out-of-balance current.

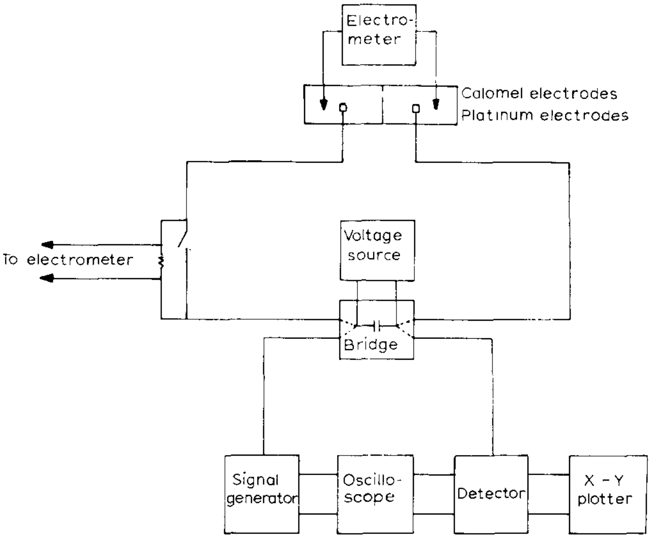


Fig. 1. Block diagram of the electrical circuit. With the transformer ratio arm bridges used, the voltage source applied a d.c. potential in the neutral line between the unknown and standard sides of the bridges and did not affect a.c. balance.

Calomel electrodes, also placed in the solutions on either side of the membrane, were connected to a Vibron electrometer which metered the transmembrane d.c. potential and was used in the measurement of the d.c. resistance of the membrane. It is to be noted that the arrangement used here was of general advantage since it enabled any polarization potential which built up on the Pt electrodes to be detected and off-set by a reverse potential bias.

RESULTS

a.c. characteristics of unbiased membranes and d.c. resistance

Measurements were made of the a.c. conductance and capacitance of membranes with zero d.c. transmembrane potential, over the frequency range 100 Hz–2 MHz and in the presence of electrolyte solutions of concentration 10^{-3}, 10^{-2}, 10^{-1}, or 1 M NaCl or KCl (with the same solution on each side of the membrane in all cases). The results, taking account of the membrane area, agreed closely with those of HANAI, HAYDON AND TAYLOR[2] and allow of the same interpretation. In particular they permitted us to regard the constant low-frequency capacitance of the system as a close approximation to the true capacitance of the membrane (see also the Appendix of ref. 7); and also to regard any membrane which showed an appropriate value of low-frequency capacitance as having a bimolecular structure.

Measurements of the d.c. resistance of membranes gave values in the range 10^2–10^3 $\Omega \cdot m^2$ (*i.e.* the measured conductance was about 10^{-8} Ω^{-1} for a membrane of area about 10^{-6} m^2). Resistances were constant (*i.e.* the voltage-current relation of the membranes was ohmic) for applied potentials up to ± 150 mV, in agreement with the results of D'AGOSTINO AND SMITH[8] but not in agreement with the results of LÄUGER *et al.*[7].

Capacitance change induced by a d.c. potential

When the membrane was electrically stable the bridge was balanced and a d.c. potential was applied, moving the system out of balance. In some cases—nearly all of which involved membranes made of commercial lecithin—a new position of stability was reached within a few minutes as illustrated in Fig. 2a. In these circumstances the bridge could be rebalanced in the presence of the applied potential, giving the result that the only measurable change was in the membrane capacitance. More commonly, as shown in Fig. 2b, the initial change following application of a potential was succeeded by a steady slow change, and the membrane did not return to its original state when the potential was removed. The bridge could not be properly balanced in slowly changing conditions, but approximate balancing—and standardly an inspection of the Lissajous figure on the oscilloscope screen—showed that in these cases, too, the only detectable changes were capacitative.

