

STUDIES WITH COMPOSITE MEMBRANES

II. MEASUREMENT OF ASYMMETRIC PROPERTIES

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ABSTRACT Electrical potentials arising across composite membranes when they separate the same concentration of a (1:1) electrolyte or electrolytes have been measured. These potentials have been shown to arise from differences in the transport number of counterions contacting the two faces of the membrane which contained in its body a high concentration of electrolyte and polyelectrolyte. When the concentration of this trapped electrolyte or polyelectrolyte is low, the asymmetry potentials are small. Although measurements of current-voltage relations provided evidence for the existence of asymmetry between the two faces of the membrane, osmotic flow of water in either direction across the membrane and the salt flow in the two directions were symmetrical. These solvent and solute flux measurements lasted more than 30 hr. Short-term (about 4 hr) flux measurements, however, using tritiated water (THO), gave flows which were different in the two directions. Similarly, the salt flows measured using ^{22}Na isotope were different in the two directions. The usefulness of the present system as a model to use for studies concerned with carrier transport problems in biology has been pointed out.

INTRODUCTION

Composite membranes have been prepared from collodion and polystyrenesulfonic acid (PSSA) by Liquori and Botre (1964, 1967). These membranes have been used by Botre et al. (1967) to simulate bioelectrical potentials observed across the squid nerve membrane. Liquori (1968) has suggested that the difference in the selectivities of the two faces of the biological membrane may form the basis for sustaining the resting potential of the living cell. This simple physicochemical proposal has been inspired by the fact that a composite membrane containing a polyelectrolyte (sulfonated polystyrene) trapped between membrane layers of increasing charge density, generated an asymmetry potential when the same concentration of an electrolyte solution was placed on either side of it. De Korosy (1968) also observed similar asymmetry potentials across his own composite membranes, but he showed that the potentials were of a temporary nature. The results of our

studies pertaining to asymmetry potentials and other transport properties of composite membranes are presented in this part.

EXPERIMENTAL

The preparation of simple and composite membranes, layer as well as sandwich types, was according to the procedures given in Part I (Lakshminarayanaiah and Siddiqi, 1971 *b*). Some membranes conforming to the specifications of Liquori and Botre (1964, 1967) were also prepared.

Membrane pieces (~ 2.5 cm squares) were converted into the Na form by immersing them in about 200 ml of a strong solution (2–3 *N*) of NaCl. They were kept gently stirred for a few hours, and left there for future use. A few pieces when required were taken out, thoroughly washed with deionized water, and equilibrated for 2 hr with gentle stirring in about 100 ml of the NaCl solution to be used in the experiment. The solution was changed frequently during equilibration.

Asymmetry, biionic, and concentration potentials arising across simple and composite membranes were measured using an electrochemical cell of the type described in Part I (Lakshminarayanaiah and Siddiqi, 1971 *b*).

The membrane current (i) and voltage (V) relations were determined using an H-cell. The membrane bisected this cell into two halves, each of which contained the same electrolyte solution and two reversible Ag-AgCl electrodes. Two of these electrodes, one on each side of the membrane, were placed a few centimeters away from the membrane. The other two were placed close to the membrane faces. A constant current (determined from measured iR drop across a known resistor) from a constant current supply was passed through the membrane using the two distant electrodes. As a consequence, a change in membrane potential occurred, and this change was measured on a microvoltmeter connected to the two near electrodes. All these experiments were carried out at room temperature (air-conditioned).

Osmotic water flow in either direction was measured using the cell described in our earlier publication (Lakshminarayanaiah, 1967). 0.5 *N* NaCl solution on one side and deionized water on the other side of the membrane were used. The assembly was kept in a water thermostat at $25 \pm 0.01^\circ\text{C}$. The flow of water was followed on a cathetometer with time. At the end of the experiment which lasted at least 30 hr, the salt content of the water compartment was estimated on a Perkin-Elmer Atomic Absorption Spectrophotometer (Perkin-Elmer Corp., Instrument Div., Norwalk, Conn.).

