THE ELECTRICAL CAPACITANCE OF PHOSPHOLIPID MEMBRANES

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ABSTRACT As one of the methods of finding out the structural change of lipid bilayers due to change of environmental solution, the capacitances of phosphatidyl choline (egg lecithin) and phosphatidyl serine (bovine brain) bilayer membranes in solutions of various pH and salt contents were measured. It was found that the capacitance of the bilayer depended upon pH and salt content. The capacitance had a minimum value around pH 4 for phosphatidyl choline and around pH 3–4 for phosphatidyl serine bilayers, respectively. The value of the capacitance increased as the pH of the solution became lower or higher. As the concentration of cholesterol in the phosphatidyl choline bilayer increased, the capacitance increased and reached a saturation value. A DC voltage across the phosphatidyl choline bilayer did not affect the value of the capacitance practically.

INTRODUCTION

To elucidate the fundamental nature of biological membranes, it is necessary to know the molecular structure of the membranes. Danielli and Davson (1935) suggested that the bimolecular lipid leaflet comprised the basic structural unit in many natural membranes, but only recently has this fundamental structure become available for direct experimental investigation (Mueller et al., 1964). There are several approaches to finding out the structure of lipid bilayer membranes, such as permeability studies, electrical impedance and capacitance measurements, electron microscopy, X-ray diffraction, surface tension, and chemical analyses. From optical and electrical measurements we may find out some physical properties such as specific resistance, specific capacitance, and dielectric constant, and macroscopic dimensions of the bilayers such as thickness of the film. The conductance and capacitance of these so-called black films have been measured by several workers. These characteristics are likely to be affected by the composition of the aqueous environment. Different investigators have reported different values of bilayer capacitance even for the same material (Hanai et al., 1964, 1965 *a*, 1965 *b*; Laüger et al., 1967; Tien and Diana, 1967). Some systematic measurements of the conductance of the bilayer in solutions of varying pH have been made by Ohki and Goldup (1968). In the present studies, observations were made of the effects of variation in pH, in the presence of varying concentrations of sodium or calcium, on the capacitance

of phospholipid bilayers. The influence of cholesterol on the capacitance of the phosphatidyl choline bilayer was also studied. The effect of applying a DC potential across the membrane on the capacitance of a phosphatidyl choline bilayer was also observed at various pH values.

MATERIALS AND METHODS

Chromatographically pure phosphatidyl choline (egg) and phosphatidyl serine (bovine brain) were purchased from Applied Science Laboratories (State College, Pa.) and also were prepared by Dr. Papahadjopoulos (Papahadjopoulos and Miller, 1967). The purity of the samples was kindly examined by Dr. Papahadjopoulos. It was found that the material was 99% pure. Phosphatidyl choline as purchased was dissolved in benzene. The benzene was driven out by blowing nitrogen gas, or evaporated in a vacuum chamber. Solutions of phosphatidyl choline were prepared in *n*-decane, either 20 mg of phosphatidyl choline in 1 ml of decane, or a mixture containing 20 mg of cholesterol and phosphatidyl choline in various proportions. Phosphatidyl serine as purchased was dissolved in chloroform solution. The chloroform was also driven out by blowing nitrogen gas, or evaporated in a vacuum chamber. The sample solutions of phosphatidyl serine were prepared by dissolving 20 mg of phosphatidyl serine in 1.0 ml of *n*-decane.

