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MEMBRANE POTENTIALS AND ION PERMEABILITY IN A CATION EXCHANGE MEMBRANE

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ABSTRACT The nonequilibrium electrical potentials across an artificial membrane bathed by solutions of a single salt have been measured and calculated using the Goldman-Hodgkin-Katz equation and the irreversible thermodynamic equation. The latter equation predicts the observed potential differences over a 2500-fold concentration range, while application of a modified Goldman-Hodgkin-Katz equation leads to difficulties.

INTRODUCTION

The relation between ion permeability and transmembrane electrical potential difference (p.d.) has been studied extensively in both artificial and biological membranes. There have been numerous attempts to evaluate the p.d. in terms of the permeability properties of the membrane and the ionic composition of the solutions. One of the expressions used frequently in consideration of biological membranes is the so-called "constant field equation" (Goldman, 1943; Hodgkin and Katz, 1949). On the other hand, an expression obtained from nonequilibrium thermodynamics has been used extensively in the study of artificial (particularly ion exchange) membranes (Spiegler, 1958; Mackay and Meares, 1959; Lakshminarayanaiah, 1965; Tombalakian and Graydon, 1966) with only occasional use in biological systems. The purpose of the present studies is to examine the application of these two approaches to the behavior of an ion exchange membrane bathed by solutions of a single electrolyte (NaCl). The ionic permeabilities and p.d. have been investigated over a 2500-fold concentration range, and the results considered in terms of the above equations. Although the system does not meet the criteria for strict validity of the Goldman-Hodkin-Katz equation, the magnitude of the expected deviations is not known.

METHODS

The membrane used for study of permeability and electrical properties was a single sample of sulfonated polystyrene cation exchange resin (Nepton 61AZL-183, formerly CR-61, Ionics, Inc., Watertown, Mass.). The membrane was sealed between two lucite chambers, each having a volume of 5.56 ml, a bore of 1 cm radius, and a single port in the top through which electrodes could be introduced and the solutions changed. The solutions were stirred by magnetically driven stirring bars and the system was maintained at room temperature (22–23°C).

Sodium ion and chloride ion permeabilities were estimated simultaneously from the fluxes of radioactive tracers across the membrane in the absence of a concentration difference. The membrane was equilibrated with nonisotopic solution for at least 1 hr and high specific activity ^{24}NaCl and Na^{36}Cl were added directly to one side. (At lower concentrations, correction was made for carrier NaCl in the tracer solution.) After a 1 hr equilibration period, the less radioactive solution was discarded; the chamber on that side was rinsed and refilled with identical solution and stirred for 5 min. The chamber was then rinsed again and fresh solution added at time zero. Duplicate 0.1 ml samples were taken from the radioactive side during the first 90 sec, and after 20 min the less radioactive solution was removed completely and saved. The chamber was then rinsed, refilled with fresh nonradioactive solution, and the procedure repeated. Several such 20 min flux determinations were made in succession. Duplicate samples of the less radioactive solution and of dilutions of the radioactive side were placed into test tubes to be counted for ^{24}Na in a well-type scintillation counter and into planchets to be counted for ^{36}Cl in a windowless flow counter after the ^{24}Na had decayed to less than one count per minute per planchet. The permeabilities were computed using the equation

$$P = -\frac{V_h V_c}{tA(V_h + V_c)} \ln\left[1 - \frac{T_c(V_h + V_c)}{T_h V_c}\right],$$

in which V_c and V_h are the volumes of the radioactively "cold" and "hot" chambers respectively, T_h the total amount of tracer at the start, and T_c the total amount of tracer that had diffused across the membrane of area A in length of time t.