In view of these findings, the out-of-balance current of the bridge detector was taken, after appropriate calibration in each instance, as a measure of a purely capacitative change induced in the membrane by the applied potential. The value of the change to be associated with any particular potential was taken by extrapolating the linear part of curves like those of Figs. 2a and 2b back to the time at which the potential was applied. Because the initial membrane area, and so capacitance, varied from one sample to another, the capacitance change was expressed as a percentage.

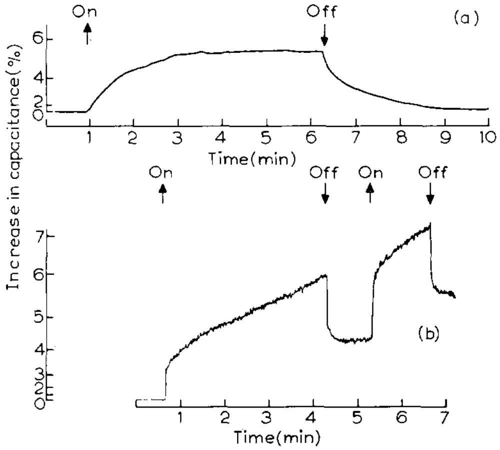


Fig. 2. Out-of-balance current of bridge detector on application and subsequent removal of a 50-mV d.c. potential. The a.c. potential was 5 mV at 500 Hz. The ordinate calibration of capacitance change was made in a subsidiary measurement after each experiment. (a) Membrane of partially purified commercial lecithin in *n*-heptane formed in 1 M NaCl solution. (b) Membrane of pure laboratory-prepared lecithin in *n*-heptane formed in 0.2 M NaCl. Different recorders were used in obtaining these two records, that used in (a) having an intrinsic time constant of approx. 30 sec.

The maximum changes recorded were 15 % of initial values usually in the range 2500–5000 pF, but the standard error in the measurements was only about ± 0.2 %.

With membranes made using pure fresh lecithin, successive application of a potential gave the same percentage capacitance change, within experimental error, for 10–12 applications and with the direction of the potential switched alternately. An indication from some early series of experiments that the amount of change fell by about a half in the course of 5–6 applications was not confirmed and was possibly a consequence of impurities in the lecithin or of oxidation effects.

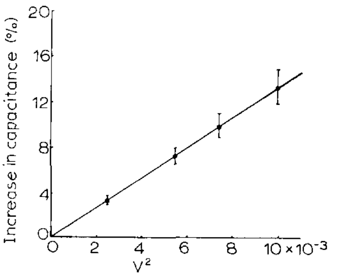
It is suggested by BABAKOV, ERMISHKIN AND LIBERMAN[5] that application of a d.c. potential across a membrane causes an enlargement of the membrane area, although their letter mentions neither the composition of their membranes nor the surrounding electrolyte solution. Increases of membrane areas corresponding to the largest capacitance changes should have been readily observable with our apparatus, but none were seen although they were carefully sought; however, the slow and usually rather small secondary changes (Fig. 2b) may have been a result of increase of membrane area.

Induced capacitance change as a function of applied potential

A study of the capacitance change as a function of the applied potential showed it to be directly proportional to the square of the potential (Fig. 3). If some polarization potential from the Pt electrodes was allowed to act on the membrane while it stabilized, the capacitance change for the applied potential V greater than 15 mV was expressible as $\Delta C/C = a + b\,V^2$, where C is the capacitance, and a and b are constants; however, the constant a is of the nature of an experimental error and was zero if the transmembrane d.c. potential was kept less than 1 mV during membrane stabilization.

Dependence of induced capacitance change on the ambient electrolyte solution

Some minor discrepancies between our results and those of BABAKOV, ERMISHKIN AND LIBERMAN[5] led us to measure capacitance changes as a function of electrolyte



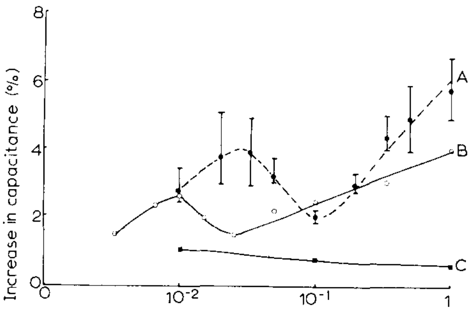


Fig. 3. Percentage change of capacitance as a function of the square of the applied d.c. potential. The a.c. signal was the same as in Fig. 2. The vertical bars give standard errors.

