# RELATION BETWEEN THE DIELECTRIC CONSTANT OF HYDROPHOBIC CATION EXCHANGE MEMBRANE AND MEMBRANE PERMEABILITY TO COUNTERIONS

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ABSTRACT Filters made of cellulose acetate-nitrate when saturated with organic solvents and interposed between aqueous solutions form membranes which behave like cation exchangers. The diffusion coefficients of counterions in such membranes are strongly dependent upon the dielectric constant of the saturating solvent. The results obtained suggest that a linear relationship between the log of the cation's diffusion coefficient (or membrane conductance) and the reciprocal value of the dielectric constant of the saturating solvent exists. There is also a good correlation between the relative membrane permeability to organic cations and the solubility of the cations in the pure solvent phase. These studies indicate that there are two routes for cation movement through the membrane: (*a*) the bulk hydrophobic phase and (*b*) continuous narrow aqueous channels.

## INTRODUCTION

Cellulose acetate-nitrate ester filters when saturated with various organic solvents were shown to behave like cation exchange membranes and discriminate between various cations (Ilani, 1965, 1966, 1970). The present study was undertaken in hope of elucidating the role of the saturating hydrophobic solvent in determining the unique properties of these membranes.

The main implication of this study is that narrow continuous aqueous channels must be present in the membrane system and that the ionic diffusion coefficients within the aqueous channel are very sharply dependent upon the polarizability of the surrounding hydrophobic medium.

The present study led us to present a theoretical model (Shohami and Ilani, 1973) which can explain many of the features of these membranes and can be of interest as an analogue of ionic routes in biological membranes.

## MATERIALS AND METHODS

Filters made of cellulose acetate and nitrate were obtained from Millipore Corp. (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 an organic solvent as specified. The two halves of the diffusion cell were then filled with aqueous solutions saturated with the appropriate solvent. The whole cell was placed in an organic solvent bath, in order to ensure complete saturation of the membrane and aqueous solution with the solvent and prevent leakage from the diffusion cell. Mixing of the aqueous solutions was effected by glass-sealed iron bars driven by a magnetic stirrer. All experiments were done at 23–25°C.

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

The AC resistance was measured by a Wheatstone bridge (Wheatstone Ceramics, Inc., Foster, Ky.) using Ag-AgCl electrodes. Bridge operation was generally at 250 cycle/s. At this frequency the measured resistance was very close (within 0–3%) to the DC resistance. The latter measurement was performed periodically. To measure the DC 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 potential difference across membranes was measured by a Keithley electrometer, type 601, (Keithley Instruments, Inc., Cleveland, Ohio), using Ag-AgCl electrodes for contact with the aqueous solutions.

The isotope $^{131}$Cs was obtained from The Radiochemical Centre (Amersham, England). Radioactivity was determined in a well-type Auto Gamma spectrometer (Packard model 5219, Packard Instrument Co., Inc., Downers Grove, Ill.). By measuring the amount of $^{131}$Cs crossing the membrane as a function of time it was possible to determine the "holdup time" of Cs in the membrane (Hellferich, 1962; Ilani, 1966). The holdup time $t_h$ is related to the diffusion constant in the membrane by the equation: $D_i = \Delta x^2/t_{h_i}$, where $\Delta x$ is membrane thickness.

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 24 h in a measured volume of 15 mM CsCl solution. A sample of the latter solution was analyzed for radioactivity.

All salts and solvents used were of analytical grade. *p*-Anisidine was recrystallized from ethanol solution and dissolved in HCl solution.

The distribution coefficients of some cations $B_i$ between water and an organic solvent in the presence of picrate was determined in the following manner (a modification of Pedersen's [1968] method): an aqueous solution containing $10^{-5}$ M picric acid and $1.5 \times 10^{-2}$ M of a particular salt was prepared. The solution was saturated with an organic solvent by adding few drops of the solvent to a relatively large volume of the aqueous solution. The optical density of the aqueous solution at 420 nm, $OD_1$, was then determined (Gilford spectrophotometer, Gilford Instrument Laboratories, Inc., Oberlin, Ohio). At this particular wavelength the absorption due to the organic cation used was negligible whereas that due to the picrate was appreciable. An aliquot of the aqueous solution was equilibrated with an equal volume of the organic solvent for 1 h. The optical density of the aqueous solution was then redetermined, $OD_2$. The change in optical density, $OD_1 - OD_2$, was proportional to the concentration of picrate, $C_{\text{pictrate}}$, in the organic phase. $OD_1$ represented the optical density of $10^{-5}$ M picrate solution and therefore the concentration of picrate in the organic phase was