The electrical potential differences in the presence of a concentration difference were measured utilizing a high input impedance volt meter (Keithley Instruments, Inc., Cleveland, Ohio) and silver–silver chloride electrodes. The net electrode junction potentials calculated from solution activities were subtracted to obtain the transmembrane p.d. In most determinations of p.d., the membrane was preequilibrated with the solution of lower concentration before one chamber was emptied and the more concentrated solution added. The electrodes were placed into the two chambers and the p.d. measured less than 15 sec after forming the cell. However, another procedure was necessary in order to obtain reliable measurements at high concentration differences. In those cases, both chambers were first rinsed five times with distilled water. One chamber was then briefly rinsed (<5 sec) with high concentration solution in order to avoid a distilled water film on the membrane, and the opposite chamber was rinsed with dilute solution. The chambers were filled with appropriate solutions, the dilute side 1 min before the high concentration side, and the electrodes were inserted for a potential measurement within 5 sec after adding the final solution. The observed potential was maximal and stable for 15–30 sec then drifted to a lower stable level over 1–2 min. The time course of a p.d. measurement for the largest concentration difference studied (0.0016 M–4.0 M) is shown in Fig. 1. No decrease in p.d. with time was noted when the concentration difference was less than or equal to that of the 0.0016 M NaCl || 0.5 M NaCl concentration cell and stirring was maximal. Although at the higher concentration differences the initial p.d. was independent of stirring rate, the final p.d. was dependent on the stirring rate in the dilute solution. The final p.d. was higher for faster stirring if the elevated stirring

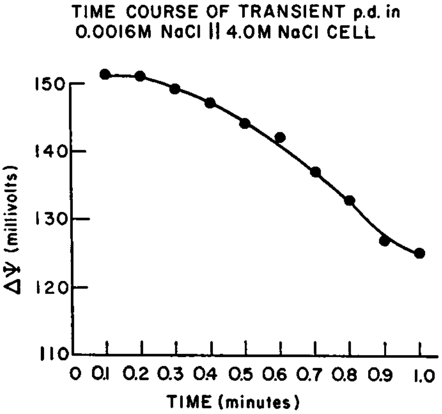

FIGURE 1 Transient p.d. after forming a concentration cell in the presence of maximal stirring.

rate in the dilute solution was continuous from the time of the cell formation or if the rate was stepped at some time during the transient.

To determine fixed charge density, separate samples of the membrane were equilibrated with solutions of various concentrations of NaCl containing ^{22}Na. The membranes were leached with deionized water to remove NaCl and counted directly in the well-type scintillation counter. Samples of the equilibrating solutions were also counted and Na content of the membrane was calculated assuming uniform specific activity. Water content of the membrane equilibrated with the various solutions was determined by drying to a constant weight at 80°C. Fixed charge concentration $[X]$ was taken as equal to Na concentration in eq/liter membrane water.

THE GOLDMAN-HODGKIN-KATZ AND NONEQUILIBRIUM THERMODYNAMIC EQUATIONS AND THEIR APPLICATION TO THE EXPERIMENTAL SYSTEM

The system is a cation exchange membrane separating two solutions (′) (″). The Goldman-Hodgkin-Katz equation for a single univalent salt may be written as

$$\Delta\psi = \psi'' - \psi' = -\frac{RT}{F}\ln\frac{P_{\mathrm{Na}}[\mathrm{Na}]'' + P_{\mathrm{Cl}}[\mathrm{Cl}]'}{P_{\mathrm{Na}}[\mathrm{Na}]' + P_{\mathrm{Cl}}[\mathrm{Cl}]''}, \qquad (1)$$

in which $\psi'' - \psi'$ is the transmembrane p.d., P_{Na}, P_{Cl} are the constant permeabilities, and $[\ ]'$, $[\ ]''$ are the concentrations of the ions on the respective sides; R, T, and F have their usual meanings. The assumptions under which this equation was originally derived (Goldman, 1943) include: (*a*) steady state, (*b*) zero current, (*c*) constant ion mobilities, (*d*) $\partial\mu/\partial x = RT\partial(\ln c_i)/\partial x$ (the effects of variations in activity coefficients and swelling pressure of the membrane on chemical potential are assumed to be negligible), and (*e*) constant electric field between the solutions. The constant field assumption, though sufficient, is not necessary, and more general assumptions have been proposed to extend the validity of an equation of the Goldman-Hodgkin-Katz form (Harris, 1960; Sanblom and Eisenman, 1967). Patlak

(1960) derived an equation of this form without the constant field or constant ion mobility assumptions and interpreted the permeability ratios in terms of unidirectional flux ratios.