The apparatus is as follows: The central vessel was machined out of a solid rod of Delrin (Du Pont de Nemours & Co.). A small part of the wall of the Delrin vessel was thinned to less than 0.5 mm and a hole 1.59 mm in diameter was punched through this thin section. The vessel was immersed in concentrated HCl solution for a few hours. Since Delrin is slightly dissolved in the concentrated HCl, the edge of the hole becomes smooth. The Delrin vessel was suspended in appropriate aqueous solution in a glass beaker. The films were observed through a low-power microscope (Unitron MSF) in light reflected by the film from a microscope illuminator (American Optical Co., Buffalo, N.Y.). The room was always kept at 25 $\pm$ 1°C, and in order to prevent temperature rise of the solution in the glass beaker due to the microscope lamp, a heat filter was set between the illuminator and the beaker. The Delrin vessel was first cleaned by washing in detergent, and then was washed and rinsed in chloroform and methanol solution, and finally rinsed in distilled water. The films were prepared by smearing a membrane-forming solution across the punched hole with a thin glass pipette. Before the vessel was immersed in the aqueous solution, a small part around the hole on the vessel was smeared with the membrane solution to facilitate bilayer formation. Capacitance was measured with a Universal Bridge B221A (frequency = 10^4) of the Wayne Kerr Laboratory Ltd. (Chessington, Surrey, England).[1] The accuracy of the measurements was generally better than 1%. Platinum electrodes were used. A membrane potential was measured with Electrometer 610B of Keithley Instruments, Inc. (Cleveland, Ohio). The film diameter (black film part) and the hole diameter were measured with the aid of crossed scales in the microscope eyepiece, for every measurement.

RESULTS

In the first series of experiments, the capacitance of pure phosphatidyl choline bilayers was measured. The aqueous solution comprised 0.1 normal sodium chloride

[1] From the values of capacitance measured with various frequencies, it was found that with this bridge (frequency = 10^4) the "low frequency" value for the capacitance of the lipid bilayer is measured.

to which various amounts of hydrochloric acid or sodium hydroxide were added to adjust the pH to values varying from 1 to 10. The exact pH value was measured before and after the measurements of capacitance, using a standard pH meter (The London Co., Westlake, Ohio). Comparison experiments using solutions in which the pH was controlled by Tris or phosphate buffer gave identical results. Curve *a* of Fig. 1 shows the specific capacitance of the phosphatidyl choline bilayer in 0.1 normal sodium chloride solution with various pH values. Attempts to form bilayers in solutions of higher pH were unsuccessful. The capacitance has a minimum value (0.36 $\mu f/cm^2$) around pH 4, and increases symmetrically toward low pH or high pH.

In the second series of experiments, the capacitance of lecithin-cholesterol bilayers was measured in the same aqueous solution as in the first series. Curves *b* and *c* of Fig. 1 show the specific capacitance obtained with phosphatidyl choline + cholesterol (1:½ and 1:1 weight ratio) bilayers. The curve for the capacitances as a function of pH has a form similar to that for pure phosphatidyl choline, but the values of the capacitance were greater than those for pure phosphatidyl choline bilayer as the amount of cholesterol in the sample solution was increased.

In the third series of experiments, the capacitance of phosphatidyl serine bilayers was measured in aqueous solution containing 0.1 normal sodium chloride at various