$$C_{\text{picrate}} = 10^{-5} \frac{OD_1 - OD_2}{OD_1} \text{ M}.$$

Since electroneutrality is assumed for the bulk solvent the concentration of the cation in the organic solvent is equal to the concentration of picrate in the organic phase. With cation concentration in the aqueous solution of $1.5 \times 10^{-2}$ M the distribution coefficient of the cation $B_i$ was calculated as

$$B_i = C_{\text{picrate}}/1.5 \cdot 10^{-2}.$$

The dielectric constants of the solvents were taken from *Handbook of Physics and Chemistry* (R. C. Weast, editor. The Chemical Rubber Co., Cleveland, Ohio. 49th edition). The dielectric constant of mixtures of organic solvents was determined by measuring the capacitance of two metal plates of 10 cm² area immersed in the organic solvent mixtures. Control determinations of dielectric constants of pure solvents yielded values which were within ±5% of their known values.

## RESULTS

### *Influence of Solvent's Dielectric Constant on Ion Mobility within the Membrane*

Fig. 1 summarizes the results of determinations of diffusion coefficients of cesium and membrane resistances using 13 types of organic solvents for saturating the

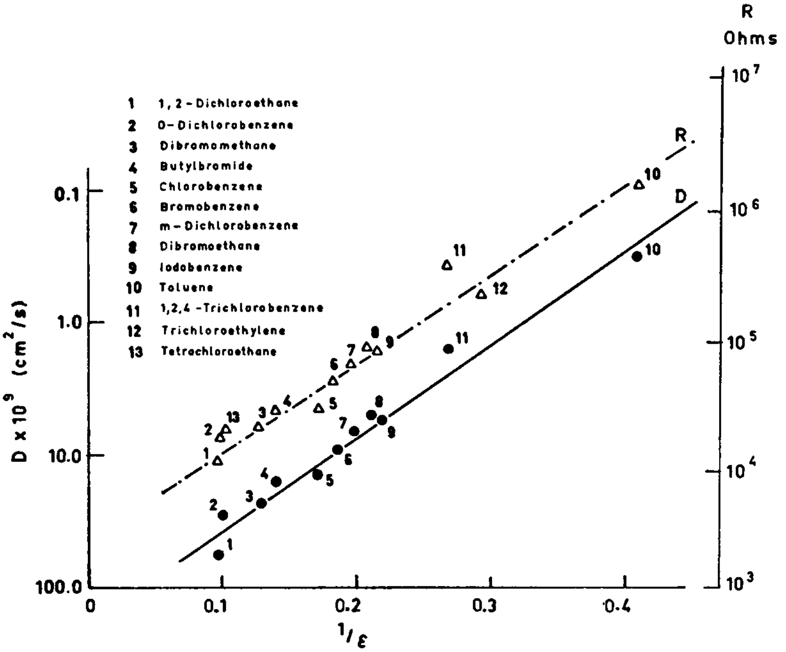

FIGURE 1 Relation between membrane resistance (△) or the diffusion coefficient of Cs within the membrane (●) and the reciprocal of dielectric constant of the saturating organic solvent. The cell consisted of a Millipore filter (GS type) interposed between 5 mM cesium chloride solutions. The filter was saturated by various solvents as indicated. The diffusion coefficient of cesium was determined by measuring the holdup time (see Hellferich, 1962; Ilani, 1966) using the radioactive tracer $^{131}$Cs. The slopes of the drawn curves correspond to about $10^6$-fold change in resistance or diffusion coefficient per unit change in $1/\epsilon$.

cellulose acetate-nitrate filters. The values of the log diffusion coefficients and log membrane resistance are plotted against the reciprocal of the dielectric constants of the organic solvents. The similarity of the slopes of the two curves in Fig. 1 indicates that variations in membrane resistance are related to changes in ion mobility within the membrane, and not to differences in counterions content, and this was verified experimentally by measuring the cesium content in the membrane (Fig. 3 C). The cation permselectivity was found to be a constant feature of the solvent-saturated filters (Fig. 3 B).

The definite relationship between the dielectric constant and membrane resistance is seen also when the dielectric constant is varied by using solutions of organic solvents for saturating the cellulose ester filters (Fig. 2).

