INTERACTION BETWEEN CATIONS IN HYDROPHOBIC SOLVENT-SATURATED FILTERS CONTAINING FIXED NEGATIVE CHARGES

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ABSTRACT Previous investigations have shown that Millipore filters bearing fixed negatively charged sites saturated with organic solvents behaved like cation exchangers. In this study the mobilities of some cations in bromobenzene-saturated filters were determined at various membrane compositions. Appreciable interaction between counterions in the membrane was demonstrated; e.g., Na^+ was about four times more mobile in a Na^+ membrane than in a K^+ one. The qualitative nature of the interaction could be summarized as follows: the bringing together of rapid and slow counterions in the membrane made the fast ion faster and the slow ion slower. Possible mechanisms for such an interaction are discussed. A quantitative estimation of K and Na mobilities and selectivity factors at various K-Na fractions in the membrane was made. The above parameters were used to compute the potential difference in a K-Na bi-ionic cell. The calculated bi-ionic potential was found to agree reasonably well with the measured value.

INTRODUCTION

In a previous study (1) it was shown that Millipore filters (porous discs made of cellulose acetate and nitrate) saturated with organic solvents sharply discriminate between cations as indicated by the presence of high bi-ionic potentials across such membranes. It was subsequently established (2, 3) that these hydrophobic membranes are of an ion exchange type owing to their fixed negatively charged groups and that the discrimination is also expressed by the selectivity of the membranes and by the different mobilities of the ions in the membrane.

The present study started with experiments aimed at measuring the relative K^+-Na^+ permeability of the membrane and correlating it with the K^+-Na^+ bi-ionic potential. In the course of the investigation it became obvious that the mobilities of the ions were significantly affected by the composition of the counterions in the membrane. The nature and extent of this kind of interaction between ions in the membrane and its relevance to the calculation of the magnitude of the bi-ionic potential are the subject of this report.

The organic solvent used in this study was bromobenzene. With this solvent the resistance of the membrane is relatively small and, therefore, the fluxes of ions through such membranes could be measured more easily (2).

METHODS

A Millipore filter (cellulose acetate and nitrate[1]) was placed between two glass chambers held together by four metal springs (see Fig. 1 in reference 1). After saturating the filter with bromobenzene the whole cell was placed in a bromobenzene bath and both sides of the cell filled with the desired aqueous solutions. The solutions could be agitated by glass-sealed magnetic stirrers. This type of diffusion cell was used both for studying the fluxes across the membrane and for measuring its electrical resistance.

In studying the properties of the membrane under equilibrium conditions (i.e., identical solutions present on both sides of the membrane) the membrane was allowed to equilibrate with the solution for at least 2 hr prior to the experiment. The results of the experiments indicate that this time interval was more than sufficient to attain equilibrium throughout the diffusion cell.

The permeability ratio of the membrane for two ions was determined by measuring the unidirectional fluxes of the ions in an equilibrium cell using radioactive isotopes. The permeability of the membrane to an ion, p_i, is defined as the ratio between the flux of the ion and its electrochemical gradient across the membrane at zero volume flux. Since the cell is in equilibrium the unidirectional flux, J_i, should be proportional only to the concentration in the water solution, C_i^w, and the permeability of the membrane namely, $J_i \sim C_i^w \cdot p_i$. Therefore, the permeability ratio, P_j^i is given by

$$P_j^i = p_i/p_j = (J_i/C_i^w)/(J_j/C_j^w). \tag{1}$$

If the rate-limiting process of diffusion is the passage through the membrane, the flux is given also by

$$J_i = SD_iC_i/l \tag{2}$$

where S is the area of the membrane, l is the thickness of the membrane, C_i is the concentraton of the ion in the membrane, and D_i is the diffusion coefficient in the membrane. Introducing equation 2 into equation 1 one obtains

$$P_j^i = (D_i/D_j)(C_j^wC_i/C_i^wC_j). \tag{3}$$

The right-hand term in equation 3 can be identified as the selectivity factor K_j^i. Thus, of the three parameters–permeability ratio, diffusion coefficient ratio, and selectivity–only two are necessary for the characterization of the membrane discrimination between two ions under equilibrium conditions. Since the Nernst-Einstein relationship seems to hold for these membranes (see section 2 of the Results), the term mobility ratio is used in this paper as identical with the diffusion coefficient ratio.

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[1] Note that uncharged polyethylene Millipore filters are also available, the properties of which, by virtue of the absence of fixed negative charges, are different from those reported here.

The flux across the membrane was always below 0.6. 10^{-11} mole/sec cm^2. The thickness of an unstirred film is not likely to be more than 0.01 cm (see Fig. 3 in reference 4). If the ion diffusion coefficient in water is about 10^{-5} cm^2/sec the concentration difference across the unstirred film is less than 0.6 10^{-8} mole/cm^3. This is less than 1% of the concentration used in the flux experiments (generally 75 10^{-8} mole/cm^3). This implies that the flux in these studies was determined by diffusion through the membrane; the effect of the unstirred layer was negligible.

The isotopes used in this study were K^{42}-, Na^{22}-, or Na^{24}-, and C^{14}-labeled TEA (tetraethylammonium). K^+ and Na^+ radioactivity was determined by conventional well type scintillation counter. C^{14} was measured in a liquid scintillation counter after dissolving the TEA in 3 cc of ethyl alcohol and adding 7 cc of toluene containing 0.57% PPO and 0.014% POPOP (Packard Instrument Company, LaGrange, Illinois). When studying fluxes Na^+ and K^+ simultaneously, the Na^{22} isotope was used. Its activity was determined after the activity due to the K^{42} had sufficiently decayed. The difference between the activity due to Na^{22} and the total activity, measured on the day of the experiment, represented the K^{42} activity.

Electrical resistance of the membrane was measured by balancing a Wheatstone bridge at 1000 CPS, using a Tektronix oscillator (Tektronix Inc., Portland, Oregon) as null point detector. The resistance measured at this frequency was very close to the resistance of the membrane as determined by studying equivalent series resistance and reactance at various frequencies and drawing the impedance locus (2). Connection with the solutions was through platinum electrodes.

The resistance as well as the fluxes of ions through the membrane, unlike the bi-ionic potentials, depended upon the type and even the batch of filters used. It was therefore important to use the same batch of filters for every set of experiments in order to make results obtained from different membranes comparable.

K^+-Na^+ selectivity was measured in the following way: filters saturated with bromobenzene were soaked in water solutions containing various amounts of Na^{22} Cl and K^{42} Cl for about 12 hr. The filters were then rinsed once with distilled water and dried by an absorbent paper. The dried filter was then placed in 1 cc KCl 15 mM solution[2] and the activity of that sample was measured and compared with the activity of Na^{22} and K^{42} in the aqueous solution. The sum of Na^+ and K^+ in membranes having an area of about 3 cm^2 was around 6.10^{-8} moles. This value represented the total ion exchange capacity of the membrane (2). Since the thickness of the membrane is around 0.015 cm, it follows that the concentration of fixed sites in the membrane was around $1.4.10^{-6}$ equivalents per cc of membrane.

RESULTS

1. K^+-Na^+, and K^+-TEA^+ Permeability Ratio. Fig. 1 shows the simultaneous movement of K^+Na^+ and K^+-TEA^+ across a bromobenzene-saturated filter in equilibrium cells. The isotopes K^{42}-, Na^{22}-, and C^{14}-labeled TEA^+ were introduced into one side of the cell and their appearance in the other

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[2] Due to the relatively low energy of the K^{42} emission, comparable counts per minute are obtained only by measuring the radioactivity of the isotope when dissolved in the same volume of solution. Therefore, the activity of K^{42} in the solution and in the membrane was determined in a 1 cc solution.