RESULTS AND DISCUSSION

No asymmetry potential E_0 , i.e. potential when the same electrolyte of the same concentration was placed on either side of the membrane, was observed across simple membranes of high or low charge density. Under similar conditions, composite membranes, both layer and sandwich types, gave potentials (high charge density side taken positive) shown in Fig. 1. Immediately on assembling the whole cell, the value for E_0 was noted (i.e., 2–3 min after mounting the membrane) and followed with time for 1–2 hr. The change in E_0 during this period was within $\pm 8\%$ of the initial value. Each point in Fig. 1 represents the mean of the initial values observed with at least four different membranes which were screened to keep the variability of the asymmetry potentials within $\pm 10\%$. The values ob-

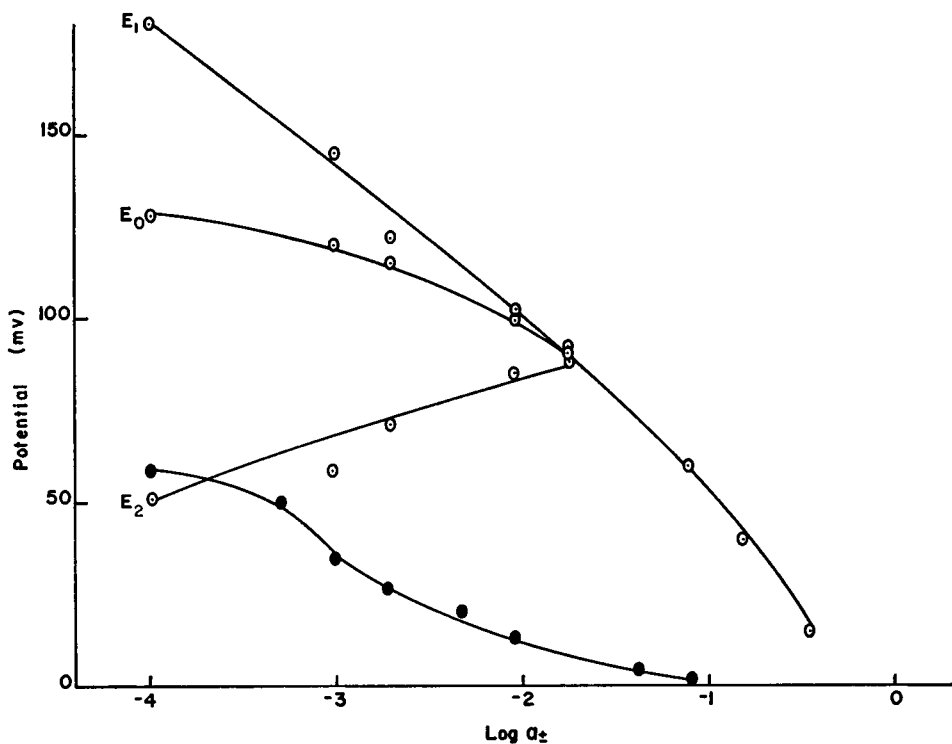


FIGURE 1 Electrical potentials arising across a composite membrane when it separates the same or different concentrations of NaCl, plotted against the activity of the external solution. ○ refers to layer-type composite membranes. E_0 refers to asymmetry potentials, i.e., potentials when the same concentration of NaCl is placed on both sides of the membrane. E_1 refers to concentration potentials with 0.02 N NaCl solution on one side of the membrane (low charge density side) and the concentration on the high charge density side is varied. E_2 refers again to concentration potentials when the concentration gradient is reversed. ● refers to asymmetry potentials arising across sandwich-type membrane.

served for E_0 across sandwich-type membranes are lower than those observed across layer types. The possible reason as to why this is so is discussed later.

The concentration potentials, i.e. potentials arising across the layer-type membranes when different concentrations of the same electrolyte solution are placed on one side keeping the concentration of the electrolyte on the other side constant, and vice versa, are also given in Fig. 1. The values of the potential are high when the electrolyte solution gradient is acting in the same direction as the asymmetry potential, whereas the values are low when the gradient is opposing the asymmetry potential. In either case, however, it is found (see Table I) that the observed value for any given concentration ratio does not agree with the value calculated (i.e., $E_0 \pm E_{\max}$) by the algebraic addition of the theoretical concentration potential

TABLE I
ELECTRICAL POTENTIALS OBSERVED ACROSS A LAYER-TYPE
MEMBRANE WHEN A CONCENTRATION POTENTIAL ACTS
EITHER TO REINFORCE OR TO OPPOSE
THE ASYMMETRY POTENTIAL

Electrolyte concn C_2 held constant	Electrolyte concn C_1 varied	Concn potential $E_{max} =$ $\frac{RT}{F} \ln \frac{a_2}{a_1}$	Asymmetry potential E_0, C_1 on both sides	Concn potential (observed) with E_0		Concn potential (calculated)	
				Rein- forcing	Oppos- ing	$E_0 + E_{max}$	$E_0 - E_{max}$
<i>eq/liter</i>	<i>eq/liter</i>	<i>mv</i>	<i>mv</i>	<i>mv</i>	<i>mv</i>	<i>mv</i>	<i>mv</i>
0.02	0.0001	130.0	128	180	51.5	258	-2.0
0.02	0.001	72.6	120	145	59.0	193	47.4
0.02	0.002	55.4	115	122	71.5	170	59.6
0.02	0.01	16.5	102	100	85.0	119	85.5
0.02	0.02	0	90	87	91.0	90	90.0

