

STUDIES WITH COMPOSITE MEMBRANES

III. MEASUREMENT OF WATER PERMEABILITY

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ABSTRACT The permeability of tritiated water (THO) across simple and layer-type composite membranes of collodion containing different amounts of polystyrenesulfonic acid has been measured and corrected for the effects of aqueous stationary layers present at the membrane-solution interfaces. It was found that the water permeabilities in the two opposite directions across the composite membranes were different, whereas they were the same across simple membranes. The theoretical permeability value for the composite membrane (formed by putting one simple membrane on top of another simple membrane of increasing charge density and gently pressing them together), calculated from the values due to simple membranes, was found to be always greater than the two measured values. It was shown that the aqueous layers trapped between membranes were not responsible for the low measured values. The factor causing this was ascribed to the mechanism which produced rectification of water flow in the composite membranes. Establishment of the THO concentration profile in the layered membranes showed that accumulation and depletion of THO in the membrane phase when the THO was flowing from the high charge density side to the low charge density side and vice versa, respectively, were responsible for the unequal flows observed across the composite membrane in the two directions.

INTRODUCTION

In part I in this series (Lakshminarayanaiah and Siddiqi, 1971 *a*), the preparation of simple and composite membranes of collodion containing polystyrenesulfonic acid and their impedances, and in part II (Lakshminarayanaiah and Siddiqi, 1971 *b*), some of the asymmetric properties of the layer-type composite membranes, have been described. Some preliminary values for the transport of salt and water across them given in part II showed that the magnitude of each of these flows in the opposite directions was different. A detailed report of the flow of THO in simple and in layer-type composite membranes is presented in this paper.

EXPERIMENTAL

The preparation of simple and layer-type composite membranes was carried out according to the procedures given in part I (Lakshminarayanaiah and Siddiqi, 1971 *a*). The composite

membranes used in this study were the same as those used in the earlier study described in Part II (Lakshminarayanaiah and Siddiqi, 1971 *b*). The concentrations of various membrane-forming solutions used in the preparation of both simple and composite membranes are given at the bottom of Table I.

Membrane pieces (~3 cm square) were converted into the Na form by immersing them in about 100 ml of 1 N NaCl solution in which they were left for future use. When they were required, a few pieces were taken out, gently stirred with deionized water with frequent changes of liquid for about 2 hr, equilibrated for 2-3 hr in about 100 ml of 0.001 N NaCl solution which also was changed often, and finally used in the experiments to be performed.

TABLE I
THO PERMEABILITY DATA FOR DIFFERENT SIMPLE MEMBRANE SYSTEMS

Membrane*	Single membrane permeability	Double membrane permeability	True membrane permeability	Stagnant layer thickness
	$P_s \times 10^6$ (equation 2)	$P_d \times 10^6$ (equation 2)	$P_t \times 10^6$ (equation 5)	δ (equation 6)
	cm/sec	cm/sec	cm/sec	μ
M_1	352.00 \pm 10.00 \dagger	182.00 \pm 5.00 \dagger	377.00	23
M_2	10.72 \pm 0.30	5.41 \pm 0.16	10.92	210
M_3	5.48 \pm 0.20	2.80 \pm 0.10	5.73	955
M_4	5.34 \pm 0.20	2.75 \pm 0.10	5.66	1330
M_5	5.11 \pm 0.20	2.60 \pm 0.10	5.30	828

* Membranes formed from 2% collodion solution (alcohol/ether ratio = 3/1) containing 2.91 (M_1), 0.41 (M_2), 0.10 (M_3), 0.04 (M_4), and 0.004 (M_5) mg of polystyrenesulfonic acid/ml of membrane-forming solution. Thickness of each single membrane = 0.002 cm.

\dagger The mean and one standard error of the mean of 10-15 values derived from at least three membranes.

The cell used in the measurement of permeability of THO was of the type described elsewhere (Lakshminarayanaiah, 1967 *a, b*). The area of the membrane exposed to the "hot" (0.001 N NaCl solution containing THO on one side) and "cold" (0.001 N NaCl solution on the other side) solutions was 5.0 cm². The volume of each of the two chambers holding the membrane between two rubber gaskets was about 40 ml and contained exactly 35 ml of the hot or cold solution. Magnetic stirrers (size: length = 2.5 cm, diameter = 1.1 cm) were used in the two chambers and were kept rotating close to the membrane faces. The rate of rotation (200 rpm) was determined by using a Strobotac, Type 1531 (General Radio Co., Concord, Mass.). The flow of THO was followed by sampling aliquots (exactly 1 ml) of liquid on the cold side at regular intervals of time. The loss in the volume of liquid due to sampling was compensated by adding 1 ml of 0.001 N NaCl solution.

