BBA 75983

MEMBRANE POTENTIAL OF PHOSPHOLIPID BILAYERS: ION CONCENTRATION AND pH DIFFERENCE

SHINPEI OHKI

Department of Biophysical Sciences, State University of New York at Buffalo, Buffalo, N.Y. 14226 (U.S.A.)

(Received March 13th, 1972)

SUMMARY

A study was made of transmembrane potentials at the initial state produced by a concentration gradient of identical ions and also different ionic species at the same concentration for various phospholipid bilayers. It was found that the potential of the salt added side was lower than that on the other side of the membrane. For the phosphatidylserine and phosphatidylethanolamine membranes, there was a significant difference between the potentials produced by the addition of the same concentrations of KCl and NaCl, respectively, on one side of the membrane, whereas, for the phosphatidylcholine membrane, there was no appreciable difference in produced membrane potentials. The produced membrane potential reached saturation with high concentration of salt on one side. For the cases of the addition of divalent ions, it was found that even a low concentration of $CaCl_2$ on one side of the phosphatidylserine membrane resulted in a large membrane potential, which suggests a strong interaction of Ca^{2+} with phosphatidylserine molecules. The presence of a pH difference between the two sides of the phosphatidylserine membrane also produced membrane potentials, but, for the phosphatidylcholine membrane, there was no appreciable change in the produced membrane potential. It is concluded that these membrane potentials observed at the initial state of the system are produced mainly by the difference between two diffused double-layer potentials formed at the interface of the membrane and the electrolyte solution on both sides and not by the ion diffusion across the membrane.

INTRODUCTION

Phospholipid membranes have recently become the subject of intensive research as a model for studying the structure and function of biological membranes[1-3]. Although phospholipid bilayers composed of purified phospholipids serve in general as a simplified model for the biological membrane, some of the properties of such artificial membranes have been shown to have striking similarities to those of the biological membrane[1-3].

Among properties of the membrane, transmembrane potential produced when there is the ion concentration gradient across the membrane is one of the important subjects in understanding the function of the biological membrane.

There have been several investigators who have observed membrane potentials produced by ion concentration gradient across the phospholipid membrane[1,5–7,10]. However, the major interest of membrane potential for lipid bilayers was to observe the transmembrane potential due to specific ion permeation in the presence of various surfactants[8], certain inorganic ions[7], proteins (excitability-inducing material)[3,9]. There have been a few investigators[7,10,11] who have observed the transmembrane potential of pure phospholipid bilayers under various salt concentration gradients.

Hopfer *et al.*[10] have studied the membrane potential produced by alkaline salt solution gradients across the membrane, and have shown that the permeability of various negative and neutral charged phospholipids to cations was varied while a positively charged phospholipid showed a marked sensitivity only to negative ion permeability.

On the other hand, Massey and McCulloch[11] have reported a possible idea that the cation induced transmembrane potential would be explained by the concept of ion adsorption.

Recently, Ohki[4] has proposed a theory of membrane potential; that is, the membrane potential may arise from the difference between two diffused surface potentials on two sides of the membrane which are produced by the fixed charges or polar groups at the membrane surface and the surrounding electrolyte solution. The following membrane system may exhibit this type of membrane potential, that is, a membrane can be considered to be impermeable to ions compared to the diffusion of ions in the aqueous phase. The membrane has a hydrophobic interior and hydrophilic surface facing electrolyte solution phases. If one surface of the membrane is different from the other membrane surface with respect to their surface charges and dipoles, two different diffused double layer potentials would be produced on two different sides of the membrane. Assuming that there is no potential gradient in the membrane, a potential on one side of the membrane away from the membrane surface would be different from that on the other side of the membrane. At the initial state, therefore, the difference between two surface potentials may be observed since the ionic conductance of the membrane is very low, although this potential difference will vanish as the stationary state of the system is attained. A phospholipid bilayer system which has asymmetrical distribution with the surface charges or polarization due to polar groups, may correspond to the one which demonstrates such a membrane potential, at the initial state.

In this report, a study was systematically made of the initial state membrane potential produced by the ion concentration gradient together with different salt contents (*e.g.* NaCl on one side and KCl on the other side) across various phospholipid bilayers.

MATERIALS AND METHODS

Bimolecular phospholipid membranes were formed in aqueous solution through an adaptation of a method used for the formation of black soap films. Phospholipids used were chromatographically pure phosphatidylcholine (egg), phosphatidylserine (bovine) and phosphatidylethanolamine (bovine) purchased from Applied Science Laboratories (State College, Pa.) and some from Supelco (Bellefonte, Pa.). By using the same type of phospholipid identical experimental results were observed within

the experimental error for both Applied Science Lab. and Supelco's samples. These phospholipids were stored in chloroform (phosphatidylserine and phosphatidylethanolamine) and benzene (phosphatidylcholine).

The membrane-forming solution was 2.5 % (w/w) phospholipid in *n*-decane. The details of the cell arrangement used to form bimolecular membranes are described in the earlier papers[12,13]. All chemicals used were reagent grade (Fisher Scientific Co.). Water was triple distilled including the process of distillation by using $KMnO_4$. The electronic circuit used for measurement of the membrane potential, is described schematically in Fig. 1, where A is the differential amplifier (each input impedance

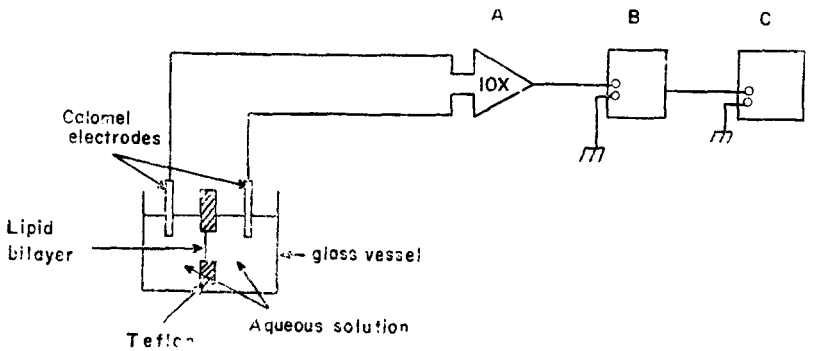

Fig. 1. Schematic diagram of instrumentation for measurements of membrane potential. A, differential amplifier; B, electrometer; C, chart recorder.

$> 10^{13}\ \Omega$, gain = 10×), B is the Keithley Instrument Model 610C electrometer and C is the strip chart recorder (Bausch-Lomb, VOM 7). Calomel half cell electrodes (standard pH electrodes) (London Co., Ohio) were used as reversible electrodes to detect the potential. Micro-syringes (Hamilton Co.) were used for injection of small amounts of solution. The temperature was maintained at 23 ± 1 °C. All aqueous solutions contained 0.2 mM Tris–HCl (Ultra Pure grade, Mann Research Co.) as a buffer. The solutions also contained 0.05 mM disodium EDTA to remove small amounts of contaminant such as bi- and multivalent metals, which are present as contaminants in the monovalent salts and are also extracted along with phospholipids from natural sources. Metal analysis of the phospholipids is described elsewhere[14].