Unlike the clear effect of the dielectric constant on ion diffusibility and membrane resistance, the discrimination between Na and K by the membrane were independent of the nature of solvent used as determined by measurement of the bi-ionic cell potentials (Fig. 3 A). The same behavior was noted for the Li-Na bi-ionic cell potential which was 67 ± 5 mV (the Na side negative) and showed no consistent dependence on the dielectric constant of the solvent used.

Since the bi-ionic cell potential is determined by the relative permeability of the membrane to the cations involved (Ilani, 1966), the implication of these observa-

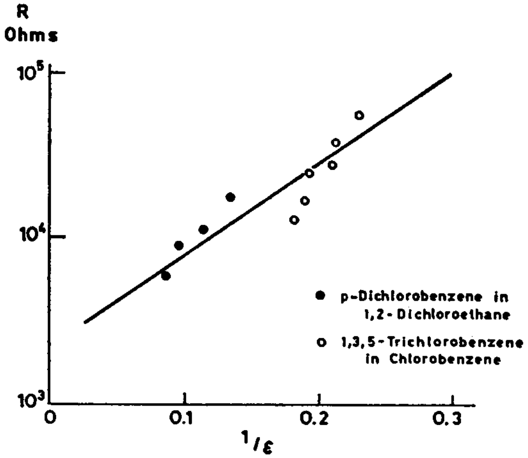

FIGURE 2 Relation between membrane resistance and the reciprocal of dielectric constant of saturating solvent. Membrane consisted of Millipore filters (GS type) saturated with various solutions of 1,3,5-trichlorobenzene in chlorobenzene (O) and *p*-dichlorobenzene in 1,2-dichloroethane (●). The composition of the solutions for the points shown is from left to right 0, 10, 20, 40% *p*-dichlorobenzene in 1,2-dichloroethane and 0, 10, 20, 30, 40, and 50% 1,3,5-trichlorobenzene in chlorobenzene. The measurements for this set of experiments were done on filters from a new batch. The resistances of these membranes were lower by a factor of about 1.3 from those shown in Fig. 1. The linear curve in the figure has the same slope as the corresponding curve in Fig. 1.

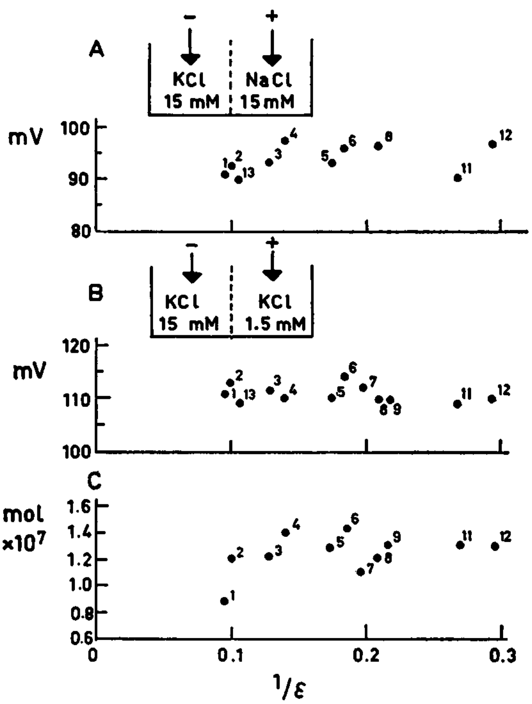

FIGURE 3 Abscissa: The reciprocal of the dielectric constant of the organic solvent used for saturating the cellulose ester membranes. The numbers identify the particular solvents used according to the list which appears in Fig. 1. Ordinate: A, K-Na bi-ionic cell potential. B, KCl concentration cell potential, measured with Ag-AgCl electrodes. Note that the value of the potential represent the sum of membrane potential and the chloride electrode potential. C, Amount of cesium recovered from membranes exposed to solutions containing 5 mM CsCl for 24 h. The points represent average of at least three measurements.

tions was that even though the absolute mobility of ions within the membrane varied by three orders of magnitude in the dielectric constant range of 2.5–10.0 (Fig. 1) the relative mobility and membrane selectivity for the alkali metal ions remained constant.

## *Permeability and Selectivity for Organic Cations in the Membrane*

The membranes of this study showed a striking selectivity for some organic cations. The presence of cations such as quinine or *p*-anisidinium in the aqueous solution sharply affected the cesium content in the membrane or its flux across the membrane (Fig. 4).