The aliquots taken for counting were transferred into polyethylene vials (capacity of each about 25 ml) to which 20 ml of Bray's solution (Bray, 1960) were added and mixed thoroughly. These were counted in a Packard Tri-Carb liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.). All these experiments were performed in an air-conditioned room at 22°C.

RESULTS

The measurement of the flow of THO across the membrane, simple or composite, is quite straightforward provided the stationary aqueous layers at the membrane-solution interfaces are eliminated. This is very difficult to carry out. One could use turbulent flow of solution to eliminate them. Such a technique, however, would require elaborate hardware (Klein et al., 1969). Other methods described in the literature (Lakshminarayanaiah, 1965, 1969 *a*) could be employed to overcome the boundary layer effects which generally lowered the value for the permeability of THO. In recent years a few more methods (Scattergood and Lightfoot, 1968; Hale and Govindan, 1969; Everitt and Haydon, 1969; Everitt et al., 1969; Andreoli and Troutman, 1971) have been described. In this study the method described by Everitt et al. (1969) has been used. This involved making two permeability measurements, one using a piece of the simple membrane and the other using two pieces of the same membrane contacting each other in series ("double" membrane). The apparent permeability P_i for the single and for the double membrane systems was calculated from the equation (Dainty and House, 1966)

$$P_i = \frac{2.303V_1V_2}{(V_1 + V_2)A\Delta t} \log \frac{C'V_1 + C''V_2 - C''_{t_0}(V_1 + V_2)}{C'V_1 + C''V_2 - C''_{t_0+\Delta t}(V_1 + V_2)}, \quad (1)$$

where V_1 and V_2 are the volumes of compartments 1 (cold) and 2 (hot). C' and C'' are the initial concentrations (taken as counting rate per milliliter) at time $t = 0$ of radioactive isotope in compartments 1 and 2. C''_{t_0} and $C''_{t_0+\Delta t}$ are the concentrations in compartment 2 at $t = t_0$ and $t = t_0 + \Delta t$ respectively. Δt is thus the time in seconds between samplings. As $V_1 = V_2 = 35$ ml and $A = 5.0$ cm², equation 1 becomes

$$P_i = \frac{8.05}{\Delta t} \log \frac{C'' - 2C''_{t_0}}{C'' - 2C''_{t_0+\Delta t}}. \quad (2)$$

A plot of $\log \left(\frac{C'' - 2C''_{t_0}}{C'' - 2C''_{t_0+\Delta t}} \right)$ against time (Δt) gave a straight line (results not shown) whose slope multiplied by 8.05 gave the value for P_i ; however in this work P_i was evaluated numerically.

Equation 1 is applicable to the steady state of flow of THO across the membrane. In all the experiments, the diffusion of THO was allowed to go on for about 5 hr. In this period, the radioactivity (counts per minute) accumulating on the cold side followed as a function of time was linear (results not given). The steady-state straight line, in the case of simple membranes, cut the time axis very close to zero and gave a value between 1 and 3 min for the holdup time, whereas in the case of composite membranes, the holdup time was between 10 and 18 min.

The apparent permeability P_s evaluated from equation 2 using a simple membrane has been related to the true membrane permeability P_t by the relation (Kedem and Katchalsky, 1963)

$$\frac{1}{P_s} = \frac{1}{P_t} + \frac{\delta_1 + \delta_2}{D}, \quad (3)$$

where δ_1 and δ_2 are the thicknesses of the stagnant boundary layers existing at the two membrane faces and D is the self-diffusion coefficient of THO whose value has been found to be $2.44 \times 10^{-5} \text{ cm}^2 \text{ sec}^{-1}$ (Wang et al., 1953). Similarly for the double membrane, equation 3 becomes

$$\frac{1}{P_d} = \frac{2}{P_t} + \frac{\delta_1 + \delta_2}{D}, \quad (4)$$

where P_d is the apparent permeability of the double membrane. The value for P_t is given by solving equations 3 and 4. Thus

$$P_t = \frac{P_s \times P_d}{P_s - P_d}. \quad (5)$$

As the hydrodynamic conditions prevailing in the two chambers are almost similar, it is assumed that $\delta_1 = \delta_2 = \delta$. Consequently equations 3 and 4 give

$$\delta = \frac{2P_d - P_s}{P_s P_d} \times \frac{D}{2}. \quad (6)$$

The various measured values of P_s and P_d and the derived values of P_t and δ are given in Table I.