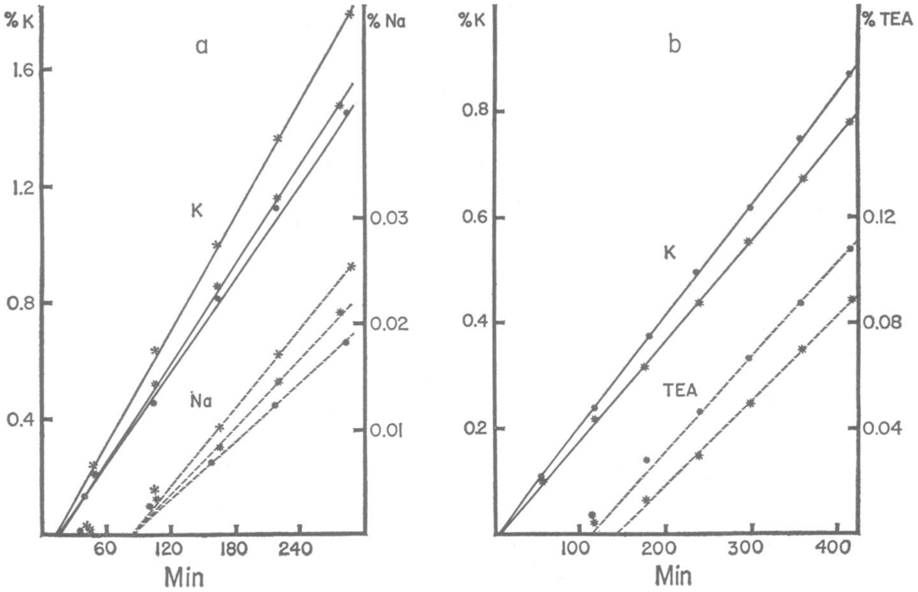

Figure 1 K^+ (solid lines) and Na^+ or TEA^+ (broken lines) fluxes across a bromobenzene-saturated membrane in equilibrium cells. The ordinate expresses the amount of radioactive material in side II as per cent of the amount in side I. The radioactive elements were introduced into side I at time zero. Each symbol represents one equilibrium cell. Composition of solution = (*a*) KCl and NaCl 0.75 mM each; (*b*) KCl and TEABr 0.75 mM each. Temperature 23°C.

side was followed with time. The slopes of the curves represent the per cent of material in side I which diffuses into side II per unit time and therefore the ratio between the slopes of two curves is the permeability ratio for the ions concerned (see equation 1). These are approximately 50 for the K^+-Na^+ permeability ratio and around 5 for K^+-TEA^+ permeability ratio.

Another obvious difference between the various ions is the rapidity with which their flux through the membrane attained a constant value. This is evident from the fact that the linear curves intersect with the time abcissa at different points. If the mobility of the ion along the axis of diffusion was constant, which is to be expected for an homogeneous membrane under equilibrium conditions, the point of intersection, known as the holdup time, would be inversely proportional to the diffusion coefficient *D* in the membrane. The relation between the two is given by the equation

$$D = l^2/6\tau \qquad (4)$$

where τ is the holdup time and l is the thickness of the membrane (5). Analysis of the curves in Fig. 1 shows that the K^+-Na^+ and K^+-TEA^+ mobility ratios are around 6 and 13 respectively. Given the permeability ratio of 50 and 5 it must be concluded (see equation 3) that the selectivity factors are about 9 and 0.4 for K^+-Na^+ and K^+-TEA^+ respectively. These figures tally quite well with the previously reported selectivity factors (2).

However, the mobility ratios reported previously were much lower; e.g., 1.3 and 2.1 for the K^+-Na^+ and K^+-TEA^+ respectively. The discrepancy is due to the fact that the mobility ratios in the present experiments have been determined at a certain membrane composition for both ions whereas in the previous report the mobility was estimated by measuring the electrical conductance of the membrane in its pure forms on the assumption that (*a*) only cations carry electrical current through the membrane and that (*b*) the mobilities of the ions are constant. The justification for the first assumption, which was implicit in previous observations (2), is reaffirmed in the next section. However, as will be shown in the other parts of this study, the second assumption is entirely invalid for these membranes. Thus, the previous estimation of the mobility ratio applies only to the particular conditions under which it was studied.

2. The Relation between K^+ Flux and Membrane Conductance. As already pointed out the Millipore filters from different batches, although always yielding the same bi-ionic potentials varied quite widely in the magnitude of their electrical resistance. If the transference number of the cations is close to 1 it would be expected that the fluxes of cations through the membrane would be proportional to the electrical conductance of the membrane. Experiments were carried out with the aim of relating K^+ fluxes to the membrane electrical conductance. Filters from different batches were saturated with bromobenzene and exposed to various solutions. Using radioactive isotope, the K^+ flux through the membrane was determined in an equilibrium cell. The electrical conductance of the same membrane was measured immediately afterwards. The results are shown in Table I. Given the

TABLE I

UNIDIRECTIONAL K^+ FLUXES, MEMBRANE CONDUCTANCE, AND K^+ TRANSFERENCE NUMBER FOR VARIOUS EQUILIBRIUM CELLS

The K^+ transference number, t_K, is determined by the equation $t_K = F^2J_K/RTk$ where J_K is the unidirectional K^+ flux in the equilibrium cell, k is the membrane conductance, F, R, and T have their usual meaning. Temperature 25°C. Membrane area about 3 cm².

Type of filter used	Composition of solution K^+	Na^+	K^+ fluxes	Electrical conductance 10^{-5} ohm^{-1}	t_K
	mH	*mM*	10^{-11} *mole/sec*		
HA_{25}	15.0		>0.4	0.7	—
VF_{25}	15.0	—	1.5	5.6	1.0
VC_{47}	15.0	—	1.04	3.97	0.93
VF_{47}	1.5	—	0.92	3.60	0.96
VC_{25}	1.5	—	0.55	2.12	0.97
VF_{47}	0.015	1.5	0.16	2.18	0.275
VF_{47}	0.015	1.5	0.17	2.22	0.29

Nernst-Einstein relation, $D_i = RT/F\ u_i$, where D and u are the diffusion coefficient and the mobility respectively in an equilibrium cells, R, T, and F have their usual meaning, it can be shown that the conductance due to the ion i, k_i, is related to its unidirectional flux, J_i, by the following expression:

$$k_i = \frac{J_i F^2}{RT}. \tag{5}$$

Therefore, the transference number, t_i, (which by definition is k_i/k where k is the membrane conductance) can be determined by measuring the conductance of the membrane and the flux of the ion i in an equilibrium cell through the equation:

$$t_i = \frac{J_i F^2}{kRT}. \tag{6}$$

The transference number for K^+, calculated by equation 6, is shown in the last column of Table I. It is evident that when the membrane is exposed to solution containing only KCl the transference number for K^+ is around 1 implying that electroconductance due to chloride or electroosmosis is negligibly small.

When solutions bathing the membrane contain NaCl as well as KCl, the transference number of K^+ is less than 1. It can be seen, however, that K^+ accounted for about 28% of the membrane conductance although it comprised only 1% of the cations in the solution. If the Na^+ accounted for the remaining 72% of the membrane conductance it follows that Na flux was around 2.5 times higher than that of K^+. Since Na^+ was 100 times more abundant than K^+ in the aqueous solution it follows that the K^+-Na^+ permeability ratio was around 40, a value which agrees reasonably well with the previous direct determination of the permeability ratio.

3. Dependence of Ion Mobility on Membrane Composition. In order to see whether or not the ion mobility was independent of counterion composition of the membrane, experiments of the type shown in Fig. 1 were conducted for various equilibrium cells and the holdup time determined. The results are shown in Table II. The diffusion coefficients were calculated by equation 4 on the assumption that the membrane thickness is 0.015 cm (thickness of the dry filter). It is clear that the mobility of each ion was markedly affected by the relative amount and the nature of the other cation present in the solution. The mobility of each ion was highest in a TEA^+ membrane and lowest in a K^+ membrane. It is also clear that the relative mobility changes much less than the absolute mobility. The qualitative nature of the interaction between the ions can be summarized as follows: mixing of two cations in the membrane makes the fast ion go faster and the slow ion go slower. Possible mechanisms for this kind of interaction are discussed below.