In contrast to the discrimination between alkali metal ions, the membrane selectivity for organic cations may vary considerably with the nature of the solvent used. Table I shows that the K-TEA (tetraethylammonium) bi-ionic cell potential varies from −83 to +28 mV. A change of that magnitude (i.e., 111 mV) implies a varia-

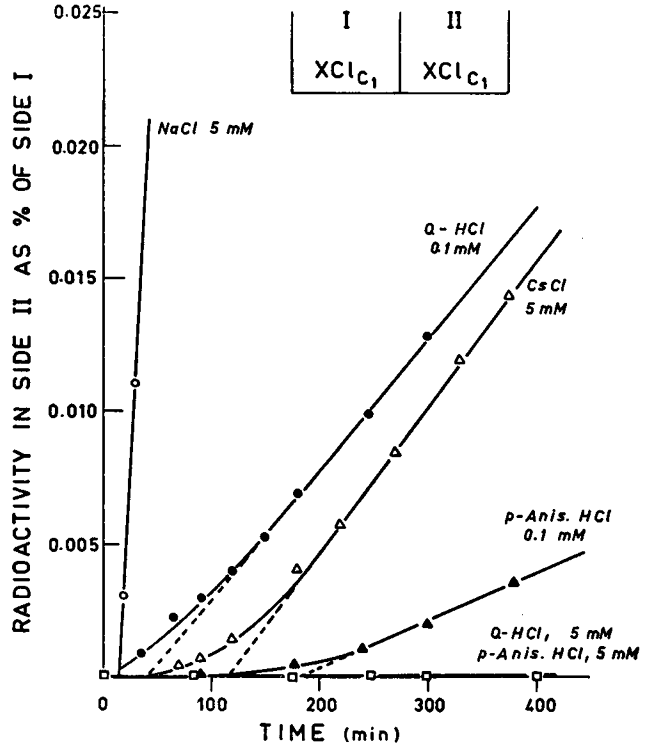

FIGURE 4 The appearance of radioactive $^{131}$Cs in side II of a diffusion cell as a function of time after adding $^{131}$Cs ("carrier-free") to side I of the cell. The composition of the solutions in the cell are indicated on each curve. Note that when the solutions contained 5 mM of either quinine hydrochloride (Q-HCl) or *p*-anisidinium chloride (*p*-Anis·HCl) there was not any detectable radioactivity in side II. The intercept of the linear curves with the abscissa is referred to as the holdup time $t_h$, and it is related to the diffusion constant of cesium in the membrane by the relation $D^{Cs} = \Delta x^2/6t_h$ where $\Delta x$ is membrane thickness.

tion of about 100-fold in the K-TEA permeability ratio. It can be seen that there is a fairly good correlation between relative membrane permeability to the TEA (as determined from the bi-ionic cell potential measurements) and the relative solubility of TEA in bromobenzene.

Table II shows the bi-ionic cell potentials of some cations measured against cesium in a bromobenzene-saturated filter. All four organic ions shown in the table have a measurable solubility in bromobenzene. Neither of the alkali metal ions (e.g., cesium) nor ammonium ions have any detectable solubility under the same conditions.

From the bi-ionic cell potential $V_B$ one can estimate the relative permeability of the ions involved, by the equation

$$V_B = (RT/F) \ln (P_{Cs}/P_i),$$

TABLE I

BI-IONIC CELL POTENTIAL AND DISTRIBUTION COEFFICIENT OF TEA

| Solvent | A<br>Potential | B<br>$B_{\text{TEA}}$ $10^{-4}$ |
|---|---|---|
| | *mV* | |
| Trichloroethylene | −83 | <0.02 |
| 1,2,4-Trichlorobenzene | −78 | 0.4 |
| Chlorobenzene | −49 | 0.45 |
| Bromobenzene | −40 | 0.34 |
| *o*-Dichlorobenzene | −26 | 1.0 |
| 1,1-Dichloroethane | −18 | 3.1 |
| 1,2-Dichloroethane | +22 | 6.0 |
| Tetrachloroethane | +28 | 6.0 |

Bi-ionic cell potential of membranes saturated with various organic solvents and the distribution coefficient of TEA between the solvent and water.

A. TEA-potassium bi-ionic cell potential in millivolts. Polarity refers to potassium side. Each figure represents average of at least three measurements which did not differ by more than ±10% of the average.

