

A Theory of Ion Permeation Through Membranes with Fixed Neutral Sites

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Summary. Some model membranes and biological membranes behave as if ion permeation were controlled by fixed neutral sites, i.e., by groups that are polar but lack net charge. By solving the boundary conditions and Nernst-Planck flux equations, this paper derives the expected properties of four types of membranes with fixed neutral sites: model 1, a membrane thick enough that microscopic electroneutrality is obeyed; model 2, same as model 1 but with a free-solution shunt in parallel; model 3, a membrane thin enough that microscopic electroneutrality is violated; and model 4, same as model 3 but with a free-solution shunt in parallel. The conductance-concentration relation and the current-voltage relation in symmetrical solutions are approximately linear for all four models. Partial ionic conductances are independent of each other for a thin membrane but not for a thick membrane. Sets of permeability ratios derived from conductances, dilution potentials, or bionic potentials agree with each other in a thin membrane but not in a thick membrane. The current-voltage relation in asymmetrical single-salt solutions is linear for a thick membrane but nonlinear for a thin membrane. Examples of potential and concentration profiles in a thin membrane are calculated to illustrate the meaning of space charge and the electroneutrality condition. The experimentally determined properties (by A. Cass, A. Finkelstein & V. Krespi) of thin lipid membranes containing "pores" of the anion-selective antibiotic nystatin are in reasonable agreement with model 3. Tests are suggested for deciding if a membrane of unknown structure has neutral sites, whether it is thick or thin, and whether the sites are fixed or mobile.

The best-known artificial membranes exhibiting permeability differences between cations and anions or among ions of the same sign, and hence providing possible models for the selective permeability of biological membranes, are ion-exchangers. These contain ionized sites with net charge, such as $-\text{COO}^-$ or $-\text{NH}_3^+$, confined within the membrane and balanced electrically by mobile ions of opposite sign from the bathing solution. Familiar examples of ion-exchange membranes include glass electrodes, collodion membranes, and liquid ion exchangers. Much of the literature on ion exchangers has been admirably summarized in a review by Teorell (1953) and in papers by Eisenman (1962; Conti & Eisenman, 1965, 1966).

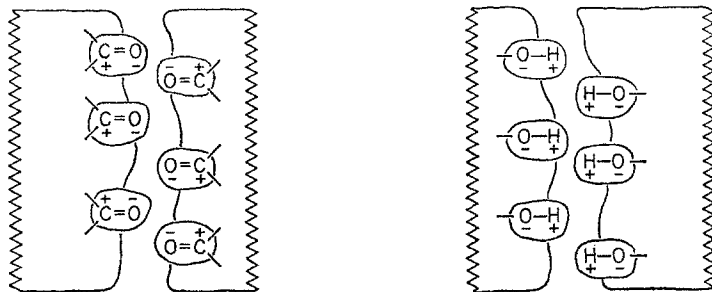


Fig. 1. Examples of neutral membranes which are ion-selective because of fixed polar sites. Left: a channel through the membrane is lined by carbonyl groups, oriented so that the oxygen (negative) ends of the dipoles point towards the center of the channel. Since ion migration takes place in a predominantly negative immediate environment, cations have lower standard chemical potentials, and possibly higher mobilities, in the membrane than do anions, and the membrane is cation-selective. Right: a channel through the membrane is lined by hydroxyl groups, oriented so that the hydrogen (positive) ends of the dipoles point towards the center of the channel. Since ion migration takes place in a predominantly positive immediate environment, anions have lower standard chemical potentials, and possibly higher mobilities, in the membrane than do cations, and the membrane is anion-selective.

More recently, marked ion selectivity has also been observed in so-called neutral membranes, i.e., ones lacking sites with net charge. The sites controlling ion permeation in these membranes prove to be multipolar (specifically, dipolar) groups, such as the carbonyl ($\delta + >C=O \delta -$), ether linkage ($\delta + \begin{array}{c} \diagup C \diagdown \\ \diagdown C \diagup \end{array} O \delta -$), or hydroxyl ($\delta - -O-H \delta +$). Selectivity arises from the fact that the change in standard chemical potential $\Delta\mu^0$ from water to the membrane (i.e., "solubility" in the membrane) may be very different for cations and anions, if the structure of the membrane compels permeating ions to be nearer one end of the polar groups than the other (see Fig. 1). In addition, if ions permeate as independent entities rather than being associated with a molecule bearing polar groups and acting as a carrier, cations and anions might have different mobilities, depending on whether their immediate environment is predominantly positive or negative. The principles governing discrimination among ions of like sign are essentially the same whether the nearest neighbors of permeating ions bear an ionic charge or a partial charge (Diamond & Wright, 1969, pp. 607-608; Eisenman, 1969, pp. 32-36). However, the forms of several of the relations between ion fluxes and external driving forces differ between ion exchangers and neutral membranes because of the electroneutrality condition. Examples of model membranes with mobile neutral sites include thin lipid mem-

branes containing carriers such as monactin, valinomycin, and cyclic polyethers (Pressman, 1968; Tosteson, 1968; Eisenman, Ciani & Szabo, 1968; Szabo, Eisenman & Ciani, 1969; McLaughlin, Szabo, Eisenman & Ciani, 1970*a*), whereas thin lipid membranes containing nystatin "pores" exemplify fixed neutral sites (Finkelstein & Cass, 1968; Cass, Finkelstein & Krespi, 1970). As for possible biological instances, "black-box" experiments suggest that cation permeation in barnacle muscle is controlled by either fixed or mobile neutral sites (Hagiwara, Toyama & Hayashi, 1971), whereas the following papers (Wright, Barry & Diamond, 1971—referred to as paper III—and Barry, Diamond & Wright, 1971—referred to as paper IV) present evidence that cation permeation in gallbladder and perhaps in other epithelia is controlled by fixed neutral sites.

A theoretical treatment of ion permeation in thin membranes with mobile neutral sites under conditions of zero net current was given by Ciani, Eisenman and Szabo (1969). The present paper derives the current-voltage relation, the conductance-concentration relation, and the voltage: concentration-gradient relation for membranes with fixed neutral sites. We consider separately the cases of membranes which are thick enough that microscopic electroneutrality is obeyed, and membranes which are thin compared to the Debye length and in which microscopic electroneutrality may therefore be violated. (The meaning of microscopic electroneutrality is discussed in more detail on pp. 310–319.) In addition, we consider the case, for both a thick membrane and a thin membrane, of a fixed neutral-site channel in parallel with a free-solution shunt. This case may approximate the real situation in some biological experiments, where a shunt may develop in parallel with the native membrane and thus alter measured properties. However, the theoretical treatment offers the possibility of extracting the properties of the native membrane from measured properties of the whole system. Our theory is compared with the experimental studies of Cass *et al.* (1970) on a model membrane containing nystatin pores on pp. 324–325, and with our experimental results on the gallbladder in a subsequent paper (paper IV). Finally, we suggest in the Discussion how to decide if the mechanism of ion permeation in a biological membrane of unknown structure is controlled by fixed neutral sites.

Model 1. Thick Membrane Without Shunt

We consider a membrane lying in a plane perpendicular to the x axis and extending from $x=0$ to $x=d$. The aqueous solutions adjacent to the membrane-solution interfaces at $x=0$ and $x=d$ are indicated by super-

scripts ' and ', respectively. We symbolize concentrations by C , activities by a , activity coefficients by γ , fluxes by J , electrical potentials by ψ , and mobilities by u . The standard chemical potential of the i^{th} ion in water and in the membrane is written as $\mu_i^0(w)$ and $\mu_i^0(m)$, respectively. Consideration is restricted to the case of two univalent cations, symbolized by subscripts 1 and 2, and one univalent anion, symbolized by subscript 3.

The two principal assumptions are as follows.

(a) The membrane contains fixed, neutral (lacking net charge), polar sites, such that the change in μ^0 between the membrane and an aqueous solution is unequal for cations and anions.

(b) The membrane is thick enough that microscopic electroneutrality must be obeyed throughout it (for discussion, *see* pp. 310–319). Since the membrane lacks charged sites, the concentration of mobile cations must equal the concentration of mobile anions at a given point in the membrane.

We further assume the following conditions.

(c) There is perfect stirring on either side of the membrane, such that the bathing solutions have the composition of bulk solution right up to within a few Debye lengths of the membrane-solution interface, and unstirred-layer effects such as the transport-number effect (Barry & Hope, 1969*a, b*; Wedner & Diamond, 1969) and altered boundary-layer concentrations owing to permeation can be neglected.

(d) The relation between the activity a_i and the concentration C_i of the i^{th} ion inside the membrane is assumed to be of the form

$$a_i = \gamma C_i^n \quad (1)$$

where n is the so-called non-ideal activity factor and can have a value other than 1. This expression has been found empirically to hold for a variety of artificial systems (Eisenman, 1962; Garrels & Christ, 1965; Doremus, 1969). The physical significance of n is discussed in paper IV.

(e) μ^0 , γ , and u for each ion, and n , are assumed uniform through the membrane.

(f) γ is assumed to be the same for all ions present, a generalized Guggenheim assumption (Barry & Diamond, 1970, p. 104).

(g) The electrochemical potential μ is assumed continuous across the membrane-solution interface; i.e., the interfacial resistance is negligible.

The total membrane potential, E , is related to the applied voltage across the membrane, E^* , and the voltage for zero current across the membrane,

E_0 by

$$E^* + E_0 = E. \tag{2}$$

E is the sum of two boundary potentials at the membrane-solution interfaces ($\psi'' - \psi(d)$, $\psi(0) - \psi'$) plus the diffusion potential in the membrane interior ($\psi(d) - \psi(0)$):

$$E = \psi'' - \psi' = \psi'' - \psi(d) + \psi(d) - \psi(0) + \psi(0) - \psi'. \tag{3}$$

The procedure is to solve the boundary conditions in order to obtain the two boundary potential terms, and then to solve for the membrane interior potential.