Fig. 4. Percentage change of capacitance on application of a 50-mV d.c. potential as a function of electrolyte concentration of the solution in which the membrane was formed. A: lecithin membrane (as in Fig. 2b) in KCl solutions; B: lecithin membrane (as in Fig. 2b) in $MgSO_4$ solutions; C: cholesterol membranes (see text) in KCl solutions. The vertical bars in Curve A give the scatter of experimental results.

concentration for several electrolytes. In these experiments the membranes always had the same solutions on each side, and the same applied potential of 50 mV was used in each measurement. The direction of the applied potential had no effect on the results.

Curve A of Fig. 4 shows the capacitance changes found in these conditions for membranes formed in KCl solutions of concentrations in the range $2 \cdot 10^{-4}$–1 M; each point on this and the other curves of the figure is the mean of 3–6 determinations and the vertical bars give the maximal scatter of the measurements. The most prominent feature of the curve is the pronounced dip in the middle. We noted that the position of the minimum occurs at a KCl concentration (0.1 M) for which the Debye length (9.6 Å) is just about equal to the dipole length of the polar group in the lecithin molecule[3,9]. To test whether or not this was a mere coincidence of numbers we repeated the experiment using $MgSO_4$ solutions. The result is shown in Curve B of Fig. 4 and we were pleased to find that although the shape of the curve was unaltered, the position of the minimum had shifted down to an electrolyte concentration having the same Debye length. It may be noted in passing, that the maximum in these two curves also occur at concentrations (0.025 M for KCl solutions) having the same Debye length, and that the value (19.2 Å) is close to the value given by LADBROOKE, WILLIAMS AND CHAPMAN[10] for the thickness of the "water layer" at the membrane surface.

Similar series of experiments with other 1–1 electrolytes (NaCl, KI) gave curves very similar to that of KCl and with the minimum in the same position. No 2–2 electrolyte other than $MgSO_4$ was used, but a series with a 2–1 electrolyte ($CaCl_2$) gave a curve with the minimum in the expected position between those for KCl and $MgSO_4$.

Membranes were formed from a solution in heptane of a sample of cholesterol which, however, had been standing in the laboratory for some months and was possibly partly oxidized. Small capacitance changes were found on application of a potential (Curve C of Fig. 4) and these changes decreased steadily with increasing electrolyte (KCl) concentration. Membranes formed of solutions of aged (oxidized?) lecithin yielded curves in which the features shown in Curves A and B of Fig. 4 tended to be flattened out, and with very old solutions the curves were like that for cholesterol, although with somewhat larger changes.

Time constant in the appearance of induced capacitance changes

It can be seen from Fig. 2 that application or removal of a d.c. potential resulted in an approximately exponential increase or decrease of the bridge detector out-of-balance current, with a time constant of the order of 1 min. For the electrical circuit in which the membrane is an element, the time constant might be expected to be given by $\tau = RC$ where R and C are the resistance and capacitance of the membrane. However, the observed values of time constant were usually about 30–100 times greater than the value calculated from the measured values of R (approx. $10^8\ \Omega$) and C (approx. 3000 pF). We therefore measured the time constant as accurately as possible (translating curves like those of Fig. 2b into capacitance changes and plotting these semilogarithmically against time) and sought a temperature dependence. With the aid of the thermostat jacket of the apparatus it was possible to keep the membrane and its surroundings at any temperature between 10° and 30°. Variation of temperature within these limits made only marginal differences (< 5 %) to the capacitance of the unbiased membrane or to its d.c. resistance. However, between the lowest and highest temperatures the time constant fell by a factor of 5, as shown in Fig. 5. The experimental results shown in the figure were obtained with membranes formed in 0.01 M KCl; the two parts of the figure show the measured time constant as a function of temperature and as a function of the ionic mobility.