The permeability results pertaining to composite membranes are given in Table II. Two types of composite membrane systems were used in these studies. Type 1 was formed in the usual way (Lakshminarayanaiah and Siddiqi, 1971 *a*) by spreading and drying different membrane-forming solutions of collodion containing varying amounts of polystyrenesulfonic acid one on top of the other. These composite membranes have been designated as M_1M_5 and $M_1M_2M_3M_4M_5$. Type 2 was formed from simple membranes by putting one equilibrated (i.e., wet) membrane, for example M_1 , on top of another simple wet membrane, for example M_5 , and pressing them together so as to make good physical contact. These have been designated as M_1-M_5 , $M_1-M_3-M_5$, and $M_1-M_2-M_3-M_4-M_5$. The values of apparent permeability P_t for the composite membrane systems were corrected for the presence of stagnant diffusion layers by using equation 3 and a value of 23μ for δ_1 (layer in contact with high charge density membrane surface) and a value of 1037μ (average of the three high values given in the last column of Table I) for δ_2 (layer in contact with low charge density membrane surface). These corrected values which correspond to P_t for the composite membranes are also given in Table II.

In the case of simple membranes M_1 through M_5 , the permeability of the membrane was the same whether the flow of THO was in one direction or in the opposite direction. On the other hand, the results given in Table II for the various composite

TABLE II
THO PERMEABILITY DATA FOR THE LAYER-TYPE
COMPOSITE MEMBRANE SYSTEMS

Membrane	Direction of THO flow	Apparent permeability $P_i \times 10^6$ (equation 2)	True permeability $P_i \times 10^6$ (equation 3)
		<i>cm/sec</i>	<i>cm/sec</i>
$M_1M_5^*$	$M_1 \rightarrow M_5$	8.82 ± 0.46	9.17
	$M_5 \rightarrow M_1$	7.49 ± 0.38	7.75
$M_1-M_5 \ddagger$	$M_1 \rightarrow M_5$	4.33 ± 0.21	4.41
	$M_5 \rightarrow M_1$	3.67 ± 0.19	3.72
$M_1-M_3-M_5$	$M_1 \rightarrow M_5$	1.73 ± 0.07	1.74
	$M_5 \rightarrow M_1$	1.55 ± 0.06	1.56
$M_1M_2M_3M_4M_5$	$M_1 \rightarrow M_5$	1.53 ± 0.05	1.54
	$M_5 \rightarrow M_1$	1.17 ± 0.05	1.18
$M_1-M_3-M_5-M_4-M_5$	$M_1 \rightarrow M_5$	1.40 ± 0.06	1.41
	$M_5 \rightarrow M_1$	1.14 ± 0.06	1.15

* Membranes designated M_iM_j are formed by spreading and drying different membrane-forming solutions of collodion containing varying amounts of polystyrenesulfonic acid one on top of the other.

‡ Membranes designated M_i-M_j are formed from simple membranes M_i and M_j by putting them one on top of the other and pressing together to make good physical contact. Equilibrated wet membranes are used.

membranes show that the permeabilities in the two opposite directions across the membrane were different. When the THO solution was in contact with the high charge density surface of the composite membrane, the flow observed was higher than that observed in the opposite direction, i.e., when THO solution was contacting the low charge density surface of the membrane. Whether the difference in the values of the two permeabilities observed in the two directions was significant or not was established by carrying out a Student's *t* test on at least 12 sets of values. In the case of all composite membranes used in these studies (see Table II), the values of *t* were always greater than 4 and thus the difference in the two permeabilities in the opposite directions for a given composite membrane was significant.

