K-Na Discrimination by Porous Filters Saturated with Organic Solvents As Expressed by Diffusion Potentials

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ABSTRACT The permeability ratio of Millipore filters saturated with organic solvents to K and to Na has been studied by measuring the potential difference across these filters. It was found that with *n*-octanol, toluene, and chloroform the membranes were more permeable to K^+ than to Na^+, the degree of discrimination being in inverse proportion to the polarity of the solvent. The dependence of NaCl and KCl diffusion potentials upon the concentration gradients across a filter soaked with about 1 : 1 toluene/*n*-butanol solution, could be expressed by the constant field equation, if it is assumed that this layer is 6 to 7 times more permeable to K^+ than to Na^+ and that the permeability to Cl^- is negligible. Elevating the fraction of toluene in *n*-butanol in the separating phase makes it more selective.

Many of the characteristics of cell permeability to non-electrolytes are explained on the assumption that the cell membrane is a barrier of lipoid nature (1). However, when the permeability of cells to different ions is considered, it has frequently been found necessary to modify the membrane model to account for its striking discriminative properties. Thus the widespread discrimination between K and Na is attributed to the presence of 4 Å pores which hinder the passage of Na and favour that of K (2).

Although many studies on the permeability of continuous hydrophobic layers have been made, a high K/Na permeability ratio on the part of such membranes has hitherto not been stressed (1, pages 212–213). Osterhout, however, who found nitrobenzene to discriminate between Na and K (3), said: "It is most interesting to find that an organic solvent can distinguish sharply between ions as similar chemically as K and Na."

The aim of this study is to examine the K/Na permeability ratio of several organic solvents put on thin porous filters, and in addition, to investigate the relation between the K concentration and the potential difference across such a membrane.

The fact that a preferentially K-permeable solvent-filter combination was found in the course of the experiments performed, made it possible to subject this "K electrode" membrane to the same type of analysis as was used by other workers to test the validity of the theory that the resting potential in biological systems is a K diffusion potential.

MATERIALS AND METHODS

The apparatus employed consists of two glass diffusing chambers each of which has three openings (Fig. 1). The large opening (1 in Fig. I) has a diameter of 23 mm and its margin is broadened and ground so as to fit that of the other chamber. A Millipore filter[1], VC-type, is introduced between the two facets of the diffusing

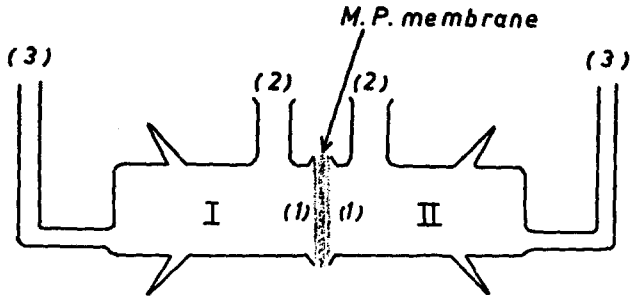

FIGURE 1. The diffusing chambers.

chambers and held tightly in position by four springs attached to hooks on the outer surface of the chambers. The other two openings (2 and 3) serve to introduce solutions and as an inlet for measuring electrodes.

After putting the Millipore filter (M.P.) between the two facets, high vacuum grease was smeared over the junction, which was then covered by a 1 cm broad rubber ring. This procedure was found to ensure complete insulation of the interior of the chambers from the water bath.

An organic solvent was then dripped onto the filter to effect complete wetting; the apparatus was placed in an empty water bath and the two chambers were filled simultaneously with the specified solutions at a desired temperature. After equalizing the levels of the solutions in the two chambers, the bath was filled with water at a constant temperature. Mixing began within 2 minutes after filling the chambers. In experiments in which one of the chambers contained water only, platinized elec-

[1] According to the manufacturer (Millipore Filter Corporation, Bedford, Massachusetts, United States) the filters are "cellulose plastic porous membrane. The pore penetrates directly through the average 150 μ depth of the filter with minimum cross-linkage. Pore volume occupies about 80 per cent of the total filter volume." The VC-type has a pore diameter of 0.1 μ. Experiments done with toluene or butanol on other types of membranes (HA-type, 150 μ thick, 0.45 μ pore diameter and TH-type, 25 μ thick and 0.45 μ pore diameter) yielded the same results.

trodes were placed in that part so as to follow the decrease of resistance of the solution in time.

