J. Membrane Biol. 3, 223–240 (1970)

Discrimination between Monovalent and Divalent Cations by Hydrophobic Solvent-Saturated Membranes Containing Fixed Negative Charges

A. ILANI

Department of Physiology, Hebrew University-Hadassah Medical School, Jerusalem, Israel

Received 9 April 1970

Summary. Cellulose acetate-nitrate filters were saturated with hydrophobic solvent and interposed between various aqueous solutions. The membranes thus formed are cation permselective. The discrimination between a monovalent cation such as K^+ and the alkaline earth group divalent cations is very sharp. The discrimination ratio is at least a few thousand times in favor of the monovalent cation. A major part of this discrimination is caused by the very low mobility of the divalent cation within the membrane compared with that of the monovalent cation. The remainder of the discrimination is caused by the selectivity of the membranes which prefer monovalent to divalent cations. There is a clear discrepancy between Ba^{++} diffusibility and mobility within the membrane. This implies that Ba^{++} may move within the hydrophobic membrane as a neutral complex. Some similarity with natural biological membranes is indicated.

Porous discs made of cellulose acetate and nitrate, when saturated with a hydrophobic solvent such as toluene or bromobenzene and interposed between two aqueous solutions, form membranes which are cation permselective and show quite interesting discrimination between monovalent cations [8–10]. It is assumed that the cation permselectivity arises because of the presence of a small amount of fixed negative charges in the skeleton of the cellulose ester filter [8, 11]. In contrast to conventional ion exchangers, the fixed-charge sites in these membranes are somehow embedded in hydrophobic solvents. The solvents are retained in the membrane even after prolonged exposure to aqueous solutions. The exact relation between the filter substance, the solvents and the water within the membrane is not clear. Yet, it is argued that the solvents are essential for determining the sharp discrimination between cations by the membranes and for the degree of ion diffusibility within the membranes [8, 9].

The present study deals with some electrochemical properties of these hydrophobic membranes when they are exposed to solutions containing divalent ions of the alkaline earth group. It appears that the hydrophobic membranes are extremely discriminative between monovalent and divalent cations; they are almost impermeable to the divalent ions. Some analogy to ion exchangers with a high degree of cross-linking is emphasized. The implication is that these hydrophobic membranes impose an extreme restriction on the hydration shell of an ion during its passage from aqueous solution into the membrane phase.

Materials and Methods

Filters made of cellulose acetate and nitrate were obtained from Millipore Filter Corporation (Bedford, Mass., GS type). The filters were inserted between the halves of a glass diffusion cell. The two parts of the diffusion cell were held together by springs attached to hooks on the outer surfaces. The filters were wetted by bromobenzene, and the halves of the diffusion cell were then filled with the appropriate aqueous solutions. The whole cell was placed in a bromobenzene bath. This latter procedure ensured complete saturation of the membrane and aqueous solution with the solvent and prevented leakage from the diffusion cell. Mixing of the aqueous solutions was effected by glass-sealed iron bars driven by a magnetic stirrer.

The membrane separating the two water solutions was 3 cm^2 in area and 0.015 cm thick.

The a-c resistance was measured by a Wheatstone bridge using Ag–AgCl electrodes. Bridge excitation was generally at 250 cycle/sec. To measure the d-c resistance, two pairs of Ag–AgCl electrodes were used: one pair served for passing the current and the other for measuring the potential difference across the membrane. The setup is shown in Fig. 1. The potential difference across membranes was measured by a Keithley electrometer, type 601, using Ag–AgCl electrodes for contact with the aqueous solutions.

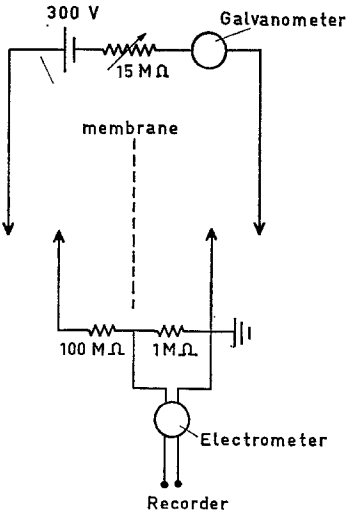

Fig. 1. Setup for measuring current-voltage relationship

Isotopes $^{45}CaCl_2$, $^{131}BaCl_2$ and "carrier-free" ^{131}Cs were obtained from the Radiochemical Centre (Amersham, England). Radioactivity was determined by a liquid scintillation counter (for ^{45}Ca) or a well-type scintillation counter (for ^{131}Cs and ^{131}Ba). In the latter case, it was possible to measure ^{131}Cs and ^{131}Ba simultaneously by using suitable "windows", since the radiation energy of ^{131}Cs is quite homogeneous and distinct from that of ^{131}Ba.

The amount of radioactivity within the membrane was determined in the following way: the membrane was released from the diffusion cell, washed slightly by being dipped into deionized water (specific resistance more than $4 \cdot 10^6$ Ω cm), blotted with absorbent paper, and immersed for 48 hr in a measured volume of salt solution. A sample of the latter solution was analyzed for radioactivity.

Results

Sensitivity of Membrane Potential to K^+ Concentration in the Presence of Divalent Cations

Fig. 2 illustrates the potential difference across a membrane separating a 15 mM solution of $CaCl_2$ or $BaCl_2$ from a similar solution containing, in addition, various concentrations of KCl. It is obvious that the membrane

