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HYDROGEN IONS IN HYDROPHOBIC MEMBRANES

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SUMMARY

Filters containing fixed negative charges were saturated with hydrophobic solvent and exposed to solutions containing K^+, Na^+, or tetraethylammonium$^+$ at various pH's. Between pH 2 and pH 7, H^+ is comparable to K^+ and tetraethylammonium$^+$ in its ability to compete for fixed negative sites in the membrane. The membranes, however, show much greater selectivity for H^+ than for Na^+. Mobility of H^+ in the membrane is dependent upon the nature of the counterion in the membrane. However, in all conditions examined, its mobility is much lower than that of K^+ or Na^+ and is approx. one-third that of tetraethylammonium$^+$. The bi-ionic potential of H^+ *vs.* tetraethylammonium$^+$ is around 70 mV, and it tallies with the observed relative mobility and selectivity for these ions. Corroborating previous findings, these facts support the view that ion movement in such membranes does not occur *via* continuous water channels but involves passage of the ions through the hydrophobic medium.

INTRODUCTION

The present communication deals with the behavior of H^+ in cation-exchange membranes embedded in hydrophobic medium. As shown previously[1,2], filters made of cellulose acetate nitrate behave like cation-exchange membranes when saturated with hydrophobic solvents. The ionic discriminative properties of such membranes can be characterized experimentally by three parameters: (1) selectivity, *i.e.* the relative equilibrium distribution ratio between water and membrane of two cations; (2) mobility of cations in the membrane; (3) bi-ionic potential across such membranes, which in turn depends upon both selectivity and mobility, *i.e.* it is a measure of the relative permeability[1,3].

Measurements of bi-ionic potentials across such membranes indicate that the relative permeability to H^+ is quite low; for instance, the K^+–H^+ bi-ionic potential (*i.e.* the potential across a membrane separating solutions of KCl and HCl of equal concentration) was approx. 110 mV with the K^+ side negative[2]. As will be shown in this paper, this low permeability is due to the very low mobility of H^+ in such systems. This is one of the conspicuous features distinguishing the hydrophobic membrane from aqueous media.

The findings described in this study bear on the question of the mechanism of ion movement in ion-exchange membranes embedded in a hydrophobic medium. The possible mechanisms are discussed in view of present as well as previous studies.

The hydrophobic nature of cell boundaries suggests that these studies may have relevance for physiology.

METHODS

Millipore filters made of cellulose acetate and nitrate were interposed between two halves of a diffusion cell. The filters were saturated with bromobenzene, and the two chambers were filled with desired aqueous solutions. Measurements of bi-ionic potential and electrical resistance of the membranes were carried out as described earlier[1,2]. In most of the experiments conducted in this series, the membranes were exposed to identical solutions on both sides, either 1.5 mM of K^+, Na^+, or tetraethylammonium$^+$ at various pH's. The nature of anion used—Cl^- or Br^-—was immaterial[1]. The pH of the solution was varied by addition of HCl. After 48 h of exposure to the solutions, labeled $^{42}K^+$, $^{24}Na^+$, or ^{14}C-labeled tetraethylammonium$^+$ was added on one side of the membrane, and its appearance on the other side was followed. After enough time has elapsed, the appearance of radioactivity on the unlabeled side of the membrane becomes a linear function of time. From the slope of this curve it is possible to calculate the unidirectional flux, J_i, and the point of intersection of the curve with the time abscissa defines the so-called "hold-up" time, t_h, for the particular ion and membrane (see ref. 3 for details). From these two quantities it was possible to calculate the following parameters: (1) the content of the ion in the membrane, m_i; (2) the contribution of the ion to the electrical conductivity of the membrane, k_i; (3) the diffusion coefficient of the ion, D_i, from the following relations (see also refs. 3, 4):

$$m_i = 6J_it_h \tag{1}$$

$$k_i = J_iF^2/RT \tag{2}$$

$$D_i = l^2/6t_h \tag{3}$$

where l is the thickness of the membrane and F, R, and T are the Faraday, the gas constant, and the absolute temperature, respectively. At the end of the experiments, the membranes were washed lightly in deionized water (specific resistance more than $4 \cdot 10^6\ \Omega \cdot$cm), and the ion content in the membrane was determined by measuring its radioactivity (for details see refs. 1 and 3). There was generally only a slight discrepancy between this value and that determined by Eqn. 1.