EXPERIMENTAL RESULTS

The experiments can be divided into three parts. In the first part, the membrane potentials produced by the addition of monovalent salt (NaCl or KCl) on one side or both sides of the membrane were measured for various phospholipid membranes prepared in an aqueous solution. In the second part of the experiments, the membrane potentials produced by the addition of divalent ions ($CaCl_2$ and $MgCl_2$) were measured using different phospholipid membranes made in monovalent salt solutions. For all cases involving the addition of salt on one side of the membrane, the electrical potential of the solution for the salt added side was lower than that of the other side of the membrane. In order to compensate for the hydrostatic pressure difference due to the addition of salt solution on one side, a small amount of the same aqueous solution in which the membrane was prepared, was added on the other side of the

membrane. In the third part of the experiments, the membrane potential created by a pH difference between the two sides of the membrane was examined. The membrane potentials were gradually developed after gentle stirring of the salt solution on one side of the membrane, and were observed as if the system reached a steady state. However, this potential was not the steady state potential. The developed potential tended to decay which should have vanished to zero after a long time. However, the potential at the initial state (0–15 min) did not show much change (a few percent charge in potential which varied depending upon the type of ions). All phospholipid membranes were prepared in pH 7.2 solution unless otherwise specified.

Asymmetrical distribution of Na^+ and K^+

Figs 2A, 2B and 2C show the electrical membrane potentials produced by the addition of NaCl (3 moles/l) on one side of phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine membranes, respectively, which were prepared in distilled water (a) and 10 mM NaCl (b), respectively. The membrane potential produced by the addition of any given concentration of NaCl on one side of the membrane showed the greatest potential for the phosphatidylserine membrane and the smallest membrane potential for the phosphatidylcholine membrane. The membrane potential produced by the addition of a given concentration of salt on one side of the membrane prepared in distilled water, was greater than that for the membrane prepared in the solution of 10 mM NaCl. It was noticed that the membrane potential increased gradually, as the concentration of the salt was increased on one side, and finally reached a saturation point for the addition of more salt.

In Figs 3A, 3B, and 3C, similar observations as in the above experiments were shown for the addition of KCl (3 moles/l) on one side of the membrane. The general

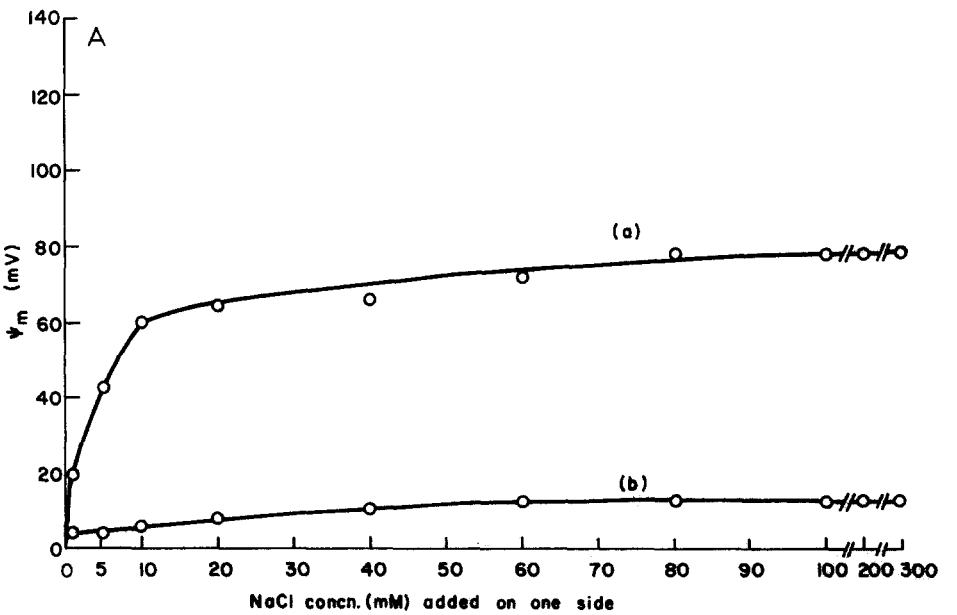

Fig. 2.

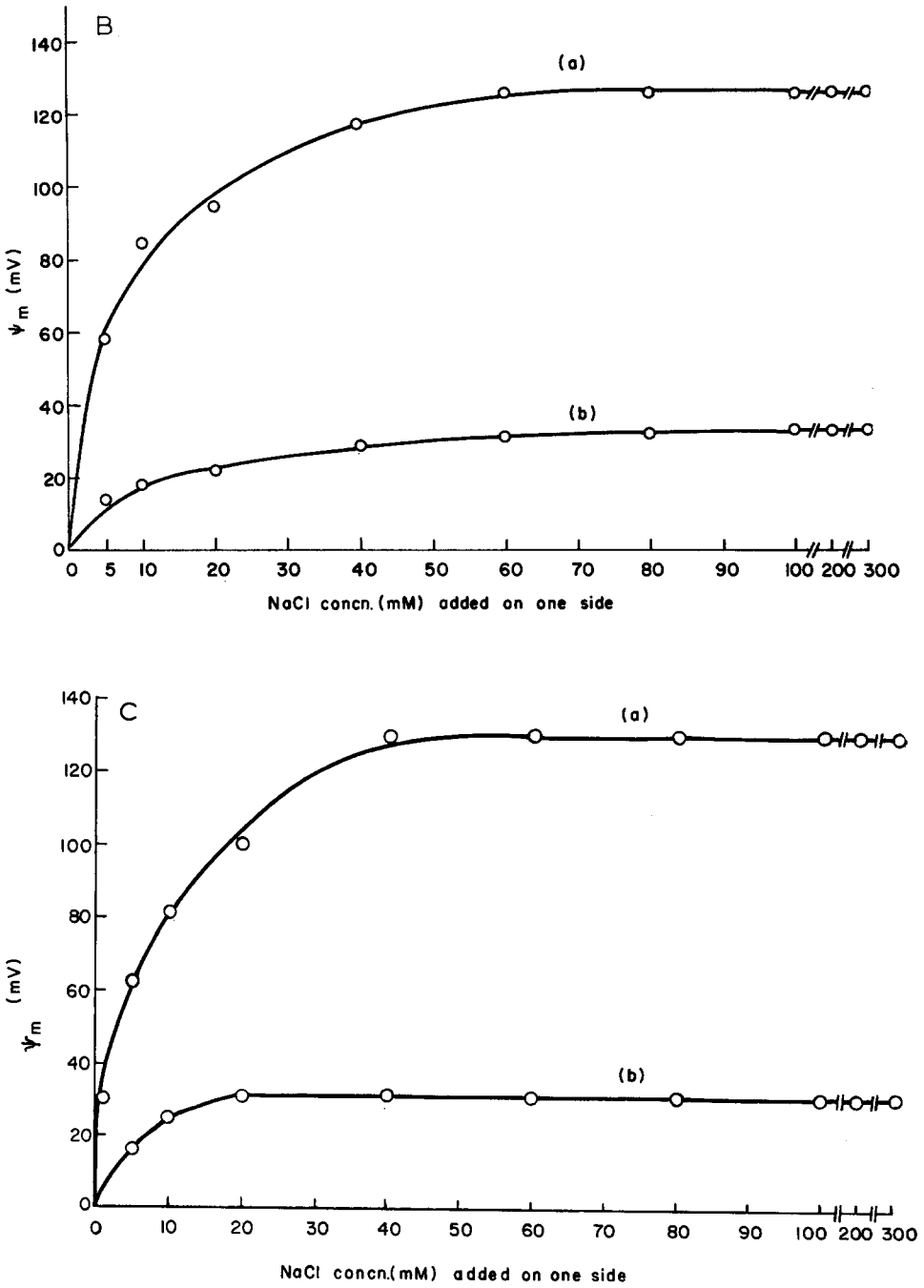

Fig. 2. Transmembrane potentials (A for phosphatidylcholine; B for phosphatidylethanolamine and C for phosphatidylserine membranes) produced by NaCl addition on one side. The salt added side showed lower potential. (a) membrane prepared in 0 mM NaCl, pH 7.2; (b) membrane prepared in 10 mM NaCl, pH 7.2.