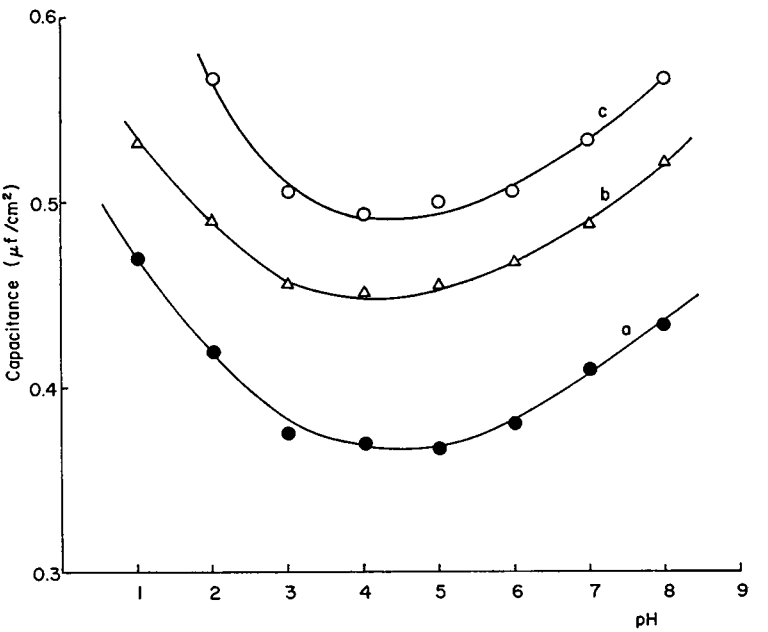

FIGURE 1. The specific capacitances of phospholipid bilayers with respect to pH of solutions. Curve *a*, phosphatidyl choline; *b*, phosphatidyl choline + cholesterol (1:½ weight ratio); *c*, phosphatidyl choline + cholesterol (1:1 weight ratio).

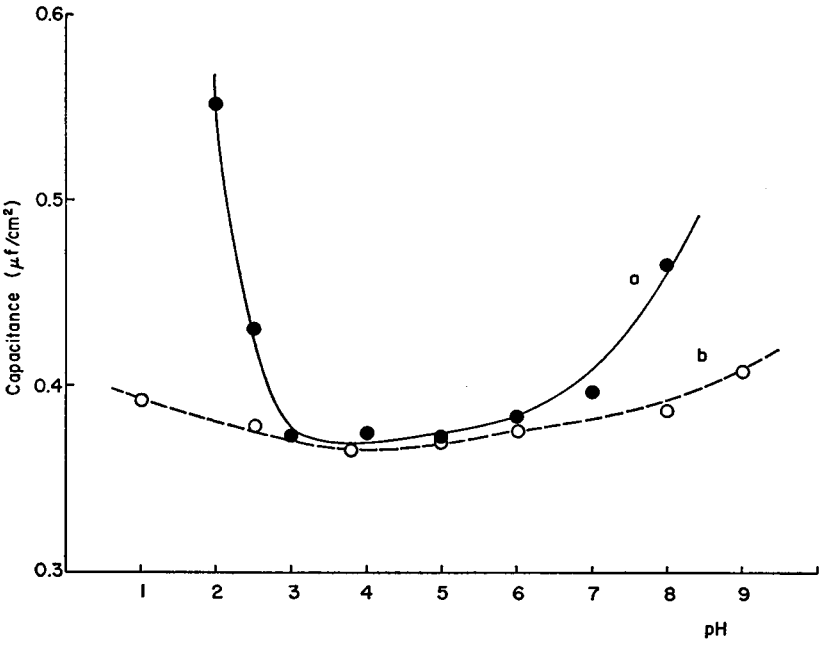

FIGURE 2. The specific capacitances of phosphatidyl serine bilayers with respect to pH of solutions. Curve *a*, in solution of 0.1 N sodium chloride; *b*, in solution of 0.1 N sodium chloride + 1 mM calcium chloride.