There are several problems involved in accomodating this equation to the system used in this study. We shall make the assumption, for the time being, that the experimental membrane is a homogeneous structure with uniformly distributed fixed charges. There is a Donnan potential at each membrane-solution interface making a discontinuity in the electric field. As a result, the constant field assumption is not valid unless the two Donnan potentials are equal and opposite as they would be if the total ion concentrations in the two solutions were equal. In the present system, equal Donnan potentials occur only when there are identical solutions on the two sides and no electrical potential difference.

As pointed out by Teorell (1935), the total membrane potential can be considered as the sum of a Donnan potential at each surface and an internal diffusion potential. We shall describe the internal diffusion potential by equation 1 and add the two Donnan potentials π' and π''. Thus,

$$\Delta\psi_{\text{total}} = \pi' - \pi'' + \Delta\psi_{\text{internal}} \tag{2}$$

$$\Delta\psi_{\text{internal}} = -\frac{RT}{F} \ln \frac{\bar{P}_+ c_+'' + \bar{P}_- c_-'}{\bar{P}_+ c_+' + \bar{P}_- c_-''}, \tag{3}$$

in which $\bar{P}_+$, $\bar{P}_-$ are the permeabilities within the water phase of the membrane for sodium and chloride respectively, and c_+, c_- are the molar ion concentrations in the water phase just inside the two surfaces of the membrane. We are thus taking as a model for the inside of the membrane the implicit assumptions of the Goldman-Hodgkin-Katz equation including constant internal permeabilities. If ion concentrations in the external solutions are denoted by a_+ and a_-, the Donnan distribution coefficient r at each boundary is given by:

$$r = c_+/a_+ = a_-/c_-. \tag{4}$$

Using the Donnan coefficient at each interface denoted by r'' and r', equation 3 becomes

$$\Delta\psi_{\text{internal}} = -\frac{RT}{F} \ln \frac{\bar{P}_+ a_+'' r'' + \bar{P}_- a_-'/r'}{\bar{P}_+ a_+' r' + \bar{P}_- a_-''/r''}. \tag{5}$$

The constant internal permeabilities can be expressed in terms of externally measurable permeabilities by considering the steady-state flux of sodium per unit area J_+ through the membrane. When $\Delta\psi = 0$ and the concentration gradient is the only driving force, $P_+ = J_+/\Delta a_+$ and $\bar{P}_+ = J_+/\Delta c_+$. In the steady state, J_+ across the surface of the membrane is equal to J_+ across an equal coplanar area in the interior of the membrane. If tracers are used to measure the flux and one solution and thus one surface of the membrane is kept at essentially zero concentration, $\Delta a = a$ and $\Delta c = c$ so that we may write

$$Pa = \bar{P}c,$$

or by equation 4,

$$P_+ = \bar{P}_+ r \text{ and } P_- = \bar{P}_-/r. \tag{6}$$

Since $r'' \neq r'$, we must superscribe the permeabilities to identify them with the solution in the chambers when that external permeability was measured. Using equation 6, equation 5 becomes:

$$\Delta\psi_{\text{internal}} = -\frac{RT}{F}\ln\frac{P''_+ a''_+ + P'_- a'_-}{P'_+ a'_+ + P''_- a''_-}. \tag{7}$$

The assumption of constant permeability within a membrane with fixed charges requires the matching of the inconstant external permeabilities with their appropriate external solutions.