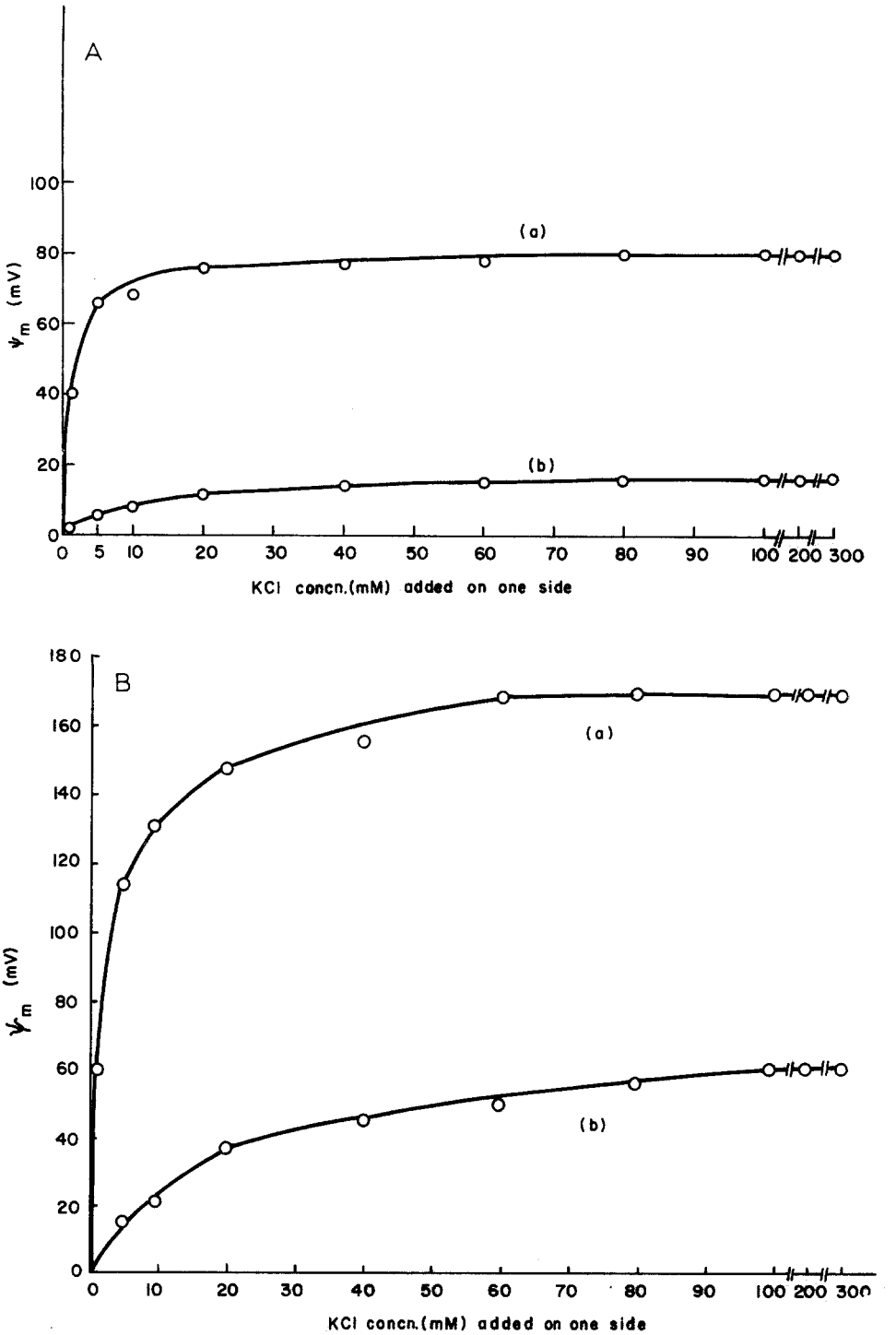
A
100
80
60
40
20
0
ψ_m (mV)
(a)
(b)
0 5 10 20 30 40 50 60 70 80 90 100 200 300
KCl concn.(mM) added on one side
B
180
160
140
120
100
80
60
40
20
0
ψ_m (mV)
(a)
(b)
0 10 20 30 40 50 60 70 80 90 100 200 300
KCl concn.(mM) added on one side

Fig. 3.

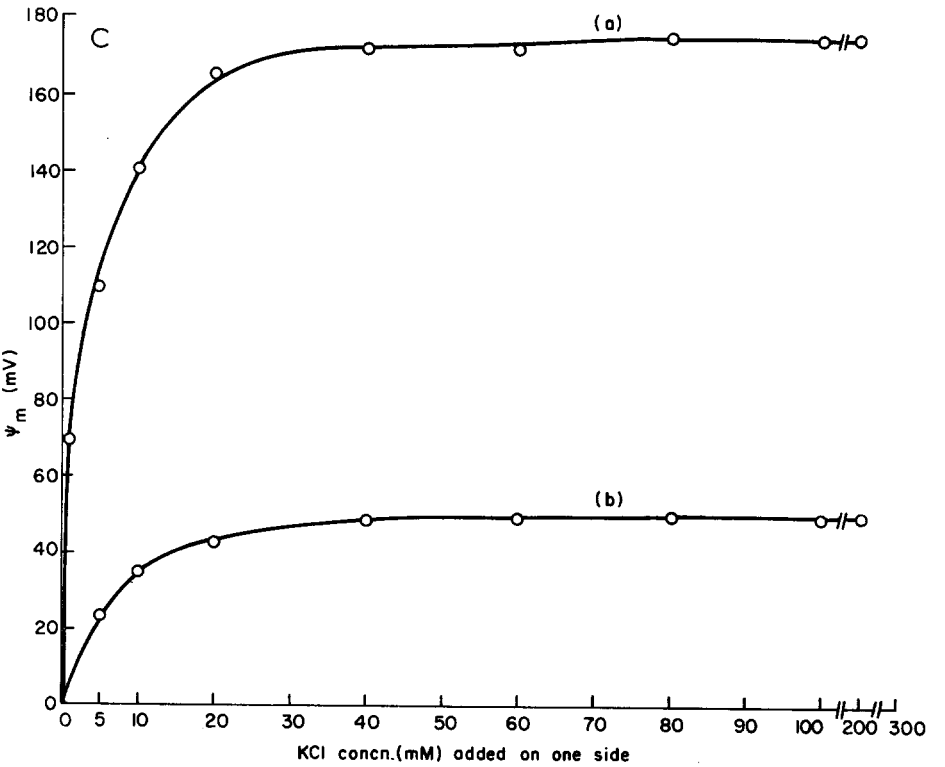

Fig. 3. Transmembrane potentials (A for phosphatidylcholine, B for phosphatidylethanolamine and C for phosphatidylserine membranes) produced by KCl addition on one side. The salt added side showed lower potential. (a) Membrane prepared in 0 mM KCl, pH 7.2; (b) membrane prepared in 10 mM KCl, pH 7.2.

behavior of the produced potentials was similar to the previous case (Figs 2A, 2B and 2C). One of the differences between NaCl and KCl injections was that the addition of KCl on one side of the phosphatidylserine and phosphatidylethanolamine membranes, respectively, produced greater potentials than those produced by the addition of the same concentration of NaCl. The potential difference at the saturation point was about 1.4-fold of that for the case of NaCl addition. Whereas, for the phosphatidylcholine membrane, almost equal membrane potentials were produced by the addition of the same amount of KCl and NaCl, respectively.