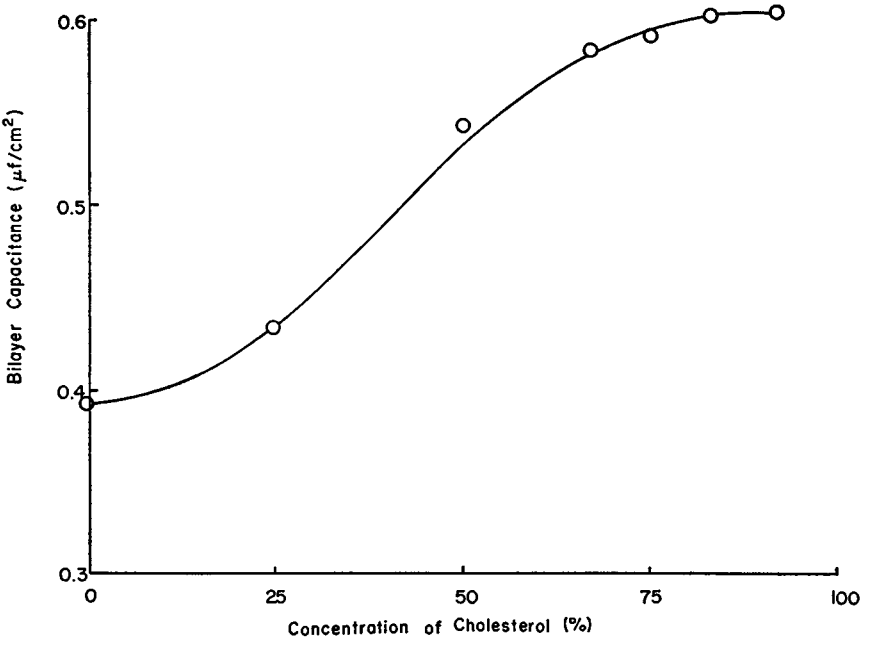

FIGURE 3. The specific capacitances of phosphatidyl choline + cholesterol bilayers with various percentages of cholesterol.

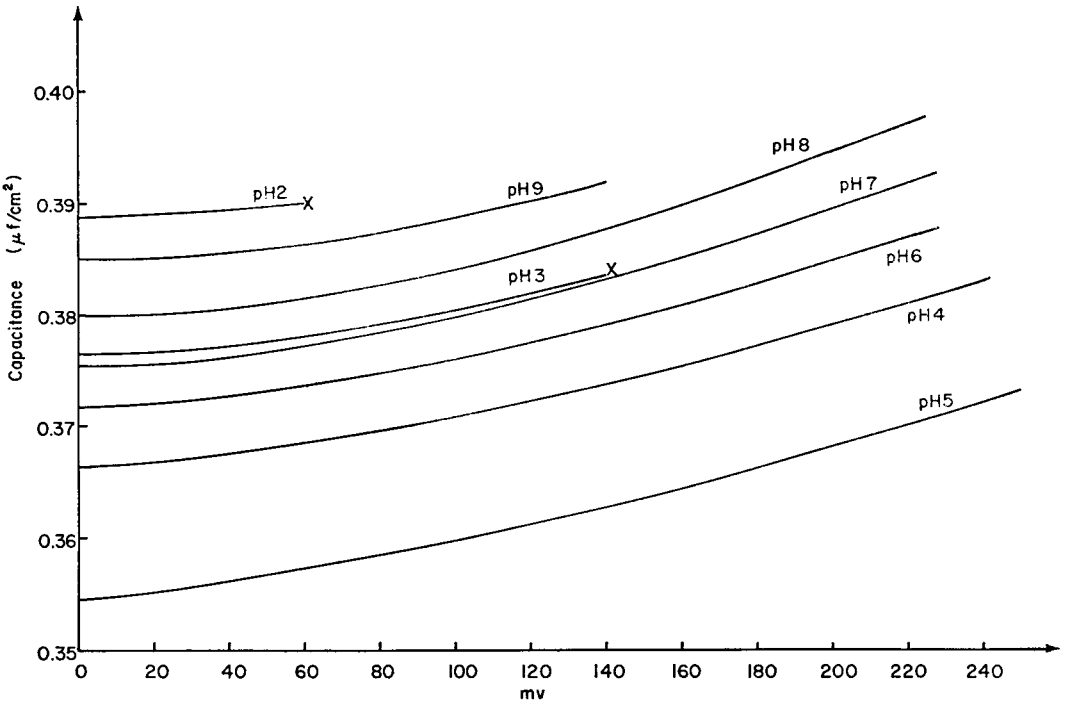

FIGURE 4. The specific capacitances of phosphatidyl choline bilayers with various DC applied potentials. *X*, breakdown potential.