To solve the boundary conditions, we begin by equating the electrochemical potentials of ion i across the $x=0$ interface:

$$\mu_i^0(w) + nRT \ln(a_i')^{1/n} + z_i F \psi' = \mu_i^0(m) + nRT \ln \gamma^{1/n} C_i(0) + z_i F \psi(0). \tag{4}$$

Rearranging, we obtain:

$$C_i(0) = (a_i' K_i / \gamma)^{1/n} e^{-z_i F [\psi(0) - \psi'] / nRT} \tag{5}$$

where the ion-site binding constant K_i is defined by

$$K_i \equiv e^{[\mu_i^0(w) - \mu_i^0(m)] / nRT}. \tag{6}$$

K_i has the significance of the potential-independent part of a partition coefficient. At the $x=d$ interface, an identical expression is obtained for $C_i(d)$, except that a_i' in Eq. (5) is replaced by a_i'' .

By inserting Eq. (5) into the electroneutrality condition,

$$C_1(0) + C_2(0) = C_3(0), \tag{7}$$

one finds that

$$C_1(0) = \theta' (K_1 a_1' / \gamma)^{1/n}, \tag{8a}$$

$$C_2(0) = \theta' (K_2 a_2' / \gamma)^{1/n}, \tag{8b}$$

and

$$C_3(0) = (K_3 a_3' / \gamma)^{1/n} / \theta', \tag{8c}$$

where

$$\theta' \equiv e^{-F [\psi(0) - \psi'] / nRT} = \sqrt{(K_3 a_3')^{1/n} / [(K_1 a_1')^{1/n} + (K_2 a_2')^{1/n}]}. \tag{9a}$$

Expressions identical to Eqs. (8a)–(8c) hold for $C_1(d)$, $C_2(d)$, and $C_3(d)$, except that θ' , a_1' , a_2' , and a_3' are replaced by θ'' , a_1'' , a_2'' , and a_3'' , respectively, where

$$\theta'' \equiv e^{-F [\psi(d) - \psi''] / nRT} = \sqrt{(K_3 a_3'')^{1/n} / [(K_1 a_1'')^{1/n} + (K_2 a_2'')^{1/n}]}. \tag{9b}$$

This completes the solution of the boundary conditions [boundary potentials, Eqs. (9a and b), boundary concentrations, Eqs. (8a–8c)] in terms of standard chemical potentials and bathing solution concentrations. Substituting Eqs. (9a) and (9b) into Eq. (3) gives for the whole potential:

$$E = \psi'' - \psi' = \psi(d) - \psi(0) + \frac{nRT}{F} \ln \frac{\theta''}{\theta'}. \quad (10)$$

We now solve as follows for the membrane interior potential $\psi(d) - \psi(0)$, assuming the validity of the Nernst-Einstein equation and the Nernst-Planck equation.

The Nernst-Planck flux equation for the i^{th} ion within the membrane reads:

$$J_i(x) = -u_i C_i(x) RT d \ln a_i(x)/dx - z_i u_i C_i(x) F d\psi(x)/dx \quad (11)$$

where z_i is the valency. Substituting Eq. (1) [$a_i = \gamma C_i^n$] into the first term on the right-hand side of Eq. (11):

$$-u_i C_i RT d \ln a_i(x)/dx = \frac{-u_i C_i RT}{a_i} \frac{d(\gamma C_i^n)}{dx} = -u_i n RT d C_i(x)/dx. \quad (12)$$

The Nernst-Planck equations thus become:

$$J_1(x) = -u_1 n RT d C_1(x)/dx - u_1 C_1(x) F d\psi(x)/dx, \quad (13a)$$

$$J_2(x) = -u_2 n RT d C_2(x)/dx - u_2 C_2(x) F d\psi(x)/dx, \quad (13b)$$

$$J_3(x) = -u_3 n RT d C_3(x)/dx + u_3 C_3(x) F d\psi(x)/dx. \quad (13c)$$

If one divides Eqs. (13a)–(13c) by u_1 , u_2 , and u_3 , respectively, and adds them, remembering that $C_1(x) + C_2(x) = C_3(x)$ and hence that

$$\frac{dC_1}{dx} + \frac{dC_2}{dx} = \frac{dC_3}{dx},$$

one obtains:

$$-2nRT \frac{dC_3(x)}{dx} = \frac{J_1}{u_1} + \frac{J_2}{u_2} + \frac{J_3}{u_3} \equiv J_+. \quad (14)$$

Since J_+ is independent of x in the steady state, Eq. (14) may be integrated between $x=0$ and $x=d$ to give:

$$C_3(x) = C_3(0) - x J_+ / 2nRT; \quad (15)$$

i. e., the concentration profile in the membrane is linear. Hence

$$C_3(x) = C_3(0) - x[C_3(0) - C_3(d)]/d \tag{16}$$

and

$$J_+ = 2nRT[C_3(0) - C_3(d)]/d. \tag{17}$$

In analogy to the derivation of Eq. (14), if one divides Eqs. (13a)–(13c) by u_1 , u_2 , and u_3 , respectively, and subtracts Eq. (13c) from Eqs. (13a) plus (13b), one obtains

$$-2C_3(x)F d\psi/dx = \frac{J_1}{u_1} + \frac{J_2}{u_2} - \frac{J_3}{u_3} \equiv J_-, \tag{18}$$

which yields upon substitution of Eq. (15) and integration:

$$\psi(d) - \psi(0) = \frac{J_- nRT}{J_+ F} \ln \frac{C_3(d)}{C_3(0)}. \tag{19}$$

Eq. (18) means that the potential gradient in the membrane is not linear, i. e., the field is not constant.

To obtain a general expression for the current requires an algebraic manipulation of the Nernst-Planck flux equations similar to that employed by Planck in deriving his expression for the liquid junction potential. In analogy with Planck [see MacInnes, 1961, p. 462, Eq. (12)], we define

$$U(x) = u_1 C_1(x) + u_2 C_2(x), \tag{20}$$

$$V(x) = u_3 C_3(x). \tag{21}$$

We also define

$$\zeta \equiv \exp \{F[\psi(d) - \psi(0)]/nRT\} \tag{22}$$

and

$$\xi_n \equiv \exp [EF/nRT]. \tag{23}$$

From Eq. (19), Eq. (22) gives:

$$\zeta = [C_3(d)/C_3(0)]^{J_-/J_+}. \tag{24}$$

From Eqs. (17) and (24),

$$J_- = \frac{2nRT[C_3(0) - C_3(d)] \ln \zeta}{d \ln [C_3(d)/C_3(0)]}. \tag{25}$$

From Eqs. (10), (19), (22), and (24):

$$\ln \xi_n = \ln \zeta + \ln \frac{\theta''}{\theta'}$$

and hence

$$\zeta = \theta' \xi_n / \theta'' . \quad (26)$$

We next relate $U(0)$, $U(d)$, $V(0)$, and $V(d)$ to the fluxes and the boundary concentrations. Combining Eqs. (13a)–(13c) with Eqs. (20) and (21):

$$J_1 + J_2 = -nRT dU/dx - FU(x) d\psi/dx , \quad (27)$$

$$J_3 = -nRT dV/dx + FV(x) d\psi/dx . \quad (28)$$

First, we solve Eq. (27). Substituting Eqs. (15) and (18) into Eq. (27):

$$\frac{dU}{dx} - J_- U(x)/2nRT [C_3(0) - xJ_+/2nRT] = -(J_1 + J_2)/nRT . \quad (29)$$

The solution to this first-order linear differential equation (*see, e.g., Ince, 1956, pp. 18–19*) can be shown to be:

$$U(x) = \frac{2(J_1 + J_2)}{(J_+ + J_-)} [C_3(0) - xJ_+/2nRT] + A_1 [C_3(0) - xJ_+/2nRT]^{-J_-/J_+} . \quad (30)$$

Evaluation of Eq. (30) at $x=0$, where $U(x) = U(0)$, gives for the integration constant A_1 :

$$A_1 = [U(0) - 2C_3(0)(J_1 + J_2)/(J_+ + J_-)] / [C_3(0)]^{-J_-/J_+} . \quad (31)$$

Evaluation of Eq. (30) at $x=d$, where $U(x) = U(d)$ and $C_3(0) - xJ_+/2nRT = C_3(d)$, gives, after substitution of Eqs. (24) and (31):

$$\frac{\zeta U(d) - U(0)}{\zeta C_3(d) - C_3(0)} = 2(J_1 + J_2)/(J_+ + J_-) . \quad (32)$$

The solution of Eq. (28) exactly parallels steps (29)–(32) and yields in analogy to Eq. (32):

$$\frac{V(d) - \zeta V(0)}{C_3(d) - \zeta C_3(0)} = 2J_3/(J_+ + J_-) . \quad (33)$$

The current I is given by

$$I = F(J_1 + J_2 - J_3) . \quad (34)$$

Substitution of Eqs. (32) and (33) into (34), and substitution of Eqs. (17) and (25) for J_+ and J_- , finally yields the general expression for the current:

$$I = \frac{nFRT [C_3(0) - C_3(d)]}{d \ln [C_3(d)/C_3(0)]} \left[\left(\frac{\zeta U(d) - U(0)}{\zeta C_3(d) - C_3(0)} \right) \left(\ln \zeta + \ln \frac{C_3(d)}{C_3(0)} \right) - \left(\frac{V(d) - \zeta V(0)}{C_3(d) - \zeta C_3(0)} \right) \left(\ln \frac{C_3(d)}{C_3(0)} - \ln \zeta \right) \right] . \quad (35)$$

Case 1. The Potential at Zero Current, E_0 . When $I=0$, Eq. (35) reduces to

$$\frac{\zeta U(d) - U(0)}{\zeta C_3(d) - C_3(0)} = \left[\left(\ln \frac{C_3(d)}{C_3(0)} - \ln \zeta \right) / \left(\ln \frac{C_3(d)}{C_3(0)} + \ln \zeta \right) \right] \left[\frac{V(d) - \zeta V(0)}{C_3(d) - \zeta C_3(0)} \right]. \quad (36)$$

This is similar to the Planck liquid-junction-potential equation (see MacInnes, 1961, p. 234, Eq. (27)) if $n=1$ and if the values of $U(0)$, $U(d)$, $V(0)$, and $V(d)$ are taken as those on the membrane side of the membrane-solution interfaces.