As a combination of the above experiments, the membrane potential was measured for various phospholipid membranes when NaCl was added on one side of the membrane and, at the same time, the same concentration of KCl was added on the other side. Fig. 4 shows these membrane potentials for the phosphatidylethanolamine and phosphatidylserine membranes, respectively, which were first prepared in distilled water. As expected from the results shown in Figs. 2 and 3, for the phosphatidylserine and phosphatidylethanolamine membranes, the KCl added side was more negative in potential, and the produced membrane potential in Fig. 4 was approximately equal to the difference between the membrane potentials produced (Figs 2B and 2C, and 3B and 3C) when KCl and NaCl were added, respectively, on one side of the phospha-

tidylserine or phosphatidylethanolamine membrane. The membrane potential increased for the first stage and gradually decreased until a saturation value was reached as the concentration of salt was increased. The saturation value was approximately equal to the difference between saturation values by KCl and NaCl added independently on one side of the phosphatidylserine or phosphatidylethanolamine membrane. However, for the phosphatidylcholine membrane, there was no appreciable membrane potential produced in this case. The membrane potential was fairly well reproducible for each phospholipid. Each experimental point in Figs 2, 3 and 4 was obtained by taking arithmetic means of measurements for more than five membranes. The error of the mean value varied with the salt concentration. The more concentrated the salt was, the less was the error. The error of the mean value of the potential in the case of the lowest salt concentration used was less than $\pm$ 10 %.

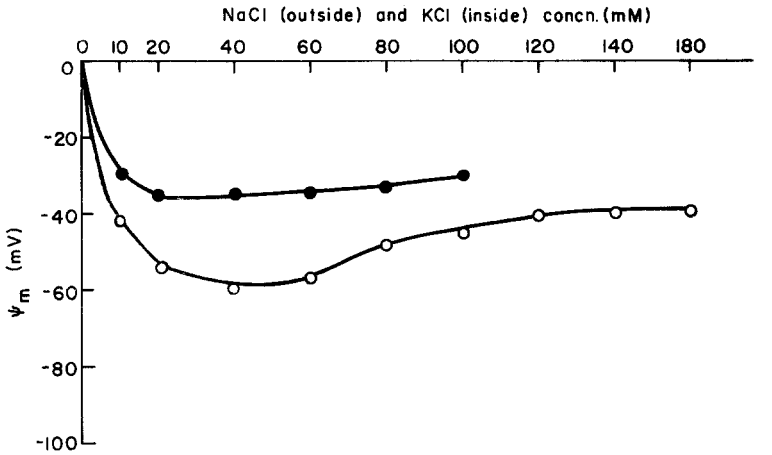

Fig. 4. Membrane potentials [for phosphatidylserine (○) and phosphatidylethanolamine (●) membranes] produced when the same concentrations of NaCl on one side and KCl on the other side were added. KCl added side was lower in potential. pH 7.2.

Asymmetrical distribution of Ca^{2+} and Mg^{2+}

In the second part of the experiments, the membrane potentials produced by the addition of divalent ions (Ca^{2+} and Mg^{2+}) were observed for various types of membrane in the solution with various concentrations of NaCl by the same procedure as in the above experiments.

Fig. 5 shows the membrane potentials produced by adding various amounts of $CaCl_2$ (3 moles/l) on one side of the phosphatidylserine, phosphatidylethanolamine and phosphatidylcholine membranes, respectively, which were prepared in 100 mM NaCl solution. In all cases, the membrane potential increased with the increase of $CaCl_2$ concentration. Especially, the membrane potentials produced for the phosphatidylserine membrane were greater than those for phosphatidylethanolamine and phosphatidylcholine membranes for the addition of the same amount of $CaCl_2$. In addition, in the case of the phosphatidylserine membrane, an instability of the membrane was observed by the addition of $CaCl_2$ on one side of the membrane. A study of this instability has been worked out in detail in earlier papers[14,15]. For example, the phosphatidylserine membrane prepared in 0.1 M NaCl at pH 7.2 became unstable by

the addition of up to 5 mM $CaCl_2$ on one side of the membrane. Beyond the above concentration (5 mM $CaCl_2$), the membrane broke. For the phosphatidylcholine and phosphatidylethanolamine membranes prepared in the same salt solution, the pro-

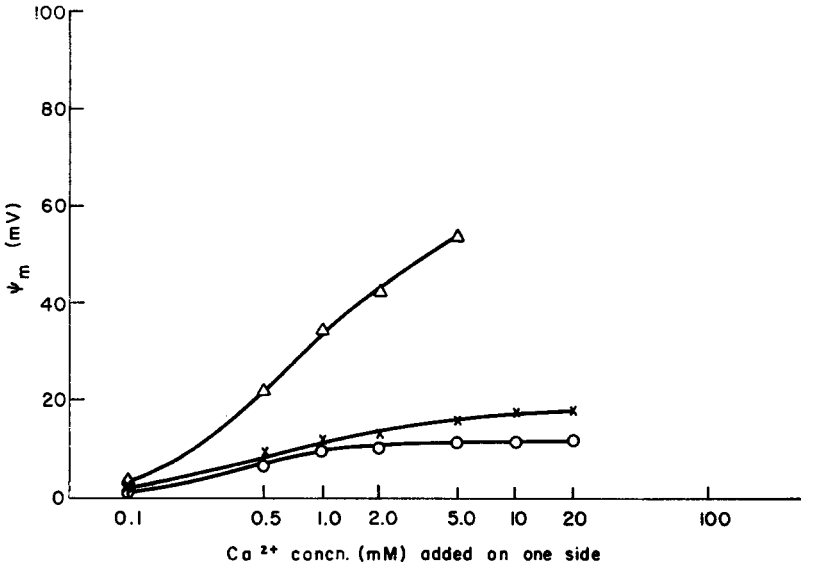

Fig. 5. Membrane potentials [for phosphatidylcholine (○), phosphatidylethanolamine (×) and for phosphatidylserine (△) membranes] produced by the addition of $CaCl_2$ on one side of the membrane prepared in 100 mM NaCl solution. pH 7.2.