DISCUSSION

The effect of the stationary boundary layers, as pointed out already, is to reduce the value of P_i , the true permeability of the species (THO) flowing through the membrane. The magnitude of the reduction in the value of P_i is determined as discussed elsewhere (Lakshminarayanaiah and Siddiqi, 1972) by (a) the size of the stirrers in relation to the area of the membrane, (b) the rate of stirring, (c) the distance at which the stirrers rotated from the membrane surfaces, and (d) the nature of the membrane. Factors a-c were adequately taken care of in this study by

using large stirrers rotating at 200 rpm as close to the membrane faces as possible. That the stirrers were fairly close to the membrane faces was indicated by the fact that a number of experiments had to be abandoned because of rupture of the membrane by rotating bars. The rotation of the bars at 200 rpm was also found satisfactory since speeds greater than 200 rpm never increased the THO permeability. With regard to factor (*d*), the results given in Tables I and II show that when the membrane resistance was low, i.e. simple membrane M_1 , even thin stagnant layers (23μ) have a significant effect on P_i . The P_i has increased in this case by about 7%. On the contrary, in the case of membranes M_3 through M_5 which have relatively high resistance compared with M_1 (see Lakshminarayanaiah and Siddiqi, 1971 *a*), the increase in the value of P_i is between 3 and 5% even though the boundary layer thickness is as high as 1000μ . In the case of the relatively thick composite membrane $M_1M_2M_3M_4M_5$, the boundary layers have very little effect ($< 1\%$) on the value of P_i . These facts are in accord with the information available in the literature (Helfferich, 1962 *a*; Lakshminarayanaiah, 1969 *b*).

In the earlier study (Lakshminarayanaiah and Siddiqi, 1971 *a*) it was shown from impedance measurements that the composite membrane M_1M_5 had the characteristics of three membranes, viz. M_1 , M_5 , and the third simple membrane formed from M_1 and M_5 and existing in between them. The permeability results also lead to the same conclusion as illustrated below.

If the composite membrane M_1M_5 retained the characteristics of M_1 and M_5 , it should have according to equation 3, i.e.

$$\frac{1}{P_{M_1M_5}} = \frac{1}{P_{t(M_1)}} + \frac{1}{P_{t(M_5)}},$$

a value of 5.23×10^{-6} cm/sec for its permeability to THO. This value is lower than either of the two observed values, i.e., 9.17 or 7.75×10^{-6} cm/sec (see Table II). Substituting the higher of the two values in the modified form of equation 3, viz.

$$\frac{10^6}{9.17} = \frac{d_1}{\bar{D}_1} + \frac{1}{P_{15}} + \frac{d_5}{\bar{D}_5}, \quad (7)$$

(where d_1 and d_5 are the thicknesses of membranes M_1 and M_5 as they prevail in the composite membrane M_1M_5 , \bar{D}_1 and \bar{D}_5 are the diffusion coefficients of THO in membranes M_1 and M_5 , and P_{15} is the permeability of the third unit to THO, the third unit being formed as stated already from M_1 and M_5), equation 7 becomes

$$P_{15} = \frac{1}{1.09 \times 10^5 - 9.55 \times 10^7 d}, \quad (8)$$

where the assumption $d_1 = d_5 = d$ and the following substitutions have been made: $\bar{D}_1 = P_{t(M_1)}d_1 = 7.54 \times 10^{-7}$, $[P_{t(M_1)} = 3.77 \times 10^{-4}$ cm/sec and $d_1 = 2 \times 10^{-3}$

cm; see Table I], $\bar{D}_5 = P_{t(M_5)}d_5 = 1.06 \times 10^{-8} \text{ cm}^2 \text{ sec}^{-1}$ [$P_{t(M_5)} = 5.3 \times 10^{-6}$ and $d_5 = 2 \times 10^{-3} \text{ cm}$; see Table I].

From equation 8, values for P_{15} may be calculated by assigning different values for d . The results of such calculations are given in Table III. It is seen from these results that when $d = 0$, $P_{15} = 9.17 \times 10^{-6}$, i.e., one uniform membrane is formed; and when $d = 1.14 \times 10^{-3} \text{ cm}$, $P_{15} = \infty$. Also when $d = 1.113 \times 10^{-3} \text{ cm}$, $P_{15} = 3.77 \times 10^{-4}$, the same value as the high charge density membrane, and the thickness of this unit is $1.774 \times 10^{-3} \text{ cm}$. That a uniform membrane is formed is not true as rectification of THO flow is observed; and that the end units have a thickness of $1.113 \times 10^{-3} \text{ cm}$ is also not true because this would give a composite membrane M_1M_5 (thickness = $4 \times 10^{-3} \text{ cm}$) containing a high charge density membrane M_1 of thickness $2.887 \times 10^{-3} \text{ cm}$, i.e. $1.774 \times 10^{-3} + 1.113 \times 10^{-3}$, and M_5 of thickness $1.113 \times 10^{-3} \text{ cm}$. The thickness of M_1 can never be greater than $2 \times 10^{-3} \text{ cm}$ because the volume of membrane-forming solution and the area it occupies are fixed. Consequently it is established that the thickness of the end units must be less than $1.113 \times 10^{-3} \text{ cm}$. What the values of P_{15} are when the end units have a thickness in the range $(1.1-0.1) \times 10^{-3} \text{ cm}$ are given in Table III.