Inserting Eqs. (20), (21), and (26) into Eq. (36):

$$\begin{aligned} & \frac{\theta' \xi_n [u_1 C_1(d) + u_2 C_2(d)] - \theta'' [u_1 C_1(0) + u_2 C_2(0)]}{\theta' \xi_n C_3(d) - \theta'' C_3(0)} \\ &= u_3 \left[\frac{\ln \frac{C_3(d)}{C_3(0)} - \ln(\theta' \xi_n / \theta'')}{\ln \frac{C_3(d)}{C_3(0)} + \ln(\theta' \xi_n / \theta'')} \right]. \end{aligned} \quad (37)$$

Inserting Eqs. (8a)–(8c), the corresponding expressions for $C(d)$, and Eqs. (9a) and (9b) into Eq. (37) and rearranging:

$$\begin{aligned} & \frac{\ln \left[\frac{(a'_1)^{1/n} + (K a'_2)^{1/n}}{(a'_1)^{1/n} + (K a'_2)^{1/n}} \right]^n + E_0 F / RT}{\ln \frac{a''_3}{a'_3} - E_0 F / RT} \\ &= \frac{u_3 \left\{ \xi_{n,0} [(a'_1)^{1/n} + (K a'_2)^{1/n}] - [(a'_1)^{1/n} + (K a'_2)^{1/n}] \right\}}{u_1 \left\{ \xi_{n,0} [(a'_1)^{1/n} + r(K a'_2)^{1/n}] - [(a'_1)^{1/n} + r(K a'_2)^{1/n}] \right\}}. \end{aligned} \quad (38)$$

Eq. (38) incorporates the new definitions

$$\xi_{n,0} \equiv e^{E_0 F / n R T} \quad (39)$$

[i.e., from Eq. (23), $\xi_n = \xi_{n,0}$ when there is no current and $E = E_0$],

$$K \equiv K_2 / K_1 \quad (40)$$

and

$$r \equiv u_2 / u_1. \quad (41)$$

Eq. (38) is the general solution, for the case of two cations and an anion, for the zero-current potential of a thick fixed-neutral-site membrane,

and must be solved numerically by computer.¹ In Eq. (38), the expression $rK^{1/n} = u_2 K_2^{1/n} / u_1 K_1^{1/n}$ has the significance of the permeability ratio for cations 1 and 2.²

Case 1a. Dilution Potential (e.g., 150 mM NaCl vs. 75 mM NaCl). For the case of a dilution potential ($a'_2 = a'_2 = 0$, $a'_1 = a'_3 = a'$, $a''_1 = a''_3 = a''$)

1 The following numerical procedure is the one we used to extract cation permeability ratios $u_2 K_2^{1/n} / u_1 K_1^{1/n}$ for biionic potentials in gallbladder epithelium from this equation (paper IV, Table 4e). u_3/u_1 may be determined from Eq. (42) by measuring the dilution potential for cation 1, u_3/u_2 by measuring the dilution potential for cation 2 hence, $r \equiv u_2/u_1$ is known. n is determined by either of two methods discussed in paper IV (p. 384 and Fig. 2) or else from the slope of $\log G$ vs. $\log a$ [see Eq. (46)]. Thus if E_0 in a two-cation situation is measured, $K \equiv K_2/K_1$ becomes the only unknown in Eq. (38). To determine K , let

$$A' \equiv \ln \left[\frac{(a'_1)^{1/n} + (K a'_2)^{1/n}}{(a'_1)^{1/n} + (K a'_2)^{1/n}} \right],$$

$$B' \equiv \left(\frac{1}{n} \right) \ln (a'_3/a'_3),$$

$$C' \equiv (u_3/u_1) [(a'_1)^{1/n} + (K a'_2)^{1/n}],$$

$$D' \equiv (u_3/u_1) [(a'_1)^{1/n} + (K a'_2)^{1/n}],$$

$$E' \equiv (a'_1)^{1/n} + r(K a'_2)^{1/n},$$

$$F' \equiv (a'_1)^{1/n} + r(K a'_2)^{1/n}.$$

Then Eq. (38) becomes

$$(A' + \ln \xi_{n,0}) / (B' - \ln \xi_{n,0}) = (C' \xi_{n,0} - D') / (E' \xi_{n,0} - F')$$

or

$$\eta_1 \equiv (A' + \ln \xi_{n,0}) / (B' - \ln \xi_{n,0}) = (\xi_{n,0} - C'') / (D'' \xi_{n,0} - E'') \equiv \eta_2 \quad (38a)$$

where $C'' \equiv D'/C'$, $D'' \equiv E'/C'$, $E'' \equiv F'/C'$, and remembering that $\ln \xi_{n,0} \equiv E_0 F / n RT$. One then computes η_1 and η_2 for a given value of K , and varies K until $\eta_1 = \eta_2$, avoiding the trivial solution of K that makes both η_1 and η_2 zero. This method is similar to that devised by Planck for solving his liquid-junction-potential equation (MacInnes, 1961, p. 235, Fig. 8).

2 Eq. (38) is identical in form to an equation we have derived, extending the treatment of Conti and Eisenman (1965), for the zero-current potential of a fixed-site ion-exchange mosaic, if one replaces u_3/u_1 in Eq. (38) with S^+/S^- in the mosaic equation. S^+/S^- equals u_3/u_1 if (in the terminology of Conti & Eisenman, 1965) $C_0^+(x) = C_0^-(x)$ and if $du/dx = 0 = dC_0^+/dx = dC_0^-/dx$. For the case of dilution potentials, the ion-exchange mosaic equation simplifies to

$$E_0 = \left(\frac{S^+ - S^-}{S^+ + S^-} \right) \frac{RT}{F} \ln \frac{a''}{a'}.$$

This is identical in form to our Eq. (42), S^+ being analogous to $1/u_1$, and is identical to Eq. (99) of Conti and Eisenman (1965) except that they have an error in sign.

Eq. (38) reduces to the form

$$E_0 = -\frac{RT}{F} \frac{(u_1 - u_3)}{(u_1 + u_3)} \ln \frac{a''}{a'}. \tag{42}$$

In Eq. (42), the mobility ratio u_1/u_3 alone has the significance of a permeability coefficient ratio for the i^{th} ion. Note that Eq. (42) is independent of both n and the K 's.

Case 1b. Bionic Potential (e.g., 150 mM NaCl vs. 150 mM KCl). For the case of a bionic potential ($a_2'' = a_3'' = a_3' = a_1'$, $a_1'' = 0 = a_2'$), Eq. (38) reduces to

$$\frac{\ln \frac{K a_2''}{a_1'} + E_0 F/RT}{-E_0 F/RT} = \frac{u_3}{u_1} \left[\frac{\xi_{n,0} (K a_2'')^{1/n} - (a_1')^{1/n}}{\xi_{n,0} r (K a_2'')^{1/n} - (a_1')^{1/n}} \right]. \tag{43}$$

Eq. (43) may be solved for K by the same numerical procedure described in footnote 1 for solving Eq. (38).

Case 2. Conductance and the Current-Voltage Relation in Single-Salt Solutions. We restrict consideration to the case where a single salt is present, though not necessarily at the same concentration on both sides of the membrane; i.e., $a_2' = a_2'' = 0$, $a_1' = a_3' = a'$, $a_1'' = a_3'' = a''$. Eqs. (9a) and (9b) simplify to: $\theta' = \theta'' = (K_3/K_1)^{1/2n}$.

Starting from Eq. (35), inserting Eqs. (8a)–(8c) plus the corresponding equations in $C(d)$ plus the electroneutrality condition plus Eq. (26), and rearranging, one eventually obtains:

$$I = \frac{nRTF}{d\gamma^{1/n}} K_3^{1/n} \left(\frac{K_1}{K_3}\right)^{1/2n} \left[\frac{(a')^{1/n} - (a'')^{1/n}}{\ln \left(\frac{a''}{a'}\right)^{1/n}} \right] \cdot \left\{ u_1 \left[\ln \left(\frac{a''}{a'}\right)^{1/n} + EF/nRT \right] - u_3 \left[\ln \left(\frac{a''}{a'}\right)^{1/n} - EF/nRT \right] \right\}. \tag{44}$$

Substituting Eq. (42) for the zero-current potential E_0 , and remembering from Eq. (2) that the applied voltage $E^* = E - E_0$:

$$I = \frac{-F^2 E^* (u_1 + u_3)}{d\gamma^{1/n}} (K_1 K_3)^{1/2n} \left[\frac{(a'')^{1/n} - (a')^{1/n}}{\ln \left(\frac{a''}{a'}\right)^{1/n}} \right]. \tag{45}$$

In Eq. (45), the expressions $u_1(K_1 K_3)^{1/2n}$ and $u_3(K_1 K_3)^{1/2n}$ have the significance of the cation and anion permeability coefficients, respectively.