In the present single salt system, the Donnan potentials are given by

$$\pi' + \pi'' = -\frac{RT}{F}\ln\frac{r'}{r''}. \tag{8}$$

The total transmembrane potential is given by the sum of equations 7 and 8

$$\Delta\psi_{\text{total}} = -\frac{RT}{F}\ln\left[\frac{r'}{r''}\left\{\frac{P''_+ a''_+ + P'_- a'_-}{P'_+ a'_+ + P''_- a''_-}\right\}\right]. \tag{9}$$

THE IRREVERSIBLE THERMODYNAMIC EQUATION

Scatchard (1953) and Staverman (1952) have derived an expression for the isothermal, isobaric diffusion potential without any limiting assumptions concerning membrane composition or structure, electric field or mobilities. These derivations are based on the assumptions of thermodynamics of irreversible processes (Prigogine, 1955; Katchalsky and Curran, 1965). For the present case, the total membrane potential is:

$$-F(\Delta\psi) = \int_{\mu'}^{\mu''} (t_+ - t_-)\, d\mu_{\text{NaCl}}, \tag{10}$$

where the integral is from one solution to the other, μ is the chemical potential, and t is the transport number of the ion.[1] If transport numbers for sodium and chloride ions are obtained at several concentrations, equation 10 may be integrated graphically assuming linearity of the transport number at interpolated points. Thus, the total potential may be computed independent of any assumptions about the membrane.

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[1] The water transport number of this membrane is relatively low, t_w = 200 ml/Faraday at 0.1 NaCl (Curran, unpublished observations). Experimentally, no change in p.d. was observed when the osmotic gradient was reversed by mannitol addition to the dilute NaCl solution. Therefore, the electroosmotic contribution to the potential has been neglected in equation 10.

The transport number t_k is defined as the fraction of total current carried by the kth ion under conditions of uniform concentration and no bulk flow of water. When there is no concentration gradient, the ion currents at a point for a NaCl system can be expressed as

$$i_+ = -u_+c_+F\frac{d\psi}{dx}; \qquad i_- = -u_-c_-F\frac{d\psi}{dx},$$

where u_+, u_- are the ion mobilities that are assumed to be functions only of concentration and are thus constant throughout the membrane when identical solutions bathe each surface. In the steady state, the ionic currents are uniform across the membrane and the total current $i = i_+ + i_-$. Thus

$$t_+ = \frac{i_+}{i} = \frac{u_+c_+}{u_+c_+ + u_-c_-}, \tag{11}$$

$$t_- = \frac{i_-}{i} = \frac{u_-c_-}{u_+c_+ + u_-c_-}. \tag{12}$$

When a single uniunivalent salt is the only mobile solute present there is a direct relation between permeabilities and transport numbers. Permeabilities have been measured when there is no chemical potential difference across the membrane. Under these circumstances each external concentration a_{NaCl} corresponds to a specific c_+ and c_- within the membrane determined by the equality of the individual electrochemical potentials between the solution and membrane water phases. There also exist an average path length between the membrane surfaces Δx, and an average area available for diffusion A_d. Since

$$u_i = \frac{A_g\Delta x}{A_d RT}\bar{P}_i, \tag{13}$$

where A_g is the geometric area of the membrane, internal permeabilities may be substituted for internal mobilities in equations 11 and 12. Then since $\bar{P}c = Pa$ for each ion and $a_+ = a_-$, we find that

$$t_+ = \frac{\bar{P}_+ r}{\bar{P}_+ r + \frac{\bar{P}_-}{r}} = \frac{P_+}{P_+ + P_-}; \qquad t_- = \frac{\frac{\bar{P}_-}{r}}{\bar{P}_+ r + \frac{\bar{P}_-}{r}} = \frac{P_-}{P_+ + P_-}. \tag{14}$$

In the multi-ion case, the external ion concentrations would not all be equal and the transport number becomes a weighted fraction dependent on the individual ion valences as well as concentration.

RESULTS

The concentration of fixed charge $[X]$ in membrane water at the various NaCl concentrations used is shown in Table I together with the value of r calculated from the relation

$$r = \frac{[X]}{2a} + \left(\left(\frac{[X]}{2a}\right)^2 + 1\right)^{1/2}. \tag{15}$$

The concentration increases progressively as external NaCl concentration increases and the water content decreases from approximately 40 to 35%.