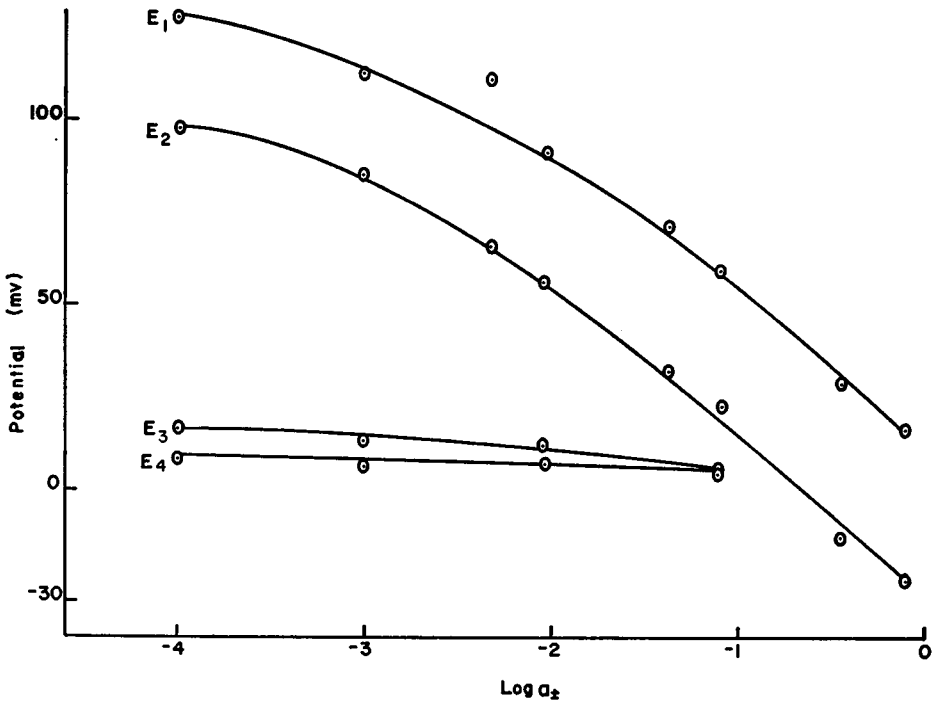


FIGURE 2 Biionic potentials observed across layer-type composite membranes when they separate the same concentration of NaCl and KCl solutions, plotted as a function of the activity of the external NaCl solution. E_1 refers to potentials when KCl solution is in contact with high charge density side and E_2 refers to potentials when KCl and NaCl are interchanged. E_3 and E_4 refer to biionic potentials observed across simple high charge and low charge density membranes.

E_{max} , viz. $E_{max} = (RT/F) \ln (a_2/a_1)$, where a_2 is the constant activity of the solution 2, and a_1 is the activity of the solution 1 which is varied, and the observed asymmetry potential E_0 . An exception exists in the case $(C_2/C_1) = 2$ where the concentration gradient is opposing the asymmetry potential. In all the other cases, the discrepancy noted reflects the fact that the *composite membrane as a whole* generates low concentration potentials and thus has low permselectivity to the Na^+ ions.

The results of asymmetry potentials across composite membranes which were prepared according to the formula of Liquori and Botre (1964, 1967) followed the same pattern of behavior shown in Fig. 1; but the actual values, in the case of layer type, were lower, and in the case of sandwich type, a little higher, than those shown in Fig. 1.

The biionic potentials generated when layer-type membranes separated NaCl and KCl solutions of the same concentration are shown in Fig. 2. The E_1 curve shows the variation of biionic potential as a function of the activity of the outside NaCl solution when KCl solution is in contact with the high charge density side of the membrane. The potentials are reduced a little (i.e., E_2 curve) when the solutions are interchanged allowing NaCl to contact the high charge density surface. In the same figure appear also the biionic potentials (E_3 and E_4 curves) observed across simple high and low charge density membranes. These potentials are very low compared to those observed across layer-type membranes.