Mixing was effected by a magnetic stirrer. The stirrers, one in each chamber, were sealed in glass tubes. When the solutions were not stirred the potential tended to be slightly lower, the difference being higher when butanol was placed on the filter and hardly noticeable with toluene as separating phase. (The butanol referred to in this study is *n*-butanol.)

Calomel electrodes were connected to the chambers by KCl-saturated agar bridges. The EMF was measured by a potentiometer using a sensitive galvanometer. With the solvent of lowest resistance (butanol) on the filter it was possible to measure the potential difference to within 0.3 mv, whereas with the highest resistance organic solvent used (toluene) the figures were reliable only to within 3 mv.

The KCl-agar bridges were generally placed in the tube on the distal side (3 in Fig. 1) in order to minimize contamination of the bulk solutions with KCl. However, when changes in the content of the solution were made during the experiment (by adding through inlet 2) it was found necessary to place the bridge in the bulk solution so as to avoid measuring the diffusion potential between the bulk solution and that of the side tube 3 (see Fig. 1), which was not mixed effectively by the stirring mechanism. In some experiments it was necessary to avoid the least contamination and the agar bridge was further displaced from the bulk solutions as described in section 3 of the Results.

Two criteria could be used to verify the persistence of the organic solvent in the membrane: (*a*) The rate of change of the resistance of the water solution. This was always nil or very low with the organic solvent; whereas in control experiments with water placed on the membrane the rate of decrease of resistance was very high. (*b*) Size, sign, and persistence of EMF. When water was placed on the membrane the potential was either zero or opposite in sign to that found when the membrane was saturated with an organic solvent. In the case of a membrane damaged during the experiment the potential fell rapidly towards zero. Damage occurred whenever the stirrer struck the membrane, though no macroscopic hole could be demonstrated in the filter. A rapid fall of potential could also be produced artificially by using solutions which were not saturated with the organic solvent.

All the aqueous solutions used were prepared from a stock solution containing 300 meq/liter of salt and from double distilled water saturated with the organic solvent used, at 15–16°C. The solvents which were placed on the membrane were of analytical grade and, unless otherwise indicated, saturated with double distilled water at 15–16°C.

When using a mixture of toluene and butanol in a certain proportion, the solubility of butanol in water, about 7 ml per 100 cc, was taken into account (the solubility of toluene is negligible). In preparing 1:1 toluene/butanol solution, the equilibrated mixture consisted of 100 cc toluene, 107 cc butanol, and 200 cc water; *i.e.*, 3.5 cc of surplus butanol was added per 100 cc of water in preparing a 50 per cent butanol solution. The exact composition of the final equilibrated solutions is, of course, uncertain, but since the effect of elevating the fraction of toluene in butanol on the EMF appeared very clearly, no accurate analysis of the solution was made.

TABLE I

EMF OF DIFFUSION CELLS IN MILLIVOLTS

The two sides of the cells were separated by an organic solvent phase. The content of salt solution was of 15 meq/liter. Temperature 15°C. Side II, positive. Figures in parentheses indicate the number of experiments performed.

Nature of diffusion cells	Solvent phase			
	Butanol	*n*-Octanol	Toluene	Chloroform*
$NaCl_I$:$water_{II}$	49–52 (5)	80–82 (2)	95–105 (7)	70–120 (2)
KCl_I :$water_{II}$	52–53 (4)	95 (2)	175–195 (4)	130–200 (2)
KCl_I :$NaCl_{II}$	2–1 (4)	15–16 (2)	90–95 (4)	65–95 (2)

* The potential difference across chloroform membranes was unsteady and the figures represent the variation of the potential in the first 10 minutes after filling the diffusion chambers.

RESULTS

1. *Diffusion Potential of NaCl and KCl across Various Organic Solvent Layers*

A number of solvents were tested for their ability to discriminate between Na and K ions.