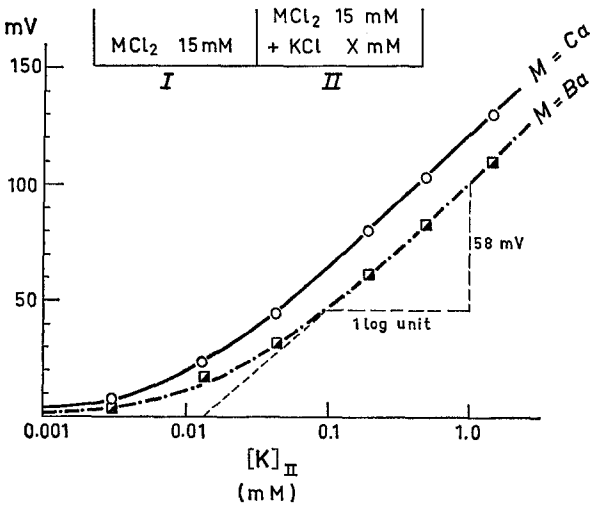

Fig. 2. The potential difference across a cellulose ester membrane saturated with bromobenzene and interposed between 15 mM $CaCl_2$ or $BaCl_2$ solutions as function of log K^+ concentration on one side of the membrane. The curve for $SrCl_2$ solution is similar to that of the Ba curve. The curve for $MgCl_2$ falls to the left of the Ca curve.—The continuous curves are theoretical (*see* equation) and consistent with either one of the following values for the permeability ratios or initial K content: for M=Ca, P_K/P_{Cl}= 3,480, P_K/P_{Ca}=6,960, and C_K=0.0086 mM; for M=Ba, P_K/P_{Cl}=1,700, P_K/P_{Ba}= 3,400, and C_K=0.018 mM

is sensitive to a concentration of KCl which is at least 500 times less than the molar concentration of the divalent cation.

In order to estimate the extent of discrimination between the mono- and divalent ions as expressed by this experiment, we use the following constant-field equation [4, 6] which relates the potential difference across a membrane to the concentration of ions across it:

$$V=\frac{RT}{F}\ln\frac{P_K(\mathrm{K})^{\mathrm{I}}+P_{\mathrm{Cl}}(\mathrm{Cl})^{\mathrm{II}}+4mP_{\mathrm{Ca}}(\mathrm{Ca})^{\mathrm{I}}}{P_K(K)^{\mathrm{II}}+P_{\mathrm{Cl}}(\mathrm{Cl})^{\mathrm{I}}+4mP_{\mathrm{Ca}}(\mathrm{Ca})^{\mathrm{II}}\exp(-FV/RT)} \tag{1}$$

where $m=\frac{1-\exp(-FV/RT)}{1-\exp(-2FV/RT)}$ in which V is the potential difference ($V^{\mathrm{I}}-V^{\mathrm{II}}$); the quantities in parentheses denote activities (assumed to equal the concentrations in our analysis) of the various ions on either side of the membrane; and P_i denotes the membrane permeability to the ion i defined as $P_i=\frac{B_iU_i}{d}$ where B_i is the distribution coefficient of the ion between the membrane and water, U_i is the mobility of the ion within the membrane and d is membrane thickness. Eq. (1) is derived similarly to that of Hodgkin and Katz [6] but is slightly more complicated owing to the introduction of a divalent cation into the system.

The experimental results shown in Fig. 2 fit a curve predicted by Eq. (1) if the membrane were 3,400 and 6,900 times more permeable to K^+ than to Ba^{++} and Ca^{++}, respectively. This is, however, the lower limit of the permeability ratio because (1) although the slope shown in Fig. 2 is indicative of cation permselectivity, it implies only that the permeability to Cl^- is negligible compared to that of K^+ but not necessarily compared to that of the divalent cation, and (2) it was tacitly assumed that the divalent ion solution does not contain any K^+ or other monovalent ion for which the membrane permeability is much higher than for the divalent cation. However, extrapolation of the curves shown in Fig. 2 to zero potential difference cuts the abcissa at K concentrations which are approximately 0.1% of the divalent ion concentration. This value approaches a level of contamination in analytical products (e.g., 0.01% K^+ in $MgCl_2$ or $SrCl_2$ according to manufacturer's catalogue). It is obvious that both a non-zero permeability to Cl^- and non-zero contamination by penetrable cations would imply even higher K-divalent ion permeability ratios. To be more precise, the curve for Ca shown in Fig. 2 is consistent with zero permeability to Ca either if the 15 mM $CaCl_2$ solution also contained 0.0086 mM K^+ or if P_K/P_{Cl} were 3,480 (or if both happen together and K contamination were less than 0.0086 and P_K/P_{Cl} were greater than 3,480).

Dependence of Resistance of Membrane on Nature of Ions in Solution

Table 1 shows the a-c electrical resistance of membranes exposed to various solutions. It is clear that the membrane resistance increases in the order Mg, Ca, Sr, Ba. Also, it is apparent that the presence of a monovalent ion leads to a decrease in membrane resistance. The relative K-membrane divalent-ion membrane resistance would appear to vary only between 1.5 (for Mg) and 10.0 (for Ba). However, the membrane resistance in the case of the divalent ion is more complicated than the measurement in an a-c. Wheatstone bridge would imply. Thus, there exists a time-dependent current-voltage relationship if the membrane is exposed to solutions containing divalent ions only (*see* Fig. 3). The voltage across a membrane in response to a constant current increases with time. Interruption of the current leads to an immediate drop of almost the whole voltage across the membrane, indicating that the major part of the increase in voltage response is caused by an increase in membrane resistance and that only a minute fraction is caused by membrane polarization.

The time-dependent increase in resistance becomes clearer as the current increases. It is abolished by addition of monovalent ions (e.g., K^+) and by stirring of the side which contains the anode (Fig. 3). Reversal of the current

Table 1. *The a-c resistance of bromobenzene-saturated membranes exposed to various solutions*

Solution and concn. (mM)	a-c Resistance (kΩ) average ±SD	No. of membranes measured
KCl 15	52.8± 4.0	6
KBr 15	54.0± 3.1	4
NaCl 15	76.0± 3.0	3
$BaCl_2$ 3	375.0± 75.0	8
$BaCl_2$ 15	517.0±123.0	14
$BaCl_2$ 15 + NaCl 1.5	245.0± 48.0	5
$SrCl_2$ 15	269.0± 47.0	9
$CaCl_2$ 3	179.0± 21.0	10
$CaCl_2$ 15	221.0± 36.0	16
$CaCl_2$ 15 + NaCl 1.5	99.0± 14.0	6
$MgCl_2$ 15	106.0± 14.0	7