Since the factors multiplying E^* in Eq. (45) are independent of I or E^* the current-voltage curves are linear, both in symmetrical and asymmetrical solutions.

In symmetrical solutions where $a' = a'' \equiv a$ and thus

$$[(a')^{1/n} - (a'')^{1/n}] / \ln \left(\frac{a''}{a'} \right)^{1/n} = -(a)^{1/n},$$

Eq. (45) simplifies to the following expression for the conductance G (defined as $-I/E^*$):

$$\begin{aligned} G &= \frac{F^2 a^{1/n}}{\gamma^{1/n} d} (K_1 K_3)^{1/2n} (u_1 + u_3) \\ &= \frac{F^2}{d} \left(\frac{\gamma_w}{\gamma} \right)^{1/n} [C(w)]^{1/n} (K_1 K_3)^{1/2n} (u_1 + u_3) \end{aligned} \quad (46)$$

where $C(w)$ is the salt concentration and γ_w the activity coefficient in the aqueous phase.

The significance of Eq. (46) is that the conductance-concentration relation is linear or nearly linear for n equal to or close to 1. Further, the partial cation conductance $(F^2/d)(\gamma_w/\gamma)^{1/n}[C(w)]^{1/n}u_1(K_1 K_3)^{1/2n}$ and the partial anion conductance $(F^2/d)(\gamma_w/\gamma)^{1/n}[C(w)]^{1/n}u_3(K_1 K_3)^{1/2n}$ are not independent of each other, since the anion equilibrium constant K_3 appears in the cation expression and the cation equilibrium constant K_1 appears in the anion expression. That is, the magnitude of the partial anion conductance depends on which cation is present, and vice-versa – in contrast to the result for a thin membrane [Eq. (102), p. 323]. Figuratively speaking, one could describe this by saying that anions are dragged into the membrane by the boundary potential established by a strongly preferred cation, to preserve electroneutrality.

We may finally note that cation permeabilities are proportional to either u_i , $u_i K_i^{1/n}$, or $u_i K_i^{1/2n}$, depending on whether they are extracted from measurements of dilution potentials [Eq. (42)], biionic potentials [Eq. (43)], or conductances [Eq. (46)], respectively. Thus, these three types of measurements will generally yield numerically different sets of cation permeability ratios, and often even different permeability sequences.³

Experiments on several epithelia (frog gallbladder, choroid plexus, and intestine: E. M. Wright, *personal communication*) suggest that their behavior is approximated by this model of a thick fixed-neutral-site membrane.

³ However, the cation-to-anion permeability ratio extracted from a conductance measurement, $u_1(K_1 K_3)^{1/2n}/u_3(K_1 K_3)^{1/2n} = u_1/u_3$, is the same as the cation-to-anion permeability ratio taken from dilution potentials.

Model 2. Thick Membrane with Shunt

This model is identical to the previous model (thick membrane with fixed neutral sites), except that, in parallel with and insulated from the fixed-neutral-site channels, there are shunts in which ion mobility ratios are the same as in free solution. The effective areas of the neutral-site channel and of the shunt channel are written as ρ_v and ρ_s , respectively. Ion mobilities are represented by u_i in the neutral-site channel and by v_i in the shunt channel. Activity coefficients are represented by γ_v in the neutral-site channel and by γ_s in the shunt channel.

For the neutral-site channel, the excess cation flux over anion flux

$$J \equiv J_1 + J_2 - J_3 = I/F \tag{34}$$

is obtained from Eq. (35) which, after substituting Eqs. (8)–(10) and (20)–(23), eventually becomes:

$$J = \frac{\rho_v n RT}{\gamma_v^{1/n} d} \frac{[(K_3 a'_3)^{1/n}/\theta' - (K_3 a''_3)^{1/n}/\theta'']}{\ln \left[\frac{\theta'}{\theta''} \left(\frac{a''_3}{a'_3} \right)^{1/n} \right]} \left\{ H - u_3 \left[\ln \left(\frac{a''_3}{a'_3} \right)^{1/n} - \ln \xi_n \right] \right\} \tag{47}$$

where

$$H \equiv \frac{\left[\ln \xi_n + \ln \frac{(K_1 a'_1)^{1/n} + (K_2 a'_2)^{1/n}}{(K_1 a'_1)^{1/n} + (K_2 a'_2)^{1/n}} \right]}{\xi_n [(K_1 a'_1)^{1/n} + (K_2 a'_2)^{1/n}]} \cdot \frac{\{ \xi_n [u_1 (K_1 a'_1)^{1/n} + u_2 (K_2 a'_2)^{1/n}] - [u_1 (K_1 a'_1)^{1/n} + u_2 (K_2 a'_2)^{1/n}] \}}{-[(K_1 a'_1)^{1/n} + (K_2 a'_2)^{1/n}]} \tag{48}$$

For the shunt channel, the excess cation flux over anion flux

$$J^* \equiv J_1^* + J_2^* - J_3^* \tag{49}$$

is given by an expression similar to Eq. (47), setting $n = 1$, $K_1 = K_2 = K_3 = 1$, and replacing ξ_n by ξ :

$$J^* = \frac{\rho_s RT}{\gamma_s d} \left[\frac{a'_3 - a''_3}{\ln \left(\frac{a''_3}{a'_3} \right)} \right] \cdot \left\{ \left[\frac{\xi (v_1 a'_1 + v_2 a'_2) - (v_1 a'_1 + v_2 a'_2)}{\xi (a'_1 + a'_2) - (a'_1 + a'_2)} \right] \left[\ln \left(\frac{\xi a''_3}{a'_3} \right) \right] - v_3 \ln \left(\frac{a''_3}{\xi a'_3} \right) \right\} \tag{50}$$

where

$$\xi \equiv e^{EF/RT} \tag{51}$$

The general current equation

$$I = F(J + J^*), \quad (52)$$

obtained from the sum of Eqs. (47) and (50), is unwieldy and does not simplify. However, several particular cases do simplify.

Case 1. Dilution Potentials. If $a'_2 = a''_2 = 0$, $a'_1 = a'_3 \equiv a'$, $a''_1 = a''_3 \equiv a''$, $I = 0$, then $\theta' = \theta'' = (K_3/K_1)^{1/2n}$, and $J = -J^*$. Eq. (52), substituting Eqs (47), (48), and (50), simplifies to:

$$E_0 = \frac{-RT}{F} \frac{(P_1 - P_3)}{(P_1 + P_3)} \ln \frac{a''}{a'} \quad (53)$$

where

$$P_1 \equiv u_1(K_1 K_3)^{1/2n} + \lambda_s A_n v_1, \quad (54)$$

$$P_3 \equiv u_3(K_1 K_3)^{1/2n} + \lambda_s A_n v_3, \quad (55)$$

$$\lambda_s \equiv \rho_s \gamma_v^{1/n} / \rho_v \gamma_s, \quad (56)$$

and

$$A_n \equiv (a' - a'')/n [(a')^{1/n} - (a'')^{1/n}]. \quad (57)$$

λ_s is a measure of the area of the shunt relative to the area of the cation channel. In Eqs. (54) and (55), the first term on the right-hand side [$u_1(K_1 K_3)^{1/2n}$ or $u_3(K_1 K_3)^{1/2n}$] represents the permeability of the neutral-site channel, and the second term ($\lambda_s A_n v_1$ or $\lambda_s A_n v_3$) the permeability of the shunt, to the cation and the anion, respectively. Note that Eq. (53) is identical in form to the corresponding equation for a fixed-neutral-site channel without shunt [Eq. (42)]. Thus, the presence of a shunt does not affect the form of the relation between dilution potentials and concentration gradient, though it changes the meaning of the permeability parameters.

Case 2. Conductance and the Current-Voltage Relation in Single-Salt Solutions. In asymmetrical solutions of a single salt ($a'_2 = a''_2 = 0$, $a'_1 = a'_3 \equiv a'$, $a''_1 = a''_3 \equiv a''$), so that $\theta' = \theta'' = (K_3/K_1)^{1/2n}$, Eqs. (47), (48), (50), and (52) simplify to:

$$I = -G_n E^* (P_1 + P_3) \quad (58)$$

where P_1 and P_3 are given by Eqs. (54) and (55), and

$$G_n \equiv \frac{\rho_v F^2}{\gamma_v^{1/n} d} \left[\frac{(a'')^{1/n} - (a')^{1/n}}{\ln(a''/a')^{1/n}} \right]. \quad (59)$$

In symmetrical solutions of a single salt ($a' = a'' \equiv a$), Eq. (58) still holds, with G_n and A_n simplified to

$$G_n = \rho_v F^2 a^{1/n} / \gamma_v^{1/n} d, \tag{60}$$

$$A_n = a^{(n-1)/n}. \tag{61}$$

Eq. (58) means that the current-voltage relation is linear in both symmetrical and asymmetrical solutions. Eqs. (60) and (61) mean that the conductance-concentration relation in symmetrical solutions is linear if $n=1$. Eqs. (58) and (60) are the same as the corresponding equations for a thick membrane without shunt [Eqs. (45) and (46)], except that the meaning of the permeability coefficient P_i is changed from $u_i(K_1 K_3)^{1/2n}$ to $[u_i(K_1 K_3)^{1/2n} + \lambda_s A_n v_i]$.

Eqs. (57) and (61) for A_n are identical if $n=1$, and yield closely similar values if n is not too far from 1. For instance, with $n=0.8$, $A_n=0.29$ from Eq. (61), whereas $A_n=0.31$ from Eq. (57) if $a''/a'=2$. Since permeability ratios are a function of A_n [Eqs. (54) and (55)], sets of ratios extracted from dilution potentials and conductances will be identical if $n=1$ and very similar if $n=0.8$.