The question of stability of the cellulose ester membranes at various pH's was studied by determining the appearance of nitrate in the solution, an expected

TABLE I

NITRATE CONCENTRATION IN 4 ml OF VARIOUS SOLUTIONS AFTER 96 h OF EXTRACTION OF A CELLULOSE ACETATE NITRATE FILTER (ABOUT 32 mg) SATURATED WITH BROMOBENZENE

Zero means < 0.15 mM (the limit of the analytical method).

Solution	*pH*	*Nitrate concn. (mM)*	*Total nitrate (mg)*
5 mM HCl	2.55–2.57	0	0
15 mM HCl	2.06–2.13	0	0
100 mM HCl	1.44–1.47	0	0
5 mM NaOH	11.02–11.15	3	0.74

concomitant of hydrolysis. Nitrate was determined by the method of Johnson and Ulrich[5]. The results are shown in Table I. Using this sensitive measure it can be seen that no detectable hydrolysis of the membrane took place in the low pH range. This finding is confirmed by the observation that the electrical resistance of a membrane was steady for up to 5 days of exposure to solution at pH 2–7. On the other hand, the membranes exhibited hydrolysis at higher pH's (Table I). This in turn corresponds to the steady decrease of the electrical resistance of membranes exposed to alkaline solutions (pH approx. 11.0) discernible from about the 1st day onward. However, no gross structural defects could be observed, even in membranes exposed to the alkaline medium.

The filters used in this study were of two batches designated as Type A and Type B. The bi-ionic potentials across these membranes were the same, but the membranes differed in their electrical resistance, as has already been pointed out[1,3]. It is evident from this study (Figs. 2 and 3) that the difference in resistance between the two membranes is due to differences in ion mobility and not to ion-exchange capacity. The ion-exchange capacity was even a little higher in the membranes of Type B, which had a higher resistance than those of Type A.

RESULTS

Ion content of the membrane as a function of pH

Fig. 1 shows the K^+, Na^+, and tetraethylammonium$^+$ content of a membrane exposed to 1.5 mM KCl or NaCl or tetraethylammonium bromide at various pH's as determined from Eqn. 1. It will be noted that the membrane content of these cations converges toward a value of about $1 \cdot 10^{-7}$–$1.2 \cdot 10^{-7}$ moles at pH 7.0. This figure agrees with the ion-exchange capacity of these membranes as determined earlier[1,4]. A decrease of pH leads to a reduction of the ion content of the membrane, presumably

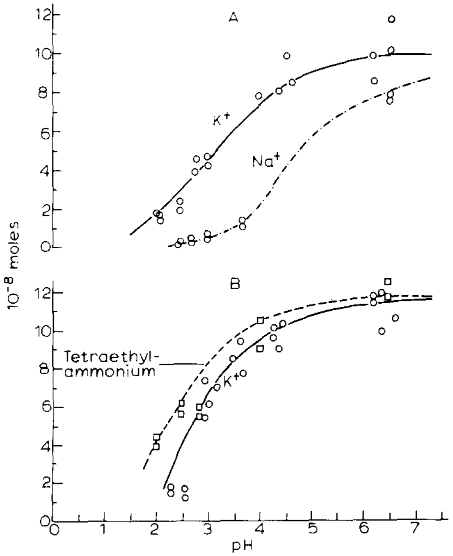

Fig. 1. The Na, K^+, or tetraethylammonium$^+$ content of Type A and Type B membranes exposed to solutions containing 1.5 mM NaCl, KCl, or tetraethylammonium bromide at various pH's.

due to competition between H$^+$ and the other cation for the fixed negative sites in the membrane. Fig. 1 also shows, that at the same low pH, the ion content in the membrane increases in the order tetraethylammonium$^+$ > K$^+$ > Na$^+$. This agrees with previous observations on the selectivity of the membranes for these ions[1].