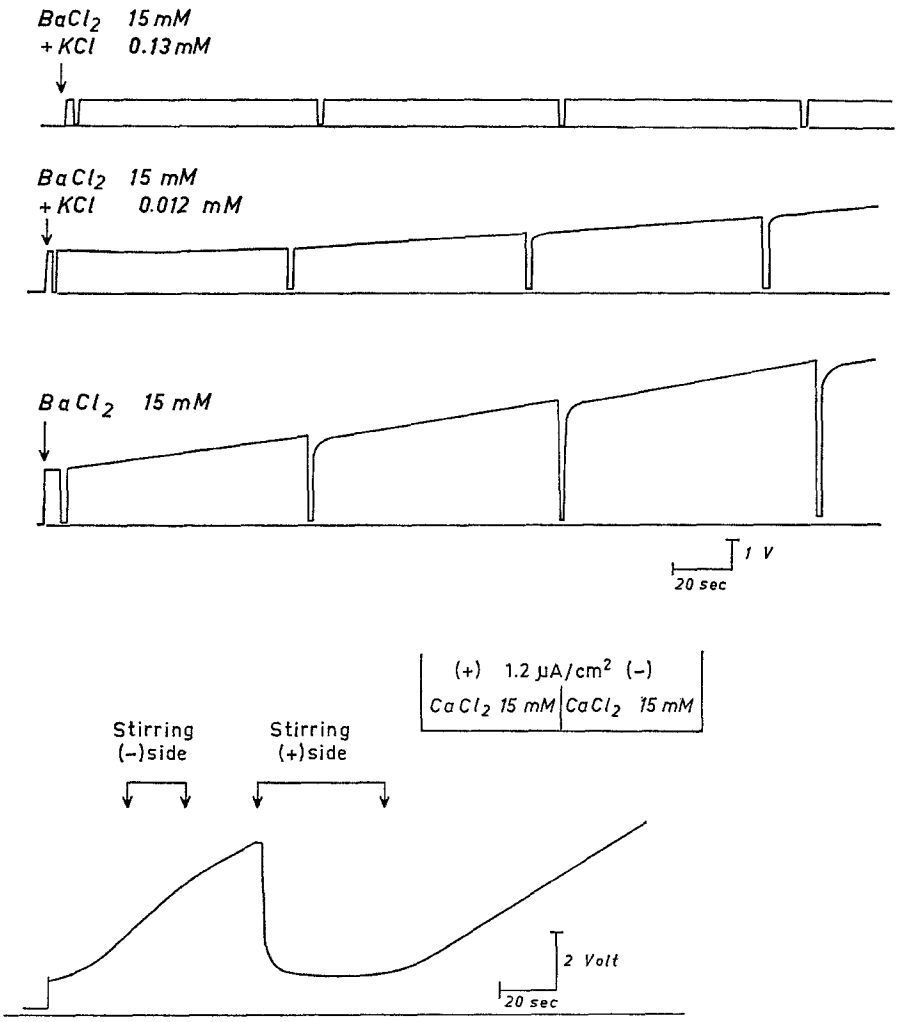

Fig. 3. The voltage across a membrane saturated with bromobenzene as a function of time after application of a constant current. *Top*: Elimination of time-dependent voltage response by consecutive addition of KCl into the $BaCl_2$ solution. Current density 0.8 μamp/cm². Effects of short current interruptions are shown. *Bottom*: Effect of stirring on voltage response

leads to a rapid decrease in resistance to approximately the starting value, and then the typical increase in membrane resistance ensues (Fig. 4).

If the current is applied for sufficiently long periods at a density which is lower than about 7.0 μamp/cm² [1], the resistance would reach a plateau.

1 If the d-c density is increased to higher levels, the resistance of the membrane falls either irreversibly or in a hysteretic way. The nature of these changes which occur at membrane electric fields of 5,000 to 20,000 V/cm are still under investigation.

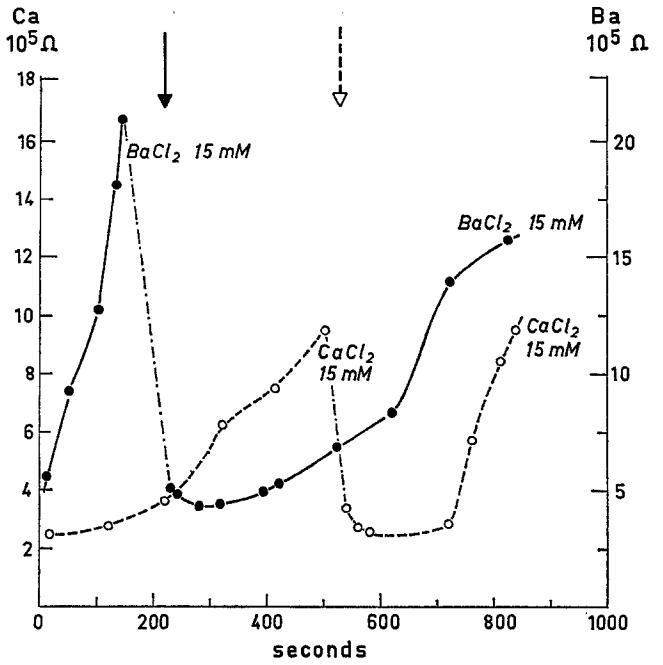

Fig. 4. The resistance of a bromobenzene-saturated membrane exposed to 15 mM $BaCl_2$ or $CaCl_2$ as function of time after application of constant current. Current density 1.2 μamp/cm². Arrows indicate reversal of current

Table 2. *Membrane a-c and plateau-level d-c resistance*

Solution (15 mM)	d-c current (μA/cm²)	Plateau-level d-c resistance ($10^6\,\Omega$)	a-c resistance ($10^5\,\Omega$)
$MgCl_2$	3.2	3.4	0.85
	5.3	3.6	0.85
	7.2	3.8	0.85
	7.25	3.1	0.76
$CaCl_2$	7.0	4.3	1.65
	6.4	4.8	1.47
$SrCl_2$	5.2	8.6	2.11
	5.3	7.6	2.52
	2.9	6.6	2.52
$BaCl_2$	1.3	36.8	3.62
	2.3	36.4	3.62
	2.7	33.5	3.63

Table 2 shows such values for various current intensities. It can be seen that the d-c resistance can reach values which are 40 to 100 times higher than the a-c resistance.