TABLE I

MEMBRANE FIXED CHARGE CONCENTRATION $[X]$ AND CALCULATED DONNAN DISTRIBUTION COEFFICIENT r AT DIFFERENT SALT CONCENTRATIONS (EQUATION 15)

[NaCl]	$[X]$	r
moles/liter	*moles/liter*	
0.0016	2.60	1625
0.0144	2.80	194
0.04	2.89	72.3
0.1	3.08	30.8
0.5	3.05	6.26
1.0	3.18	3.47
4.0	3.60	1.55

TABLE II

SODIUM AND CHLORIDE PERMEABILITIES AND TRANSPORT NUMBERS

Concentration	P_{Na}	Coefficient of variation ($\sigma/\bar{x}$)	No. of determinations	t_{Na}
moles/liter	*cm/min*			
0.0016	0.069	0.044	8	0.9997
0.0144	0.037	0.027	8	0.997
0.04	0.021	0.034	10	0.988
0.1	0.0097	0.027	8	0.960
0.5	0.0031	0.013	11	0.818
1.0	0.0020	0.016	9	0.744
4.0	0.00095	0.009	11	0.686

Concentration	P_{Cl}	Coefficient of variation ($\sigma/\bar{x}$)	No. of determinations	t_{Cl}
moles/liter	*cm/min*			
0.0016	0.00002	0.250	10	0.0003
0.0144	0.000105	0.122	10	0.003
0.04	0.00026	0.053	11	0.012
0.1	0.00041	0.045	11	0.040
0.5	0.000685	0.028	11	0.182
1.0	0.000685	0.030	12	0.256
4.0	0.00044	0.035	12	0.314

The arithmetic means of the tracer permeability measurements of P_{Na} and P_{Cl} at seven concentrations over a 2500-fold range are given in Table II, together with the coefficients of variation. The higher coefficients of variation for P_{Cl} at the low concentration reflect the difficulties of the tracer method at very low ion fluxes. Inasmuch as the subsequent calculations of p.d. and transport number depend only on the order of magnitude of these small permeabilities, the large variations do not compromise the value of the data.

Because of the presence of an unstirred layer at the surfaces of the membrane, partial or complete film control (Helfferich, 1962) will occur when the permeabilities are comparable to diffusion rates in the unstirred layer. Thus, the true permeabilities for sodium when the membrane is bathed in the dilute solutions will be greater than those measured. In order to evaluate this effect we have proceeded as follows. A preliminary estimate of the thickness δ of the unstirred layers was obtained by assuming complete film control for the most dilute solution. The observed P_{Na} would then be given by D_{Na}/δ where D_{Na} is taken as the diffusion coefficient of Na in free solution (1.33×10^{-5} cm²/sec). The calculated value of δ is 116 μ (58 μ on each side of the membrane). Using this value, $\bar{P}_{Na}$ was calculated from equation 6 as well as the relation

$$\frac{1}{P_{obs}} = \frac{1}{P_\delta} + \frac{1}{P_{memb}},$$

using values of r given in Table I. These calculations indicated that $\bar{P}_{Na}$ was approximately constant at 0.0144, 0.04, and 0.1 M. This condition provides a more precise estimate of δ (or P_δ) from the observed values of P_{Na} at these concentrations and the assumption that $\bar{P}_{Na}$ is the same at the three concentrations. The resulting value of δ is 102 μ and this value was used to calculate corrected values of P_{Na} for the membrane which are given in Table III together with calculated values of internal permeability ($\bar{P}_+$ and $\bar{P}_-$). Such corrections are not necessary for chloride because for this ion $1/P_{memb} \gg 1/P_\delta$. The resulting $\bar{P}_+$ increases slightly at high NaCl concentrations but $\bar{P}_-$ varies markedly over the entire concentration range.