Although it is generally assumed that the negative fixed sites in cellulose membranes are of a carboxylic nature[6,7], the results shown in Fig. 1 suggest that in the membranes of this study the fixed sites involved are of a more strongly acidic nature. Thus, with an aqueous Na$^+$ concentration of only 1.5 mM at pH 6.0, around 80% of membrane ion-exchange capacity is occupied by Na$^+$ (Fig. 1A), whereas for carboxylic acid resins, even at an aqueous Na$^+$ concentration of 10 mM, the Na$^+$ membrane content is close to zero at pH 6.0 (Fig. 3 of ref. 8). With K$^+$, such a contrast is more obvious. In cellophane, where carboxylic acid residues are presumably the functional groups, at aqueous K$^+$ concentration of about 2 mM and pH 6.0, only 25% of the fixed sites are occupied by K$^+$ (ref. 7) as compared to about 100% in the membranes of this study.

It is plausible that trace amounts of sulfonic groups are responsible for the ion-exchange properties of these membranes at pH's below 7.0. Thus, SOLLNER AND ABRAMS[9] have found sulfate in various collodion (*i.e.* cellulose nitrate) preparations from 0.03 to 3.1 mg sulfate per g dry collodion. Samples of the Millipore filters sent for sulfur analysis (Galbraith Laboratories, Inc., Knoxville, Tenn.) showed a sulfur content of 0.01 and 0.012% in duplicate determinations. Since a filter of 25 mm diameter weighs around 32 mg, the above figures would correspond to $1 \cdot 10^{-7}$–$1.2 \cdot 10^{-7}$ moles of sulfur per filter. The area of filter exposed to the water solution in the experiments of this study was 21–22 mm, the outer margins of the filter being pressed by the opposing surfaces of the diffusing chambers. One would expect, therefore, that these membranes would contain about $0.7 \cdot 10^{-7}$–$0.93 \cdot 10^{-7}$ mole of sulfur as compared to $1 \cdot 10^{-7}$–$1.2 \cdot 10^{-7}$ moleequiv of fixed negative sites found by ion-exchange capacity measurements. Considering the possible experimental errors, especially in trace determinations of sulfur, the fit between the sulfur content and the ion-exchange capacity of the membrane must be considered good.

The distribution coefficient of tetraethylammonium bromide and KCl between bromobenzene and water was studied and was found in each case to be less than $7 \cdot 10^{-5}$, as shown in Table II. This means that the amount of tetraethylammonium$^+$

TABLE II

AMOUNT OF RADIOACTIVITY IN counts/min FOUND IN 1 ml OF BROMOBENZENE EQUILIBRATED FOR 24 h WITH 1.5 mM AQUEOUS SOLUTION OF ^{42}KCl OR ^{14}C-LABELED TETRAETHYLAMMONIUM BROMIDE

The radioactivity in 1 ml of 1:1000 dilution of the aqueous solution is also shown.

	1 ml bromobenzene	*1 ml of 1:1000 dilution of aq. solution*	*Ratio of radioactivity in bromobenzene to water*
^{14}C-Labeled tetraethylammonium bromide	> 20	380	< $7 \cdot 10^{-5}$
^{42}KCl	> 25	2650	> 10^{-5}

or K^+ found in the membrane, even at pH 2, is more than 10^3 times higher than the amount that could be accounted for by solubility in bromobenzene.