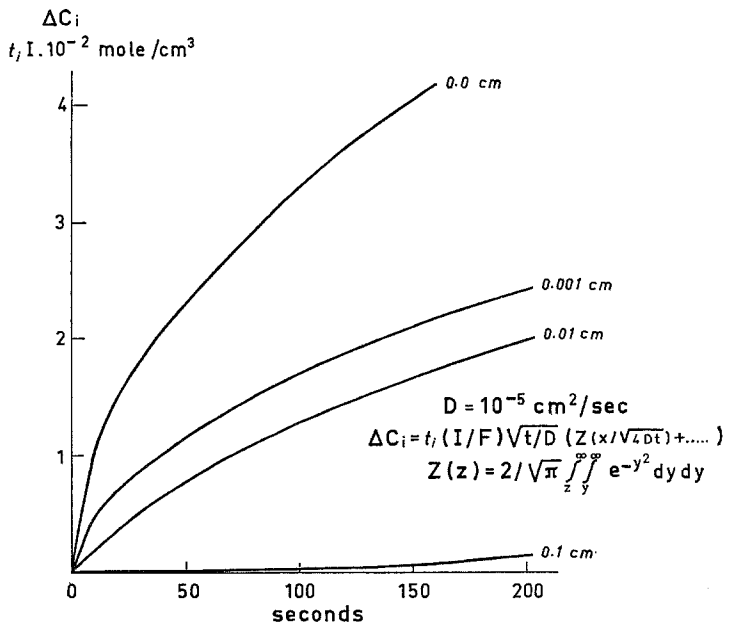

Fig. 5. Change in concentration, ΔCi, of an ion i in an unstirred layer near a membrane surface in response to an applied current density I (amp/cm^2). t_i is the transference number of the ion. The theoretical equation is taken with slight modification from the work of Macey [12]

These phenomena could be explained by the assumption that trace amounts of monovalent ions, such as K^+ or Na^+ are responsible for the a-c membrane resistance. A direct current, according to this assumption, would cause depletion of these ions in the aqueous film adjacent to the membrane and hence would lead to an increase in the membrane resistance. Fig. 5 shows the theoretical change in the concentration of an ion at various distances from the membrane surface as a function of time after application of a direct current. Clearly, this will depend on the current density and on the transference number of the ion (*see* ordinate of figure). From this figure, it can be gathered that after 100 sec of 0.8 μamp/cm^2 current density, the change in concentration near (i.e., 0 to 0.001 cm) the membrane would be 1.2 to 2.5 10^{-8} mole/cc or 0.012 to 0.025 mM for an ion with transference number 1.0. These figures are roughly consistent with the observation that addition of 0.13 mM KCl leads to complete disappearance of the time-dependent resistance, whereas it is still discernible if the K^+ level is only 0.012 mM (Fig. 3).

According to this notion, the plateau-level d-c conductance reflects the divalent ion mobility within the membrane. Thus the order of increase in

Table 3. *Membrane a-c resistance before and 48 hr after addition of $MgCl_2$ or $BaCl_2$, to a concentration of 10 mM, to solution originally containing the indicated salt at 15 mM*

Original solution (15 mM)	Membrane a-c resistance (kΩ)					
	Addition of $MgCl_2$			Addition of $BaCl_2$		
	before	after	% change	before	after	% change
$MgCl_2$	102	105	+ 3	121	540	+450
	110	116	+ 5	114	484	+340
$CaCl_2$	273	260	− 6	205	770	+250
	268	250	− 6	129	333	+160
$SrCl_2$	270	238	−14	333	584	+ 77
	274	232	−21	291	500	+ 75
$BaCl_2$	700	600	−18	620	575	− 5
	622	599	− 5	—	—	—

mobility within the membrane is (according to Table 3) Ba <Sr <Ca <Mg. The fact that the a-c membrane conductance follows the same line (Table 1) is not related to the divalent ions' mobilities within the membrane but, as will be shown in the next section, to the selectivity factor.

Part of the time-dependent increase in voltage represents membrane polarization, as can be seen from the fact that interruption of the current does not result in a return of the voltage to its initial zero value (Fig. 3). This polarization may represent the sensitivity of the membrane to changes in concentration of ions such as K^+, as exemplified in Fig. 2.

Do Divalent Ions Enter the Membrane?

The data shown so far indicate that the permeability of the membrane to the divalent ions is extremely low and that the extent of membrane a-c conductance in the case of exposure to solutions containing only divalent ions is determined by the presence of contaminants. Yet even the a-c level of resistance changes consecutively in the order Mg, Ca, Sr, Ba. Two possible explanations for this fact are apparent. (1) The order of increase in resistance reflects the order of decrease, fortuitous or otherwise, in concentration of penetrable contaminants in the particular divalent-cation salts. (2) The order of increase in resistance reflects increase in selectivity of the membrane for the divalent ions; e.g., Ba^{++} is taken up preferentially to Mg^{++} and therefore there are less contaminants in a membrane exposed to Ba^{++} than in a membrane exposed to Mg^{++}. The results of two types of experiments shown in Tables 3 and 4 prove that the second possibility

Table 4. *Relative ^{131}Cs content in bromobenzene-saturated membranes exposed to various solutions*

Solution (15 mM)	Relative ^{131}Cs content[a]
$BaCl_2$	1.00
$SrCl_2$	1.15 ± 0.03
$CaCl_2$	2.20 ± 0.15
$MgCl_2$	7.40 ± 2.30

[a] The content in a Ba membrane was arbitrarily considered as 1. $CsCl = 6 \times 10^{-6}$ M. Six measurements were made for each solution.

is more valid. First, addition of Ba^{++} to solutions of divalent ions leads to a substantial increase in membrane resistance, whereas similar addition of Mg^{++} has a negligible effect (Table 3); and secondly, the relative amounts of ^{131}Cs found in membrane exposed to a solution of divalent ions containing small amounts of $^{131}CsCl$ decreases in the order Mg, Ca, Sr, Ba. The conclusions, therefore, are: (1) divalent ions probably enter the membrane; and (2) they compete for occupancy of the fixed sites within the membrane in the order Mg, Ca, Sr, Ba, and yet the presence of small amounts of contaminants within the membrane accounts for the major part of the membrane conductivity, (i.e., the mobility of the divalent ions within the membrane is extremely low).