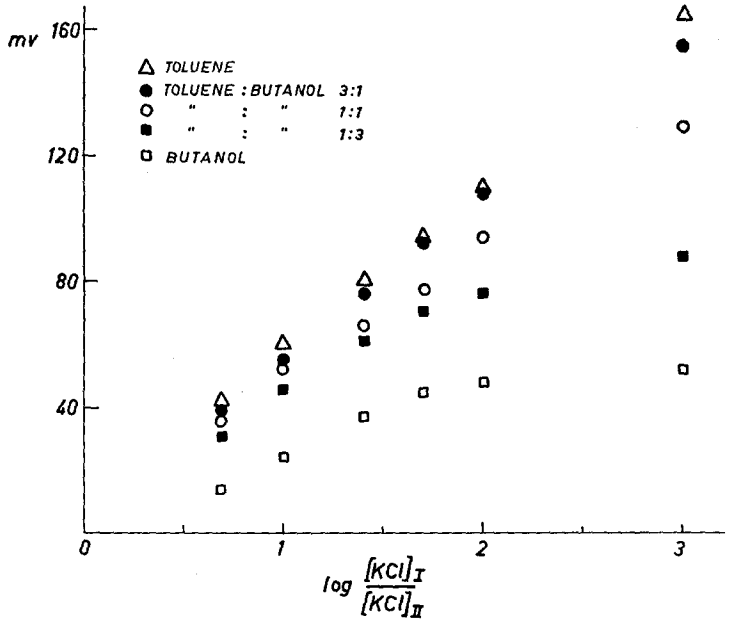

FIGURE 2. Diffusion potential of KCl across an organic solvent phase. $[KCl]_I$ is of 15 meq/liter. $[KCl]_{II}$ varies between 0.015 and 3 meq/liter. Temperature 15°C. Polarity refers to side II.

It is apparent from Table I that all the solvents used are preferentially permeable to cations. In addition it can be seen that the more hydrophobic solvents are also selectively more permeable to K than to Na.

In experiments in which the difference in osmotic pressure between the two sides of a cell (*e.g.* KCl:water) was compensated by adding glucose, there was no significant change in the measured potential. It can thus be inferred that differences in osmotic pressure in this range do not affect the diffusion potential.

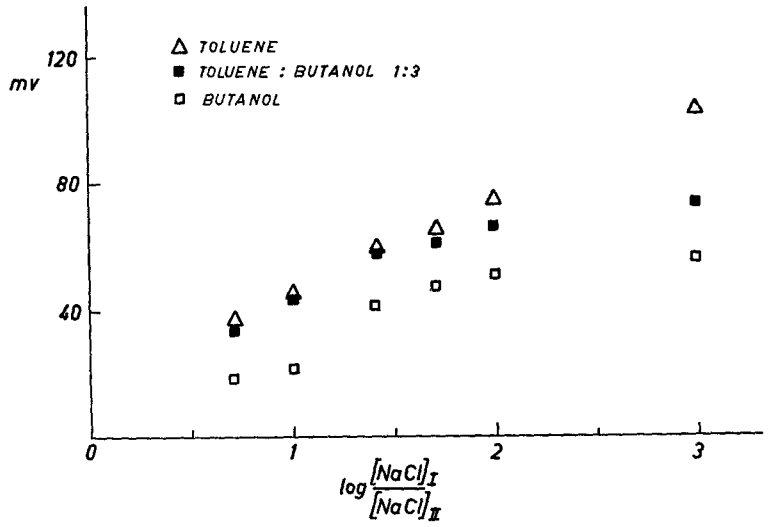

FIGURE 3. Diffusion potential of NaCl across an organic solvent phase. Details as in Fig. 2.

It is noteworthy that the cell KCl:NaCl gives rise to a potential which is very close to the difference between the cells KCl:water and NaCl:water. This point is dealt with further in the Discussion.

2. *Diffusion Potential of NaCl and KCl across a Phase Composed of Toluene and Butanol in Different Proportions*

From Figs. 2 and 3 it appears that elevation of the toluene fraction in butanol makes the membrane more discriminative between cation and anion. In addition, by comparing curves *B* and *C* of Fig. 4 it can be seen that the increase in discrimination between anion and cation is greater for K than for Na. This is explicitly shown by curve *A* (Fig. 4) from which it can be implied that elevation of the toluene fraction is also responsible for the greater permeability of the membrane to K than to Na.