Unlike the composite membrane M_1M_5 , the composite membrane M_1-M_5 formed by laying and pressing M_1 on top of M_5 has no intermediate unit in the sense in which it is supposed to exist in the membrane M_1M_5 . Still there might be a thin liquid layer between the two membranes M_1 and M_5 . The theoretical value for the permeability of this membrane system should still be $5.23 \times 10^{-6} \text{ cm/sec}$ as the contribution of the thin liquid layer to the over-all permeability of the whole membrane system would be very small. This theoretical value is higher than the

TABLE III
THICKNESS OF END UNITS OF M_1 AND M_5 AS THEY EXIST
IN THE COMPOSITE MEMBRANE M_1M_5 AND THE PERMEABILITY
 P_{15} OF THE MIDDLE UNIT FORMED FROM M_1 AND M_5
CALCULATED ACCORDING TO EQUATION 8

Thickness of end units of composite membrane M_1M_5 $d \times 10^3$	Permeability of middle unit formed from M_1 and M_5 of the composite membrane M_1M_5 P_{15}
cm	cm/sec
1.14	∞
1.12	5.00×10^{-4}
1.113	3.77×10^{-4}
1.10	2.50×10^{-4}
1.00	7.40×10^{-5}
0.67	2.21×10^{-5}
0.10	1.01×10^{-5}
0.00	9.17×10^{-6}

two observed values (see Table II). This discrepancy might arise from (a) the presence of not so thin a liquid layer between M_1 and M_5 or (b) the same factor which caused the rectification of the THO flows. A simple calculation using equation 3, i.e.

$$\frac{10^6}{4.41} = \frac{10^6}{5.23} + \frac{10^5 d}{2.44},$$

(where 4.41×10^{-6} cm/sec is the observed permeability of THO across the composite membrane M_1 - M_5 [see Table II], 5.23×10^{-6} cm/sec is the theoretical permeability value, 2.44×10^{-5} cm²/sec is the self-diffusion coefficient of THO, and d is the thickness of the trapped aqueous layer between membranes M_1 and M_5) gives a value of 0.88 cm for the thickness of the trapped aqueous layer. This value is too high (several orders higher than the thickness of the membranes put together) to be true. Similarly in the case of the other composite membrane systems, M_1 - M_3 - M_5 and M_1 - M_2 - M_3 - M_4 - M_5 , the observed permeabilities (1.74 and 1.41×10^{-6} cm/sec respectively) are found to be lower than the calculated (theoretical) values (2.74 and 1.58×10^{-6} cm/sec). In these cases also, the thicknesses of the aqueous layers trapped between membranes are unbelievably high (5.1 and 1.7 cm). So the factor a mentioned above cannot be responsible for the discrepancy between the observed and the calculated values of THO permeability. It is very interesting to find that the observed permeability across the composite membrane $M_1M_2M_3M_4M_5$, i.e. 1.54×10^{-6} cm/sec, is close but still lower than the theoretical value of 1.58×10^{-6} cm/sec. Consequently it is believed that the factor b mentioned above is probably involved in generating low values for the THO permeability in composite membranes. So the important question raised by the above discussion is what is really causing the composite membranes to allow more THO to flow in one direction (i.e. $M_1 \rightarrow M_5$) than in the reverse direction, i.e., $M_5 \rightarrow M_1$.

The two faces of the composite membranes are asymmetrical in that one surface is more highly charged (M_1) than the other surface (M_5); besides, surface M_1 is more porous than surface M_5 (see Lakshminarayanaiah and Siddiqi, 1971 *a*). In general the flow of an uncharged species like THO through the composite membrane will not be influenced by the fixed charges present in the membrane (Helfferich, 1962 *b*). On the other hand, the porosity, i.e. the amount of water present in the membrane, will affect the flow of THO. This is very well reflected in the values of permeability given in the fourth column of Table I. M_1 which is more porous than M_5 is nearly 70 times more permeable to THO than is M_5 . The permeability sequence of the individual units used in the composite membrane is as $M_1 > M_2 > M_3 \geq M_4 \geq M_5$.