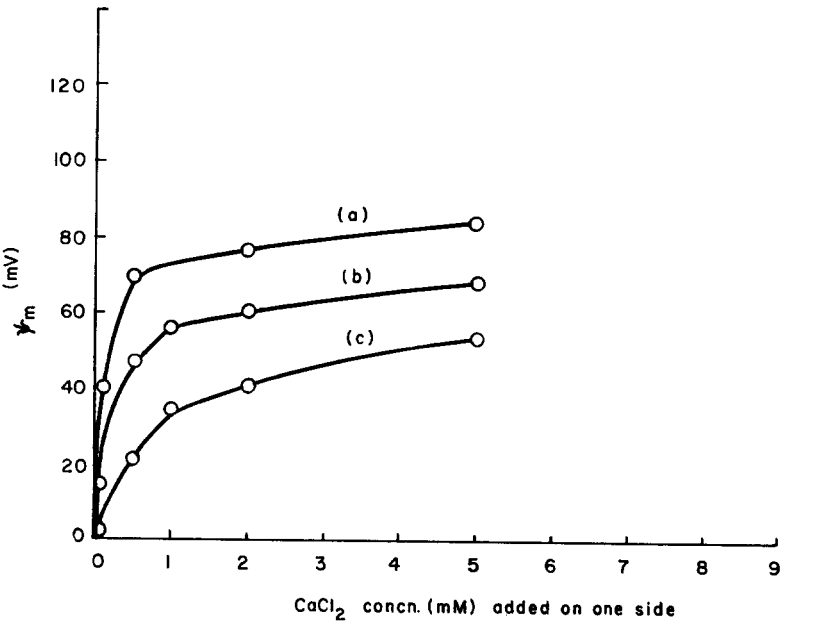

Fig. 6. Membrane potentials [for phosphatidylserine membranes prepared in (a) 1 mM NaCl, (b) 10 mM NaCl and (c) 100 mM NaCl, respectively] produced by the addition of $CaCl_2$ on one side. $CaCl_2$ added side resulted in a negative potential. pH 7.2 for all cases.

duced membrane potentials reached 10 mV with the addition of 1 mM $CaCl_2$ on one side of the membrane and there was no appreciable increase of the membrane potential for concentrations of more than 1 mM $CaCl_2$. The membrane potentials produced by the addition of $CaCl_2$ (3 moles/l) on one side of the phosphatidylserine membrane

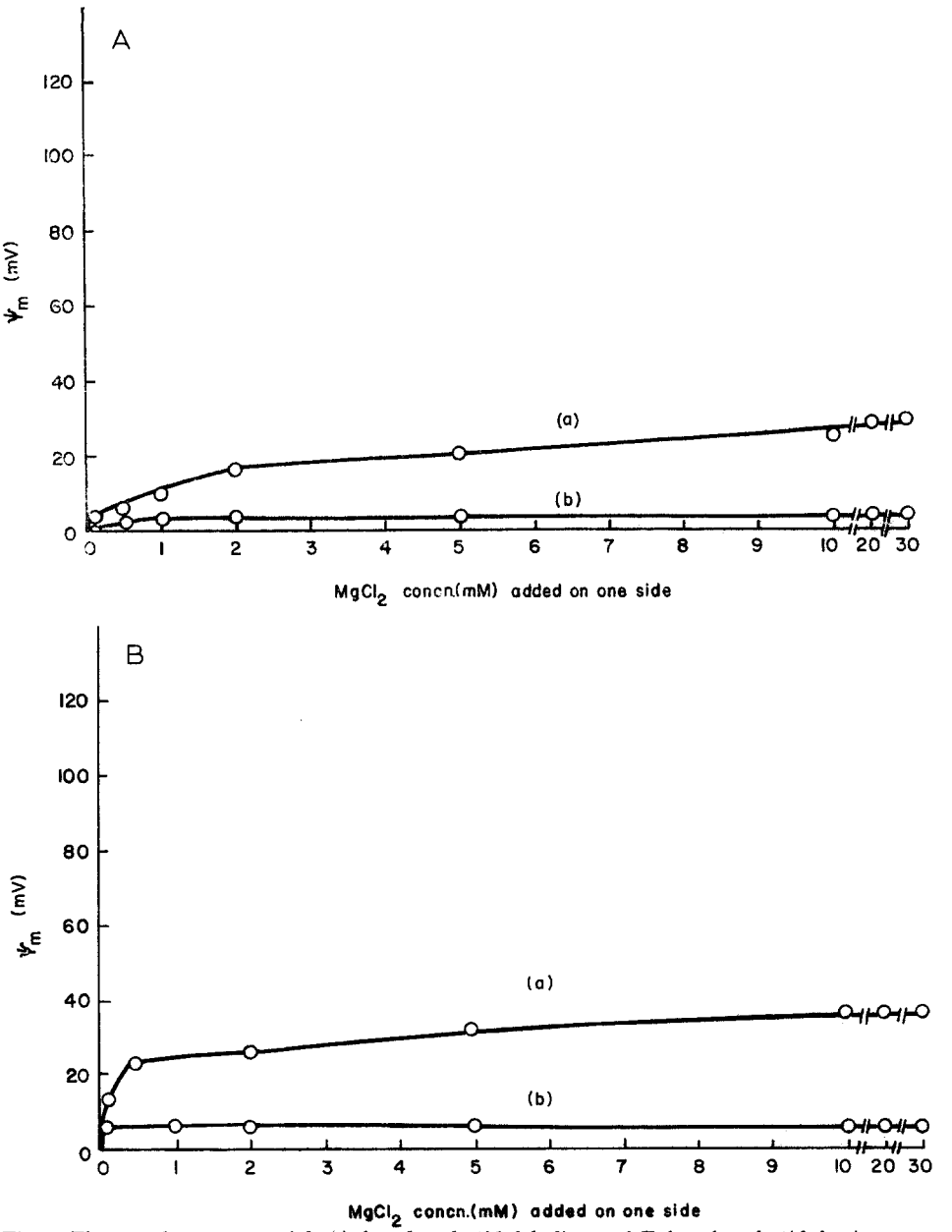

Fig. 7. The membrane potentials (A for phosphatidylcholine and B for phosphatidylserine membranes) produced by the addition of $MgCl_2$ on one side. $MgCl_2$ added side was negative in potential. (a) Membrane prepared in 10 mM NaCl, pH 7.2; (b) membrane prepared in 100 mM NaCl, pH 7.2.

prepared in various NaCl solutions are shown in Fig. 6. As the concentration of NaCl solution was decreased, the produced membrane potential with the same concentration of $CaCl_2$ was greater. Especially, in low NaCl concentrations, (10 mM and 1 mM NaCl), appreciably large membrane potentials were observed for the addition of very low concentrations of $CaCl_2$ (0.1 mM) on one side.

It was also noticed that for the membrane potentials produced by asymmetrical distribution of Ca^{2+}, there was no good reproducibility for some phosphatidylserine samples. Each point for the phosphatidylserine membrane in Figs 5 and 6 is the average value of more than five measurements for different membranes, and it may contain about 10% error in the magnitude of the potential. However, it is significant that there are large differences in produced membrane potentials between phosphatidylserine and phosphatidylcholine (or phosphatidylethanolamine) membranes.

The membrane potentials produced by adding various amounts of $MgCl_2$ (3 moles/l) on one side of the membrane are shown in Figs 7A and 7B, for the phosphatidylcholine and phosphatidylserine membranes which were prepared in 10 and 100 mM NaCl, respectively. For all cases, the addition of $MgCl_2$ on one side of the membrane did not produce a membrane potential as great as for the addition of the same concentration of $CaCl_2$.

The effects of $CaCl_2$ addition on one side of the phosphatidylserine membrane were strikingly different from the others (phosphatidylethanolamine and phosphatidylcholine). The former case (phosphatidylserine–$CaCl_2$) resulted in a greater membrane potential with low concentration of $CaCl_2$ (1–5 mM), and instability due to Ca^{2+} was observed. It is interesting to note that as far as the membrane potentials are concerned, the addition of monovalent ions (Na^+ and K^+) produced almost equal membrane potentials for both phosphatidylserine and phosphatidylethanolamine membranes, and Ca^{2+} produced a particularly large membrane potential for only the phosphatidylserine membrane.