The basis for the existence of asymmetry potential across a composite membrane has been explored by Liquori and Botre (1964) from the standpoint of the concepts of the theory of membrane potential developed by Teorell (1935 *a, b*), and Meyer and Sievers (1936 *a, b, c*). They showed that the asymmetry potential arose as a result of the differences in the Donnan and diffusion potentials existing at the two asymmetric faces of the membrane. Later "a simple straightforward explanation" was given in terms of the model system shown in Fig. 3 (Liquori and Botre, 1967). The model system was composed of two simple membranes M_H and M_L which contained respectively high and low concentrations of fixed negative

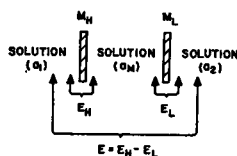


FIGURE 3 The model system for the composite membrane. M_H and M_L are simple membranes of high charge (PSSA = 2.5 mg/ml) and low charge (PSSA = 0.025 mg/ml) density separating three solutions of the same electrolyte of activity a_1 , a_M , and a_2 contained in the three compartments. E_H is the concentration potential arising across the membrane M_H , and E_L is the concentration potential arising across the membrane M_L . E is the potential acting across the whole cell.

groups. The membranes were assembled in series to form a three-chambered cell. The middle chamber, which represented the interior of the composite membrane, contained a known concentration of the electrolyte (e.g., NaCl) solution. In the two outer compartments, NaCl solution of the same or different concentration was placed to measure asymmetry and/or concentration potentials across the whole membrane system.

In terms of the model system, the asymmetry potential is given by the algebraic sum of the membrane potentials which exist across the two simple membranes M_H and M_L . The membrane potential across M_H (i.e., E_{H_0}) is given by (Lakshminarayanaiah, 1969)

$$E_{H_0} = \frac{RT}{F} (2\bar{i}_{+(H)} - 1) \ln \frac{a_M}{a_1}. \quad (1)$$

Similarly the membrane potential E_{L_0} across M_L is given by

$$E_{L_0} = \frac{RT}{F} (2\bar{i}_{+(L)} - 1) \ln \frac{a_M}{a_2}, \quad (2)$$

where $\bar{i}_{+(H)}$ and $\bar{i}_{+(L)}$ are the transport numbers of counterions in membranes M_H and M_L respectively. a_1 and a_2 are the activities of the two solutions in the outer compartments. a_M is the activity of the solution used in the middle compartment. The net potential E across the composite membrane system (Fig. 3) is given by

$$E = E_{H_0} - E_{L_0} = \frac{RT}{F} \left[(2\bar{i}_{+(H)} - 1) \ln \frac{a_M}{a_1} - (2\bar{i}_{+(L)} - 1) \ln \frac{a_M}{a_2} \right]. \quad (3)$$

When $a_1 = a_2 = a$, the asymmetry potential E_0 is given by

$$E_0 = E_{H_0} - E_{L_0} = \frac{2RT}{F} [\bar{i}_{+(H)} - \bar{i}_{+(L)}] \ln \frac{a_M}{a}. \quad (4)$$

When $a_M = 0.35$ (i.e., $C_M = 0.5$ N NaCl solution), E_{H_0} , E_{L_0} and E_0 have been measured using a high charge density membrane (collodion containing 2.5 mg/ml of PSSA) and a low charge density membrane (collodion containing 0.025 mg/ml of PSSA) as a function of the activity of the external NaCl solution (see Fig. 4). Similarly values for E_0 when $a_M = 0.08$ (i.e., 0.1 N) and 0.009 (i.e., 0.01 N) have been determined also and are given in the same figure.

The data given in Fig. 4 show that the algebraic addition of the values of E_{H_0} and E_{L_0} agree with the values given for the curve E_0 (i.e., for 0.5 N NaCl). At other values of a_M similar agreement was obtained (values not given). So it is inferred that equations 1, 2, and 4 are valid for the model system. For the case when $a_1 \neq a_2$, equation 3 has been assumed to be valid. This is based on the fact that the values of E_1 or E_2 (see Fig. 4) measured across the whole membrane system were found to