Case 3. Bionic Potentials. If $a'_1 = a'_2 = 0$, $a'_1 = a'_3 \equiv a'$, $a''_2 = a''_3 \equiv a''$, and $I=0$, then $\theta' = (K_3/K_1)^{1/2n}$, $\theta'' = (K_3/K_2)^{1/2n}$, and $J = -J^*$. Eq. (52), substituting Eqs. (47), (48), and (50), becomes:

$$\frac{[(K_1 K_3)^{1/2n} (a')^{1/n} - (K_2 K_3)^{1/2n} (a'')^{1/n}] \left[H - u_3 \ln \frac{a''}{\xi_0 a'} \right]}{\ln \left[\left(\frac{K_2}{K_1} \right)^{1/2n} \left(\frac{a''}{a'} \right)^{1/n} \right]} = \frac{\lambda_s v_3 (a'' - a')}{\ln \left(\frac{a''}{a'} \right)} \left[\frac{(\xi_0 a'' v_2 / v_3 - v_1 a'_1 / v_3) \ln \left(\frac{\xi_0 a''}{a'} \right)}{\xi_0 a'' - a'} - \ln \frac{a''}{\xi_0 a'} \right] \tag{62}$$

where now

$$H = \left(\ln \frac{\xi_0 K_2 a''}{K_1 a'} \right) \left[\frac{\xi_{n,0} u_2 (K_2 a'')^{1/n} - u_1 (K_1 a')^{1/n}}{\xi_{n,0} (K_2 a'')^{1/n} - (K_1 a')^{1/n}} \right], \tag{63}$$

$$\xi_0 \equiv e^{E_0 F / RT}, \tag{64}$$

$$\xi_{n,0} \equiv e^{E_0 F / nRT}. \tag{65}$$

Eq. (62) is too complex to be of use for extracting permeability ratios from experimental potential differences, even when simplified by setting $u_3 = 0$ (as in the gallbladder: see paper IV, p. 382).

In the following papers (papers III and IV), we shall show that ion permeation across rabbit gallbladder epithelium corresponds well to the predictions of this model of a thick fixed-neutral-site membrane with shunt

Differences Between "Thick" Membranes and "Thin" Membranes

The differences between a thick membrane and a thin membrane may be discussed in terms of the concepts of microscopic electroneutrality and the so-called Debye length. In a salt solution, the tendency of ions to disperse randomly is opposed by the electrical work required to separate positive and negative charges. Thus, the concentration of cations as a function of distance from an anion follows a Poisson-Boltzmann distribution from which the Debye length L may be defined as a measure of the average distance between cations and anions. L^2 varies directly as the dielectric constant and inversely as the salt concentration of the medium, and is given by Eq. (80) (in the bathing solutions) or Eq. (72) (within the membrane). If cations and anions are unequally soluble in a membrane [i.e. have different values of $\mu^0(w) - \mu^0(m)$] because of carriers, multipolar sites or charged sites, then the membrane region within a few Debye lengths of the membrane-solution interface will contain a net excess of charges of one sign, balanced by an excess of oppositely charged ions in the aqueous solution within a few Debye lengths of the interface. This excess space charge is a function of the bathing solution ion concentrations and the ion equilibrium constants of the membrane sites. A consequence of the space charge is that the electrical potential ψ immediately at the solution side of the interface is not identical to ψ in the bulk solution.

If, now, the membrane is sufficiently thin that its thickness is comparable to or less than its Debye length, then this space-charge region extends through the whole membrane thickness, and the space-charge density represents a significant fraction of the total ion concentrations in the membrane. Furthermore, the change in ψ across the interface is sufficiently gradual compared to the membrane thickness that it is meaningless to refer to boundary potentials; and $\psi(x)$ and ion concentrations $C_i(x)$ are not uniform throughout the thickness even when the membrane separates identical solutions.

If, on the other hand, a membrane phase is very thick compared to the Debye length within it, then the space-charge region at the interfaces occupies an insignificant fraction of the membrane thickness. The membrane as a whole, and any microscopic portion of it that is also thick compared

to the Debye length, is virtually electroneutral; i.e., the difference between cation and anion concentrations is a negligible fraction of the concentrations. From this it follows, as assumed in the previous two models and discussed further below, that there is a change in ψ across the interface within a distance that is a very small fraction of the membrane thickness, making it possible to refer meaningfully to this change as a boundary potential; it also follows that in symmetrical solutions $\psi(x)$ and $C_i(x)$ are uniform throughout virtually the whole membrane thickness.

In this section we derive $\psi(x)$ and $C_i(x)$ for a thin membrane with fixed neutral sites in symmetrical solutions at zero current. Our treatment considers the membrane to be homogeneous, but the conclusions may also be qualitatively applicable to a predominantly low-dielectric-constant membrane in which the sites are confined to high-dielectric-constant pores (e.g., nystatin-treated bilayers: pp. 324–325). A derivation for a thin membrane with neutral carriers has been given by Ciani *et al.* (1966).

Assumptions and notation are the same as for model 1, except that for mathematical convenience the center of the membrane is taken as $x=0$ so that the interfaces become $x=-d/2$ and $x=+d/2$; activities are equated with concentrations; and consideration is restricted to symmetrical solutions of a single cation (subscript 1) and a single anion (subscript 3), i.e., $a'_1 = a'_3 = a''_3 = a'_3 = a'_1 \equiv a$. Across the membrane-solution interfaces, the electric potential ψ , the electric displacement vector D , and the electrochemical potential μ are continuous; the standard chemical potentials μ_i^0 , the relative dielectric constant ϵ , the ion concentrations C_i , and the electric field or potential gradient $d\psi/dx$ are discontinuous. The rationalized MKS system of electrical units is used. The problem is to derive $\psi(x)$, $C_1(x)$, and $C_3(x)$ as a function of the bathing-solution activity a , the standard chemical potential of the cation $\mu_1^0(m)$ and of the anion $\mu_3^0(m)$ in the membrane, and the relative dielectric constant in the membrane ϵ_m and in the aqueous solution ϵ_w .

We begin by deriving an expression for $\psi(x)$ in the membrane phase ($0 \leq |x| \leq |d/2|$).

Within the membrane

$$a_1(x) = K_1 a e^{-F[\psi(x) - \psi(\infty)]/RT}, \tag{66}$$

$$a_3(x) = K_3 a e^{+F[\psi(x) - \psi(\infty)]/RT} \tag{67}$$

where K_1 and K_3 are related to $\mu_1^0(m)$ and $\mu_3^0(m)$ by Eqs. (6a) and (6c), and $\psi(\infty)$ and $\psi(-\infty)$ are taken as zero.

The excess space charge concentration in the membrane, ρ , is given by

$$\rho(x) = F[a_1(x) - a_3(x)] \quad (68)$$

where concentrations have been approximated by activities.

The Poisson equation reads

$$\frac{d^2 \psi}{dx^2} = -\rho / \epsilon_m \epsilon_0 \quad (69)$$

where ϵ_0 is the permittivity of free space.

Substituting Eqs. (66) and (67) into Eq. (68) and then Eq. (68) into Eq. (69) yields the Poisson-Boltzmann equation:

$$\frac{d^2 \psi}{dx^2} = \frac{aF}{\epsilon_m \epsilon_0} (K_3 e^{F\psi/RT} - K_1 e^{-F\psi/RT}). \quad (70)$$

Provided the space charge is sufficiently low that $\psi(x) \ll RT/F$ (see p. 319) one may introduce the approximation $e^{\pm F\psi/RT} \sim 1 \pm F\psi/RT$ so that Eq. (70) becomes

$$\frac{d^2 \psi}{dx^2} = \frac{\psi}{L_m^2} + \frac{(K_3 - K_1)aF}{\epsilon_m \epsilon_0} \quad (71)$$

where L_m , the effective Debye length in the membrane, is defined by

$$L_m \equiv \sqrt{RT \epsilon_m \epsilon_0 / F^2 a (K_1 + K_3)}. \quad (72)$$

The solution to Eq. (71) is

$$\psi(x) = \frac{RT(K_1 - K_3)}{F(K_1 + K_3)} + A_1 e^{-x/L_m} + A_2 e^{x/L_m} \quad (73)$$

where A_1 and A_2 are constants of integration.

Combining Eq. (73) with the symmetry condition $\psi(-d/2) = \psi(+d/2)$ yields

$$A_1 = A_2 \quad (74)$$

so that Eq. (73) becomes

$$\psi(x) = \frac{RT(K_1 - K_3)}{F(K_1 + K_3)} + 2A \cosh(x/L_m), \quad \text{for } 0 \leq |x| \leq |d/2|. \quad (75)$$

We next derive an expression for $\psi(x)$ in the aqueous phase ($|x| \geq |d/2|$)

The conditions

$$a_1(x) = a e^{-F\psi/RT}, \tag{76}$$

and

$$a_3(x) = a e^{F\psi/RT} \tag{77}$$

combined with Eqs. (68) and (69) give the Poisson-Boltzmann equation:

$$\frac{d^2 \psi}{dx^2} = \frac{aF}{\epsilon_w \epsilon_0} (e^{F\psi/RT} - e^{-F\psi/RT}) \tag{78}$$

where ϵ_w is the relative dielectric constant of the aqueous phase. The condition $\psi \ll RT/F$ simplifies Eq. (78) to:

$$\frac{d^2 \psi}{dx^2} = \psi/L_w^2 \tag{79}$$

where L_w , the Debye length in the aqueous phase, is defined by

$$L_w \equiv \sqrt{RT \epsilon_w \epsilon_0 / 2F^2 a}. \tag{80}$$

The solution to Eq. (79) is

$$\psi(x) = B_1 e^{x/L_w} + B_2 e^{-x/L_w}. \tag{81}$$

As $x \rightarrow \infty$, $\psi(x) \rightarrow 0$, so that $B_1 = 0$ and Eq. (81) becomes

$$\psi(x) = B_2 e^{-x/L_w}, \quad \text{for } |x| \geq d/2. \tag{82}$$

To solve for A_1 in Eq. (75) and B_2 in Eq. (82), we invoke the continuity of the electric displacement vector D at the interfaces $x = \pm d/2$ (Peck, 1953), assuming surface charge and surface dipole potentials to be negligible:

$$D = \epsilon_m \left. \frac{d\psi}{dx} \right|_m = \epsilon_w \left. \frac{d\psi}{dx} \right|_w \tag{83}$$

where $\left. \frac{d\psi}{dx} \right|_m$ and $\left. \frac{d\psi}{dx} \right|_w$ refer to the value of $\frac{d\psi}{dx}$ on the membrane side and the aqueous side, respectively, of the interface.