Asymmetrical distribution of H^+

The third part of the experiments was to measure the membrane potentials produced when there was a difference in pH between the two sides of the membrane. The membrane was first formed in 0.1 M NaCl solution at pH 7.2 and the pH of the solution on one side of the membrane was changed subsequently by titration with HCl. The results are shown in Fig. 8. For the phosphatidylserine membrane, as the difference of pH between the two sides of the membrane was increased, greater transmembrane potential was observed and for pH lower than 2.5, the potential difference did not change and seemed to reach a saturation value (67 mV). Whereas, for the phosphatidylcholine membrane, an appreciable membrane potential change was not observed for the subsequent change of pH of one side of the membrane (pH 7.2–2.5), as seen in Fig. 8. In the case of the phosphatidylethanolamine membrane, similar results to those for the phosphatidylcholine membrane were observed. In order to test that the above membrane potentials observed were produced at the membrane-solution phase and not at the electrode–solution interface (liquid junction potential), the change in calomel electrode (a standard pH electrode) junction potential was measured by using a glass electrode as a reference electrode in various concentrations of KCl or NaCl at a constant pH.

There was no significant change in terms of potential difference between calomel and glass electrodes over a wide range of KCl or NaCl concentrations (0–300 mM) at pH 7.2.

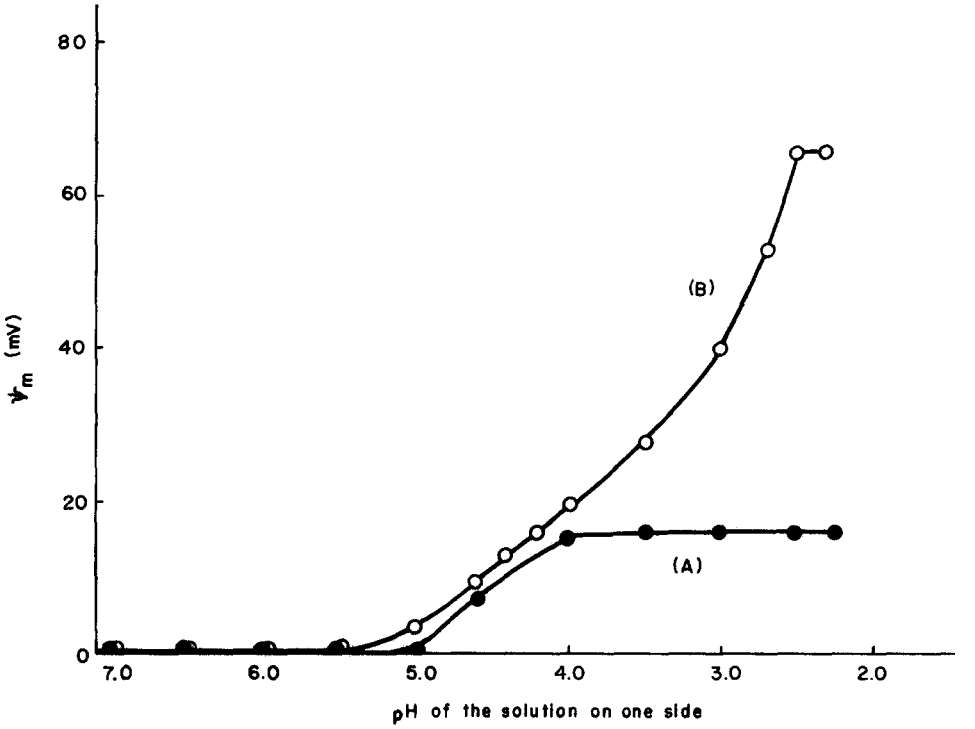

Fig. 8. The membrane potentials (A for phosphatidylcholine and B for phosphatidylserine membranes) produced by a difference of pH between two sides of the membrane. The abscissa shows the pH of one side, while pH of the other side was kept at 7.2. The side having lower pH was negative in potential.

Also, the potential difference between two compartments having no membrane across the cell was measured by adding salt (KCl or NaCl) on one side. It was found that after the addition of salt and stirring the solution, the salt added side attained a positive potential relative to the other side (*e.g.* 20 mV for 100 mM NaCl addition) for a while. The potential difference gradually decreased with time. After concentrations in both compartments apparently reached equilibrium (20–30 min), the potential difference of both sides was diminished. Since the mobility of Cl^- is usually greater than that of K^+ or Na^+ in bulk solution, this may indicate that the observed time dependent potential is produced by the ion diffusion potential across the diaphragm between two compartments.

DISCUSSION

From the results shown in Figs 2, 3 and 4, the following is evident; that is, for the phosphatidylserine and phosphatidylethanolamine membranes, there was a

difference in membrane potentials between those produced by the addition of the same concentration of NaCl and KCl, respectively, on one side of the membrane. Since Cl^- concentration was the same for both cases, and also the change of the junction potential of calomel electrodes in KCl or NaCl solution of various concentration (0–300 mM) was not relevant, this difference was due to the different interaction between K^+ and Na^+ with these phospholipid membranes, probably with their polar groups. On the other hand, there was no appreciable difference in the produced membrane potentials with the same concentration of KCl and NaCl addition, respectively, on one side of the phosphatidylcholine membrane. It is apparent that there is no appreciable difference in interaction between K^+ and Na^+ with the phosphatidylcholine membrane. It was also observed that the side with the more concentrated salt solution (either KCl or NaCl) was lower in potential than the other side of the membrane. The observed potential gradually increased from zero with the increase of salt concentration on one side[32]. This suggests that this potential is not due to the ion diffusion potential across the membrane which is expressed by the Nernst–Planck diffusion potential.

Moreover, the relations between membrane potential and the logarithm of the concentration ratio of one side to the other side ($[salt]_{out}/[salt]_{in}$), are shown in Fig. 9 for phosphatidylcholine and phosphatidylserine membranes (from data in Figs 2 and 3) which were prepared in 10 mM NaCl (or KCl) solution. For example, the potential value versus logarithm of the concentration ratio of salt (KCl_{out}/KCl_{in}) was almost a linear relation up to the ratio (2 for the phosphatidylserine membrane and 5–7 for the phosphatidylcholine membrane) and above this ratio, the relationship was no longer linear.