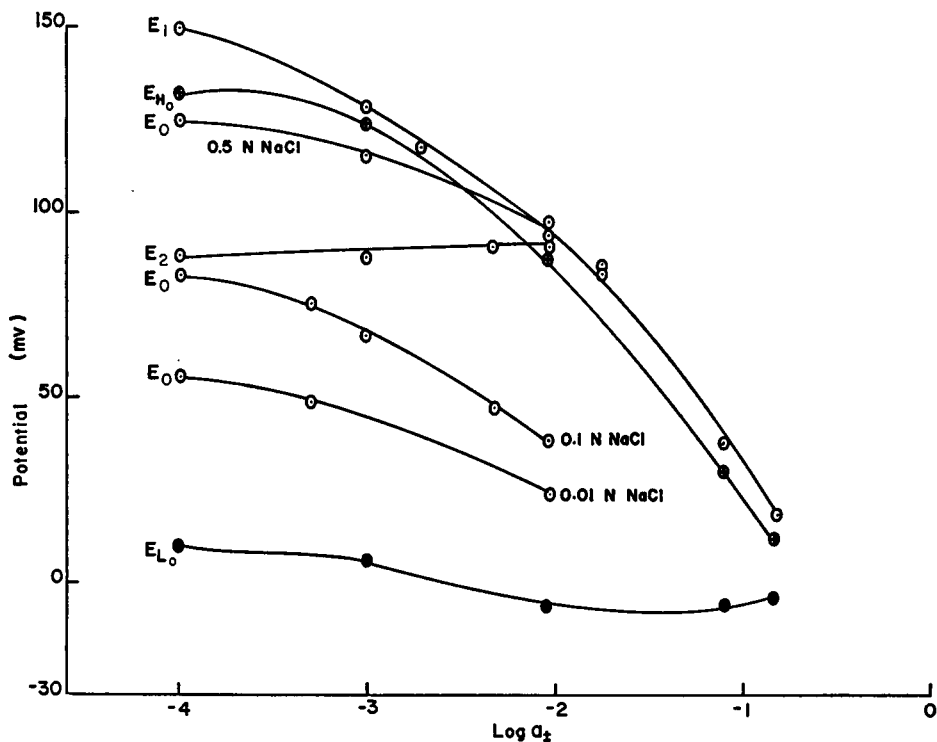


FIGURE 4 Electrical potentials arising across the model membrane system of Fig. 3, plotted as a function of the activity of the external NaCl solution. E_0 's refer to the asymmetry potentials (i.e., $a_1 = a_2$) measured across the complete cell when the middle chamber contained 0.5, 0.1, and 0.01 N NaCl solution (values of a_M are 0.35, 0.079, and 0.009). E_{H_0} and E_{L_0} are the concentration potentials observed across simple membranes M_H and M_L when they separated 0.5 N NaCl solution on one side (middle chamber) and the other NaCl solution whose concentration is varied on the other side. Algebraic addition of E_{H_0} and E_{L_0} gave E_0 (0.5 N NaCl solution in the middle chamber). E_1 refers to the concentration potentials measured across the complete membrane cell when the middle compartment contained 0.5 N NaCl solution and compartment 2 contained 0.02 N NaCl solution and compartment 1 contained NaCl solution whose concentration is varied. E_2 refers to concentration potentials when the concentration gradient is reversed.

be equal to the values obtained by the algebraic addition of the potentials measured across the simple membranes M_H and M_L (these values not given). The measured values of E_1 in Fig. 4 correspond to the case when the concentration potential was acting in the same direction as the asymmetry potential. The measured values of E_2 pertain to the other case when the concentration potential opposed the asymmetry potential. The results of Fig. 4 simulate those observed across layer-type composite membranes (see Fig. 1). Equations 1-4 thus could be applied to explain the results of Fig. 1. To do this, one should know the values for a_M , $\bar{i}_{+(H)}$, and $\bar{i}_{+(L)}$ for the layer-type membrane. Values for $\bar{i}_{+(H)}$ and $\bar{i}_{+(L)}$ can be calculated from the

values of E_{H_0} and E_{L_0} (see Fig. 4) using equations 1 and 2. These results are given in Table II. Assuming that these values are appropriate for the two sides of the composite membrane, values for the asymmetry potential can be calculated from equation 4 substituting different values for a_M . Only for a value of 0.4 for a_M (i.e., concentration ≈ 0.6 N), results agreeing with the experimental values for the asymmetry potential in the region of low concentration are obtained (see Table III). At higher concentrations, the calculated values are lower than the observed values. This discrepancy could arise from (a) the actual values of $[\bar{i}_{+(H)} - \bar{i}_{+(L)}]$ being different from those used in the calculations, and (b) the value substituted for a_M in the calculations being too low. A simple calculation using equation 4 shows that the value for $[\bar{i}_{+(H)} - \bar{i}_{+(L)}]$ must be 0.77 for the solutions 0.5/0.1 N or 0.98 for the solutions 0.5/0.2 N to obtain agreement between calculated and observed

TABLE II
VALUES OF TRANSPORT NUMBER FOR HIGH AND LOW CHARGE DENSITY MEMBRANES M_H AND M_L CALCULATED FROM EQUATIONS 1 AND 2 WHEN 0.5 N NaCl SOLUTION IS KEPT IN THE MIDDLE COMPARTMENT

NaCl solution on two sides	$i_{+(H)}$	$i_{+(L)}$	$[\bar{i}_{+(H)} - \bar{i}_{+(L)}]$
<i>eq/liter</i>			
0.0001	0.82	0.52	0.30
0.001	0.91	0.52	0.39
0.002	0.92	0.50	0.42
0.01	0.97	0.46	0.51
0.02	0.96	0.46	0.50
0.10	0.85	0.45	0.40
0.20	0.77	0.36	0.41