The condition that $\psi(x)$ is continuous at the interfaces gives, equating Eqs. (75) and (82) and letting $x = d/2$:

$$B_2 e^{-d/2 L_w} = \frac{RT(K_1 - K_3)}{F(K_1 + K_3)} + 2A_1 \cosh(d/2 L_m). \tag{84}$$

The condition Eq. (83) gives, substituting Eqs. (75) and (82) and letting $x = d/2$:

$$\frac{-\varepsilon_w B_2}{L_w} e^{-d/2 L_w} = \frac{2\varepsilon_m A_1}{L_m} \sinh(d/2 L_m). \quad (85)$$

Combining Eqs. (84) and (85) to solve for A_1 :

$$A_1 = \frac{-RT(K_1 - K_3)}{2F(K_1 + K_3) \left(\cosh \frac{d}{2L_m} + \frac{\varepsilon_m}{\varepsilon_w} \frac{L_w}{L_m} \sinh \frac{d}{2L_m} \right)}. \quad (86)$$

Inserting Eq. (86) into Eq. (75):

$$\psi(x) = \frac{RT(K_1 + K_3)}{F(K_1 + K_3)} \left[1 - \left(\cosh \frac{x}{L_m} \right) / \left(\cosh \frac{d}{2L_m} + \eta \sinh \frac{d}{2L_m} \right) \right], \quad (87)$$

$$0 \leq |x| \leq |d/2|,$$

where η is defined by

$$\eta \equiv \frac{\varepsilon_m L_w}{\varepsilon_w L_m} = \sqrt{(K_1 + K_3) \varepsilon_m / 2 \varepsilon_w} \quad (88)$$

since, from Eqs. (72) and (80),

$$L_w/L_m = \sqrt{(K_1 + K_3) \varepsilon_w / 2 \varepsilon_m}. \quad (89)$$

Inserting Eq. (86) into Eq. (85) to solve for B_2 and inserting the resulting expression for B_2 into Eq. (82):

$$\psi(x) = \frac{\eta RT(K_1 - K_3) \left(\sinh \frac{d}{2L_m} \right) e^{(d/2 - x)/L_w}}{F(K_1 + K_3) \left(\cosh \frac{d}{2L_m} + \eta \sinh \frac{d}{2L_m} \right)}, \quad |x| \geq |d/2|. \quad (90)$$

Note that Eqs. (87) and (90) assume the same value at $x = \pm d/2$, as required by the assumption that $\psi(x)$ is continuous. Inserting Eq. (87) into Eqs. (66) and (67), and approximating $C_1(x)$ and $C_3(x)$ by $a_1(x)$ and $a_3(x)$, respectively, gives expressions for ion concentrations in the membrane.

Eqs. (66), (67), (87), and (90) have been used to calculate the typical potential and concentration profiles in a thin membrane depicted in Fig. 2, the effect of salt concentration on the potential profile (Fig. 3), the effect of ion equilibrium constants K_i on potential profiles (Figs. 4 & 5), and the effect of membrane thickness on the potential profile (Fig. 6). The following qualitative conclusions about thin membranes emerge from Figs. 2-6:

1. Provided that cations and anions have unequal equilibrium constants (standard chemical potentials) in the membrane, they also have unequal

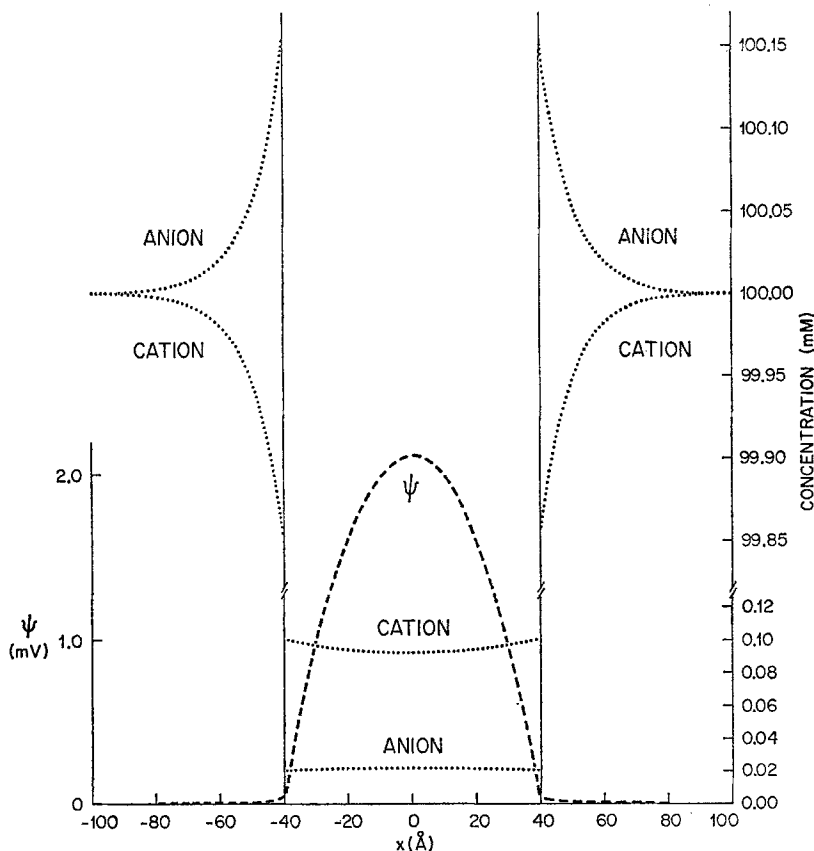


Fig. 2. Calculated potential profile (dashed line) and concentration profiles (dotted lines) in a thin membrane with fixed neutral sites (ordinate), as a function of x (abscissa), the distance in angstroms from the center of the membrane. The membrane thickness d is 80 \AA , so that the membrane extends from $x = -40$ to $x = +40 \text{ \AA}$, while the aqueous solutions are at $|x| \geq 40 \text{ \AA}$. The cation equilibrium constant K_1 is taken as 1×10^{-3} , the anion equilibrium constant K_3 as 0.2×10^{-3} ; the cation and anion concentrations in the bulk bathing solutions are 100 mM , the temperature 20°C , and the relative dielectric constants in water and in the membrane ϵ_w and ϵ_m are taken as 80 and 3 , respectively. $\psi(x)$ was calculated from Eq. (87) inside the membrane and Eq. (90) outside, the cation concentration from Eq. (66) inside and Eq. (76) outside, and the anion concentration from Eq. (67) inside and Eq. (77) outside. The calculated Debye length is 76.1 \AA in the membrane (L_m), and 9.6 \AA outside the membrane in the aqueous solution (L_w). The figure has been condensed by interrupting the concentration axis between 0.12 and 99.85 mM . Note that there is no boundary potential, and that the cation concentration exceeds the anion concentration in the membrane, whereas the reverse is true in the aqueous solutions within ca. 100 \AA of the interfaces. For all sets of values in Figs. 2-6, the calculated space charge inside the membrane [i.e., the area between the cation and anion curves, or the integral of $a_1(x) - a_3(x)$] equals the space charge outside the membrane within the error of the numerical integration procedure used ($\pm 5\%$); an agreement that would not have been obtained had the approximation $\psi \ll RT/F$ introduced significant error