3. *Dependence of EMF on KCl Concentration Gradient*

Since the relation between the size of the potential and the concentration gradient has an important bearing on related biological potentials, a thorough study of this relation was made.

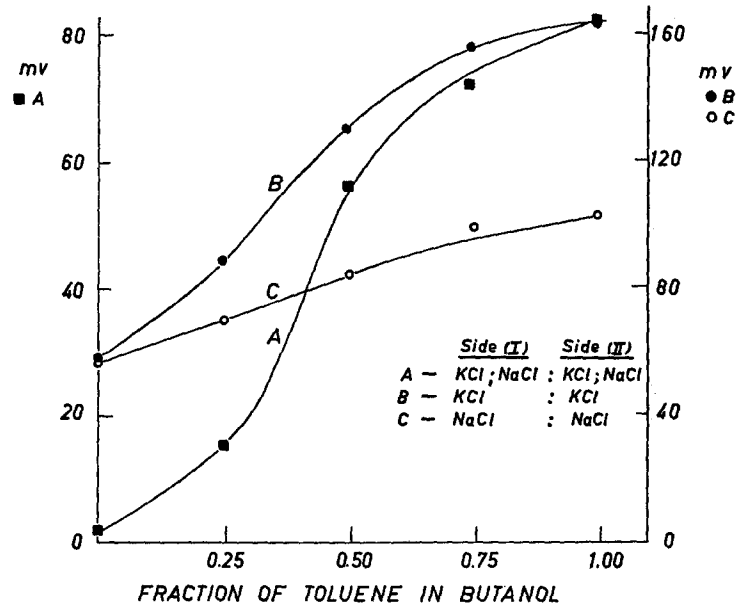

FIGURE 4. EMF of diffusion cells as function of the membrane composition.

$$\text{A, } \frac{[\text{KCl}]_{\text{I}}}{[\text{KCl}]_{\text{II}}} = \frac{[\text{NaCl}]_{\text{II}}}{[\text{NaCl}]_{\text{I}}} = 100$$

$$\text{B, } \frac{[\text{KCl}]_{\text{I}}}{[\text{KCl}]_{\text{II}}} = 1000$$

$$\text{C, } \frac{[\text{NaCl}]_{\text{I}}}{[\text{NaCl}]_{\text{II}}} = 1000.$$

The higher concentration is 15 meq/liter. Temperature, 15°C. Polarity refers to side II.

In order to avoid any change in the composition of the solution as a result of introducing the agar bridges, solutions identical with those on each side of the membrane were placed in two separate containers connected through narrow inverted U tubes to each side of the cell *via* openings labeled 3 in Fig. 1, and the agar bridges were put in the external containers. The electrodes were connected through a cathode follower having an input resistance of 10^{11} ohms to the DC-preamplifier of a Grass polygraph. Changes in concentration of the contents of the diffusion cells were produced by injecting

measured amounts of KCl solution (300 meq/liter). Since the mobilities of K and Cl are the same in aqueous solution, there was no problem of an interference arising from a diffusion potential at the boundary of the inverted U tube and the bulk solution.

A typical picture of the time course of the change in potential brought about by varying the KCl concentration is shown in Fig. 5. A steady potential is attained within 1 to 2 minutes after changing the concentration, a fact which leads to the assumption that a steady state is rapidly reached in the system. This assumption appears the more justified as the diffusion potential remains unaltered whether the solvent placed on the membrane is saturated with double distilled water or with a KCl solution of 60 meq/liter.