A quantitative expression of the selectivity of the membranes for H^+ was made by determining the selectivity factor, $K^i_{H^+}$, which is defined by the following equation:

$$K^i_{H^+} = (X_i/X_{H^+})\,(c_{H^+}/c_i) \tag{4}$$

where X_i is the fraction of fixed sites occupied by the ion i, and c_i is the concentration of the ion in the water solution.

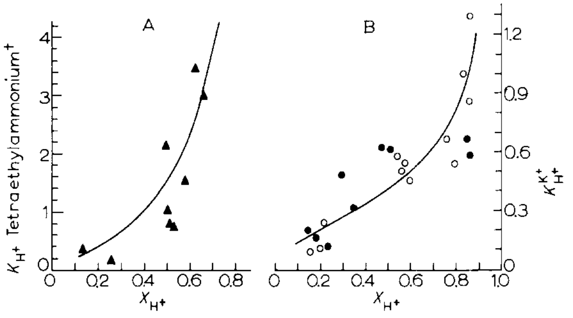

Fig. 2. $K^{TEA^+}_{H^+}$ and $K^{K^+}_{H^+}$ as function of H^+ fraction in the membrane. The curves represent a theoretical relationship if the following equation is applicable: $\bar{K}^i_{H^+} = (X_i/X_{H^+})^n\,(c_{H^+}/c_i)$ where $\bar{K}^{TEA^+}_{H^+} = 1.5$ and $n = 2$ for the tetraethylammonium$^+$–H^+ pair, and $\bar{K}^{K^+}_{H^+} = 0.4$ and $n = 1.5$ for the K^+–H^+ pair. Open and solid symbols represent data from Type A and Type B membranes, respectively.

Fig. 2 shows the selectivity coefficient between tetraethylammonium$^+$ and H^+, and between K^+ and H^+, as a function of X_{H^+}. These coefficients were computed from results shown in Fig. 1 using Eqn. 4. It is obvious that the selectivity coefficients are not constant. The same is true for the selectivity factors between other cations[3]. The curves in Fig. 2 depict the relation between $K^i_{H^+}$ and X_{H^+} if a constant selectivity factor, $\bar{K}_{H^+}$, exists which is defined by an empirical equation of the type:

$$\bar{K}^i_{H^+} = (X_i/X_{H^+})^n\,(c_{H^+}/c_i) \tag{5}$$

where $\bar{K}^i_{H^+}$ and n are constants. Such an equation was found applicable to the description of the selectivity of various ion-exchange resins[10]. With such an approximation and with the estimation of the diffusibility of H^+ and tetraethylammonium$^+$ which is made in the next section, it will be possible to compute the tetraethylammonium$^+$–H^+ bi-ionic potential and to compare it with the measured value, as was previously done for the K^+–Na^+ pair[3].

The diffusibility of K^+, Na^+, tetraethylammonium$^+$, and H^+

From the measurements of the ion content, its unidirectional flux, its hold-up time, and the electrical conductance of the membrane, it was possible to compute the diffusibility of the ions in the membrane in the following way. The ion-exchange capacity of the membranes was considered to be $1 \cdot 10^{-7}$ (Type A) or $1.2 \cdot 10^{-7}$ (Type B) moleequiv. The difference between this figure and the amount of K^+, Na^+, or tetraethylammonium$^+$ found in the membrane was taken as the amount of H^+ in the membrane, assuming electroneutrality. The diffusion coefficient, D_i, and the contribution of the ion, i, to the electrical conductance, k_i, were determined for K^+, Na^+, or tetraethylammonium$^+$ by Eqns. 3 and 2, respectively. The difference between the measured membrane electrical conductance and the calculated electrical conductance due to K^+, Na^+, or tetraethylammonium$^+$ was taken as the contribution of H^+ to the electrical conductance of the membrane, k_{H^+}. From these parameters it was possible to calculate the diffusibility of H^+ in the membrane by the equation:

$$D_{H^+} = D_i \, (k_{H^+}/k_i) \, (X_i/X_{H^+}) \tag{6}$$

Examples of such calculations are shown in Table III. Note that when K^+ was used as a competing cation almost all the conductivity of the membrane was accounted for by this cation, even though more than 70% of the membranes fixed sites were occupied by H^+. Thus the diffusibility of the H^+, D_{H^+}, in a membrane containing K^+ as well as H^+, was so low that it was impossible to estimate. Only when the more slowly diffusing tetraethylammonium$^+$ was used as a competing cation was it possible to estimate D_{H^+} over a reasonable range of H^+ content (Fig. 3B).

The accuracy of the determined value of D_{H^+} (by Eqn. 6) was dependent on the accuracy of the straight line drawn through the points depicting the accumulated radioactivity in the unlabeled side of the diffusion cell. The slope of this line determined k_i and hence k_{H^+}. The intercept with the abscissa determined D_i, and the product of slope and intercept determined X_i and hence X_{H^+}. In some of the better experiments, the slope of the determined line was better than ±5%, and in the worse ones it was better than ±30%. The effect of this uncertainty on the D_{H^+} determined was greater the smaller X_{H^+} was. The points shown in the center of Fig. 3B have an accuracy of more than ±50%. The point at the extreme right is accurate to within ±10%, and the point at the extreme left has an upper limit of +100%, whereas the lower limit is undefined (*i.e.* the real D_{H^+} at low X_{H^+} may be as close to zero as possible).

The following qualitative conclusions can be drawn from these studies: (1) The diffusibility of H^+ in the membrane is extremely low compared with that of K^+ or Na^+. (2) The diffusibility of the ions is dependent upon the counterion composition of the membrane. The nature of counterion interaction in the membrane is similar to that already noted with other cations[3], namely: when both a rapid ion (*i.e.* tetraethylammonium$^+$) and a slow ion (*i.e.* H^+) are mixed in the membrane, the fast ion becomes faster and the slow one slower.

The fact that H^+ becomes faster as its content in the membrane increases

TABLE III

TWO EXAMPLES OF THE CALCULATION OF THE DIFFUSION COEFFICIENT OF H^+ IN THE MEMBRANE

Membranes of Type A (K^+) and Type B (tetraethylammonium$^+$) exposed to 1.5 mM KCl and tetraethylammonium bromide, respectively, at pH 2.5 and 25°.

Ion present	*pH*	*Ion membrane content* × *10*^{8} *(moles)*	X_{H^+}	*Ion flux* × *10*$^{-12}$ *(moles/sec)*	$k_i \times 10^5$ (Ω^{-1})	$k \times 10^5$ (Ω^{-1})	$D_i \times 10^9$ *(cm²/sec)*	$D_H \times 10^9$ *(cm²/sec)*
K^+ (A membrane)	2.5	2.7	0.73	4.55	1.71	1.73	38.0	$D_{K^+} \gg D_{H^+}$
Tetraethylammonium$^+$ (B membrane)	2.5	4.9	0.58	1.55	0.585	0.75	7.1	1.85

(Figs. 3A and B) also implies that the ion-exchange groups are strongly acidic. If they were of weakly acid groups, they would be expected to dissociate less as more of them are in the H^+ form, and thus the H^+ diffusibility, as judged by its contribution to the electrical conductance of the membrane, should be lowered with an increase in X_{H^+}. The slowing of H^+ at decreased H^+ content is clear in the tetraethylammonium$^+$- as well as in the Na^+-containing membranes.