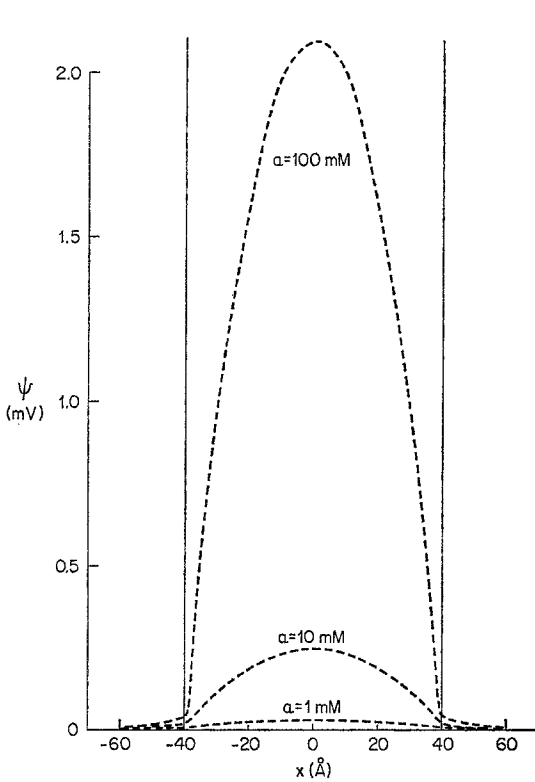


Fig. 3

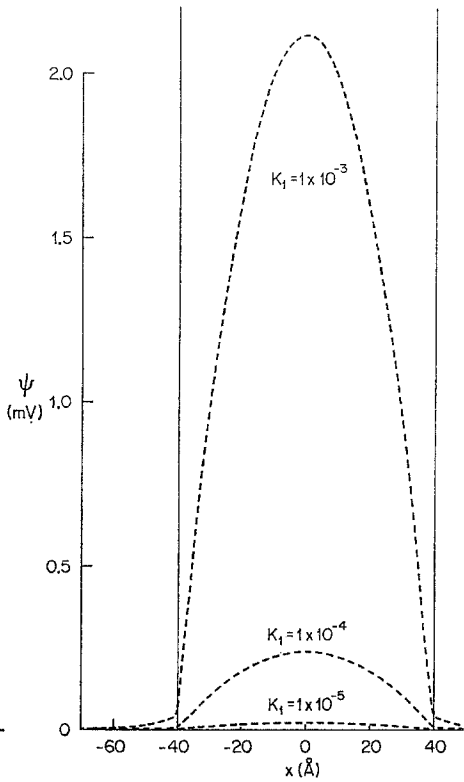


Fig. 4

Fig. 3. Potential profiles in a thin membrane with fixed neutral sites (dashed lines, ordinate), calculated from Eqs. (87) and (90), as a function of bulk bathing-solution concentration. The abscissa is x , the distance from the center of the membrane. The membrane thickness d is taken as 80 \AA , the equilibrium constants K_1 as 1×10^{-3} and K_3 as 0.2×10^{-3} , the bulk-solution concentrations a as 1, 10, or 100 mM, and the other parameters as in Fig. 2. The calculated Debye length is 761.3, 240.7, and 76.1 \AA inside the membrane, and 96.3, 30.5, and 9.6 \AA outside, at 1, 10, and 100 mM, respectively.

Note that ψ increases with concentration

Fig. 4. Potential profiles in a thin membrane with fixed neutral sites (dashed lines, ordinate), calculated from Eqs. (87) and (90), as a function of ion equilibrium constants. The abscissa is x , the distance from the center of the membrane. The membrane thickness d is taken as 80 \AA , the bulk bathing-solution concentration a as 100 mM, the equilibrium constant K_1 as 1×10^{-5} , 1×10^{-4} , or 1×10^{-3} , K_3 as $0.2 K_1$ in each case, and the other parameters as in Fig. 2. The calculated Debye length is 761.3, 240.7, and 76.1 \AA inside the membrane at $K_1 = 10^{-5}$, 10^{-4} , and 10^{-3} , respectively, and 9.6 \AA outside the membrane in all three cases. Note that ψ increases with K

concentrations; i.e., the membrane bears a net charge (Fig. 2). The excess cation concentration in the membrane is balanced by an excess anion concentration immediately adjacent to the membrane, as seen by comparing

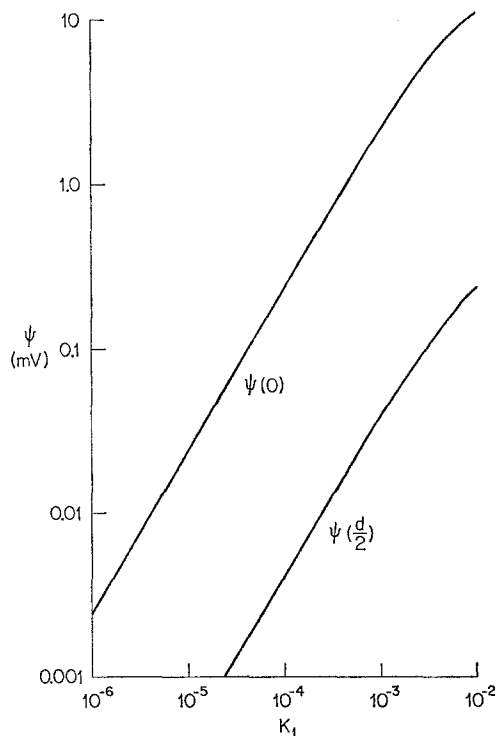


Fig. 5. Potentials in a thin membrane with fixed neutral sites (ordinate), calculated from Eq. (87), as a function of the cation equilibrium constant K_1 (abscissa). Both axes are logarithmic. The curve $\psi(0)$ gives the potential at the center of the membrane, whereas $\psi(d/2)$ is the potential at the membrane-solution interface. The membrane thickness d is taken as 80 \AA , the bulk bathing-solution concentration a as 100 mM , K_3 as $0.2 K_1$, and the other parameters as in Fig. 2. As K_1 goes from 10^{-6} to 10^{-2} , the calculated Debye length goes from 2407 to 24.1 \AA in the membrane and remains at 9.6 \AA outside. Note that $\psi(0)$ is always much greater than $\psi(d/2)$, and that both increase with K_1 .

the areas between the cation and anion curves of Fig. 2 inside and outside the membrane. (This statement would also apply to a thick membrane, but the excess cation concentration would then be an insignificant fraction of the total concentration, whereas the cation concentration is approximately five times the anion concentration in the thin membrane depicted in Fig. 2.) For most of the curves of Figs. 2–6, the Debye length is comparable to or greater than the membrane thickness.

2. The potential within the membrane is not uniform but reaches a maximum in the center, and rises so gradually from the interfaces to the center that it would be meaningless to speak of a boundary potential (Figs. 2–4, and the lower curves of Fig. 6). The potential immediately

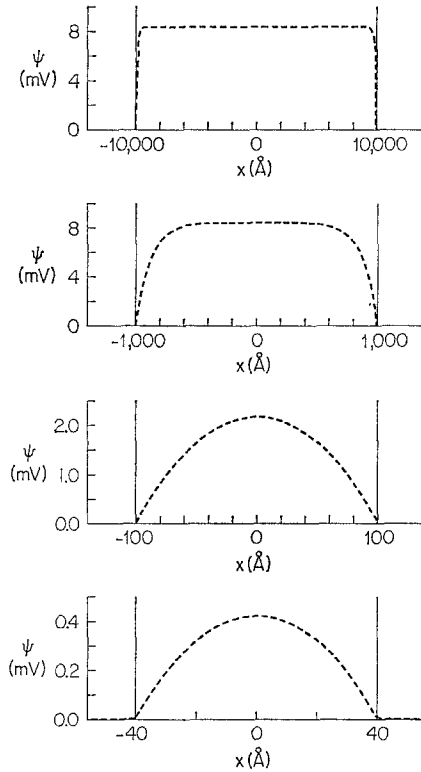


Fig. 6. Potential profiles in a thin membrane with fixed neutral sites, for four different membrane thicknesses, calculated from Eq. (87). In each case, ψ in mV (ordinate) is plotted as a function of the distance x from the center of the membrane in angstroms (abscissa). The membrane thickness d is taken as 20,000, 2,000, 200, and 80 Å in the top, upper-middle, lower-middle, and bottom curve, respectively. K_1 is taken as 2×10^{-4} , K_3 as 1×10^{-4} , a as 150 mm, and the other parameters as in Fig. 2. The calculated Debye length is 124.3 Å inside the membrane and 7.9 Å outside in all cases. Note that in the thickest membrane (top) virtually the whole potential is developed within a small fraction of the membrane thickness near the interface and may be considered a boundary potential, but that the potential change becomes increasingly smoothly distributed through the membrane as the membrane becomes thinner; also note that the potential increases with increasing thickness until a limiting value of 8.4 mV is reached, the boundary potential for a thick membrane with $K_1/K_3 = 2$

adjacent to the membrane differs slightly from the bulk-solution value (best illustrated in Fig. 5, lower curve).

3. For low values of membrane space charge, the potential is low (≤ 10 mV, often $\ll 1$ mV, in Figs. 2–6). If $\psi \ll RT/F$, ψ is too small to influence ion concentration profiles in the membrane, which are nearly flat (e.g., in Fig. 2 the cation concentration dips and the anion concentration rises very slightly toward the center of the membrane, associated with a

positive potential of ≤ 2 mV). ψ increases with increasing bathing-solution salt concentration (Fig. 3), increasing membrane thickness (Fig. 6), and increasing values of the ion-site binding constants (Figs. 4 & 5).

4. If the membrane is thick enough that its thickness is large compared to the Debye length, and if ψ inside it becomes large compared to $[\mu^0(w) - \mu^0(m)]/F$ for anions, then the potential (due ultimately to preferential cation solubility) drags anions into the membrane in sufficient numbers nearly to balance the cations, the potential profile through the membrane is virtually flat, and most of the potential change is within a small fraction of the membrane thickness near the interfaces and may be described as a boundary potential (top curve, Fig. 6). Under these conditions, the potential depends only on the ratio K_1/K_3 as in Eq. (9) for a thick membrane, and is independent of concentration and of further increases in thickness.

The assumption of low space charge [$\psi(0) \ll RT/F$] introduces two simplifications into the mathematical treatment of permeation in thin membranes (pp. 319–327). First, one may assume that the difference between the potential at the membrane-solution interfaces and in the bulk solution is negligible ($|\psi(\pm d/2)| \ll RT/F$). Secondly, any potential due to a concentration gradient across the membrane or to an applied current, if its magnitude is significant compared to RT/F , will be virtually unperturbed by the space charge. Therefore, the potential gradient or electric field in the membrane will be virtually constant (Walz, Bamberg & Lauger, 1969, p. 1155; see Neumcke & Lauger, 1970, for discussion of the effects of high space charge). A further consequence of this assumption, to be derived below [Eq. (102)], is that the conductance-concentration relation should be linear. The reason why we have introduced the simplifying assumption of low space charge is that the conductance-concentration relation is in fact linear in thin lipid membranes containing nonactin carriers after correction for ionic-strength effects (Szabo *et al.*, 1969, Fig. 10) and in thin lipid membranes containing nystatin “pores” up to ion concentrations of about 0.1 M (Cass, Finkelstein & Krespi, 1970, Fig. 3), suggesting that the space charge is low in these model systems.