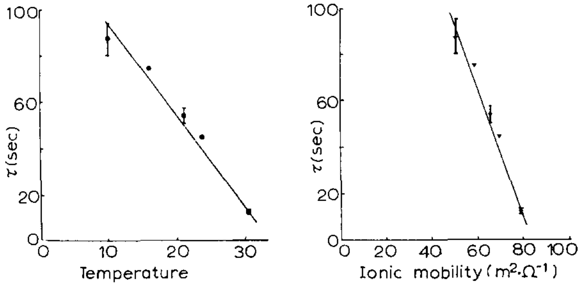


Fig. 5. Time constant (τ) of the appearance of capacitance changes following application of a d.c. potential of 50 mV to lecithin membranes formed in 0.01 M KCl. Time constants were determined using traces produced by the recorder used in Fig. 2b. Vertical bars give standard error. (a) τ as a function of temperature. (b) τ as a function of μ, the ionic mobility of K^+.

DISCUSSION

The experimental results reported here show that the application of a d.c. potential across a bimolecular lecithin membrane formed in electrolyte solution caused a marked change in the membrane's capacitance, but no change in its d.c. resistance and no measurable change in its a.c. resistance. The chief problem is to explain the capacitative change, but it is one to which we do not yet have a satisfactory answer.

Babakov, Ermishkin and Liberman[5] suggest that the applied potential, acting in conjunction with the surface tension of the membrane, causes the rim of the membrane to collapse and the membrane area to grow. This mechanism seems improbable to us for two reasons: we have sought but failed to observe the proposed increase of area; and we know of no evidence which might correlate the surface tension of a lecithin interface in electrolyte solution with the curves given in Fig. 4.

There is the possibility that pressure resulting from the electric field (about 10^7 V/m, if all the potential fall is directly across the membrane) causes an electrostriction, reducing the membrane thickness; and it is to be noted that the electrostrictive effect is, like the present effect (Fig. 3), proportional to the square of the applied potential. However, calculation using the standard formulae shows that the pressure involved is about a tenth of an atmosphere. Assuming that the material of the membrane has a modulus of bulk compressibility of the same order as usual organic liquids (approx. 10^{-4} bar^{-1}), there would be a negligible effect on the membrane thickness. This is a weak argument against an electrostrictive effect because the situation is complicated by the rim of the membrane experiencing a smaller electrostrictive pressure than the planar region. But a much more significant objection is that electrostriction would be expected to be constant with electrolyte concentration since the electrolyte always acts as a good conductor between the electrodes and the membrane surfaces: here again there is no explanation for the curves of Fig. 4.

The minima in Curves A and B of Fig. 4, the fact that these minima both occur at a Debye length approximately equal to the distance between the phosphate and trimethylammonium groups of the lecithin molecule, and the correlation between the time constant of appearance of the capacitative change and the ionic mobility which determines solution conductivity (Fig. 5), are three strong indications of the complexity of the conditions at the membrane solution interface. A qualitative consideration of these conditions suggests that they are sufficient to explain the observed capacitative changes in terms of the applied potential rearranging the ionic distribution near the interface in an energetically reversible manner. This concept is supported by the fact that cholesterol membranes, interacting with the solution only by means of the OH group of each intact cholesterol molecule, and so involving much less ionic interaction, show the effect to a much smaller degree (Fig. 4, Curve C).

However, we have not yet been able to obtain satisfactory quantitative expectations from any of the models we have considered and in particular we have not been able to determine whether application of a potential brings about a rotation of the lecithin dipole head either into or out of the membrane plane.

The experimental results presented here suggest two specific requirements in a model of the bimolecular membrane. (i) Electric charges, both in the membrane material and in the solution, must be localized, rather than smeared out as a charge density function. (ii) The stereochemistry of the interface must allow considerable ionic movement, more than would appear to be possible if the lecithin dipoles were firmly and permanently bound in the plane of the membrane. A major difficulty is to describe this situation in a mathematically tractable form.

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