It is generally recognized in the case of artificial membranes that the permeabilities of counterion, coion, and nonelectrolytes are all concentration dependent (Helfferich, 1962 *b*). We may include water also to show this behavior, although it is difficult to come up with a direct demonstration in the way the dependence of permeability

on concentration of a nonelectrolyte solute, noninteracting with the membrane matrix, can be demonstrated.

When a gradient of THO acts across a composite membrane it takes, as stated already, 10–18 min for the flow of THO to reach a steady state. During this period, i.e. holdup time, THO would move through the different units constituting the composite membrane at rates proportional to the corresponding permeabilities in those units. When the gradient is acting in the direction $M_1 \rightarrow M_5$, the rate at which THO moves through the M_1 unit is faster than the rate at which it is removed through the M_5 unit. Consequently one might expect, during the time the flow is reaching the steady state, accumulation of THO at some border line in the membrane phase. Similarly for the same reason, there will be depletion of THO at some border line in the membrane phase when the direction of the THO gradient is reversed, i.e., $M_5 \rightarrow M_1$. Following this, the steady state is reached when the rates of flow in the different units would be equal. When the flow has attained this state, the

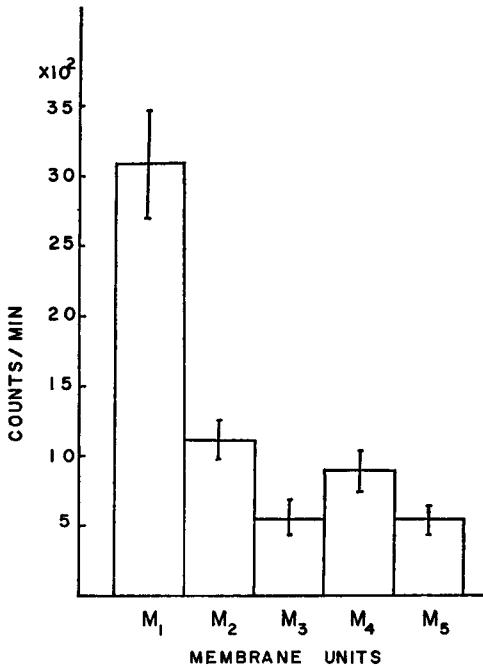


FIGURE 1

FIGURE 1 Concentration profile of THO in composite membrane (M_1 - M_2 - M_3 - M_4 - M_5) formed by putting one simple membrane on top of another and pressing them together when the THO gradient was acting in the direction $M_1 \rightarrow M_5$, where M_1 was the high charge density membrane and M_5 was the low charge density membrane.

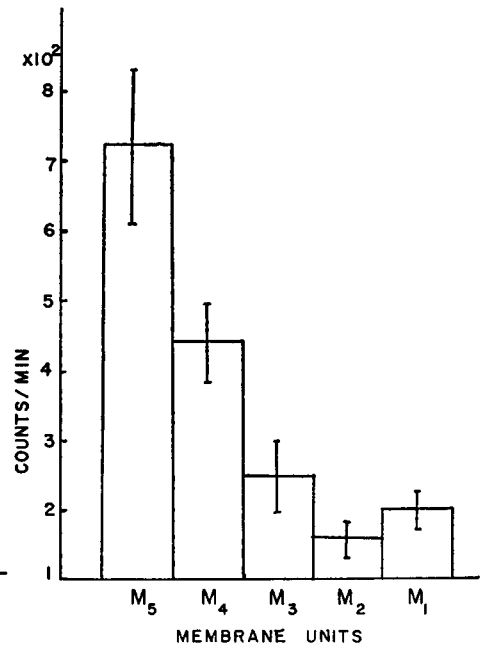


FIGURE 2

FIGURE 2 Concentration profile of THO in composite membrane (M_5 - M_4 - M_3 - M_2 - M_1) formed by putting one simple membrane on top of another and pressing them together when the THO gradient was acting in the direction $M_5 \rightarrow M_1$, where M_5 was the low charge density membrane and M_1 was the high charge density membrane.

concentration profile in the membrane would have become stationary probably with a hump at some distance in the membrane when the gradient is in the direction $M_1 \rightarrow M_5$ and with a dip at some distance in the membrane when the gradient is acting in the reverse direction $M_5 \rightarrow M_1$. If this is true, the flow of THO in the direction $M_1 \rightarrow M_5$ would be greater than the flow in the reverse direction provided the over-all permeability is controlled by the nature of the concentration profile. This necessarily implies dependence of THO permeability on concentration, a fact already referred to above. In order to check these points, the concentration profile of THO in the composite membrane $M_1-M_2-M_3-M_4-M_5$ was determined. This was carried out as follows.