Direct Measurements of Membrane Permeability to Divalent Cations

The kinetics of Ca^{++} entry into a membrane was studied. Fig. 6 shows the amount of ^{45}Ca recovered from a membrane exposed to a solution of $CaCl_2$ as a function of the square root of the time after adding ^{45}Ca to the solution. For comparison, a similar study with Cs^+ is also shown. The continuous curves show the theoretical Ca^{++} or Cs^+ entry into the membrane with the Ca^{++} and Cs^+ self-diffusion coefficients being $0.97 \cdot 10^{-10}$ and $0.87 \cdot 10^{-8}$ cm^2/sec, respectively. The curves are based on the solution of Fick's second equation with appropriate boundary conditions. The generalized solution is presented in Fig. 9 of Crank's book [2].

Note that addition of CsCl into the solution drastically lowers the amount of Ca^{++} found in the membrane. This is consistent with the notion that Cs^+ and Ca^{++} compete for the negative sites within the membrane.

Another noteworthy point is that the course of ^{45}Ca entry into a membrane exposed to a 3 mM $CaCl_2$ solution is indistinguishable from that ex-

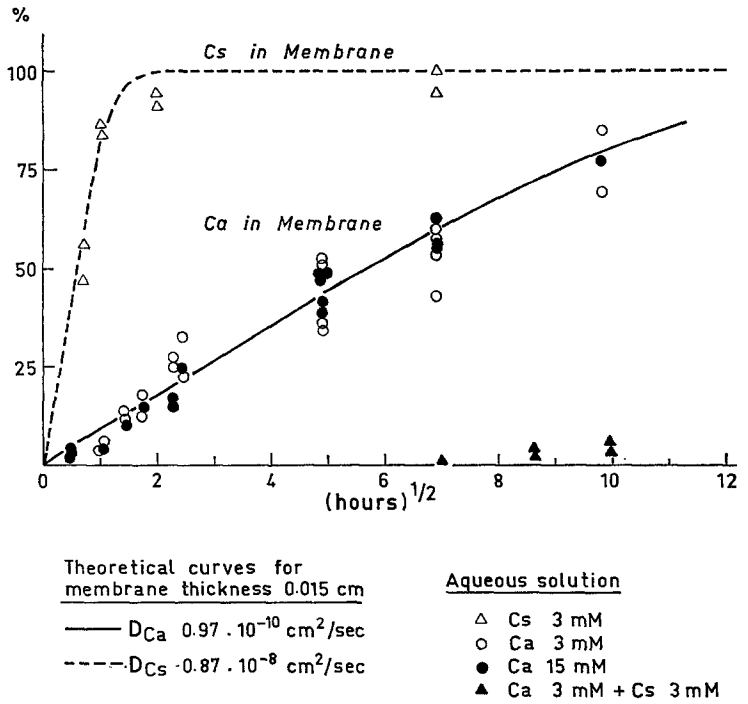

Fig. 6. Amount of ^{45}Ca and ^{131}Cs recovered from a membrane exposed to various solutions as function of the square root of time after addition of the labelled isotopes. The curves shown are theoretical; they are based upon the solution of Ficks' second equation using the appropriate boundary conditions and the indicated values for membrane thickness and Cs and Ca self-diffusion coefficients. The generalized solution is shown in Fig. 9 of Crank's book [2]. 100% for Cs and Ca is 1.5 and 1.25 10^{-7} mole respectively

posed to a 15 mM solution, a fact which is also consistent with the ion-exchange behavior of the membrane. Yet the apparent 100% Ca^{++} content is slightly over 80% of the Cs^+ content (*see* legend of Fig. 6). This may imply that most of the Ca^{++} within the membrane is present in a hitherto unidentified $(Ca\,X)^+$ form.

Fig. 7 shows measurement of passage of radioactive Ba^{++} or Ca^{++} across a membrane. Experiments with Sr^{++} gave results similar to that of Ca^{++}; namely, no labelled Sr^{++} could be shown to cross the membrane up to 72 hr of diffusion time. Yet there are measurable amounts of Ba^{++} crossing the membrane. The flux curve for Ca^{++} is calculated theoretically from the curve shown in Fig. 6. In comparison, the simultaneously measured passage of carrier-free ^{131}Cs across the membrane is also shown.

Two problems are raised by these experiments. (1) Ba^{++} is more permeable than Ca^{++} (and Sr^{++}) and is also much more mobile (holdup time