Fig. 2 presents the observed transmembrane potentials and those calculated from

TABLE III

CORRECTED P_{Na} AND INTERNAL PERMEABILITIES $\bar{P}_{Na}$ AND $\bar{P}_{Cl}$ ASSUMING THE TOTAL UNSTIRRED LAYER IS 102 μ THICK

Concentration	P_{Na}	$\bar{P}_{Na}$	$\bar{P}_{Cl}$
moles/liter	*cm/min*	$\times 10^{-3}$ *cm/min*	$\times 10^{-3}$ *cm/min*
0.0016	0.59	0.36	32.5
0.0144	0.070	0.36	20.4
0.04	0.029	0.40	18.7
0.1	0.011	0.36	12.6
0.5	0.0032	0.51	4.3
1.0	0.0020	0.59	2.4
4.0	0.00096	0.62	0.7

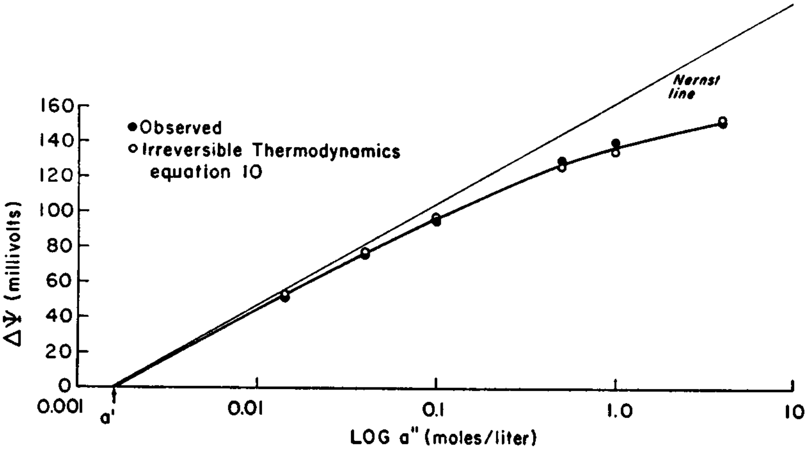

FIGURE 2 The observed and calculated membrane p.d. using equation 10 where a' is the fixed activity of NaCl on the dilute side of the membrane and a'' the activity on the opposite side.

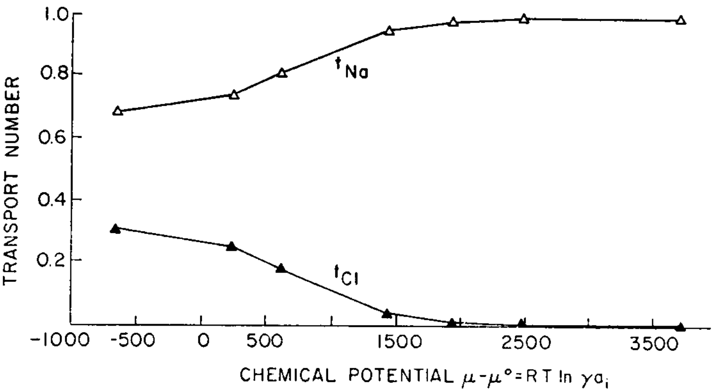

FIGURE 3 Ion transport numbers as a function of solution chemical potential.

the thermodynamic equation 10. Fig. 3, which shows t_{Na} and t_{Cl} as function of $\mu - \mu^0$ (where μ^0 is the standard chemical potential and where γ is the solution activity coefficient), was used to integrate equation 10 and obtain the potentials. The agreement between observed and predicted p.d. values is good over the entire range of concentration; the largest discrepancy was 3.2% at 1.0 M. As shown in Table IV, equation 9 gives an overestimate of the p.d. at all concentrations with the largest discrepancy (22%) at the highest concentration. As might be expected, the Donnan potentials make the major contribution to the calculated values. The calculations using equation 9 are somewhat dependent on evaluation of the effect of film control because the choice of a value for δ influences the values of P_{Na}. However, calculations indicate that different choices for δ from 58 μ (the maximum value) to 10 μ do not have a major effect. Decreasing δ increases the discrepancy between the observed and predicted values.