4. K^+-Na^+ Selectivity and Mobility Ratio as Function of Membrane Composition. A more thorough investigation of the K^+-Na^+ interaction in the membrane was carried out by studying the K^+-Na^+ selectivity factor and membrane

TABLE II

HOLDUP TIME FOR K^+-Na^+, K^+-TEA^+, AND Na^+-TEA^+ IN VARIOUS EQUILIBRIUM CELLS

In determining the holdup time and in calculating the average, a fraction of a minute was neglected. It is obvious that as the holdup time decreases the accuracy of its determination decreases too. The K^+-Na^+ equilibrium cells were sampled every 20 or 30 min, the other cells every 60 min. The diffusion coefficient was computed by the equation $D = l^2/6\tau$ where τ the holdup time and l is thickness of the membrane (0.015 cm).

Composition of solution mM		No. of experiments	Holdup time in minutes				Diffusion coefficient 10^{-8} cm²/sec	
			average-range		average-range			
K^+	Na^+		K^+		Na^+		K^+	Na^+
1.5	—	1	16	—	—	—	4.00	—
0.75	—	2	16	—	—	—	4.00	—
0.75	0.75	5	14	12–16	90	83–98	4.45	0.70
0.30	0.75	1	—	—	66	—	—	0.95
0.15	0.75	1	—	—	55	—	—	1.15
0.075	0.75	2	8	6–10	45	43–47	6.7	1.40
0.030	0.75	1	—	—	38	—	—	1.65
0.015	1.5	1	7	—	—	—	9.0	—
—	0.75	3	—	—	23	21–25	—	2.70
K^+	TEA^+		K^+		TEA^+		K^+	TEA^+
0.75	0.75	2	9	—	130	110–150	7.0	0.48
0.30	1.5	1	~2	—	—	—	~31.0	—
—	0.75	2	—	—	38	34–42	—	1.64
Na^+	TEA^+		Na^+		TEA^+		Na^+	TEA^+
3.0	0.075	1	20	—	62	—	3.1	1.0
1.5	0.075	1	14	—	51	—	4.4	1.2
1.5	0.75	1	10	—	38	—	6.2	1.64

conductance as function of membrane composition (Figs. 2 and 3). K^+-Na^+ selectivity factor is defined by

$$K_{Na}{}^{K} = \frac{X_K}{X_{Na}} \cdot \frac{a_{Na}}{a_K} \tag{7}$$

where X_i is the fraction of the ion i in the membrane, and a_i is the activity of the ion in the aqueous solution. Although there was some variability in the selectivity factor as determined it is evident from Fig. 2 that as the membrane became more in the form of the "selected" ion (K^+ ion) the selectivity factor decreased and vice versa. This is quite a common feature for many ion exchangers. Karreman and Eisenman (6) have collected evidence from literature showing that for many resins the exchange equilibrium of two ions, A and B, can be expressed by an equation of the type

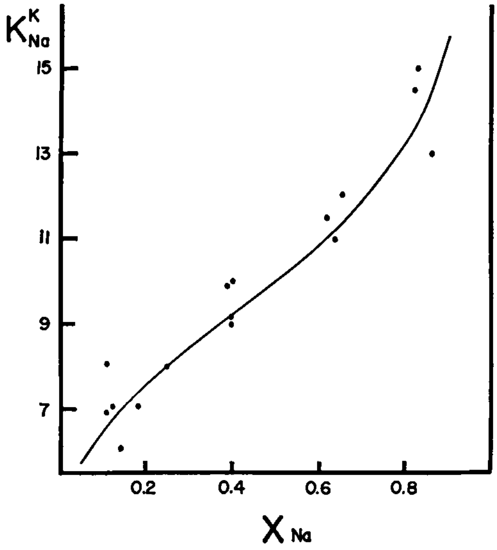

FIGURE 2 K^+-Na^+ selectivity factors for a bromobenzene-saturated membrane as function of the fraction of Na^+ in the membrane, X_{Na}. Temperature 23° to 25°C.

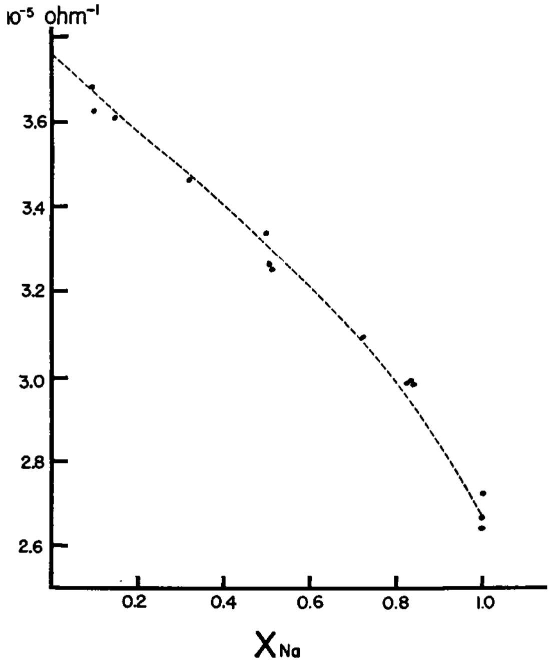

FIGURE 3 Conductance of bromobenzene-saturated membrane as function of Na^+ fraction in the membrane, X_{Na}. The only other cation present was K^+. Thus, $X_K + X_{Na} = 1$. Temperature 23° to 25°C.

$$\bar{K}_B{}^A = \left(\frac{X_A}{X_B}\right)^n \frac{a_B}{a_A} \tag{8}$$

where $\bar{K}_B{}^A$ and n are constants. The curve in Fig. 2 is a plot of $K_{\text{Na}}{}^{\text{K}}$ as a function of X_{Na} assuming the applicability of equation 8 to these membranes with $\bar{K}_{\text{Na}}{}^{\text{K}} = 10$ and $n = 1.2$. These values were chosen so as to get the best fit with the experimental points. As can be seen this equation expresses reasonably well the dependence of the K+-Na+ selectivity factor on the membrane composition.

The points in curve 3 were obtained by measuring the conductance of the membrane exposed to NaCl solution and its alteration by adding KCl into the solutions facing the membrane. The corresponding membrane composition was determined by the smooth curve in Fig. 2.

By the aid of the curve in Fig. 2 the Na+ diffusion coefficient as shown in Table II, could be plotted against membrane composition (dashed lower curve in Fig. 4). Knowing the Na diffusion coefficient and membrane conductance as functions of membrane composition (lower dashed curve in Figs. 4 and 3 respectively) it was possible to calculate the K diffusion coefficient as function of membrane composition (upper dashed curve in Fig. 4).[3]

The curves in Fig. 4 are used in the discussion for estimation of the diffusion potential in a K+-Na+ bi-ionic cell. As will be noted below the conclusions drawn from their use are not very sensitive to the uncertainty due to their approximative nature.