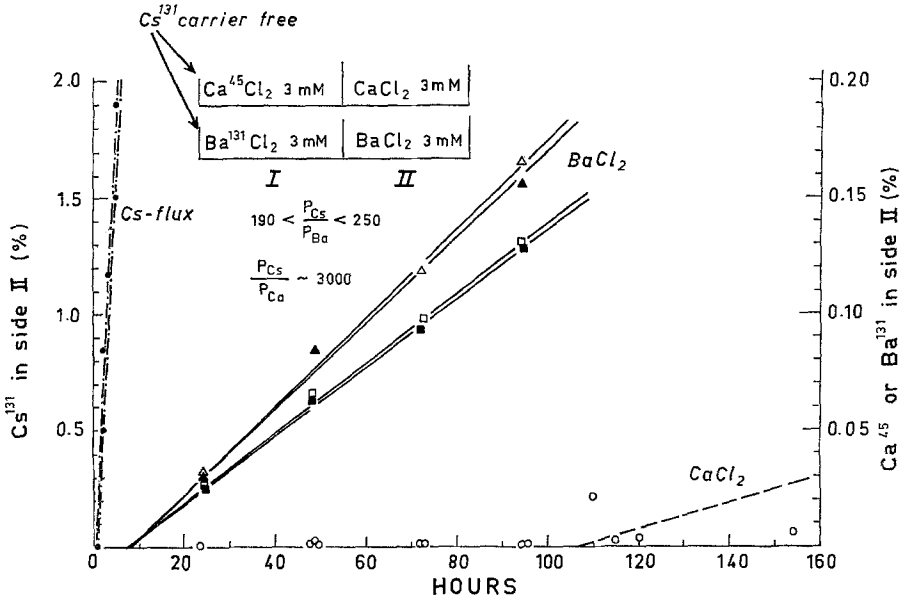

Fig. 7. Passage of ^{45}Ca or ^{131}Ba across a cellulose ester membrane saturated with bromobenzene. The simultaneous passage of "carrier-free" ^{131}Cs is also shown. Four experiments with Ba (different symbols) and three experiments with Ca (empty circles) are shown. Passage of ^{131}Cs (full circles) was measured in one of the Ca and Ba cells, the two being indistinguishable on the time scale shown. The theoretical Ca passage across the membrane (dashed line) was calculated from the study of the rate of entrance of Ca into the membrane (Fig. 6). Estimates of the K-divalent ion permeability ratios (e.g., P_K/P_{Ba}) are indicated

of 7 to 8 hr compared to a calculated holdup time of 107 hr for Ca^{++}). Yet the resistance of a Ba^{++} membrane is higher than that of a Ca^{++} membrane (Tables 1 & 3). (2) The Cs–Ca and Cs–Ba permeability ratios are much smaller than the K-divalent ions' permeability ratios calculated from measurements of the K^+ concentration-dependent membrane potential (*see* Fig. 2 and first section of Results). This could not be because of the difference between Cs^+ and K^+ since these membranes do not discriminate between these monovalent cations [8]. These facts imply lack of correspondence between Ba^{++} diffusibility and mobility.

Ba Mobility and Ba Diffusibility

The discrepancy between measurable Ba^{++} passage through the membrane and slow Ba^{++} mobility within the membrane is brought to light by

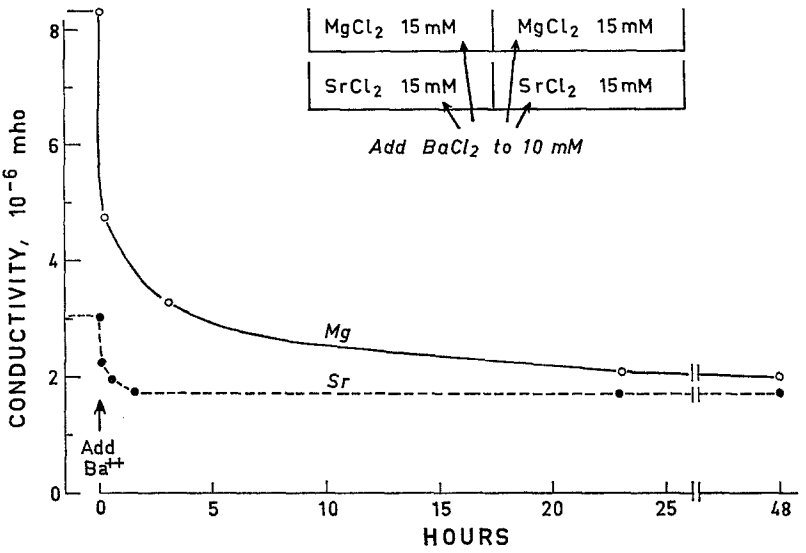

Fig. 8. Time course of change in membrane a-c conductance in response to addition of Ba^{++} into solutions containing initially Mg^{++} or Sr^{++}

following the change in membrane conductance brought about by the addition of Ba^{++} into Mg^{++} or Sr^{++} solution (Fig. 8). It will be noted that more than 75% of the change in conductance is attained within 3 hr. Yet the membrane conductance is very low, and even this low conductance is contributed mostly by the remaining monovalent contaminants (*see* second section of Results).

The implication is that Ba^{++} can move and exchange with counterions within the membrane in a form of a neutral complex, e.g., (Ba* X_2) mobile + (Ba $X)^+$ immobile $\rightleftharpoons$ (Ba X_2) mobile + (Ba* $X)^+$ immobile. This is proven quite clearly by the fact that the Cs–Ba discrimination determined under conditions where the ions are electrically driven through the membrane is at least two orders of magnitude higher than the diffusionally determined permeability ratio (Fig. 9).

Discrimination between Quinine$^+$ and Quinine^{++}

Quinine has, hitherto, been considered an ion for which the membrane permeability is highest [8]; thus a K^+-quinine$^+$ bi-ionic cell yields a potential difference of about 50 mV, the quinine$^+$ side negative. However, successive addition of acid into the two sides of a K^+-quinine$^+$ bi-ionic cell

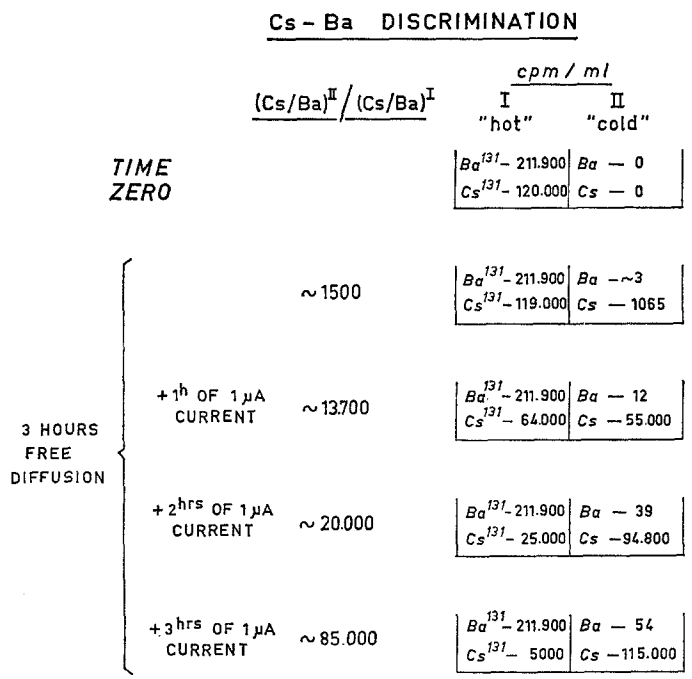

Fig. 9. Schematic presentation of the sharp Cs–Ba discrimination by a bromobenzene-saturated membrane. The membrane was exposed on both sides to 15 mM $BaCl_2$ solution. At time zero, ^{131}Ba and ^{131}Cs ("carrier free") were added to side *I* of the diffusion cell. After 3 hr of free diffusion, a current was passed through the membrane, the negative electrode put in the "cold" side. Figures denote radioactivity owing to ^{131}Cs and ^{131}Ba during the course of this experiment