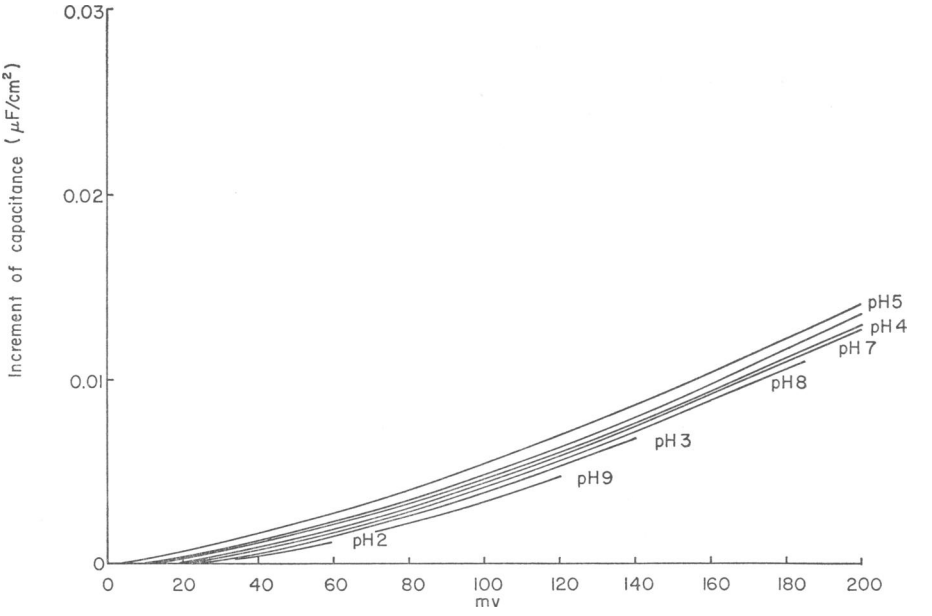

FIGURE 5. The increment of specific capacitance of phosphatidyl choline bilayers with respect to various applied potentials.

pH values. Curve *a* of Fig. 2 shows the specific capacitance of phosphatidyl serine. The values of the capacitance were almost the same as those for phosphatidyl choline bilayers, but the pH value corresponding to the minimum value of the capacitance was shifted to around pH 3. The stability of phosphatidyl serine bilayers was more sensitively dependent than that of phosphatidyl choline upon pH; that is, in a solution of pH lower than 2 or greater than 8, the bilayer cannot be formed. Presumably, the large electrostatic forces due to the dissociation of polar groups at the interface may prevent the formation of a stable bilayer.

In the fourth series of experiments, the capacitance of phosphatidyl serine bilayers was measured in 0.1 normal sodium chloride plus 1 mM calcium chloride solution at various pH values. Curve *b* of Fig. 2 shows the specific capacitance obtained for the phosphatidyl serine bilayers. The capacitance did not vary over a wide pH range (1–9). The bilayer formed easily even in a solution of low pH or high pH where in the absence of calcium ions the phosphatidyl serine bilayer was not formed at all.

In the fifth series of experiments, the capacitance of phosphatidyl choline plus various amounts of cholesterol bilayers was measured. Fig. 3 shows the specific capacitance of these bilayers. The capacitance increased as the concentrations of cholesterol in the sample solution increased until the concentration reached 80% in weight.

In the sixth series of experiments, the effect of applying direct potential across the bilayer on the capacitance of a phosphatidyl choline bilayer was observed in 0.1 normal sodium chloride solutions of various pH values. The specific capacitances are shown with respect to applied voltage in Figs. 4 and 5. Approximately 1–3% changes in the membrane capacitance with applied potential of $\sim$100 mv were observed, depending upon the pH of the solution.[2] The breakdown potential of the membrane depended upon the pH of the solution. The breakdown potential was over 200 mv for the membrane in solutions of the range pH 4–8. However, in solutions of pH 3 or 9, the breakdown potential was less than 140 mv, and it was even less than 60 mv in the solution of pH 2.

DISCUSSION

In order to obtain the capacitance of the bilayer, it is necessary to find out an effective equivalent electric circuit for the system used for the measurements (Fig. 6 *a*). After Hanai et al. (1964), the following equivalent circuit is considered (Fig. 6 *b*). C_o' is the stray capacitance for the whole system of the vessel, electrodes, and leads without electrolyte solution in the vessel. C_o' was less than 100 pf. The corresponding parallel conductance was less than 10^{-15} mho. When the vessel is filled with electrolyte solution, it is assumed that the aqueous phases and the vessel parts, except

[2] The experimental error would be at most 3% of the absolute value, to judge from the error of the estimation of area of the membrane.