TABLE III
ASYMMETRY POTENTIALS ACROSS LAYER-TYPE COMPOSITE MEMBRANES

NaCl solution on two sides	E_0 (observed)	E_0 (calculated)
<i>eq/liter</i>	<i>mv</i>	<i>mv</i>
0.0001	128	128
0.001	120	121
0.002	115	116
0.01	102	99.5
0.02	90.0	80.4
0.1	64.2	33.5
0.2	49.8	21.0

asymmetry potentials. These values for the term $[\bar{i}_{+(H)} - \bar{i}_{+(L)}]$ are too high to be true. Therefore, factor b may be responsible for the discrepancy. That this is so is apparent from the nature of the function $\log (a_M/a)$ of equation 4. In dilute solutions (e.g., $a = 0.002$ N) the value of $\log (a_M/a)$ changes little ($< 10\%$) for a given change in the value of a_M (say from 0.6 to 0.4 N) compared with a similar change ($> 30\%$) in the value of the function $\log (a_M/a)$ for the same change in a_M in strong solutions (e.g., $a = 0.2$ N).

The asymmetry potentials across sandwich-type membranes (see Fig. 1), unlike similar potentials observed by Liquori and Botre (see their Fig. 3, 1967), are low. We experienced considerable difficulty in preparing these membranes. Deposition of PSSA between M_H and M_L membranes always gave good sandwich membranes, which, when equilibrated with solutions, separated into the two membranes M_H and M_L . The results presented in Fig. 1 refer to those sandwich membranes which remained intact after repeated equilibrations with the electrolyte solutions. In Fig. 4 are given the values of E_0 realized with the three-chamber model system when 0.1 or 0.01 N NaCl solution was used in the middle chamber. Comparison of these different values of E_0 with those given in Fig. 1 for the sandwich type indicates a value of about 0.009 for a_M . It is apparent therefore that the magnitude of the asymmetry potential is controlled by the amount of electrolyte and polyelectrolyte trapped in the body of the composite membrane.

The results of biionic potentials (see Fig. 2) can be explained also in terms of equation 4. The transport numbers now correspond to the cations Na^+ and K^+ contacting the two membrane faces. As the membrane exhibits little discrimination between Na^+ and K^+ ions (see curves E_3 and E_4 of Fig. 2), the potential arising on that account is absent and all the observed potential therefore becomes attributable to the terms a_M and $[\bar{i}_{+(H)} - \bar{i}_{+(L)}]$.

The asymmetry potentials existing across layer-type membranes should exert some influence on the flow of charged species through them. To investigate this, current-voltage relations have been determined and these results are shown in Fig. 5. There is more current flowing through the membrane in one direction (positive current towards the high charge density side) than there is in the reverse direction. Because of the presence of more charges in the membrane when it is in contact with a stronger solution (i.e., 10^{-2} N compared with 10^{-4} N solution), more current is carried across the membrane. Similar experiments performed with simple membranes of high and low charge densities gave i - V relationships which were linear and nonrectifying. The values for the resistances derived from the slopes of the straight lines were 2.2×10^5 , 6.8×10^8 ohms for high charge density membrane, and 9.6×10^7 , 3.3×10^7 ohms for low charge density membrane when they were in contact with 10^{-4} and 10^{-2} N NaCl solutions respectively. The membrane area involved was 2.84 cm². The corresponding resistance values for the composite membrane were 2.6×10^6 ($-ve$ direction) and 4.8×10^5 ohms ($+ve$ direction) for 10^{-4} N solution and 2.0×10^5 and 3.5×10^4 ohms for 10^{-2} N solution. These