leads finally to a reversal of the potential difference (Fig. 10). Since previous experiments have shown that the membrane is much less permeable to H^+ than to K^+ [11], it can be concluded from Fig. 8 that monovalent quinine is roughly nine times more permeable than K^+ whereas divalent quinine is nine times less permeable than K^+. In comparison, it can be seen that the potential difference in a K^+-trimethyl-phenylammonium$^+$ bi-ionic cell is independent of pH (Fig. 10).

Addition of carrier-free ^{131}Cs shows that a membrane exposed to 5 mM quinine^{++} contains about five times more Cs^+ than a membrane exposed to 5 mM quinine$^+$; this happens in spite of the fact that the higher concentration of H^+ ion in a quinine^{++} solution would by itself tend to displace some of the Cs^+ from the membrane [11]. Thus it is clear that part of the membrane discrimination between mono- and divalent quinine occurs because of the selectivity factor; namely, quinine$^+$ is preferred over quinine^{++}.

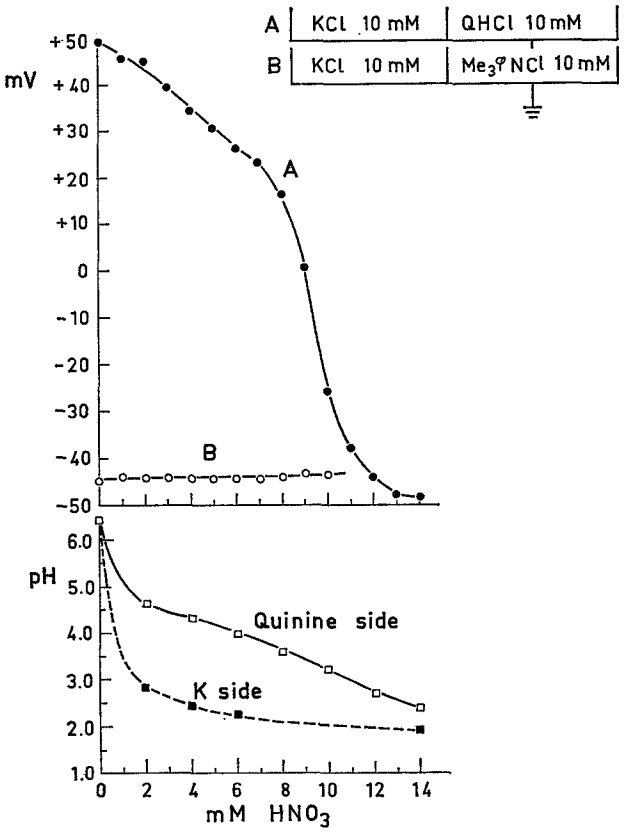

Fig. 10. The potential difference across a membrane separation a 10 mM solution of KCl from a 10 mM solution of either quinine hydrochloride (A) or trimethylphenylammonium chloride (B) as function of amount of HNO_3 added to both sides of the membrane. The simultaneous change in pH in the K^+ and quinine side is also shown

Discussion

The picture that emerges of the membranes described in this study concerning their discriminatory properties among the alkaline earth group and between this group and the monovalent ions can be summarized as follows. (1) The membrane contains approximately $1.5 \cdot 10^{-7}$ moles of fixed negative charge as judged by the monovalent cation content in the membrane (Fig. 6 and references 8 & 9). (2) Divalent ions, such as Ca^{++}, enter the membrane and compete for occupancy of the fixed negative sites with monovalent ions (Fig. 6). The order of increased selectivity is $Mg < Ca < Sr < Ba$ (Table 4). (3) The mobility of the divalent ions in the membrane is extremely low and increases in the order $Ba < Sr < Ca < Mg$ – as judged from the

plateau-level d-c resistance (Table 2). (4) The divalent ions do not accoun for more than 5% of the membrane a-c conductance. It is plausible tha trace amounts of monovalent contaminants are responsible for the majo part of the membrane a-c conductance (Fig. 3 and second section of Results) (5) The membrane a-c resistance follows the selectivity series $Mg < Ca < Sr < Ba$ since the more preferred the divalent ion, the less contaminant will be present in the membrane and the greater the membrane resistance will be (Table 1). (6) Since the permeability is determined by the selectivity and the mobility, it is possible *a priori* to find any membrane permeability relationship among the alkaline earth group. From the K^+-sensitive potential-difference study, it may be concluded that the permeability increases slightly in the order $Mg < Ca < Sr \leqq Ba$ (*see* legend of Fig. 2). (7) The Ca^{++} or Sr^{++} fluxes through the membranes are extremely slow (Fig. 7) and have a very high holdup time. The Ba^{++} fluxes are unexpectedly much higher and have a low holdup time. This implies that Ba^{++} can move within the membrane as a neutral complex. It is possible that this may apply also to the other divalent ions but to a smaller extent. If this is so, the mobility of the divalent ions will be even lower than that determined from the holdup time study; this possibility is consistent with the very low permeability of the membrane to divalent cations estimated from the study of the K^+-sensitive potential difference across the membrane (Fig. 2).