B. The distribution coefficient of TEA, $B_{\text{TEA}}$, between solvent and water under the condition described in the Methods section.

where $P_i$ and $P_{\text{Cs}}$ are the membrane permeabilities to the *i*th ion and to cesium, respectively. The above equation is based upon the constant field equation assuming that the permeability to anions is negligible (Ilani, 1966). The values of the permeability ratios are shown in column B of Table II. An estimate of the relative mobilities of the ions in the membrane are based on (*a*) the relative resistance of the membranes exposed to the respective cations (column C) and on (*b*) the relative diffusion coefficients of cesium in *i*th ion-containing membrane and in a cesium-containing membrane, $D_i^{\text{Cs}}/D_{\text{Cs}}^{\text{Cs}}$ (column D). The first parameter (column C) is proportional to the ratio between the diffusibility of cesium in a cesium-containing membrane $D_{\text{Cs}}^{\text{Cs}}$ and the diffusibility of *i*th ion in an *i*th ion-containing membrane $D_i^i$. Due to the peculiar interactions between counterions in these membranes (Ilani, 1966) the ionic mobility ratio is larger than the conductance ratio of membranes exposed to the respective ions. By dividing the permeability ratio with the mobility ratio one arrives at an estimate of the relative selectivity of the membrane for the ions concerned (shown in columns F and G). It can be seen that there is a definite relationship between selectivity of the bromobenzene-saturated filters and solubility of the cations in a pure bromobenzene phase (columns G and H). The somewhat exceptional position of choline which has solubility comparable with that of TEA but is clearly less selective will be reconsidered in the Discussion.

The observations describe in this section suggest very strongly that some organic cations can enter the membrane by dissolving in the bromobenzene phase and in so doing displace alkali metal cations from the membrane.

TABLE II

BI-IONIC CELL POTENTIALS OF SOME CATIONS MEASURED AGAINST CESIUM IN A BROMOBENZENE-SATURATED FILTER

|  | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| Cation present, $i$ | Potential* | $\frac{P_{Cs}}{P_i}$ | Relative membrane resistance* | $\frac{D_i^{Cs}}{D_{Cs}^{Cs}}$ | $\frac{D_i^{Cs}}{D_s^i}$ | Membrane selectivity for Cs | Selectivity for $i$th ion | $B_i$ $10^{-4}$ |
|  | $mV$ |  |  |  |  |  |  |  |
| $K^+$ | $+2 \pm 2$ | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | ~1.0 | <0.006 |
| $Na^+$ | −92 | 40 | 1.3 | 4 | 5.2 | 7.8 | ~0.1 | <0.005 |
|  |  |  |  | 2.5‡ | 3.3‡ | 12‡ |  |  |
| $Li^+$ | −160 | 570 | 2.3 | 6 | 13.8 | 41 | ~0.28 | <0.006 |
|  |  |  |  | 8.2‡ | 19.0‡ | 30‡ |  |  |
| $NH_4^+$ | −32 | 3.5 | 0.95 | 0.66 | 0.63‡ | 5.5‡ | ~0.18 | <0.006 |
| $TEA^+$ | −40 | 4.9 | 1.9 | 8.9‡ | 16.9‡ | 0.29‡ | 3.4 | 0.32 |
| $Choline^+$ | −175 | 1050 | 6.7 | 16 | 107 | 10 | ~0.09 | 0.265 |
|  |  |  |  | 13.3‡ | 80‡ | 13.0‡ |  |  |
| $p$-Anisidine-$H^+$ | +93 | 0.025 | 0.75 | 0.6 | 0.45 | 0.055 | 18 | 1.7 |
| Quinine-$H^+$ | +60 | 0.092 | 3.1 | 2.7 | 8.4 | 0.01 | ~120 | 5.3 |
|  |  |  |  | 4.5‡ | 13.9‡ | 0.0066‡ |  |  |

A. Bi-ionic cell potential in millivolts. One side of the cell contained 15 mM of cesium chloride and the other side 15 mM of chloride of the indicated cation. Polarity refers to Cs side. The membrane was saturated with bromobenzene. Potassium is indistinguishable from cescium by these membranes.

B. Relative membrane permeability as determined by the bi-ionic cell potential assuming the permeability to chloride is negligible.

C. Relative resistance of membranes exposed to 15 mM solution of the chloride of the indicated cation. The resistance of membranes exposed to 15 mM cesium or potassium chloride solutions was arbitrarily set to 1. The actual resistance values are not quoted since it was variable for various filter batches. The resistance of a potassium membrane varied between 9–21 × $10^4$ Ω $cm^2$. Membrane thickness: 0.015 cm.