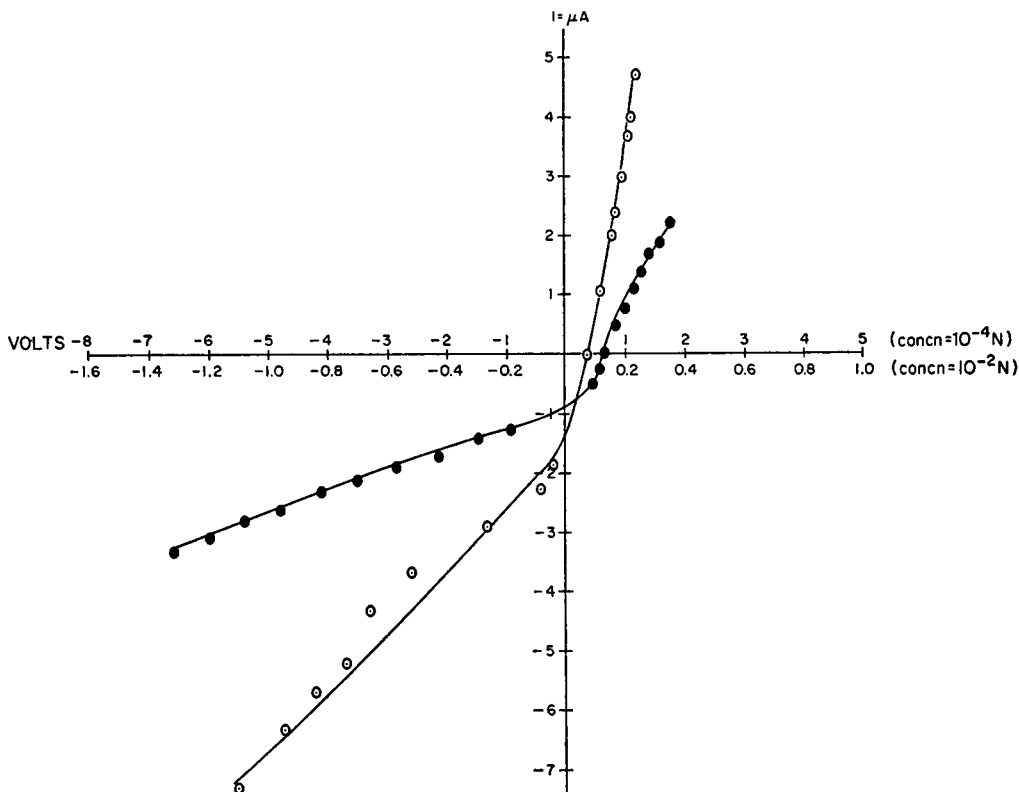


FIGURE 5 A plot of current i (μA) flowing through an area of 2.84 cm^2 layer-type membrane against voltage V (volts) when there was 10^{-4} (\bullet) and 10^{-2} N (\circ) NaCl solution on either side of the membrane.

values refer to the total resistance of the membrane and the solution films existing between the Ag-AgCl electrodes and the membrane faces. Since the solutions used in the measurements were dilute, the resistance of the solution alone was of the same order of magnitude as that due to membrane plus solution films. By this method reliable values for the resistance of the membrane only could not be derived.

Although the flow of current across the composite membrane is different in the two directions, the osmotic flow of water and the diffusional flow of salt, each of these measured in the two directions across the membrane, remained practically the same. The osmotic flow of water (0.5 N NaCl solution on high charge density side) was $(1.10 \pm 0.13) \times 10^{-3} \text{ ml/hr}$ through 2.84 cm^2 membrane in one direction and $(0.96 \pm 0.12) \times 10^{-3} \text{ ml/hr}$ in the reverse direction. The salt flow was $5.4 \times 10^{-9} \text{ eq/cm}^2 \cdot \text{hr}$ in one direction and $5.7 \times 10^{-9} \text{ eq/cm}^2 \cdot \text{hr}$ in the other direction. The average of these salt fluxes used in the unintegrated form of Fick's equation

(Stewart and Graydon, 1956), viz.,

$$\bar{D}_{\text{app}} = \frac{\Delta C V d}{A C_i t}, \quad (5)$$

(where \bar{D}_{app} = apparent diffusion coefficient of the electrolyte, ΔC = change in concentration on either side of the membrane in time t , V = volume of the solution on the low concentration side, d = membrane thickness, A = membrane area, and C_i = concentration on the high concentration side at the end of time t) gave a value of 1.6×10^{-11} ($\text{cm}^2 \cdot \text{sec}^{-1}$) for \bar{D}_{app} . Thus the diffusion of salt in these composite membranes is very low.

The results pertaining to water and salt flows are not in accord with the facts already considered about the behavior of the composite membranes, i.e., asymmetry potential and current rectification. The experiments carried out to measure significant flows of salt and water lasted more than 30 hr. Recent experiments reported elsewhere (Lakshminarayanaiah and Siddiqi, 1971 *a*) show that the asymmetry potentials when followed over this long period of time decay to low values. This is probably because of some structural change in the membrane. At this stage we do not know definitely what this structural change is. It may be that PSSA migrates from a region of high concentration to a region of low concentration. As a result, it is possible that the asymmetry of the membrane faces is reduced, if not completely destroyed. If this is true, salt flows in either direction will not be unequal. Similarly water flows in the two directions also may not be unequal. In order to obviate this difficulty involved in long-term experiments, we conducted some short-term experiments using suitable isotopes to measure both water and salt flows. THO with NaCl (10^{-3} N) on one side and only 10^{-3} N NaCl solution on the other side of the composite membrane, were used to follow the movement of THO for about 4 hr. The amount of THO that came to the "cold" side in this period of time was small. However, the average of a number of experiments gave the following results: flux of THO with THO on high charge density side = $(9.5 \pm 1.5) \times 10^{-3}$ ml/hr; flux of THO with THO on low charge density side = $(6.5 \pm 1.0) \times 10^{-3}$ ml/hr across 2.84 cm^2 membrane.