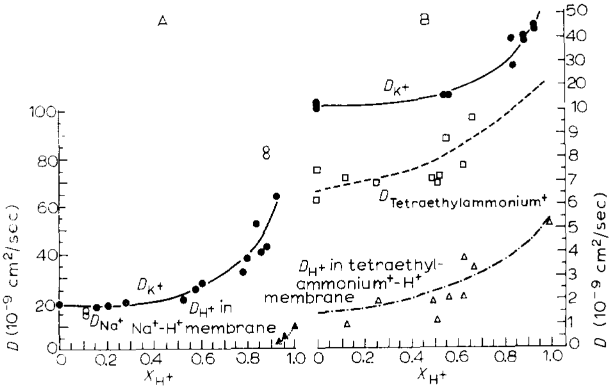

Fig. 3. The diffusion coefficient of Na^+, K^+, tetraethylammonium$^+$, and H^+ in Type A or Type B membranes as a function of X_{H^+}. The diffusion coefficients of Na^+, K^+, and tetraethylammonium$^+$ are determined by measuring the hold-up time. D_{H^+} is calculated (see text). The curves represent visual estimates of the function $D_i(X_{H^+})$.

H^+-tetraethylammonium$^+$ and H^+-K^+ bi-ionic potentials

The electrical potential across a membrane separating 15 mM solutions of tetraethylammonium bromide and HCl was measured. The result of such measurements gave a potential difference of 70 ± (S.D.)3 mV, the tetraethylammonium$^+$ side being negative. Because $\overline{K}_{H^+}^{TEA^+}$ is about 1.5 (Fig. 2A), it can be concluded that the interfacial potential difference in a tetraethylammonium$^+$–H^+ bi-ionic cell will contribute about 10 mV to the measured potential[3,10]. The diffusion potential due to differences between the mobility, U, of the ions in the bulk of the membrane will be given by:

$$V_{H^+}^{TEA^+} \text{ (diffusion)} = nRT/F\,(\log U_{TEA^+}/U_{H^+}) \qquad (7)$$

(see refs. 3 and 10) where n is defined by Eqn. 5; TEA^+ = tetraethylammonium ion. The mobility ratio and the diffusion coefficient ratio are the same. With $n = 2$ (see Fig. 2A) and U_{TEA^+}/U_{H^+} varying between 3.0 and 3.5 (Fig. 3B), the expected $V_{H^+}^{TEA^+}$ (diffusion) is between 55 and 63 mV. Adding the 10 mV due to interfacial potential difference, the tetraethylammonium$^+$–H^+ bi-ionic potential is expected to fall between 65 and 73 mV. Thus, the measured tetraethylammonium$^+$–H^+ bi-ionic potential is in good agreement with the theoretical value.

The K^+–H^+ bi-ionic potential is about 110 mV, the K^+ side being negative[2]. As noted before, it was impossible to determine H^+ mobility in the presence of K^+. An estimation of the K^+–H^+ mobility ratio can be made in the following way:

Because $\bar{K}^{K^+}_{H^+}$ is about 0.4 (Fig. 2B), it can be deduced that the difference between the interfacial potential drop in the K^+–H^+ bi-ionic system is about 23 mV, the K^+ side being positive. Thus, the K^+–H^+ diffusion potential would be $110 + 23 = 133$ mV, the K^+ side being negative. For the pair K^+–H^+, *n* in Eqn. 5 would be about 1.5 (legend of Fig. 2B). Assuming that the K^+–H^+ mobility ratio is constant and using an equation like 7, it can be concluded that the K^+–H^+ mobility ratio is around 40.

DISCUSSION

The results presented in previous studies as well as in this one show that the ion-exchange properties of these hydrophobic solvent-saturated membranes are due to the presence of about 10^{-7} moleequiv of fixed charge sites in the membrane. The membrane has an area of about 3.4 cm^2 and a thickness of about 0.015 cm. Thus, the fixed charge sites' concentration is about $2.0 \cdot 10^{-6}$ moleequiv/cm^3 or 2.0 mmoles/l volume of membrane. This is an extremely low value of ion-exchange capacity which could hardly be discerned if the filters were not saturated with the hydrophobic solvents. However, the presence of this small number of fixed charge sites in the membrane does determine to a significant degree its electrochemical properties. A prominent property affected by these fixed charge sites is electrical conductivity. Thus, a polethylene porous filter of comparable dimensions, saturated with toluene, has an infinite resistance (more than 10^{14} Ω) compared with about 10^6 Ω of the cellulose ester membranes containing the fixed charge sites. When bromobenzene is the saturating solvent, the relative resistance is more that 10^4-fold. This is hardly surprising if one recalls that the solubility of ions in such solvents is extremely low. Thus although the fixed charge sites in the membrane are present only in minute concentration, they dominate the electrochemical behavior of the membrane.