TABLE IV

OBSERVED AND CALCULATED POTENTIALS USING GOLDMAN-HODGKIN-KATZ EQUATION FOR INTERNAL P.D. PLUS DONNAN P.D.'S AT SURFACE (EQUATION 9) AS A FUNCTION OF ONE SOLUTION CONCENTRATION WHILE THE OTHER SOLUTION WAS FIXED AT 0.0016 M NaCl

Concentration	Observed p.d.	Calculated p.d. (equation 9)	Donnan p.d. (equation 8)
moles/liter	*mv*	*mv*	*mv*
0.0144	53.05	53.8	53.5
0.04	77.2	81.7	78.4
0.1	96.1	101.1	99.9
0.5	130.0	144.2	140.0
1.0	140.0	159.1	154.9
4.0	151.0	183.4	183.4

DISCUSSION

Transients

When the concentration cells were formed using the most dilute (0.0016 M) solution on one side of the membrane and one of the more concentrated solutions (1 M and 4 M) on the opposite side, the observed potential was stable for 15–30 sec and then drifted to a stable lower value over 1–2 min. There are at least two possible mechanisms for this transient behavior: film control and a changing concentration profile. Film control occurs when diffusion across the membrane elevates the salt concentration in the unstirred layer on the dilute solution side, thus reducing the concentration difference across the membrane. This effect is more prominent at greater concentration differences. A second possible cause of transient potentials is a changing concentration profile within the membrane. In dilute solutions the membrane is almost exclusively permeable to sodium and as Conti and Eisenman (1965) have shown, there is no transient when ions of only one sign permeate the membrane. At higher concentrations when both chloride and sodium permeate the membrane the absence of transients is not proven, nor are the magnitude or duration known. Thus a changing concentration profile within the membrane, which undoubtedly occurs since the membrane is equilibrated with the dilute solution just before constituting the cell, might cause the observed transients. This mechanism, however, implies that the steady-state potentials are those observed after, say, 30 sec and not the instantaneous potential. The following evidence is against this and supports the appearance of film control as the mechanism of the transients.

The initial 15–30 sec stability was approximately the time before diffusion could transfer sufficient ions to an unstirred layer and lower the observed potential difference. Experimentally, the value of the steady-state potential was dependent on the rate of stirring the dilute solution only. Increased stirring increased the final potential both when the stirring rate was a step function and when the rate was elevated but uniform during the formation of the cell. The third point in favor of filmcontrolled transients is simply that the thermodynamic potentials calculated by graphic integration of the transport numbers agrees with the instantaneous potentials and not with the steady-state potentials which were 10–25% lower even with the most vigorous stirring. We conclude that the time course, the effect of stirring and the agreement between the initial potentials and those calculated from the thermodynamic equation all support the thesis that the appearance of film control causes these transients.

Membrane Potentials

The equations for membrane potentials that are evaluated in this study are discussed above. The measurements were undertaken with the membrane in two states: (*a*) in equilibrium with two identical solutions, and (*b*) nonequilibrium between two dissimilar solutions. The permeability measurements made under the first circumstances thus correlate a specific external solution with the properties of a specific but unknown internal composition. The measured p.d. on the other hand reflects a profile of internal compositions corresponding to some composite permeability. While Patlak (1960) has shown that the composite permeability as measured by unidirectional flux can be substituted into a Goldman-Hidgkin-Katz equation and yield the observed potentials, the usual procedure has been to use quasi-equilibrium measurements of permeability to deduce the dynamic potential difference. Here we have considered the behavior of an ion exchange membrane in a concentration cell using quasi-equilibrium permeabilities to evaluate two models, the Goldman-Hodgkin-Katz form of equation assuming constant internal ion mobilities with correction for Donnan potentials and the irreversible thermodynamic equation.