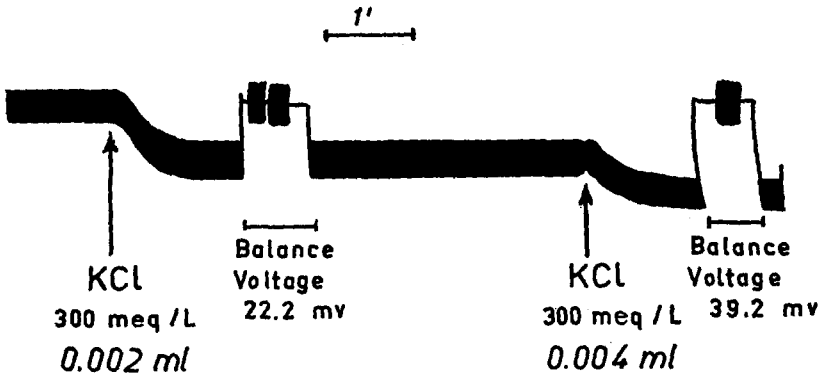

FIGURE 5. Time course of the change in potential brought about by varying the KCl concentration. The exact EMF was determined by returning the pen to the zero level with a balance voltage.

Fig. 6 expresses the relation between the diffusion potential and the KCl concentration. Curves *A* and *B* of this figure, while differing in absolute magnitude, are similar in their slope which amounts to 56 mv per tenfold change in KCl concentration. At concentrations below 0.15 meq/liter both curves deviate from linearity.

Fig. 7 illustrates an experiment in which the KCl concentration on side I was constantly maintained at about 54 meq/liter while the concentration on side II was varied. The concomitant change in the diffusion potential, reflected by curve *A* of this figure, shows a slope identical with those observed in Fig. 6, and a similar deviation from linearity at concentrations below 0.15 meq/liter. However, when NaCl is present on the same side (curve *B*) the slope is less steep (50 mv per tenfold change in KCl concentration) and below 2 meq/liter the curve deviates from linearity to the extent that the EMF becomes independent of the KCl concentration.

DISCUSSION

As a result of our experiments two points of interest are raised: (*a*) To what characteristics of the membrane may the ion discrimination be ascribed?

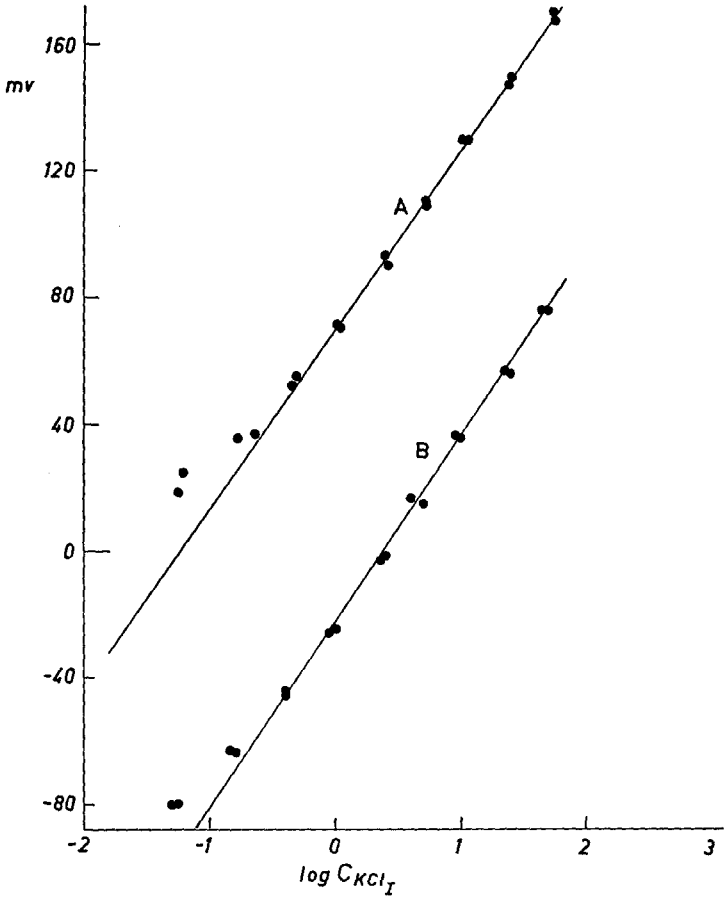

FIGURE 6. Relation between EMF and KCl concentration. Side I, of the cell, KCl concentration varied. Side II, curve *A* contains double distilled water; curve *B*, 15 meq/liter of NaCl. Membrane, about 1:1 toluene/butanol. Temperature, 15°C. Polarity refers to side II.

(*b*) The relation between KCl concentration gradient and diffusion potential.