The membranes M_1 to M_5 were stacked in series to form a seven-member stack as $M_1-M_1-M_2-M_3-M_4-M_5-M_5$. Hot and cold solutions of 0.001 N NaCl were placed on the M_1 and M_5 sides respectively. The diffusion of THO was allowed to take place in the usual way for a period of about 3 hr, at the end of which the cell was emptied thoroughly. The membrane stack was removed from the cell carefully. The end membranes, the first M_1 and the last M_5 , were discarded. The other five membranes M_1 through M_5 were carefully separated from one another and each was placed in a polyethylene vial into which 20 ml of Bray's solution were added, mixed, and counted in a liquid scintillation counter. This procedure was repeated by reversing the direction of THO flow, i.e., hot and cold solutions were placed on M_5 and M_1 sides respectively. The results realized are shown in Figs. 1 and 2.

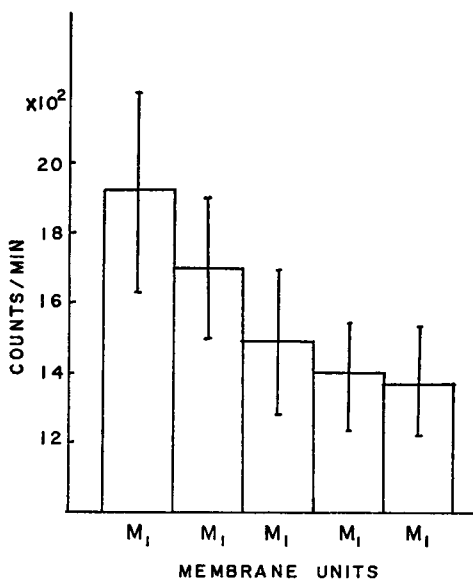


FIGURE 3 Concentration profile of THO in a uniform membrane formed from a stack of seven M_1 membranes. M_1 membrane contained 2.91 mg of polystyrenesulfonic acid/ml of membrane-forming collodion solution.

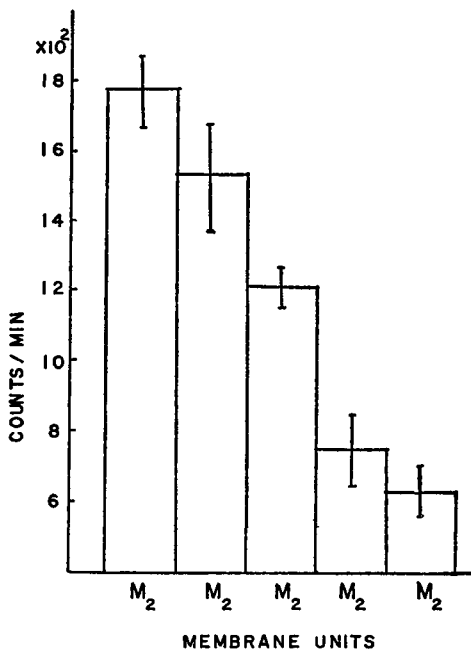


FIGURE 4

FIGURE 4 Concentration profile of THO in a uniform membrane formed from a stack of seven M_2 membranes. M_2 membrane contained 0.41 mg of polystyrenesulfonic acid/ml of membrane-forming collodion solution.

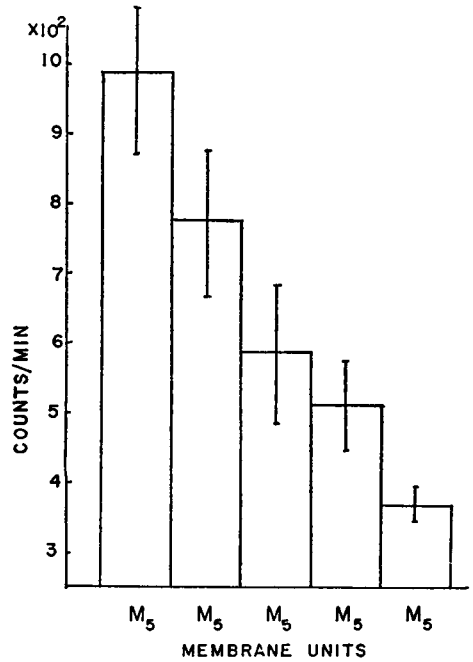


FIGURE 5

FIGURE 5 Concentration profile of THO in a uniform membrane formed from a stack of seven M_5 membranes. M_5 membrane contained 0.004 mg of polystyrenesulfonic acid/ml of membrane-forming collodion solution.