The irreversible thermodynamic equation predicts the observed potentials over the entire 2500-fold concentration range. The maximum deviation is 4.2 mv in the 0.0016 M NaCl‖1.0 M NaCl cell where the observed potential was 140 mv. The linear approximation of the transport numbers between the experimental values is probably sufficient to account for this small deviation. However, the general agreement of theory and observation over such a wide range of concentration lends credence to the method of graphical integration using linear interpolation of the transport numbers. The irreversible thermodynamic equation has previously been tested using only twofold concentration differences for each p.d. (Tombalakian and Graydon, 1966; Lakshminarayanaiah, 1969). In this study the p.d. was measured between 0.0016 M NaCl and each of the other concentrations with highest p.d. at a 2500-fold concentration ratio. Although the reason why the apparent transport number for water was negligible in this system is not known, making potential measurements with one solution very dilute may be a factor. Using a similar membrane Tombalakian and Graydon (1966) reported large contributions to the p.d. due to water transport only at high salt concentrations. Furthermore, the remarkable agreement between the observed p.d.'s and those calculated by equation 10 may in part reflect the use of tracers to measure transport numbers. In this system, where probably no carrier-mediated exchange diffusion occurs, the tracer measurement has advantages over the usual current and concentration measurements. Back diffusion of tracer may be easily corrected for and concentration polarization does not occur. In addition, membrane breakdown at high currents (Lakshminarayanaiah, 1966) may interfere in the usual measurements of transport numbers.

The discrepancies in Table IV between the observed p.d.'s and those calculated with equation 9 would appear to be because of incorrect assumptions in the derivation of the equation or incorrect evaluation of the Donnan contribution. There are two alternative ways of attempting to estimate the Donnan contribution to the p.d. According to equation 6, r'/r'' should be given by

$$\frac{r'}{r''} = \frac{P_{\mathrm{Na}'}}{P_{\mathrm{Na}''}}. \qquad (16)$$

Alternatively,

$$\left(\frac{r'}{r''}\right)^2 = \frac{P_{\mathrm{Na}'}P_{\mathrm{Cl}''}}{P_{\mathrm{Na}''}P_{\mathrm{Cl}'}}. \qquad (17)$$

However, both these expressions depend explicitly on the assumption that internal permeabilities $\bar{P}_{\mathrm{Na}}$ and $\bar{P}_{\mathrm{Cl}}$ are constant and the results in Table II suggest quite strongly that this assumption is incorrect. Nonetheless, if equations 16 and 17 are used to estimate r'/r'' and the expected p.d.'s are calculated from equation 9, in neither case do the calculated values agree with those observed. Use of equation 16 leads to an overestimate of 4.5–13.3% at the higher concentrations while equation 17 leads to an *underestimate* at all concentrations with a maximum deviation of 14.6% at the highest concentration. The best estimate of r under equilibrium conditions would appear to be given by equation 15, particularly in view of the inconsistency of internal permeabilities. However, use of these equilibrium values to calculate an effective value of r'/r'' when the membrane is bathed with different solutions on the two sides may not be justified. Because of these difficulties, it is not possible to determine exactly why equation 9 does not provide entirely satisfactory estimates of the p.d. However, the discrepancy between observed and predicted p.d. may be explained by the considerations of MacGillivary and Hare (1969). They showed that the Goldman-Hodgkin-Katz equation is the asymptotic solution of the Nernst-Planck-Poisson equations as the membrane becomes infinitely thin, while the Nernst electroneutrality equation approximates the solution for very thick membranes. While no membrane is infinitely thin and the constant field equation is never strictly valid (Zelman, 1968), it may be approximately true if the membrane is thinner than the Debye length (MacGillivary and Hare, 1969; Agin, 1969) although at this level the assumption of uniform composition in the plane of the membrane becomes questionable. Experimentally, this membrane may be too "thick" for validity of the Goldman-Hodgkin-Katz equation.

The variations in internal permeabilities found in this study appear to be in agreement with previous observations on similar membranes. For example, Mackay and Meares (1959) have found that the self-diffusion coefficients of the counterion increased with external concentration while that of the coion decreased. There appears, at present, to be no completely satisfactory explanation for these changes of ion mobilities within the membrane.

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