5. Time Course of Ion Exchange in the Membrane. Another way of studying the interaction of ions in the membrane is by following the time course of the ion exchange between water and membrane. If only two monovalent cations are involved the flux of each cation at a certain plane in the membrane can be given by an expression similar to Fick's law

$$-Ji = D_{in} S \frac{dCi}{dx} \quad \text{or} \quad \frac{-Ji}{SC} = D_{in} \frac{dXi}{dx} \tag{9}$$

where C is the total capacity of the membrane in equivalents per liter, S is the area and D_{in} is the interdiffusion coefficient given by

$$D_{\text{in}} = \frac{D_A D_B}{D_A X_A + D_B X_B}\left[1 - (X_A - X_A{}^2)\frac{d \ln K_B{}^A}{d X_A}\right] \tag{10}$$

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[3] The membrane conductance, k, is given by $k = SF/l\ (C_{\text{Na}}u_{\text{Na}} + C_{\text{K}}u_{\text{K}}) = SFC/l\ (X_{\text{Na}}u_{\text{Na}} + X_{\text{K}}u_{K})$ where C is the total membrane capacity and S is the membrane area. Using the Nernst-Einstein relationship one obtains $k = SF^2C/lRT\ (X_{\text{Na}}D_{\text{Na}} + X_{\text{K}}D_{\text{K}})$. The factor $SF^2C/l.RT$ can be determined by knowing membrane conductance and the Na$^+$ diffusion coefficient at zero K$^+$ in the membrane. Thus, $SF^2C/lRT = k^0/D_{\text{Na}}{}^0$ where k^0 and $D_{\text{Na}}{}^0$ are the membrane conductance and the diffusion coefficient of Na$^+$ respectively at $X_{\text{K}} = 0$. On rearranging the equation for the conductance of the membrane, one obtains $D_K = kD_{\text{Na}}{}^0/k^0\ (1 - X_{\text{Na}}) - X_{\text{Na}}D_{\text{Na}}/1 - X_{\text{Na}}$. This equation enables one to solve for D_{K} if k and D_{Na} are known.

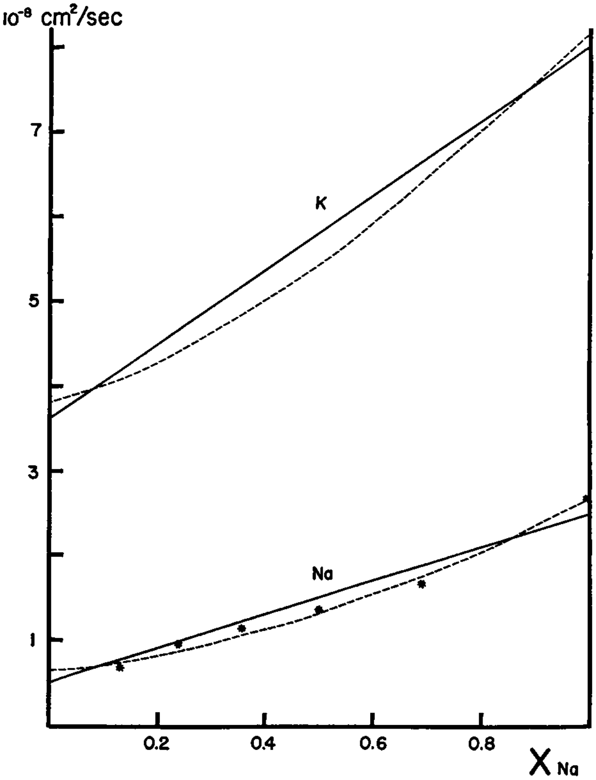

FIGURE 4 The diffusion coefficients of K$^+$ and Na$^+$ in a bromobenzene-saturated membrane as function of membrane composition, X_{Na}, where $X_{Na} + K_K = 1$. The Na$^+$ diffusion coefficient (points) are taken from Table II using the curve in Fig. 2 to calculate the corresponding X_{Na}. The dashed lower curve is a visual estimate of the function: D_{Na} (X_{Na}). The dashed upper curve is the function, D_K (X_{Na}), calculated by the aid of the lower dashed curve and by the curve in Fig. 3. The linear (solid) curves having the following formulas:

$$D_{Na} = (0.6 + 2\ X_{Na})\ 10^{-8}\ \text{cm}^2/\text{sec}$$
$$D_K = (3.6 + 4.4\ X_{Na})\ 10^{-8}\ \text{cm}^2/\text{sec}$$

are used in the discussion for estimation of the diffusion potential in a K$^+$-Na$^+$ bi-ionic cell.

where

$$K_B{}^A = \frac{X_A}{X_B} \cdot \frac{a_B}{a_A}$$

(equation 10 can be derived from equation 3.10 in reference 7 if one considers only univalent cations and remembers that

$$\frac{d \ln (f_A/f_B)}{dx} = \frac{d \ln K_B{}^A}{d X_A} \frac{d X_A}{dx}$$

where f is the activity coefficient in the membrane). If the selectivity factor, $K_B{}^A$, is constant the interdiffusion coefficient will be given by the well-known expression outside the parenthesis of equation 10 (8). Therefore, if a membrane in an A form is converted into a membrane of a B form in successive steps it is to be expected that the first step will be determined by the diffusability of ion B in a pure A membrane and the last step by the diffusability of ion A in a B membrane.

In the experiments performed here a membrane was exposed on both sides to a solution containing one type of cation, say A, and converted into the B form by adding successively small amount of B ion (more selected ion) into the solutions bathing the membrane. The exchange of A for B was accompanied by a change in membrane electrical conductance, k. After each addition of the ion B, the time course of the B-A exchange was followed by measuring the membrane conductance and sufficient time ($t = \infty$) was allowed for the system to attain a new equilibrium. It was assumed that during each step the membrane conductance is a linear function of the fraction of each ion in the membrane. (This is obviously true as the steps of change becomes increasingly small.) Therefore, $\Delta k_t/\Delta k\infty = Q_t/Q\infty$ where Q_t is the amount of ion B which entered the membrane during the time t after introducing B into the water solution. If the range of the change in membrane conductance, $K_0 - k_\infty$, for each step becomes small the interdiffusion coefficient could be considered constant for the entire step. In this case the ratio $\Delta k_t/\Delta k\infty$ as a function of time could be evaluated as follows: the differential equation $dX_i/dt = D_{\text{in}}d^2X_i/dx^2$ is solved. Introducting the appropriate boundary conditions the function $X_i(x, t)$ is obtained. The amount entering the membrane is given by $-C\cdot S\, D_{\text{in}}\, (dX_i/dx)_{x=0}\, dt$ and therefore

$$\Delta k_t/\Delta k_\infty = \int_0^t (dX_i/dx)_{x=0}\, dt \Big/ \int_0^\infty (dX_i/dx)_{x=0}\, dt.$$

A solution to this expression is given by Crank (reference 5 p. 55). In Fig. 5 the time course of the ratio $\Delta k_t/\Delta k\infty$ for a step in Li+-K+ exchange is shown and compared with the time course expected for an interdiffusion coefficient equal to 2.10^{-8}cm^2/sec and membrane thickness of 0.015 cm. (The curve in Fig. 5 was constructed from the curve designated 0% uptake in Fig. 4.6 of reference 5 remembering that the exchange in these experiments proceeds through both surfaces of the membrane and therefore the l appearing in that reference corresponds to half the thickness of these membranes.) Although the interdiffusion coefficient cannot be considered constant throughout the whole macroscopic interval it is evident that $\Delta k_t/\Delta k\infty$ as a function of time can give some idea about the magnitude of the interdiffusion coefficient at every step. In Table III the time required for attaining $\Delta k_t/\Delta k_\infty = 0.5$ for various ion exchanges is shown with the corresponding equivalent interdiffusion coefficients which can be computed with the aid of Fig. 5; i.e., $D_{\text{in}} = 2.10^{-8}\ 9/t$. (If D_{in} is 2.10^{-8}cm^2/sec it would take 9 min to accomplish half

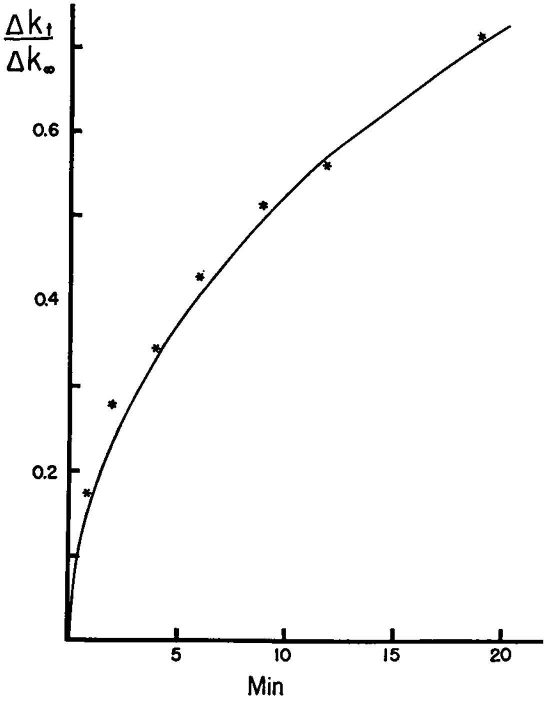

FIGURE 5 The fractional change in membrane conductance ($\Delta k_t/\Delta k_\infty$) as function of time. The change in membrane conductance was brought about by introducing K^+ into a solution containing mainly Li^+ at time zero. The solid line is a theoretical curve for an interdiffusion coefficient equal to 2.10^{-8} cm^2/sec and membrane thickness of 0.015 cm (see text).