As to the first question three possibilities should be considered:

1. The solvent layer is the discriminating factor while the filter material serves only as an inert skeleton. If this is the case, the organic solvents used have to different degrees the properties of nitrobenzene described by Osterhout (3, 4), in that they are preferentially cation-permeable and also more

permeable to K than to Na. It is, however, difficult to see why solvents such as toluene and butanol should discriminate so sharply between anion and cation. Moreover, studies by Rosano *et al.* (5, 6) show that the order of permeability to ions of a thin layer of butanol is Cl > K > Na. The curves in Fig. 4 of the article by the above authors (5) show a polarity contrary to the

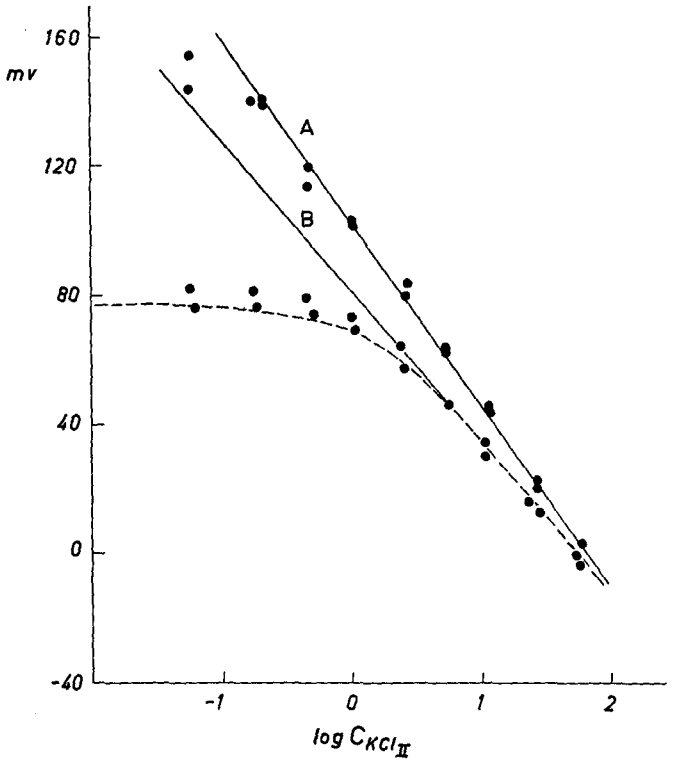

FIGURE 7. EMF of a cell, when Side I, constant KCl concentration of 54 meq/liter. Side II, curve *A*, KCl concentration varied; curve *B*, KCl concentration varied + NaCl 15 meq/liter. Membrane, about 1:1 toluene/butanol. Temperature, 15°C. Polarity refers to side II. The interrupted line has been drawn in accordance with the equation

$$V = 56 \log \frac{54}{[K]_{II} + 2.3}$$

see Discussion.

corresponding points (butanol) in Figs. 2 and 3 of this study. This contradiction could not arise from differences in thickness of the butanol layer, since the same value was found whether the membrane used was 150 or 25 μ thick; therefore this possibility must be eliminated.

2. The filter material is discriminating and the solvent placed on it is an

inert insulating material which prevents short circuiting of the EMF. In such a case the discriminative properties might be considered analogous to those described by Michaelis (7) for a dried collodion membrane, with the choice of interpretation left open (1, pages 205–206; 8). If this line of reasoning is used, then, with toluene placed on the membrane instead of water or butanol, the EMF arising from the selectivity of the filter material would become more evident owing to the better insulating properties of toluene. However, from Fig. 4, curves *B* and *C*, it is apparent that with butanol placed on the membrane the potential expressing the anion/cation permeability ratio is reduced by 50 to 75 per cent compared to the value when toluene is placed on the membrane. Conversely, it becomes clear from curve *A* of the same figure that the potential expressing K/Na permeability ratio is lowered by more than 95 per cent. This could be explained if butanol alone were much more permeable to the cation, or more permeable to Na than to K, which is not the case, as can be gathered from the work of Rosano *et al.* (5).