It has been reported[33,10] that the majority of Cl^- does not cross the lipid membrane whereas the alkaline ions go through the lipid bilayers with the charge. There-

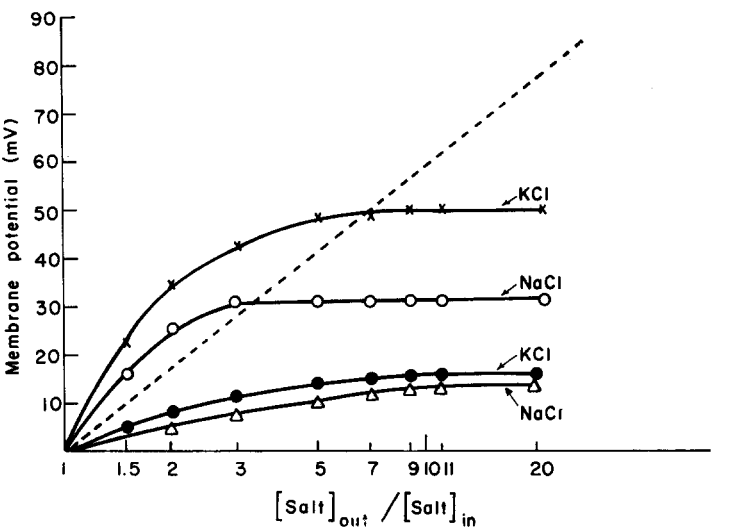

Fig. 9. The relationships between the membrane potential and the logarithm of the concentration ratio of one side to the other side (c_{out}/c_{in}) for phosphatidylcholine (●, △) and phosphatidylserine (×, ○) membranes, respectively, which were first prepared in 10 mM NaCl (or KCl) solution at pH 7.2. The salt concentration of the inside was kept at 10 mM NaCl.

fore, according to the Nernst–Planck equation the membrane potential ψ_m is approximately expressed by

$$\psi_m = \frac{RT}{F}\frac{U^+ - U^-}{U^+ + U^-}\ln\frac{c_o}{c_i} \cong \frac{RT}{F}\ln\frac{c_o}{c_i}$$

where U^+ and U^- are the mobilities of positive and negative ions, respectively, and c_0 and c_i are the concentration of salts on outside and inside of the membrane, respectively. The dotted line in Fig. 9 shows membrane potential according to the potential equation.

This is more evident in the experiment of Fig. 6, that is, in this case the concentration difference should be produced by Ca^{2+} and Cl^-. Since the Ca^{2+} is supposed to be much less permeable than the sodium or potassium ions through a lipid bilayer membrane, it may be considered that Ca^{2+} is practically impermeable through the lipid bilayer membrane compared with Cl^- transport. Therefore, the membrane potential is expressed by the same relation as for the previous case in terms of Cl^- concentrations.

$$\psi_m = \frac{-RT}{F}\ln\frac{[Cl]_o}{[Cl]_i}$$

where $[Cl]_0$ and $[Cl]_i$ are the concentrations of Cl^- for the outside and inside solution. Since $CaCl_2$ solution was added on one side (outside) of the phosphatidylserine membrane (in 0.1 M NaCl) up to a concentration of 5 mM, $[Cl]_0$ will be 110 mM and $[Cl]_i$ will be 100 mM. Therefore, the produced diffusion potential is supposed to be less than 2.5 mV and the sign of potential is opposite to that of the observed membrane potential. This membrane potential is much less than those observed (approx. 50 mV) in our experiments. Even if accepting that the Cl^- fluxes do not contribute to the membrane potential, there was a great difference in the produced membrane potentials between those for monovalent alkaline ions and Ca^{2+} additions on one side of the phosphatidylserine membrane, respectively. As the concentration of salt on one side was increased, a saturation of the membrane potential was observed for all cases. From the above evidence, it is difficult to explain the observed membrane potential fully in terms of only ion diffusion potential theory.

The phospholipid membrane has a hydrocarbon interior and hydrophilic surfaces facing the electrolyte solution phases. It is a fact that this type of membrane is very impermeable to ions, but it is still electrically conductive. Therefore, it is probable[4] that there is no appreciable electrical potential gradient inside the membrane and the membrane potential may arise mainly from the difference between two surface potentials of two sides of the membrane which are produced by the fixed charges at the membrane surfaces and the surrounding electrolyte solution (see Fig. 10). If one surface of the membrane is different from the other membrane surface with respect to their surface charges or ion binding with specific polar groups, two different surface potentials would be produced on the two sides of the membrane. It should be noted that changing the ionic strength will change the surface potential *via* a screening mechanism. A phospholipid bilayer system which has such an asymmetrical distribution with the surface charges or dipoles or ion adsorption with surface polar groups may demonstrate such a transmembrane potential

at it's initial state. The observed transmembrane potential can be explained in the following way: (For example) since the phosphatidylserine molecule has a negatively charged surface, the potential profile of the phosphatidylserine membrane in aqueous solution would be as shown in Fig. 10a. If positive ions (K^+, Na^+ or Ca^{2+}) are adsorbed more strongly than the negative ion (Cl^-) on one surface by the addition of salt on one side, the negative fixed charge of this side will be reduced. The membrane potential is measured as the difference in diffused double layer potentials between the two sides of the membranes (see Fig. 10b). A phosphatidylcholine bilayer is considered to have an electrically neutral surface as a whole in aqueous solution at neutral pH. Therefore, the potential profile would be as shown in Fig. 10c. If salt is added on one side and positive ions are adsorbed strongly on the surface, the positively charged surface will be created on that side, and consequently, the membrane potential can be formed as seen in Fig. 10d.

Interaction of divalent ions with phospholipids could be also interpreted with the same concept described above. Especially the interaction of Ca^{2+} with the phosphatidylserine molecule is an interesting subject to investigate. It is suggested that a large difference in potential produced between Ca^{2+} and Mg^{2+} additions, respectively, on one side of the phosphatidylserine membrane, shows that Ca^{2+} interacts much more strongly with phosphatidylserine than Mg^{2+}. This fact is true not only for the phosphatidylserine membrane but also for most phospholipid membranes. That is, Ca^{2+} seems to interact more strongly than Mg^{2+} with the phospholipid membranes (see Figs 5–7). This tendency corresponds to earlier measurements of conductance of phos-

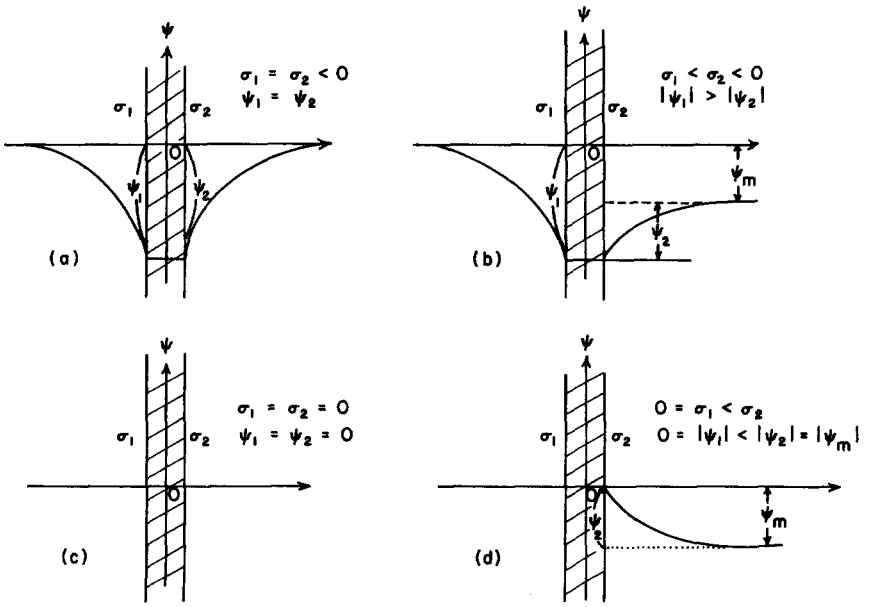

Fig. 10. A schematical sketch of the transmembrane potential due to the difference in potentials at a distance between the two sides of the membrane. σ: surface charge density. (a) Symmetrical distribution of net negative charge on the membrane surface. (b) Asymmetrical distribution of net negative charge on the membrane surface. (c) Symmetrical distribution of net zero charge on the membrane surface. (d) Asymmetrical distribution of net positive charge on the membrane surface.