the exchange.) It can be seen that the fourth stage in the Na^+-K^+ exchange proceeds 4 to 5 times more slowly than the first stage. From Fig. 4 it can be gathered that the infinitesimal last step in the exchange of Na^+ for K^+ should be about 12 times slower than the infinitesimal first step. It can also be seen that the interdiffusion coefficients for the K^+-Na^+ exchange as shown in Table III are within the limits predicted from Fig. 4; i.e., between 0.7 to 8.0 $10^{-8}cm^2/sec$. Table III indicates also that the exchange of Li^+ for K^+ proceeds more rapidly at the first stage of the exchange. The opposite is true for the K^+-quinine exchange. If the mobilities of these ions were considered constant the expected ratios between the interdiffusion coefficients at the begining and at the end of the quine-K^+ and Li^+-K^+ exchanges would be only around 2 and 4 respectively compared with over 7 and 100 as found experimentally. It is therefore very probable that these ions affect the mobility of each other in the same way as shown previously namely, the slow ion becomes slower as more of the rapid ion is present in the membrane and the rapid ion becomes quicker as more of the slow ion appears in the membrane.

TABLE III

TIME REQUIRED TO ATTAIN HALF THE CHANGE IN MEMBRANE CONDUCTANCE ($\Delta k_t/\Delta k_\infty = 0.5$) CAUSED BY A STEPWISE CHANGE IN COMPOSITION OF THE SOLUTION FACING THE MEMBRANE

The limits of the conductance change for each step, k_0 and k_∞, are indicated. See text for explanation of way of calculating the equivalent interdiffusion coefficient (D_{in}).

Step No.	Na^+-K^+ exchange				Li^+-K^+ exchange				K^+-Quinine exchange			
	10^{-5} ohm^{-1}		t for $\Delta k_t/\Delta k_\infty = 0.5$	"Equivalent" D_{in} 10^{-8} cm^2/sec	10^{-5} ohm^{-1}		t for $\Delta k_t/\Delta k_\infty = 0.5$	"Equivalent" D_{in} 10^{-8} cm^2/sec	10^{-5} ohm^{-1}		t for $\Delta k_t/\Delta k_\infty = 0.5$	"Equivalent" D_{in} 10^{-8} cm^2/sec
	k_0	k_∞	min		k_0	k_∞	min		k_0	k_∞	min	
1	2.61	2.89	3	6.0	1.7	2.09	5	3.6	3.75	2.77	130	0.13
2	2.89	3.25	7	2.6	2.09	2.60	9	2.0	2.77	2.21	18	1.0
3	3.25	3.47	8	2.2	2.60	2.90	16	1.1	2.21	1.80	3	6.0
4	3.47	3.61	15	1.2	2.90	3.31	27	0.73	1.80	1.54	~1.5	~12.0
5					3.31	3.55	34	0.55	1.54	1.4	~1.0	~18.0

It is possible of course that some peculiar behavior of the expression $d \ln K_B{}^A / dX_A$ may also be affecting the magnitude of the interdiffusion coefficients. If the exchange equilibrium of the two ions is expressed by equation 8 it is clear that:

$$\frac{d \ln K_B{}^A}{dX_A} = \frac{1 - n}{(X_A - X_A{}^2)} \tag{11}$$

Introducing equation 11 into equation 10, equation 10 becomes equation 12.

$$D_{\text{in}} = n \frac{D_A D_B}{D_A X_A + D_B X_B} \tag{12}$$

In this case, therefore, the relative magnitudes of the interdiffusion coefficients remains identical with those predicted for a constant selectivity factor, while the absolute magnitude is different by a factor of *n*.

6. The Composition of a Membrane in a Bi-Ionic Cell. Hellferich (7) has shown that in a bi-ionic cell with membrane limited diffusion the less mobile counterion will be accumulated by the membrane; the selectivity of the membrane being irrelevant. For the case of constant diffusion coefficients, D_A and D_B, the extent of accumulation of the slower ion can be evaluated as follows: when a steady state is reached the fluxes of *A* and *B* through each plane of the membrane are equal and constant; i.e.,

$$D_{\text{in}} \frac{dX_A}{dx} = D_{\text{in}} \frac{dX_B}{dx} = \text{constant.} \tag{13}$$

Integrating and introducing the boundary conditions it is found that

$$X_A = \frac{D_A \left(\frac{D_B}{D_A} \right)^{x/l} - D_B}{D_A - D_B} \tag{14}$$

(see also reference 7) The fraction of ion *A* in the membrane, F_A, is

$$F_A = \int_0^l \frac{X_A \, dx}{l}. \tag{15}$$

Introducing equation 14 into equation 15 it is found that

$$F_A = \frac{1}{\ln \frac{D_A}{D_B}} - \frac{1}{\frac{D_A}{D_B} - 1} \tag{16}$$

In Table IV, the conductance of a membrane in K+, Na+, or Li+ pure forms and in a K+-Li+ and Na+-Li+ bi-ionic cells are shown. If the mobilities of these ions would be constant the relative mobilities of K+/Li+ and Na+/Li+ would be around 2.3 and 1.6 respectively. For such relative mobilities the fraction of Li+ in the membrane would have been according to equation 16, 0.57 and 0.53 respectively. However, if the mobilities of the ions were constant the conductance of the

TABLE IV

CONDUCTANCE OF MEMBRANE EXPOSED TO 0.015 M $LiCl_2$ (k_{Li}), THE CONDUCTANCE AFTER REPLACING THE SOLUTION ON ONE SIDE WITH 0.015 M NaCl OR KCl ($k_{Li\text{-}Na}$ AND $k_{Li\text{-}K}$ RESPECTIVELY), AND THE CONDUCTANCE AFTER REPLACING THE OTHER SIDE TOO WITH 0.015 M NaCl OR KCl (k_{Na} AND k_K RESPECTIVELY)

Membrane conductance 10^{-5} ohm^{-1}					
k_{Li}	$k_{Li\text{-}Na}$	k_{Na}	k_{Li}	$k_{Li\text{-}K}$	k_K
1.65	2.2	3.75	1.71	2.10	2.78
			1.65	2.00	2.50

membranes in the bi-ionic cells would indicate that the fraction of the Li^+ in the membrane is more than 0.7 and 0.6 in the Li^+-K^+ and Li^+-Na^+ cells respectively. Such high fractions of Li^+ correspond according to equation 16 to relative mobilities of about 15 and 5 respectively. This fact implies Li^+-K^+ and Li^+-Na^+ affect the mobilities of each other in much the same fashion as indicated in the preceding sections.

DISCUSSION

Correlation between the K^+-Na^+ Bi-Ionic Potential and the other Discriminative Properties of the Membrane. In a previous communication (2) a semiquantitative correlation between the bi-ionic potential, $V_B{}^A$, and selectivity and relative mobility of the ions was based upon the equation $V_B{}^A = RT/F \ln K_B{}^A u_A/u_B$. This equation is implicit, with slight variation in the work of many authors (6, 9, 10). In the derivation of this equation the assumption was made that the mobilities of the ions are constant. Since this assumption is clearly incorrect for these membranes, the previous correlation is invalid. In the following, equations based upon more realistic assumptions for these membranes are derived. Using these equations and the data about K^+-Na^+ selectivity and mobilities as presented in Figs. 2 and 4, the bi-ionic potential will be computed and compared with the measured value which is 92± 3 mv (2).