3. The third assumption would be the formation of the discriminating entity by some interaction between the filter material and the solvent. It is plausible to assume that the filter material imparts to the membrane fixed negative charges thus rendering it more permeable to cation. Furthermore, the vicinity of the anionic sites of the filter material might be "modified" by toluene so as to endow it with K/Na discriminating properties. "Modification" of the charge site by the hydrophobic solvent might imply a change in the amount of water adsorbed. Under this condition the K/Na discrimination would be analogous to the discrimination by an optimal sized aqueous pore, as suggested by Mullins (2). Alternatively, as indicated by Eisenman (9, pages 296–300), the magnitude of selectivity of an anionic site towards alkali metal ions is inversely proportional to the "water swelling" of the anionic site.

It might be interesting to use membranes with defined ionic groups surrounded by hydrophobic medium and to study their selectivity towards alkali metal ions. The hydrophobic fluid could render the ionic sites less susceptible to water swelling and thereby contribute to the high degree of discrimination as found with Na- or K-sensitive glass electrodes (9, 10).

It is probable that a membrane model with fixed charges in an hydrophobic medium would be more relevant to biology since it possesses the two remarkable features of many of the resting excitable membranes, namely high K/Na discrimination and a relatively high resistance.

As to the second point of interest, the relation of KCl concentration to EMF, the results of our experiments show that the potential deviates from the curve of the K electrode membrane at low K concentrations. A similar deviation is also encountered in experiments involving the relation between the outside K concentration and the resting potential (11–13). It has been shown by

Hodgkin and Horowicz (13) that such a relation could conform to a membrane slightly permeable to other ions to which the "constant field" equation—in the form given by Hodgkin and Katz (14) based on Goldman's analysis (15) is applicable.

$$V = \frac{RT}{F} \ln \frac{[K]_I + \alpha_{Na}[Na]_I + \alpha_{Cl}[Cl]_{II}}{[K]_{II} + \alpha_{Na}[Na]_{II} + \alpha_{Cl}[Cl]_I}$$

where α_i is defined by P_i/p_K and $p_i = \beta_i u_i(RT/a)$.
β_i is the distribution coefficient of the i'th ion between the membrane and the aqueous solution and u_i the mobility of the i'th ion through the membrane; a is the thickness of the membrane; R, T, and F have their usual meaning.

Considering that in our experiments α_{Cl} is negligible (as can be judged from curves A in Figs. 6 and 7), α_{Na} can be estimated from curves B of the above figures. Thus curve B of Fig. 6 crosses the level of zero potential at a point corresponding to a K concentration of 2.3 meq/liter. It follows that at such a point $\alpha_{Na}[Na]_{II} = [K]_I$; *i.e.*, $\alpha_{Na} \times 15 = 2.3$ and $\alpha_{Na} = 0.153$.

The interrupted line in Fig. 7 has been drawn according to the constant field equation using the value of 0.153 for α_{Na}. It follows that $V = \frac{RT}{F} \ln \frac{54}{[K]_{II} + 2.3} = 56 \log \frac{54}{[K]_{II} + 2.3}$. As can be observed the calculated curve fits quite well the experimental results.

The application of the constant field equation to the solvents used in the experiments described could also explain the fact that a cell of the type KCl:NaCl gives rise to a potential which is close to the difference between the potentials of the cells KCl:water and NaCl:water (Table I). Therefore, the diffusion potentials of these cells would be expressed by the following equations respectively:

$$V_{KCl:NaCl} = \frac{RT}{F} \ln \frac{[K]_I}{\alpha_{Na}[Na]_{II}}$$

$$V_{KCl:water} = \frac{RT}{F} \ln \frac{[K]_I}{\Sigma \alpha_i c_i}$$

$$V_{NaCl:water} = \frac{RT}{F} \ln \frac{\alpha_{Na}[Na]_I}{\Sigma \alpha_i c_i}$$

where i denotes other ions present in the system, *e.g.* H^+. The result is that the difference between the potentials of the last two cells is equal to the potential of the first cell.

Experiments are now in progress to determine the α_{Na} directly and to see to what extent it corresponds to the potentiometric calculations.

The author wishes to express his gratitude to Professor J. Magnes, for his interest in this work and encouragement during the study.
Received for publication, August 14, 1962.

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