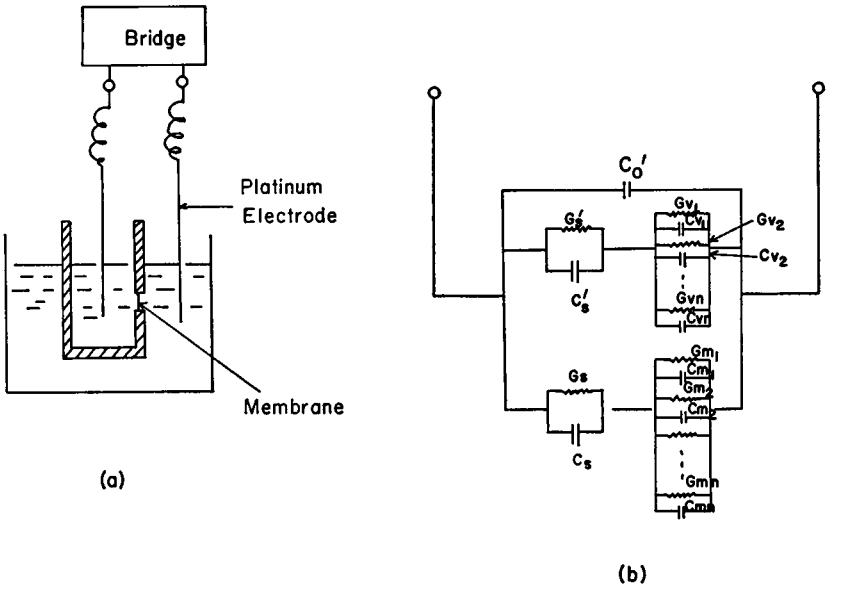

FIGURE 6. *a*. Scheme of the system of capacitance measurements. *b*. An effective equivalent electric circuit for the system. See text.

for the region of the hole, have parallel combinations of capacitance and conductance (C_s' and G_s' for the aqueous solution; C_v and G_v for the vessel) which are combined in series. Since the electrical field near the wall is not uniform, owing to the hole, C_v and G_v would comprise the parallel combination of various C_{vi} and G_{vi}. Since G_v has a very small value ($G_v < 10^{-15}$ mho), the effective equivalent circuit for the vessel and solution parts, except for the hole region, may be replaced by a small capacitance C_o, which is of the order of a few hundred picofarads (pf).

For the hole part, each of the membranes and its adjacent aqueous phase may have parallel combinations of capacitance and conductance, which are combined in series with each other (C_s and G_s for the aqueous phase; C_m and G_m for the membrane). Since the electrical field is not uniform over the whole region of the membrane, the capacitance and conductance of the membrane comprise the sum of various kinds of capacitance elements and conductance elements connected in parallel. The total impedance for the hole part is in parallel with the rest of the elements. Therefore, the effective equivalent circuit is reduced to the circuit shown in Fig. 7.

In this circuit, the total capacitances of the system without a membrane and with a membrane are expressed by

$$C_{to}^{*} = C_s + C_o + \frac{G_s}{jw}$$

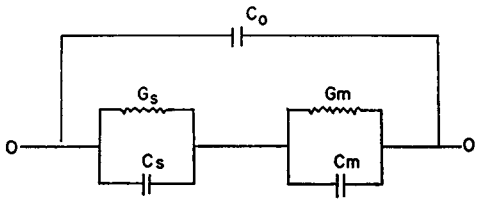

FIGURE 7. An effective equivalent electric circuit for Fig. 6 *b*. See text.

and

$$C^*_{tm} = C_h + C_s + \frac{C_l - C_h}{1 + w^2\tau^2} - j\left[\frac{(C_l - C_h)w\tau}{1 + w^2\tau^2} + \frac{G_l}{w}\right]$$

respectively, where

$$C_h = \frac{C_s C_m}{C_s + C_m}$$

$$C_l = \frac{C_s G_m{}^2 + G_s{}^2 C_s}{(G_s + G_m)^2}$$

$$G_l = \frac{G_s G_m}{G_s + G_m}$$

$$\tau = \frac{C_s + C_m}{G_s + G_m}$$

$$j = \sqrt{-1}$$

w = frequency.