The filters saturated in hydrophobic membranes behave qualitatively like the ion-exchange membranes with a high degree of cross-linking. For example, if one considers the equilibrium properties of these membranes with respect to the monovalent ions Li^+, Na^+, NH^+, K^+, and Cs^+, it can be seen that the selectivity between these ions increases with increase in degree of cross-linking; e.g., the selectivity factors for the above series are 1, 1.58, 1.9, 2.27, and 2.67 for 3% divinyl benzene (DVB) content, 1, 2.37, 3.34, 4.50, and 4.6 for 16% DVB content [1], and approximately 1, 2.5, 5.2, 30.0, and 30.0 for the bromobenzene-saturated filters [8]. As far as the membrane selectivity toward the divalent ions is concerned, the order found Mg, Ca, Sr, Ba is similar to that found for the sulfonate ion exchangers [1].

The similarity between the hydrophobic membranes and sulfonated resins with a high degree of cross-linking is not surprising since both membranes impose a change in the hydration energy of an ion on its passage from the water into the membrane phase, along a model suggested by Eisenman [3].

The relation between the monovalent and divalent ion series is interesting: in sulfonate resins with a small degree of cross-linking, the relation is $\ldots NH_4^+ < K^+ < Mg^{++} < Ca^{++} \ldots$. The selectivity for the cation of higher valency is predicted from the Donnan effect and has been referred to also

as electroselectivity [10]. In the membranes of this study, the relation between the two series is reversed, i.e., ... $Sr^{++} < Ba^{++} < Li^{+} < Na^{+} ...$.

What causes the difference between the two types of membranes, such that in the sulfonate resins the divalent ions are preferred over the monovalent alkali metal ions and vice versa for the hydrophobic membranes? One difference between the two types of membranes which could account for the preference of monovalent ions by the hydrophobic membranes is the apparent presence of the divalent ion in the hydrophobic membrane in almost equimolar concentration to the fixed sites. As shown by the analysis in Fig. 6, the full Ca^{++} content in the hydrophobic membrane appears to be slightly over 80% of the Cs^{+} content. This implies that a greater part of Ca^{++} in the membrane is in the form of a $(Ca\ X)^{+}$ complex. It is plausible that the distribution of the distances between the fixed sites in these membranes is such that the probability of neutralization of two fixed sites by one divalent ion is reduced. This effect is so pronounced that even a big tertiary amine such as quinine is less competitive in its divalent form than in its monovalent one (*see* last section of Results).

The other factor in the discrimination between monovalent and divalent ions is their mobility within the membrane. In this respect, the situation is again similar qualitatively to that found in ordinary cation exchangers with a high degree of cross-linkings. For instance, in polystyrene sulfonic acid membranes with 16% DVB content, the diffusibility of Na^{+} is 10 to 20 times higher than that of Zn^{++} [13]. An estimate of the relative mobility of the mono- and divalent ions in the membranes of this study can be made in the following way. The resistance of a K-membrane is about $5 \cdot 10^{4}\ \Omega$ compared with 3 to $30 \cdot 10^{6}\ \Omega$ for divalent ion membranes (Table 2). Thus the relative mobilities of K^{+} compared to those of alkaline earth divalent ions would appear to vary between 60 and 600. However, it has already been shown that mobilities of the monovalent ions depend very much on the nature of other counterions present in the membrane [10]. Also, in these membranes it was found that the holdup time for ^{131}Cs in a Ba^{++} membrane is about 10 times less than its holdup time in a Cs^{+} membrane (*unpublished results*). Thus the Cs–Ba mobility ratio in a Ba^{++} membrane is probably of the order of 6,000. The additional one to two orders of magnitude of discrimination (Fig. 9) is probably contributed by the selectivity factor.

It is interesting to note that some of the biological membranes studied have a very low permeability to divalent ions. An estimate (based on measurements of isotopic fluxes) of the K–Ca permeability ratio for a giant axon of *Loligo* gives a figure of 600 [7] which is smaller by a factor of five than the Cs–Ca permeability ratio determined for these membranes (Fig. 7). Another

interesting observation relates to the discrepancy between the Ca diffusi bility and mobility within the axoplasm [7]. The conclusion that Ca^{++} ma be present within the cell as a neutral complex [7] may also apply to th way in which Ca^{++} crosses the membrane. If this is true, the K–Ca electrica permeability ratio may be even higher than the estimate from isotopic flu measurements, in a way analogous to that found for Ba^{++} in the membrane of this study.

This research was supported by the Office of Saline Water of the U.S. Departmen of the Interior.

The technical help of Mr. L. Katz is gratefully acknowledged.

References

1. Bonner, O. D., Smith, L. L. 1957. A selectivity scale for some divalent cations o Dowex 50. *J. Phys. Chem.* **61**:326.
2. Cranck, J. 1956. The Mathematics of Diffusion. Clarendon Press, Oxford.
3. Eisenman, G. 1962. Cation selective glass electrodes and their mode of operation *Biophys. J.* **2** *suppl*:259.
4. Goldman, D. E. 1943. Potential, impedance and rectification in membranes. *J. Gen Physiol.* **27**:37.
5. Helfferich, F. 1962. Ion Exchange. p. 156. McGraw-Hill, New York.
6. Hodgkin, A. L., Katz, B. 1949. The effect of sodium on the electrical activity o the giant axon of the squid. *J. Physiol.* **108**:37.
7. — Keynes, R. D. 1957. Movements of labelled calcium in squid giant axons. *J Physiol.* **138**:253.
8. Ilani, A. 1965. Ion discrimination by "Millipore" filters saturated with organi solvents. I. Cation selectivity, mobility and relative permeability of membrane saturated with bromobenzene. *Biochim. Biophys. Acta* **94**:405.
9. — 1966*a*. Ion exchange membranes saturated with hydrophobic solvents. *Israel J Chem.* **4**:105.
10. — 1966*b*. Interaction between cations in hydrophobic solvent-saturated filters con taining fixed negative charges. *Biophys. J.* **6**:329.
11. — Tzivoni, D. 1968. Hydrogen ions in hydrophobic membranes. *Biochim. Biophys Acta* **163**:429.
12. Macey, H. H. 1940. Clay-water relationship. *Proc. Phys. Soc.* **52**:625.
13. Soldano, B. A., Boyd, G. E. 1953. Self-diffusion of cations in heteroionic catio exchangers. *J. Amer. Chem. Soc.* **75**:6107.