It was already pointed out that the negative fixed sites present in these membranes must be strongly acidic. The inference that sulfonic groups are responsible for the ion-exchange properties of these membranes is reasonable. It is, therefore, interesting to compare the properties of the hydrophobic membranes of this study with those of ordinary water-soaked ion exchangers containing strong acidic groups. In contrast to the membranes studied here, it is generally found that H^+ falls between Li^+ and Na^+ in the selectivity sequence of ion exchangers containing strong acidic groups[11]. In polystyrene sulfonic acid resins $\bar{K}^{K^+}_{H^+}$, $\bar{K}^{Na^+}_{H^+}$ and $\bar{K}^{Li^+}_{H^+}$ are 1.2, 1.03, and 0.74, respectively[12]. In the membranes of this study, $\bar{K}^{K^+}_{H^+}$ is about 0.4 (Fig. 2B) and $\bar{K}^{Na^+}_{H^+}$ is at least 10 times smaller (see Fig. 1A). It is conceivable that the presence of a medium of a low dielectric constant would increase the selectivity for H^+ due to increased electrostatic interaction between H^+ and the negative fixed sites.

The increased electrostatic interaction can also account for the very low H^+ mobility observed in the membranes of this study. It was shown that H^+ is about 3–4 times less mobile than tetraethylammonium$^+$ (Fig. 3B), about 10 times less mobile than Na^+ (Fig. 3A), and about 40 times less mobile than K^+. On the other hand, in water-soaked ion-exchange membranes, H^+ is more mobile than other cations. In polystyrene sulfonic acid resins, H^+ diffusibility is more than 4 and 7 times higher than that of Cs^+ and Na^+, respectively[12]. In phenol sulfonic acid membranes, the H^+ is about 10 times more mobile than Na^+ (ref. 13).

Three models which could explain the ion content and movement in these membranes are apparent: (1) The movement of the counterions in the membrane occurs through continuous water channels bounded by hydrophobic medium. This possibility is analogous to the well-known pore theory which accounts for ion permeation through cell membranes. According to this concept the concentration of the relevant fixed negative sites is the concentration in the water channels. (2) The counterions and fixed negative sites are present in the hydration shell bounded by the hydrophobic solvent. The movement of counterions through the membrane entails crossing through the hydrophobic solvent. (3) The counterions and fixed sites are present in the continuous hydrophobic medium of the membrane. According to this possibility, the counterions with a limited hydration shell are present in a medium which generally dissolves very minute amounts of ions. However, due to the presence of the fixed negative sites, the potential in the bulk of the membrane is sufficiently negative so that the electrochemical potential for cations in the membrane is reduced compared to a similar medium with no negative sites in it. A theoretical analysis of the potential course and the cation concentration profile across the interface of such a model is given elsewhere[14].

The last two possibilities may be termed "fixed associated" models in terms of the classification of EISENMAN, SANDBLOM AND WALKER[15].

In considering these possibilities it should be remembered that the diffusibility of ions in these membranes is 10^3–10^4 times slower than in water solution (see Fig. 3 and ref. 3). In a toluene-saturated membrane, the diffusibility is even lower, *e.g.* about 10^5 times slower than in water solution[4]. The activation energy of ion movement varies from 4000 to 10000 cal/mole depending on the counterion and hydrophobic solvent used[4]. These facts exclude the first possibility, in its simple version. One could hardly expect such a low mobility of ions in water channels.