It is assumed that the bi-ionic potential is the sum of the difference in the equilibrium interfacial (or Donnan) potentials, $\Delta\Psi_e$, and the diffusion potential, $\Delta\psi_d$. (6, 7, 11).

The difference in interfacial potential. The interfacial equilibrium potential is given for each interface by the equation 17.

$$\psi_e = \frac{-RT}{F} \ln \frac{C_i f_i}{a_i} \tag{17}$$

where f_i is the activity coefficient for the ion i in the membrane. Since in a bi-ionic cell of the type used in these experiments $C_A = C_B$ and $\alpha_A = \alpha_B$ it follows that the difference between the two interfacial potentials is

$$\Delta\psi_e = \frac{-RT}{F} \ln \frac{f_B(X_B = 1)}{f_A(X_A = 1)}. \tag{18}$$

If the selectivity factor is constant it is clear (considering also Gibbs-Duhem relation) that $f_B/f_A = K_B{}^A$ and therefore $\Delta\psi_e = -RT/F \ln K_B{}^A$. Eisenman (12) has shown that where the exchange equilibrium is given by equation 8 the difference between the interfacial potentials is $\Delta\psi_e = -RT/F \ln \bar{K}_B{}^A$. Since in these membranes the $\bar{K}_{\text{Na}}{}^{\text{K}}$ is about 10 (sec Fig. 2) it follows that at 25°C the difference between the interfacial potentials in a K-Na bi-ionic cell is about 58 mv with the K side negative.

The diffusion potential. The following assumptions are made in order to arrive at equation 19 (see Appendix): (*a*) Only two monovalent cations, *A* and *B*, are present; i.e., $X_A + X_B = 1$. (*b*) Coions are excluded completely. (*c*) Each cation is acted upon only by its own electrochemical gradient. (*d*) There is no electroosmotic flow in the membrane.

$$\frac{-F}{RT} \Delta\psi_d = \int_{X_A \text{ side I}}^{X_A \text{ side II}} \frac{1 - (X_A - X_A{}^2)(d \ln K_B{}^A/dX_A)}{X_A + u_B/(u_A - u_B)} \, dX_A \tag{19}$$

In the following integrations of equation 19 it will be assumed that $K_B{}^A$ is constant; i.e., $d \ln K_B{}^A/d X_A = 0$. If the exchange equilibrium of the ions is expressed by equation 8, $d \ln K_B{}^A/dX_A$ is given by equation 11. Introducing equation 11 into equation 19, equation 19 becomes:

$$\frac{-F}{RT} \Delta\psi_d = n \int_{X_{A}\text{I}}^{X_{A}\text{II}} \frac{1}{X_A + u_B/(u_A - u_B)} \, dX_A \tag{20}$$

Thus any solution of equation 19 for the case $d \ln K_B{}^A/dX_A = 0$ has to be multiplied by the factor *n* if equation 8 is applicable.

If u_A/u_B is constant the solution of equation 19 yields the well-known expression for the diffusion potential in bi-ionic cells (with zero mobility of anions).

$$\frac{-F}{RT} \Delta\psi_d = \ln \frac{u_A}{u_B} \tag{21}$$

Equation 21 multiplied by *n* is identical with the equation derived by Karreman and Eisenman (6).

In order to arrive at conditions which approach more closely those prevailing in these membranes it is assumed that the mobility of each ion is a linear function of the fraction of the ion in the membrane. Thus,

$$\begin{aligned} u_A &= b_1 + a_1 X_A \\ u_B &= b_2 + a_2 X_A \end{aligned} \tag{22}$$

where a_1, a_2, b_1, and b_2 are constants. Introducting equation 22 into equation 19 equation 19 becomes equation 23.

$$\frac{-F}{RT} \Delta\psi_d = \int_{X_{A\text{I}}}^{X_{A\text{II}}} \frac{s + rX_A}{rX_A^{\,2} + mX_A + b_2} \, dX_A \tag{23}$$

with the following substitutions: $r = a_1 - a_2$, $s = b_1 - b_2$, and $m = b_1 - b_2 + a_2$ the general solution of equation 23 for bi-ionic cells is

$$\frac{-F}{RT} \Delta\psi_d = \frac{1}{2} \ln \frac{r + m + b_2}{b_2} + \frac{s - m/2}{g} \ln \frac{(2r + m - g)(m + g)}{(2r + m + g)(m - g)} \tag{24}$$

where $g = (m^2 - 4\,rb_2)^{1/2}$. In Fig. 4 the diffusion coefficients of Na^+ and K^+ as function of the Na^+ fraction in the membrane were approximated by linear curves. Using equation 24 the diffusion potential is found to be around 32 mv. Multiplying by the factor $n = 1.2$ to account for the fact that K_K^{Na} is not constant (see previous paragraph), the expected diffusion potential in the K^+-Na^+ bi-ionic cell is around 36 mv. The total K^+-Na^+ bi-ionic cell potential is therefore around $58 + 36 = 94$ mv which is in good agreement with the measured value of 92 ± 3 mv.

An estimate of the limits of the K^+-Na^+ diffusion potential was made by drawing two extreme linear curves about the dashed lines in Fig. 4. The diffusion potential was calculated taking the highest curve for D_K and the lowest one for D_{Na} and vice versa; the diffusion potential was then found to be (for $n = 1, 2$) 40 mv and 32 mv respectively. It is obvious therefore that in spite of the approximative nature of the curves in Fig. 4 the K-Na diffusion potential is not likely to differ by more than ±5 mv from the calculated value.

It is worthwhile to note that in the evaluation of the diffusion potential, i.e. the solution of equation 19, it is only necessary to specify the boundary conditions; the profile of counterions in the membrane is immaterial. This is true if the mobilities of the ions and the selectivity factor depend on x only through the dependence of X_A on x, and hence it would be the case for any membrane that is homogeneous with respect to the x axis. Therefore, any shift in potential subsequent to a change in the composition of the solution facing the membrane should attain a constant value as long as equilibrium or steady-state conditions prevail at the interfaces, even before a new concentration gradient is established within the membrane.[4] This is true for the diffusion potential of any liquid junction in which the boundary is of the "mixture" type. [Henderson integration of the diffusion potential (14).] This conclusion is borne out by experiments on these membranes. Thus, the potential of a K^+-Na^+ bi-ionic cells was changed promptly by adding K^+ to the Na^+ side and the potential attained a constant value within 1 to 2 min (2) although experiments

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[4] In a recent paper Conti and Eisenman (13) have shown that if the mobility ratio is constant the diffusion potential would be independent of the concentration profile even if the ion mobility depends on x.

reported here would indicate that it would take much longer for the Na+ in the membrane to be replaced by K+. This is particularly impressive for the toluene saturated Millipore filters where the Na+ holdup time is in the order of a day (15), and yet the membrane potential responds in stepwise manner to a change in the composition of the surrounding solutions (1).

Mechanism of Interaction between Ions in the Membrane. The interaction between ions in these membranes is interesting not only because of its magnitude but also because of its qualitative nature. In water solution the bringing together of rapid and slow ions makes the rapid ions slower and the slow ions faster due to the relaxation effect. This type of interaction is also found in ordinary ion exchangers (16, 17) where the ions move presumably in water-filled capillaries. It is obvious that the interaction between the ions in these hydrophobic membranes is just of the opposite nature. Two possible mechanisms will be considered in explanation of this phenomenon.

(*a*) It is assumed according to the first hypothesis that the mobilities of the ions is affected by the degree of water swelling or water content of the membrane (3). Thus a Na+ membrane swells more than a K+ membrane (18) making it easier for any cation to move in a Na+ membrane. According to this assumption it would be expected that ion mobilities would be related to membrane forms as follows: Li>Na>K, as actually found. The position of the substituted ammonium ions is, however, not in the appropriate place. Although TEA will swell ordinary ion exchangers more than Na+ (18) it seems that for these hydrophobic membranes the TEA and quinine forms contain less water than the Na+ one (19) and yet they form membranes which are more favorable for the movement of ions.