pholipid membranes in the presence of divalent ions[12]. Another observation is that the interaction of divalent ions (Ca^{2+} or Mg^{2+}) with phospholipid membranes is suppressed by increasing monovalent salt concentrations in which the membrane is prepared. Namely, the effect of divalent ions on membrane potential is more suppressed when the membrane is prepared in monovalent salt solution of higher concentration. This suggests that monovalent salts compete with divalent ions when interacting with phospholipids. This also corresponds to earlier work done by other investigators[16–18]. Especially strong interaction of Ca^{2+} with acidic phospholipids may relate to some function (such as nerve excitation) as suggested previously[14,17,19]. The membrane potential is also observed when there is a pH difference between the two sides of the membrane. If we assume that the membrane potential observed for the phosphatidylserine membrane with a pH difference is produced by the diffusion potential from the different concentrations of H^+ on both sides of the membrane, the same potential for the phosphatidylcholine membrane with the same pH difference would be expected to be produced, provided that H^+ is much more permeable[20] than Cl^-, and H^+ permeability is approximately equal for both phosphatidylserine and phosphatidylcholine membranes. However, the result is quite different. When phosphatidylserine membrane is made in 0.1 M NaCl of pH 7.2, both sides of the membrane may carry a net negative charge per molecule[21]. By changing the pH of the solution on one side toward the lower pH, this side of the membrane would lose the net negative charge, and around pH 3.0 phosphatidylserine should be near the isoelectric point. Therefore, there will be a difference in charges of ion dissociation between the two sides of the membrane surface. The membrane potential shown in Fig. 8 may be produced by this charge difference between the two sides of the membrane surfaces[21,22]. This explanation may correspond to that of the membrane instability due to the pH distribution[14,15].

Since a phospholipid molecule has dissociable polar groups which have different ionization with various salt concentration and pH of the environmental solution, the phospholipid bilayer membrane will alter its surface properties like ion adsorption[17,23,24] in response to variation of these environmental parameters. Consequently, physico-chemical properties of the membrane based on surface phenomenon such as selective ion permeability[5,10,25–27], electrical conductance[7,12,28] and capacitance[13] should be affected by a change in the above environmental parameters. Surface potential measurements on phospholipid monolayers[21,29,30] and studies of the electrophoretic mobility of phospholipid dispersed systems[31] have demonstrated that the presence of a charged polar group produces a substantial potential at the lipid–solution interface. Such a potential also should influence the entry of ions at the interface[28].

The above discussion described in this paper is based on a qualitative analysis. However, for the membrane which has low permeability for ions or molecules, it seems reasonable that the observed membrane potential at the initial state could be due to the difference between two surface potentials produced at the interface of the membrane and the electrolyte solution on both sides. This potential difference however, will vanish gradually as the system reaches the stationary state. On the other hand, the surface potential measured for the monolayer interface is the stationary state potential and does not vanish.

ACKNOWLEDGEMENTS

The author would like to acknowledge Dr T. Seimiya for his valuable comments, and to Miss C. Balazs for her technical assistance in the experiments.

This work was supported by grants from U.S. National Institutes of Health (R01-NS 08739). Secretarial assistance of the manuscript was supported by grant from National Aeronautics and Space Administration (NGR-33-015-002).

REFERENCES

1 P. Mueller, D. Rudin, H. Tien and W. Wescott, in J. F. Danielli, K. Parkhurst and A. Riddiford, *Recent Progress in Surface Science*, Vol. 1, Academic Press, New York, 1964, p. 379.
2 H. T. Tien, *J. Phys. Chem.*, 71 (1967) 3395.
3 A. Goldup, S. Ohki and J. F. Danielli, in J. F. Danielli, A. C. Riddiford and M. Rosenburg, *Recent Progress in Surface Science*, Vol. 3, Academic Press, New York, 1970, p. 193.
4 S. Ohki, *J. Colloid Interface Sci.*, 37 (1971) 318.
5 A. A. Lev, V. A. Gotlib and E. P. Puzhinsky, *Z. Evolyu. Biokhim. Fiziol.*, 2 (1966) 109.
6 P. Mueller and D. O. Rudin, *Biochem. Biophys. Res. Commun.*, 26 (1967) 398.
7 P. Lauger, W. Lesslauer, E. Marti and J. Richter, *Biochim. Biophys. Acta*, 135 (1967) 20.
8 W. D. Seufert, *Nature*, 207 (1965) 174.
9 R. C. Bean, W. C. Shepherd and C. D. D'Agostino, *Fed. Proc.*, 25 (1966) 656.
10 U. A. Hopfer, L. Lehninger and W. J. Lennarz, *J. Membrane Biol.*, 2 (1970) 41.
11 T. H. Massey and R. J. McCulloch, *Biochim. Biophys. Acta*, 241 (1971) 661.
12 S. Ohki, *J. Colloid Interface Sci.*, 30 (1969) 413.
13 S. Ohki, *Biophys. J.*, 9 (1969) 1195.
14 S. Ohki and D. Papahadjopoulos, in M. Blank, *Surface Chemistry of Biological Systems*, Plenum Press, New York, 1970, p. 155.
15 S. Ohki, *Biochim. Biophys. Acta*, 255 (1972) 57.
16 J. F. Danielli, *J. Exp. Biol.*, 20 (1944) 167.
17 H. Hauser and R. M. C. Dawson, *Biochem. J.*, 109 (1968).
18 J. M. Tobias, D. P. Agin and R. Pawlowski, *J. Gen. Physiol.*, 45 (1962) 989.
19 J. M. Tobias, *Nature*, 199 (1964) 78.
20 E. A. Liberman and V. P. Topally, *Biochim. Biophys. Acta*, 163 (1968) 125.
21 D. Papahadjopoulos, *Biochim. Biophys. Acta*, 163 (1968) 240.
22 A. D. Bangham and D. Papahadjopoulos, *Biochim. Biophys. Acta*, 126 (1966) 181.
23 E. Rojas and J. M. Tobias, *Biochim. Biophys. Acta*, 94 (1965) 394.
24 T. Seimiya and S. Ohki, *Div. of Colloid and Surface Chemistry No. 95, at 162nd Am. Chem. Soc. Meeting, Washington, D.C. (1971)*.
25 T. E. Andreoli, J. A. Bangham and D. C. Tosteson, *J. Gen. Physiol.*, 50 (1967) 1792.
26 D. Papahadjopoulos and A. D. Bangham, *Biochim. Biophys. Acta*, 135 (1967) 639.
27 T. J. Gutknecht and D. C. Tosteson, *J. Gen. Physiol.*, 55 (1970) 359.
28 S. McLaughlin, G. Szabo, G. Eisenman and S. M. Ciani, *Proc. Natl, Acad. Sci. U.S.*, 67 (1970) 1268.
29 D. O. Shah and J. H. Schulman, *J. Lipid Res.*, 8 (1967) 227.
30 F. Vilallonga, M. Fernandez, C. Rotunno and M. Cereijido, *Biochim. Biophys. Acta*, 183 (1969) 98.
31 A. D. Bangham, in *Progress in Biophysics and Molecular Biology*, Pergamon Press, New York, 1968, p. 29.
32 S. Ohki, *Ann. N.Y. Acad. Sci.*, in the press.
33 R. Pagano and T. E. Thompson, *J. Mol. Biol.*, 38 (1968) 41.