D. The ratio between the diffusibility of Cs in a membrane which contains mostly the $i$th cation $D_i^{Cs}$ and the diffusibility of Cs in a cesium membrane $D_{Cs}^{Cs}$. These figures were either measured directly (by measuring the holdup times, see Fig. 4) or calculated. The calculated values (noted with double dagger) are based upon the experimental values in column F, in the same way as the calculated values in column F are based upon the measured values quoted in this column.

E. The ratio between the diffusibility of cesium in an $i$th ion-containing membrane $D_i^{Cs}$ and diffusibility of $i$th ion in an $i$th ion-containing membrane $D_i^i$. These values are arrived at by multiplying the figures in columns C and D (see text). These values are as close as possible to the average mobility ratio of cesium and $i$th ions in the membrane.

F. Membrane selectivity. These values are a measure of the membrane preference for the Cs or K over the $i$th ion. The product of the "average" selectivity constant and average mobility ratio should be equal to the permeability ratio (Ilani, 1966). The figures are either quoted experimental values (with double dagger) from previous publication (Ilani, 1965) or calculated by dividing column B by column E.

G. The average reciprocal of column F which is a measure of the membrane preference of $i$th ion with respect to cesium ion.

H. The distribution coefficient of $i$th ion $B_i$ between bromobenzene and water as measured under the conditions described in the Materials and Methods section.

* Values represent average of at least five measurements which never exceeded ±10% of the average. (Except in the case of K bi-ionic potential, where the variations were ±2 mV.)

‡ The meaning of double dagger is explained in legend.

## DISCUSSION

The principal conclusions of this study is that the organic solvents used to saturate the cellulose ester filters are directly involved in the mechanism of ion movement and interaction in the membrane system. This conclusion is evident from looking at the effect of the organic solvents on the mobility of ions in the membrane (Figs. 1 and 2) and from observing the correlation between the selectivity of the membrane for some organic cations and their solubility in a pure solvent phase (Tables I and II). The latter observation seems to indicate that the organic cations may enter and pass through the membrane in the organic solvent phase. On the other hand, this does not appear to be the case for the alkali metal ions. It can be gathered from Table II that quinine is preferred by the bromobenzene-saturated membrane some 120 times over cesium, yet its solubility in a pure bromobenzene phase is at least 800 times higher. If the energetics of the situation are considered it is very unlikely that alkali metal ions will be able to enter a medium of low dielectric constant from an aqueous solution at room temperatures.

An estimate based on the Born equation of the change in energy due to ion movement from water ($\epsilon = 80$) to a medium of dielectric constant 2 is of the order of 40 kcal/mol (Parsegian, 1969). As will be shown in the following theoretical paper (Shohami and Ilani, 1973) the expected resistance of a membrane containing fixed negative charges in a homogeneous medium of such a low dielectric constant is few orders of magnitude higher than the resistance observed in the membranes of this study. Therefore, it is highly plausible that continuous aqueous channels must be present in the membrane system. If the aqueous channels are sufficiently narrow they would exclude ions due to medium polarization effects as shown in an illuminating paper on this subject by Parsegian (1969). In the following paper (Shohami and Ilani, 1973) it will be shown that the mobility of ions in narrow nonuniform aqueous channels are dependent upon the dielectric constant of the surrounding medium. Moreover if the membrane contains fixed charges the electrostatic attraction between the fixed sites and its counterion will be influenced by the surrounding medium. This may also be the mechanism by which the medium surrounding the aqueous channels affects the diffusibility of the counterions in the channels.

It is possible that some organic cations such as TEA can enter the membrane both through the organic phase and through the aqueous channels. The discrepancy that is found between TEA and choline which have comparable solubilities in bromobenzene and yet are distinctly different in terms of membrane selectivity (Table II) may be explained by the assumption that TEA can enter the membrane through both the solvent phase and the aqueous channels whereas choline is limited to the bromobenzene phase.

Insofar as ion movements through cellular membranes involve passage through pores with or without fixed charges the study of the organic solvent-saturated filters may be helpful in unraveling unexpected interactions in systems containing narrow aqueous channels.

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## REFERENCES

HELLFERICH, F. 1962. Ion Exchange. McGraw-Hill Book Company, New York.
ILANI, A. 1965. *Biochim. Biophys. Acta.* 94:404.
ILANI, A. 1966. *Biophys. J.* 6:329.
ILANI, A. 1970. *J. Membrane Biol.* 3:223.
PARESEGIAN, A. 1969. *Nature* (*Lond.*). 221:844.
PEDERSEN, C. J. 1968. *Fed. Proc.* 27:1305.
SHOHAMI, E., and A. ILANI. 1973. *Biophys. J.* 13:1242.