Since the value of G_s is much greater than that of G_m, $(G_m/G_s \approx 10^{-4}\text{–}10^{-5})$, we have have

$$G_s + G_m = G_s\left(1 + \frac{G_m}{G_s}\right) \cong G_s,$$

and also, with the value $w = 10^4$ for the frequency, we have

$$1 + w^2\left(\frac{C_m + C_s}{G_s}\right)^2 \cong 1.$$

Then the total capacitance for the system with the membrane is

$$C^*_{tm} = C_m + C_o - \frac{1}{j}\left[\frac{(C_m - C_h)w\tau}{1 + w^2\tau^2} + \frac{G_m}{w}\right].$$

The Universal Bridge shows the value for the unknown in terms of the equivalent parallel components of conductance and capacitance:

$$C_t^* = C_t + \frac{G_t}{jw}.$$

The value C_t read with the bridge is equal to the value of the sum $(C_m + C_o)$. Since C_o is less than several hundred pf, we have

$$C_t \cong C_m .$$

In the first four series of experiments, it is evident that the capacitance of the bilayers has greater value in a solution of lower pH or higher pH than in one of medium pH. Since phosphatidyl choline has a zwitterionic group and phosphatidyl serine has three dissociable polar groups, at those lower and higher pH values the polar groups would be charged positively or negatively. Recent monolayer studies have shown (Papahadjopoulos, 1968) that the surface potentials changed at pH 4 for phosphatidyl choline and at pH 3 for phosphatidyl serine. We may assume that the dissociation of phosphate groups takes place in these pH ranges in the bilayer state. The polar groups do not contribute to AC capacitance of the bilayer (Hanai et al., 1965 *a*). The variation in capacitance with pH is quite large, when the membrane is in sodium chloride solutions. The capacitance must vary in a complex manner, since change in thickness will involve change in hydrocarbon chain orientation and hence in the dielectric constant perpendicular to the membrane. However, the variation in capacitance with pH greatly exceeds any effect which could arise from the dielectric constant variation (Ohki, 1968, 1969 *a*). Consequently most of the change must result from variation in thickness with pH—a variation clearly to be expected as charge density increases. But in the presence of calcium ions the membrane is stable, and the thickness may not change easily because of the chelating of calcium ions with the polar groups of the phospholipids. This will both reduce charge density and introduce cross-binding effects. Results of conductance measurements for the phospholipid bilayers agree with this interpretation (Ohki and Goldup, 1968).

From Figs. 1 and 2, it is seen that the capacitance for phosphatidyl choline bilayers increases symmetrically toward low pH or high pH, whereas the capacitance for phosphatidyl serine bilayers increases asymmetrically. This may be because phosphatidyl choline has a zwitterionic group (positive and negative ionized groups), whereas phosphatidyl serine has asymmetrical dissociable ionic groups (one positive and two negative ionizable groups). Our fourth series of experiments was similar to those of Hanai et al. (1965 *b*). We obtained similar results. However, there is a slight difference in the capacitance with various ratios of cholesterol to lecithin. Addition of cholesterol to a phospholipid sample increases the capacitance of the membranes. The capacitance increases gradually with the amount of cholesterol and reaches a value of close to 0.6 μf/cm^2. At over 80 % cholesterol no further change

in capacitance was found. This fact suggests that saturation of phospholipid bilayers with cholesterol occurs. A similar saturation phenomenon has been reported by Bruckdorfer et al. (1968) for the red cell membrane lipids. The cholesterol molecule oriented normally to an interface with its hydroxyl group in the aqueous phase is approximately equivalent in length to a normal alkyl chain of 14 carbon atoms, while the number of the chains of the lecithin is about 18 carbon atoms.