The normal concentration profile in a high, medium, or low charge density membrane system was also established using again a seven-membrane stack formed of seven high, medium, or low charge density membranes. These results are given in Figs. 3-5.

In the above experimental procedure, the amount of liquid trapped between membranes has been taken into account. Two other alternate treatments at the end of the experimental run were also followed to determine the amount of THO present in each membrane only of the stack. Treatment one consisted of giving each membrane after removal from the stack a quick dip in deionized water. Then it was transferred to the vial as usual. Treatment two consisted of gently pressing each membrane of the stack between filter papers and transferring it to the vial. These treatments removed the superficial liquid from the membranes. These procedures also gave profiles (results not given) similar to those shown in Figs. 1-5 although the counts were relatively low.

The results of Figs. 3-5 indicate that in a "uniform" membrane, whether it contained high, medium, or low density of fixed charges, there is a gradual decrease in

the concentration of THO in the membrane phase as THO moved down its gradient. On the other hand, the picture is different in the case of the composite membrane (see Figs. 1 and 2). When the THO gradient is in the direction $M_1 \rightarrow M_5$, the concentration of THO decreases up to the third unit (Fig. 1), then increases, and finally decreases. The maximum (i.e., accumulation) is located between M_3 and M_5 units of the composite membrane. When the gradient is reversed (i.e., $M_5 \rightarrow M_1$), the concentration of THO decreases, reaches a minimum, and then increases. The minimum (i.e., depletion of THO) is located between M_3 and M_1 units. Thus these conditions should lead to unequal flows of THO across the composite membrane in the opposite directions. The data given in Table II confirm this.

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REFERENCES

- ANDREOLI, T. E., and S. L. TROUTMAN. 1971. *J. Gen. Physiol.* **57**:464.
 BRAY, G. A. 1960. *Anal. Biochem.* **1**:279.
 DAINY, J., and C. R. HOUSE. 1966. *J. Physiol. (London)*. **185**:172.
 EVERITT, C. T., and D. A. HAYDON. 1969. *J. Theor. Biol.* **22**:9.
 EVERITT, C. T., W. R. REDWOOD, and D. A. HAYDON. 1969. *J. Theor. Biol.* **22**:20.
 HALE, D. K., and K. P. GOVINDAN. 1969. *J. Electrochem. Soc.* **116**:1373.
 HELFFERICH, F. 1962 a. Ion Exchange. McGraw-Hill Book Company, New York. 348.
 HELFFERICH, F. 1962 b. Ion Exchange. McGraw-Hill Book Company, New York. 350.
 KEDEM, O., and A. KATCHALSKY. 1963. *Trans. Faraday Soc.* **59**:1941.
 KLEIN, E., J. K. SMITH, and R. P. WENDT. 1969. *J. Polym. Sci. Part C.* **28**:209.
 LAKSHMINARAYANAI AH, N. 1965. *Chem. Rev.* **65**:499.
 LAKSHMINARAYANAI AH, N. 1967 a. *Biophys. J.* **7**:511.
 LAKSHMINARAYANAI AH, N. 1967 b. *J. Appl. Polym. Sci.* **11**:1737.
 LAKSHMINARAYANAI AH, N. 1969 a. Transport Phenomena in Membranes. Academic Press, Inc., New York. 132.
 LAKSHMINARAYANAI AH, N. 1969 b. Transport Phenomena in Membranes. Academic Press, Inc., New York. 131.
 LAKSHMINARAYANAI AH, N., and F. A. SIDDIQI. 1971 a. *Biophys. J.* **11**:603.
 LAKSHMINARAYANAI AH, N., and F. A. SIDDIQI. 1971 b. *Biophys. J.* **11**:617.
 LAKSHMINARAYANAI AH, N., and F. A. SIDDIQI. 1972. *Z. Phys. Chem. (Frankfurt am Main)*. In press.
 SCATTERGOOD, E. M., and E. N. LIGHTFOOT. 1968. *Trans. Faraday Soc.* **64**:1135.
 WANG, J. H., C. V. ROBINSON, and I. S. EDELMAN. 1953. *J. Amer. Chem. Soc.* **75**:466.