(*b*) The other explanation is connected with a possible mechanism of ion movements in these membranes. According to this hypothesis ion movement in such membranes consist of "jumps" of the counterions from their position near fixed charged sites to adjacent vacant sites. Therefore, the diffusability of the ion will be determined both by the activation energy of the "jump" and by the probability that an adjacent site is vacant; i.e., by the relative concentration of vacant sites. It is further assumed that vacancies are formed by movement of counterions from their position near fixed sites to an interstitial position. This reaction can be symbolized as follows $A_s \leftrightarrows A_i + v$ where A is a counterion, subscripts s and i refer to its position near a fixed site and interstitially respectively, and v denotes vacancy. The number of vacancies will depend upon the free energy change of the above reaction. This type of diffusion occurs in crystals and this type of vacancy formation is known in solids as Frenkel's disorder (20). A similar mechanism has been suggested for the movements of ions is glasses (21).

Following this hypothesis the change of the mobility with change in membrane composition might be due to the change in number of vacancies in the membrane; the ions being more mobile in a membrane which contains more vacancies. This would imply that ions which form the more favorable environment for diffusion are those which produce more vacancies. It is necessary, therefore, to examine the plausability of the implication that the free energy of vacancy formation for the ions tested in increasing in the order: quinine $\simeq$, $TEA^+ > Li^+ > Na^+ > K^+$. A possible way of approaching this problem is by considering the relative distribution coefficients of the ions between interstitial medium and water as against the selectivity of the fixed sites; the higher the distribution coefficient and the lower the selectivity the lower the free energy of vacancy formation to be anticipated. Since the interstitial medium in these membranes is of hydrophobic nature it is reasonable to assume that the free energy of vacancy formation is lower for quinine or TEA^+ in comparison with the alkali metal ions. This would account therefore for the higher mobility of the ions in the TEA^+ or quinine membranes. As far as the relation between the alkali metal ions themselves is concerned it should be emphasized that the relative solubility of these ions is hydrophobic solvents depends greatly upon the nature of the solvent. Thus, aliphatic alcohols will dissolve $Li^+ > Na^+ > K^+$ (22) while the reverse is true for nitrobenzene (23). In these membranes the interstitial medium is presumably bromobenzene and the cellulose ester skeleton and it is impossible to infer from available solubility data what would be the order of solubility of alkali metal ions in such a medium. It should be noted, however, that even if the distribution coefficient of K^+ for this particular hydrophobic medium is 5 times higher than that of Na^+ it is still the Na^+ which will form more vacancies, since the anionic sites prefer K^+ over Na^+ some 10 times. In conclusion it would seem plausible that the higher mobility of ions in the Na^+ as against K^+ membrane is due to the presence of more vacancies in the Na^+ membrane.

However, if the only way in which the mobility of the ions is affected were by the variation in concentration of vacancies, it would be expected that the mobility ratio would remain constant which is not the case for the K^+-Na^+ mobility ratio. It is clear from Fig. 4 that the K^+-Na^+ mobility ratio varies from around 6 in a K^+ membrane to around 3 in a Na^+ membrane. It is likely therefore that both of the above mentioned mechanisms act together to produce the kind of interaction observed.

K^+-Na^+ Discrimination. From the biological point of view the most interesting feature of these hydrophobic membranes is their ability to discriminate sharply between K^+ and Na^+, a fact already emphasized in previous publications (1, 2). The contribution of this study is the demonstration that a significant part of this discrimination is due to difference between the mobilities of the two cations. Unlike the previous estimated mobility ration of 1.3 (2) these studies show that the K^+/Na^+ mobility ratio can attain a value as high as 6. The high mobility ratio together with the high selectivity of the membrane for K^+ lead to the sharp K^+-Na^+ discrimination as expressed by the permeability ratio (see Fig. 1) and by the magnitude of the bi-ionic potential. In this respect, an ion being both more

selected and more mobile in these membranes, they differ from ordinary ion exchangers. Thus, ion exchangers containing dipicrylaminelike groups as fixed negative charges are highly selective for K^+ but the K^+ in these membranes is relatively less mobile (see pp. 42 and 304 in reference 8). Wyllie and Kannaan (24) have collected values for the K^+-Na^+ selectivities and mobilities in various ion exchangers. With selectivity factors, $K_{Na}{}^{K}$, reaching up to 2.1 the mobility ratio as estimated by measuring the resistance of membranes in the pure forms varied from 1.2 to 1.6. The actual mobility ratio as estimated from the bi-ionic potential is even lower (24). This mobility ratio, of the same order of magnitude as found in water solution, may be attributed to the difference in friction with the medium due to difference in the size of the hydrated ions. It is very unlikely that the mobility ratio observed in these membranes is related to difference in friction of the ions with a continuous medium. For this to be the case the Na^+ radius would have to be up to 6 times as large as that of K^+; a relationship which is untenable. In view of the idea that diffusion in these membranes consists of jumps of ions from one site to another, the lower mobility of Na^+ would be related to its stronger binding to the fixed sites. Thus, the energy of activation required to take the Na^+ out of its energy well near a fixed site would be higher than that for K^+. The high selectivity for K^+ might be attributed according to Eisenman's model (12) to the differential free energy change of hydration which accompanies the movement of ions from water into the membrane.

It would seem, therefore, that the hydrophobic medium surrounding the fixed charged sites allows only dehydrated or partially dehydrated ions to enter the membrane. By virtue of this restriction K^+ is selected over Na^+. However, the Na^+ which entered the membrane is bound more tightly to the fixed site and therefore its mobility is lower. It is tempting to speculate that this is the essence of the K^+-Na^+ discrimination elicited by cellular membranes.

An important reservation applying to inferences from work on artificial membranes to biological ones is connected with the difference in thickness typical of the two kinds of membranes. If the membrane is thick enough it is permissible conceptually to divide the membrane into many thin planes and to consider the mobility of an ion at each plane as independent of the direction of the force acting on the ion. This assumption is implicit in the derivation of equation 19. However, in very thin membranes, say of the order of 5 to 10 diameters of an ion, it is necessary to know also the mechanism of interaction between the ions in order to be able to predict the diffusion potential across such a membrane. In order to illustrate this point, consider membranes having a similar kind of K^+-Na^+ interaction as shown in this study and assume that the mechanism of interaction is related to difference in the free energy of vacancy formation between the two ions (see previous discussion). Thus, assume, that the probability that K^+ will move out of its position near a fixed site is 5 times higher than that for Na^+ and therefore the

K^+/Na^+ mobility ratio at any particular membrane composition is 5. However, in a Na^+ membrane the absolute magnitude of the mobilities is 4 times higher than that in a K^+ membrane since there are 4 times as many vacancies in a Na^+ membrane. Now suppose that an ultra thin membrane (say only two rows of fixed sites present) of that type separates a Na^+ from a K^+ solution. If diffusion through the membrane is still the limiting process, one row of the fixed sites will be occupied by K^+ and the other by Na^+. It is obvious that under these conditions the probability of K^+ moving into the Na^+ side is 20 times higher than the probability of Na^+ moving into the K^+ side. Since the condition of electroneutrally requires that K^+ flux be equal to Na^+ flux, a potential difference will develop to enhance Na^+ and inhibit K^+ movements. The diffusion potential in such a thin membrane would correspond, therefore, to an apparent mobility ratio of 20 as against 5 for the thick membrane.

Thus, it is possible that discrimination by a very thin membrane would be very different from that of the thick membrane if judged by the magnitude of the bi-ionic potential.