^{22}Na in 10^{-2} N NaCl solution was used to follow the salt flux. In this case also there was a small increase in the activity on the cold side. A number of experiments performed in 4-hr periods gave the following values: flux of salt with ^{22}Na on low charge density side = $(7.4 \pm 1.0) \times 10^{-9}$ eq/ $\text{cm}^2 \cdot \text{hr}$; flux of salt with ^{22}Na on high charge density side = $(5.0 \pm 0.7) \times 10^{-9}$ eq/ $\text{cm}^2 \cdot \text{hr}$. There is a definite trend towards rectification of water and salt flows, although the magnitude of the difference between the flows in the two directions is not as high as the initial membrane asymmetry demands.

The relevance of these studies to the behavior of the biological membrane may not be obvious. The composite membranes mimic none of the physical charac-

teristics of the living membrane, whereas in terms of function they show some resemblance, because of the asymmetry of the two faces, in generating asymmetry potentials when the same electrolyte concentration is placed on either side. This aspect has been well demonstrated by Botre et al. (1967) who were able to reproduce with the composite membrane the resting potentials observed across the squid axon membrane. Similar work performed with our membranes gave unsatisfactory results. The reasons for this are being investigated.

The present model seems to be an improvement over other models of the type, for example, ion exchange membranes, liquid ion exchangers, phospholipids deposited on Millipore filters, etc. (Lakshminarayanaiah, 1965). The distinct advantage of the present model is the fact that one can incorporate either into the body of the composite membrane or into the middle chamber of the three-compartment cell (Fig. 3) various enzymes or other carriers and investigate the properties of these carriers. The model could be improved by using membranes containing carboxylic, phosphate, or amino groups in place of M_H and M_L membranes, maintaining an asymmetry not only in regard to charge density but also in regard to the nature of the group. Although the biological membrane is known to contain phosphate, carboxylic, and amino groups, how they are distributed between the inner and outer surfaces of the membrane is unknown. An insight into this group distribution may be possible with the present model by measuring asymmetry potentials using membranes containing phosphate, carboxylic, and amino groups in different combinations. Studies of this type are in progress.

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REFERENCES

- BOTRE, C., S. BORCHI, and M. MARCHETTI. 1967. *Biochim. Biophys. Acta.* **135**:162.
DE KOROSY, F. 1968. *J. Phys. Chem.* **72**:2591.
LAKSHMINARAYANAIAH, N. 1965. *Chem. Rev.* **65**:554.
LAKSHMINARAYANAIAH, N. 1967. *J. Appl. Polym. Sci.* **11**:1737.
LAKSHMINARAYANAIAH, N. 1969. *Transport Phenomena in Membranes*. Academic Press, Inc., New York. 199.
LAKSHMINARAYANAIAH, N., and F. A. SIDDIQI. 1971 a. *In Membrane Processes in Industry and Biomedicine*. M. Bier, editor. Plenum Publishing Corporation, New York. 301.
LAKSHMINARAYANAIAH, N., and F. A. SIDDIQI. 1971 b. *Biophys. J.* **11**:603.
LIQUORI, A. M. 1968. *Farm. Ed. Sci.* **23**:999.
LIQUORI, A. M., and C. BOTRE. 1964. *Ric. Sci.* **34**(6):71.
LIQUORI, A. M., and C. BOTRE. 1967. *J. Phys. Chem.* **71**:3765.
MEYER, K. H., and J. F. SIEVERS. 1936 a. *Helv. Chim. Acta.* **19**:649.
MEYER, K. H., and J. F. SIEVERS. 1936 b. *Helv. Chim. Acta.* **19**:665.
MEYER, K. H., and J. F. SIEVERS. 1936 c. *Helv. Chim. Acta.* **19**:987.
STEWART, R. J., and W. F. GRAYDON. 1956. *J. Phys. Chem.* **60**:750.
TEORELL, T. 1935 a. *Proc. Soc. Exp. Biol. Med.* **33**:282.
TEORELL, T. 1935 b. *Proc. Nat. Acad. Sci. U. S. A.* **21**:152.