It could be argued, however, that the water in the channel bounded by the hydrophobic medium is not a simple solution bur rather is arranged in an "ice-like" pattern similar to the supposed "icebergs" present around hydrophobic moieties in water[16,17]. To test this possibility, H^+ diffusibility was studied. The data presented in this study show, that although the functional negative sites in these membranes are strongly acidic, the diffusibility of H^+ is very low both in absolute terms and in comparison with the other cations. It is generally accepted that H^+ diffusibility in ice is higher than in ordinary water solution[18]. It seems, therefore, that the idea of a continuous water channel through the membrane is extremely unlikely for the membranes described in these studies.

Other data also indicate that there are no continuous water channels in these membranes. It has been shown that the hydrophobic solvents do affect the ion mobility in the membranes although they do not affect the ion-discrimination pattern by the membranes[2,4]. The more polar the liquids are, the more favorable they are for ion movement[2]. This suggests that the hydrophobic solvent is concerned directly with ionic movement in the membrane.

Thus, one is left with the other two possibilities, both of which require that the ions move through the hydrophobic solvent. It is the aim of further studies on these membranes to resolve the problem as to which of these two possibilities is valid.

A possible implication of this study for physiology is that ionic permeability of cell membranes does not necessarily depend on the presence of water-filled "pores". Movement of ions through hydrophobic media is most probably feasible and might account for the electrochemical properties of the cell membrane. The suggestion that H^+ mobility (and hence permeability) in such membranes is extremely low is also interesting in view of the fact that change in pH of extracellular space does not express itself rapidly in a corresponding change intracellularly[19]. It also implies that cellular membranes capable of actively transferring H^+ might withstand appreciable gradients of pH without much energy expenditure.

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REFERENCES

1 A. Ilani, *Biochim. Biophys. Acta*, 94 (1965) 405.
2 A. Ilani, *Biochim. Biophys. Acta*, 94 (1965) 415.
3 A. Ilani, *Biophys. J.*, 6 (1966) 329.
4 A. Ilani, *Israel J. Chem.*, 4 (1966) 105.
5 C. M. Johnson and A. Ulrich, *Anal. Chem.*, 22 (1950) 1526.
6 K. Sollner and I. Abrams, *J. Gen. Physiol.*, 24 (1941) 1.
7 S. M. Neale and P. T. Standring, *Proc. Roy. Soc. Ser. A*, 213 (1952) 530.
8 D. E. Conway, J. H. S. Green and D. Reichenberg, *Trans. Faraday Soc.*, 50 (1954) 511.
9 K. Sollner and I. Abrams, *J. Gen. Physiol.*, 24 (1941) 467.
10 G. Karreman and G. Eisenman, *Bull. Math. Biophys.*, 24 (1962) 413.
11 F. Helfferich, *Ion Exchange*, McGraw-Hill, New York, 1962, p. 168.
12 A. S. Tombalakian, C. Y. Yeh and W. F. Graydon, *J. Phys. Chem.*, 71 (1967) 435.
13 D. Mackay and P. Mears, *Trans. Faraday Soc.*, 55 (1959) 1221.
14 A. Ilani, *Biophys. J.*, 8 (1968) 556.
15 G. Eisenman, J. P. Sandblom and J. L. Walker, Jr., *Science*, 155 (1967) 965.
16 H. S. Frank and W. E. Evans, *J. Chem. Phys.*, 13 (1945) 507.
17 I. M. Klotz, in M. Kasha and B. Pullman, *Horizons in Biochemistry*, Academic Press, New York, 1962, p. 523.
18 E. Eigen and L. De Maeyer, in W. J. Hamer, *The Structure of Electrolytic Solutions*, Wiley, New York, 1959, p. 64.
19 P. C. Caldwell, *J. Physiol. London*, 142 (1958) 22.