APPENDIX

DERIVATION OF EQUATION 19

Assumptions

(*a*) Only two monovalents cations A and B are present on two sides of membrane I and II. (*b*) The rate-limiting process is diffusion through the membrane; i.e., equilibrium conditions prevail at the interfaces. (*c*) Complete exclusion of co-ions by membrane. (*d*) Each ion is acted upon only by its electrochemical gradient. (*e*) Electroosmotic flow negligible. (*f*) Macroscopic electroneutrality so that at each plane of the membrane $J_A + J_B = 0$. (*g*) Membrane homogenous with respect to x axis, and no electrochemical gradient in the y or z coordinates.

By assumption (*d*)

$$\frac{-J_A F}{S} = u_A C_A \frac{d\mu_A}{dx} + u_A C_A \frac{F d\psi}{dx} \tag{1}$$

u_i is mobility, C_i is concentration of ion, μ_i, chemical potential, ψ the electric potential, F the Faraday, S the area, and J_i the ion flux. Neglecting effect of pressure we have:

$$d\mu_i = RT\, d \ln f_i C_i \tag{2'}$$

where f_i is the activity coefficient in the membrane. R is the gas constant and T the absolute temperature.

By assumptions (*c*) and (*f*)

$$C_A = X_A C \qquad C_B = X_B C \tag{3'}$$

where C is the concentration of fixed sites which is independent of x and X_i is the fraction of fixed sites occupied by the ion i.

Introducing equations 3′ and 2′ into equation 1′

$$-\frac{J_A F}{SC} = RTu_A X_A \frac{d \ln X_A}{dx} + RTu_A X_A \frac{d \ln f_A}{dx} + u_A X_A F \frac{d\psi}{dx} \tag{4′}$$

By assumption (f)

$$0 = RTu_A \frac{dX_A}{dx} + RTu_B \frac{dX_B}{dx} + RTu_A X_A \frac{d \ln f_A}{dx} + RTu_B X_B \frac{d \ln f_B}{dx} \tag{5′}$$

$+ (u_A X_A + u_B X_B) F \, d\psi/dx$ or remembering that $dX_A = -dX_B$

$$0 = RT(u_A - u_B) \frac{dX_A}{dx} + RT\left(u_A X_A \frac{d \ln f_A}{dx} + u_B X_B \frac{d \ln f_B}{dx}\right) + (u_A X_A + u_B X_B) F \frac{d\psi}{dx} \tag{6′}$$

We shall derive now expressions for $d \ln f_A$ and $d \ln f_B$ using the Gibbs-Duhem relation and the selectivity factor of the membrane. By definition

$$K_B{}^A = \frac{X_A}{1 - X_A} \frac{a_B}{a_A} \tag{7′}$$

where a is the activity in water solution. We expect that there is some thermodynamic equilibrium constant for an exchange reaction defined by

$$K_T = \frac{X_A f_A}{(1 - X_A) f_B} \frac{a_B}{a_A} \tag{8′}$$

from equations 7′ and 8′ we obtain

$$K_B{}^A = K_T \frac{f_B}{f_A} \quad \text{or} \quad \ln K_B{}^A = \ln K_T + \ln \frac{f_B}{f_A} \tag{9′}$$

since K_T is constant we have

$$d \ln K_B{}^A = d \ln f_B - d \ln f_A \tag{10′}$$

from Gibbs-Duhem relation we have

$$\left(\frac{d\mu_A}{d\mu_B}\right)_{T,p} = -\frac{1 - X_A}{X_A} \tag{11′}$$

or using equaution 2′

$$\frac{d \ln C_A + d \ln f_A}{d \ln C_B + d \ln f_B} = -\frac{1 - X_A}{X_A} \tag{12′}$$

since

$$\frac{d \ln C_A}{d \ln C_B} = \frac{d \ln X_A}{d \ln X_B} = \frac{dX_A X_B}{dX_B X_A} = -\frac{X_B}{X_A} = -\frac{1 - X_A}{X_A}$$

in order for the equality in equation 12′ to hold it is necessary either that $d \ln f_A = d \ln f_B = 0$ (in which case the middle term of equation 6′ drops) or

$$\frac{d \ln f_A}{d \ln f_B} = -\frac{1 - X_A}{X_A} \tag{13'}$$

or

$$d \ln f_A = -\frac{1 - X_A}{X_A} d \ln f_B \tag{14'}$$

introducing equation 10′ into equation 14′

$$d \ln f_A = -(1 - X_A) d \ln K_B{}^A \tag{15'}$$

$$d \ln f_B = X_A d \ln K_B{}^A \tag{16'}$$

Introducing equations 15′ and 16′ into equation 6′

$$\begin{aligned} \frac{-F}{RT}\frac{d\psi}{dx}(u_A X_A + u_B X_B) &= (u_A - u_B)\frac{dX_A}{dx} \\ &\quad - u_A X_A(1 - X_A)\frac{d \ln K_B{}^A}{dx} + u_B(1 - X_A)X_A \frac{d \ln K_B{}^A}{dx} \end{aligned} \tag{17'}$$

$$\frac{-F}{RT} d\psi = \frac{(u_A - u_B) - (u_A - u_B)(X_A - X_A{}^2)\cdot(d \ln K_B{}^A/dX_A)}{X_A(u_A - u_B) + u_B} dX_A \tag{18'}$$

Thus,

$$-\int_{\psi I}^{\psi II} \frac{F}{RT} d\psi = -\frac{F}{RT}\Delta\psi = \int_{X_A I}^{X_A II} \frac{1 - (X_A - X_A{}^2)(d \ln K_B{}^A/dX_A)}{X_A + u_B/(u_A - u_B)} dX_A \tag{19'}$$

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REFERENCES

1. Ilani, A., *J. Gen. Physiol.*, 1963, **46**, 839.
2. Ilani, A., *Biochim. et Biophysica Acta*, 1965, **94**, 405.
3. Ilani, A., *Biochim. et Biophysica Acta*, 1965, **94**, 415.
4. Mackay, D., and Mears, P., *Kolloid Z.*, 1959, **167**, 31.
5. Crank, J., The Mathematics of Diffusion, Oxford, Claredon Press, 1956, 48.
6. Karreman, G., and Eisenman, G., *Bull. Math. Biophysics*, 1962, **24**, 413.
7. Hellferich, F., *Discussions Faraday Soc.*, 1956, **21**, 83.
8. Hellferich, F., Ion exchange, New York, McGraw-Hill Book Company, Inc., 1962, 269.
9. Sollner, K., *J. Physics and Colloid Chem.*, 1949, **53**, 1211.
10. Wyllie, M. R. J., *J. Phys. Chem.*, 1954, **58**, 67.
11. Teorell, T., *Progr. Biophysics and Biophysic. Chem.*, 1953, **3**, 305.
12. Eisenman, G., *Biophysic. J.*, 1962, **2**, No. 2, pt. 2, 259.
13. Conti, F., and Eisenman, G., *Biophysic. J.*, 1965, **5**, 274.
14. MacInnes, D. A., The Principles of Electrochemistry, New York, Reinhold Publishing Corp., 1939, 231.

15. Ilani, A., unpublished.
16. Mackay, D., and Mears, P., *Tr. Faraday Soc.,* 1959, **55,** 1221.
17. Soldano, B. A., and Boyd, G. E., *J. Am. Chem. Soc.,* 1953, **75,** 6107.
18. Gregor, H. P., *Ann. New York Acad. Sc.,* 1953, **57,** 87.
19. Ilani, A., in preparation.
20. Barrer, R. M., Diffusion in Solids, London, MacMillan & Co., 1941, 249.
21. Charles, R. J., *J. Appl. Physics,* 1961, **32,** 1115.
22. Stephen, H., and Stephen, T., Solubilities of Inorganic and Organic Compounds, Vol. I, Part I, London, Pergamon Press, Ltd., 642, 1963.
23. Davies, J. T., *J. Physics and Colloid Chem.,* 1950, **54,** 185.
24. Wyllie, M. R. J., and Kanaan, S. L., *J. Phys. Chem.,* 1954, **58,** 67.