It is speculated here that as the amount of cholesterol in the membrane increases, the membrane becomes thinner and the corresponding capacitance increases until the concentration of cholesterol in the phospholipid reaches the saturation value.

In the experiments on the effect of applying direct potential on the capacitance, the circuit used is as shown in Fig. 8 *a*. For this circuit the effective equivalent circuit is considered to be as shown in Fig. 8 *b*. For the voltage range 0–260 mv, the values of C_p and G_p were practically unchanged ($C_p = 0.7$ pf, $G_p \sim 4 \times 10^{-10}$ mho). The total capacitance is

$$C_{tm}^{**} = C_m + C_o + C_p + \frac{C_l - C_h}{1 + w^2\tau^2} - j\left[\frac{(C_l - C_h)w\tau}{1 + w^2\tau^2} + \frac{G_l}{w} + \frac{G_p}{w}\right].$$

As discussed previously, since $G_m \ll G_s$ and $C_m \gg C_o$, C_p, the parallel component of the capacitance read with the bridge, is equal to the capacitance of the membrane.

Babakov et al. (1966) have reported the effects of applying direct current on the capacitance of a phospholipid bilayer. They obtained an increase of capacitance of the same order of magnitude that we found. They deduced that the changes in capacitance caused by the applied voltage were due to reversible transformations of lipid solution to a bimolecular film at the border of the ring. Apparently they measured the total increment of the capacitance. However, we measured the specific capacitance, which still showed the increase of capacitance up to 3% for applied

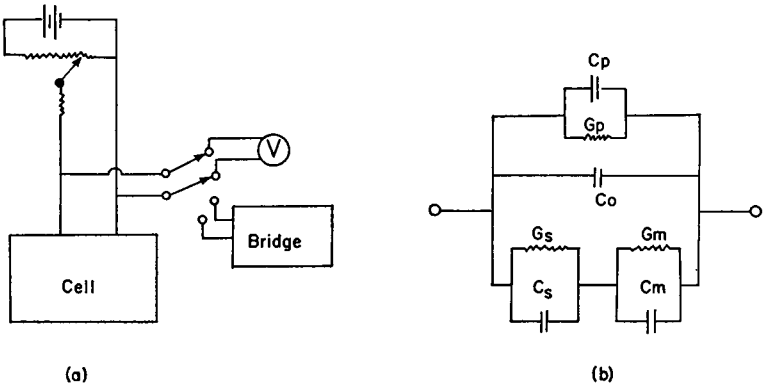

FIGURE 8. *a*. Scheme of the system of capacitance measurements with an applying voltage. *b*. An effective equivalent electric circuit for Fig. 8 *a*. See text.

potential of 100 mv, depending on the pH value of the solution. It is seen in Fig. 5 that the increment of the capacitance changes with respect to the pH value of the solution. Therefore, we propose that the cause of the increase in capacitance is not the change in area of the bimolecular region, but the interaction between electrodes and the electrolyte or the interaction between the electrolyte and the phospholipid polar groups (double layer). However, since the change in capacitance due to the applied voltage is not large (at most 3 % for applied potential of 100 mv), it may be suggested that applying voltage across the phospholipid membrane does not significantly affect the value of the capacitance of the membrane. This agrees with the fact that there is no significant change in the capacitance of the biological membranes during their excitation. With the results of conductance measurements in solutions of various pH values and various salt contents (Ohki, 1969 *b*), we conclude here that phospholipid bilayer membranes would change their thickness with solutions of various pH values, but that there is no large change (over 3 % for applied potential of 100 mv) of thickness when DC voltage is applied across the membrane.[3]

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[3] Rosen and Sutton (1968) have measured the effect of applied potential on the capacitance of phosphatidyl choline bilayers. They found that for a given potential, the percentage change of capacitance depended on the electrolyte concentration in which the membrane was formed, reaching a minimum at a concentration of 0.1 M of univalent electrolytes or 0.025 M of a divalent electrolyte.