Model 3. Thin Membrane Without Shunt

We retain from model 1 the symbols; minor assumptions (c), (d), (e), (f), and (g) concerning perfect stirring, uniformity of μ^0 , γ , and u through the membrane, the relation $a_i = \gamma C_i^n$, and interfacial continuity of the electrochemical potential; and the major assumption that the membrane

contains sites that are polar but have no net charge. In particular, we return to the convention that the axis $x=0$ coincides with the membrane-solution interface, rather than with the center of the membrane as on pp. 310–319.

The major change in assumption from model 1 is that the membrane is now considered sufficiently thin to violate microscopic electroneutrality (*cf.* pp. 310–311). Thus, it is not necessarily true within the membrane that $C_1(x) = C_2(x) = C_3(x)$. In addition, we restrict consideration to conditions under which the space charge of the membrane is sufficiently low that $|\psi(0) - \psi'|$ and $|\psi(d) - \psi''| \ll RT/F$ and the field within the membrane may be approximated as constant (*see above*).

We begin by relating ion activities on the membrane side of the membrane-solution interface to activities in the bulk bathing solutions, assuming continuity of the electrochemical potential across the interfaces and defining K 's as in Eq. (6):

$$a_i(0) = K_i a_i' e^{-z_i [\psi(0) - \psi'] F/RT} \quad a_i(d) = K_i a_i'' e^{-z_i [\psi(d) - \psi''] F/RT}. \quad (91)$$

Since the condition $|\psi(0) - \psi'| \ll RT/F$, $|\psi(d) - \psi''| \ll RT/F$ follows from the assumption of low space charge, the exponential factor is approximately 1, and Eq. (91) becomes:

$$a_i(0) = K_i a_i' \quad a_i(d) = K_i a_i''. \quad (92)$$

Since $a_i = \gamma C_i^n$, the Nernst-Planck flux equations assume the form [*see Eqs. (11)–(13)*]:

$$J_1 = -u_1 n RT dC_1/dx - u_1 C_1 F d\psi/dx, \quad (93a)$$

$$J_2 = -u_2 n RT dC_2/dx - u_2 C_2 F d\psi/dx, \quad (93b)$$

$$J_3 = -u_3 n RT dC_3/dx + u_3 C_3 F d\psi/dx. \quad (93c)$$

Adding Eqs. (93a) and (93b) and defining (*cf.* p. 301)

$$U(x) = u_1 C_1(x) + u_2 C_2(x) \quad (20)$$

yields:

$$J_1 + J_2 = -n RT dU/dx - UF d\psi/dx. \quad (94)$$

Under conditions of low space charge, $d\psi/dx$ is approximately constant:

$$d\psi/dx \doteq E/d. \quad (95)$$

Substituting Eq. (95) into Eq. (94) and integrating between $x=0$ and $x=d$ gives:

$$J_1 + J_2 = \frac{n RT}{d} \frac{(\ln \xi_n) [U(0) - \xi_n U(d)]}{(\xi_n - 1)} \quad (96)$$

where

$$\xi_n = e^{EF/nRT}. \tag{23}$$

Similarly, substituting Eq. (95) into Eq. (93c) and integrating gives:

$$J_3 = \frac{nRT}{d} \frac{(\ln \xi_n)(u_3 C'_3 \xi_n - u_3 C''_3)}{(\xi_n - 1)}. \tag{97}$$

Using the definition of current

$$I \equiv F(J_1 + J_2 - J_3) \tag{34}$$

and substituting Eqs. (1) and (92) into Eqs. (20), (96) and (97), and then Eqs. (96) and (97) into Eq. (34), gives the general expression for the current:

$$I = \frac{nFRT}{d\gamma^{1/n}} \left(\frac{\ln \xi_n}{\xi_n - 1} \right) \{ [u_1(K_1 a'_1)^{1/n} + u_2(K_2 a'_2)^{1/n} + u_3(K_3 a'_3)^{1/n}] - \xi_n [u_1(K_1 a''_1)^{1/n} + u_2(K_2 a''_2)^{1/n} + u_3(K_3 a''_3)^{1/n}] \}. \tag{98}$$

Case 1. The Potential at Zero Current, E_0 . When $I=0$, Eq. (98) reduces to

$$E_0 = \frac{-nRT}{F} \ln \left[\frac{u_1(K_1 a'_1)^{1/n} + u_2(K_2 a'_2)^{1/n} + u_3(K_3 a'_3)^{1/n}}{u_1(K_1 a''_1)^{1/n} + u_2(K_2 a''_2)^{1/n} + u_3(K_3 a''_3)^{1/n}} \right]. \tag{99}$$

The expression for dilution potentials is obtained by dropping the terms $u_2(K_2 a'_2)^{1/n}$ and $u_2(K_2 a''_2)^{1/n}$; the expression for biionic potentials is obtained by dropping the terms $u_2(K_2 a'_2)^{1/n}$ and $u_1(K_1 a'_1)^{1/n}$.

When $n=1$, Eq. (99) is similar in form to the Goldman-Hodgkin-Katz equation or "constant-field equation" [see paper IV, Eq. (1)], modified by the partition coefficients K_i . The expression $u_i(K_i)^{1/n}$ has the significance of a permeability coefficient for the i th ion.

Case 2. Conductance and the Current-Voltage Relation in Single-Salt Solutions. If there is only a single salt, then $a'_2 = a''_2 = 0$, $a'_1 = a'_3 \equiv a'$, $a''_1 = a''_3 \equiv a''$. Substituting Eq. (23) into Eq. (98) gives the current-voltage relation in asymmetrical solutions ($a' \neq a''$):

$$I = \frac{EF^2}{d\gamma^{1/n}} \frac{\{u_1(K_1 a')^{1/n} + u_3(K_3 a'')^{1/n} - e^{EF/nRT} [u_1(K_1 a'')^{1/n} + u_3(K_3 a')^{1/n}]\}}{(e^{EF/nRT} - 1)}. \tag{100}$$

Since the factor multiplying E on the right-hand side of Eq. (100) contains two exponential terms in E and is not voltage-independent, the current-

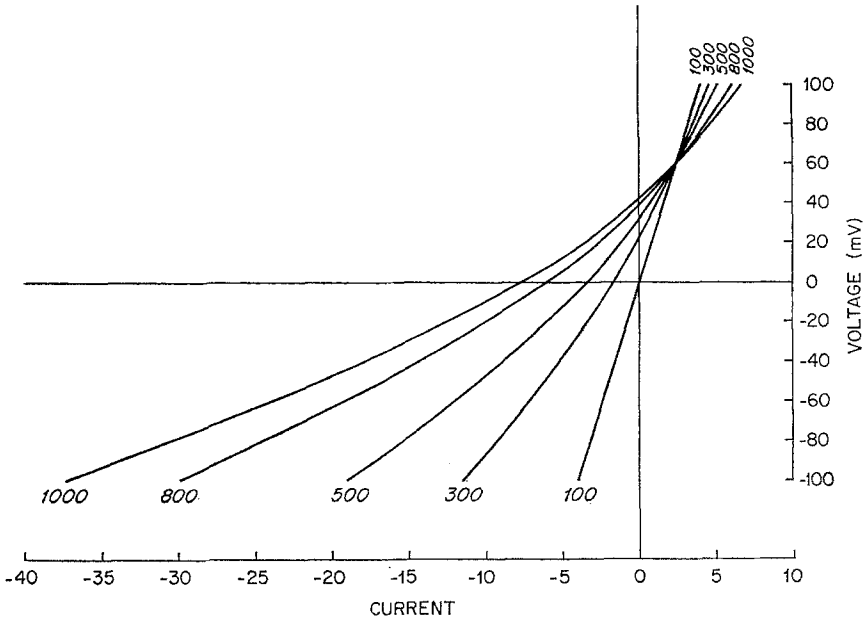


Fig. 7. Current-voltage relations for a thin membrane with fixed neutral sites in asymmetrical single-salt solutions, calculated from Eq. (100). Voltage is in mV, current in arbitrary units. n was taken as 1, the cation-to-anion permeability ratio $[u_1(K)^n/u_3(K_3)^n]$ as 0.1. The activity of one bathing solution (a') was taken as 100 mm; the activity of the other (a'') as 100, 300, 500, 800, or 1000 mm, indicated beside the appropriate curve. The potential is that of side '' with respect to side '. Note that when $a' \neq a''$, the curves are nonlinear, the greater slope (lower conductance) being for currents tending to make the concentrated solution positive. The curves were normalized so that the 100:100 mm curve goes through the point $I=2, V=50$ mV

voltage relation in asymmetrical solutions is nonlinear, provided that $u_1 K_1^{1/n} \neq u_3 K_3^{1/n}$, i.e., provided that the permeability of the cation and anion are unequal. If the anion is more permeant than the cation, the conductance is lower for applied voltages making the concentrated solution positive than for voltages making it negative, as illustrated in Fig. 7. If the cation is more permeant than the anion, the reverse is true, and if the cation and anion have equal permeabilities, the current-voltage relation is linear (Fig. 8).

In symmetrical solutions ($a' = a'' \equiv a$), Eq. (100) reduces to:

$$I = \frac{-EF^2 a^{1/n}}{d\gamma^{1/n}} (u_1 K_1^{1/n} + u_3 K_3^{1/n}). \tag{101}$$

The factor multiplying E is now voltage-independent, so that the current-voltage relation in symmetrical solutions is linear.

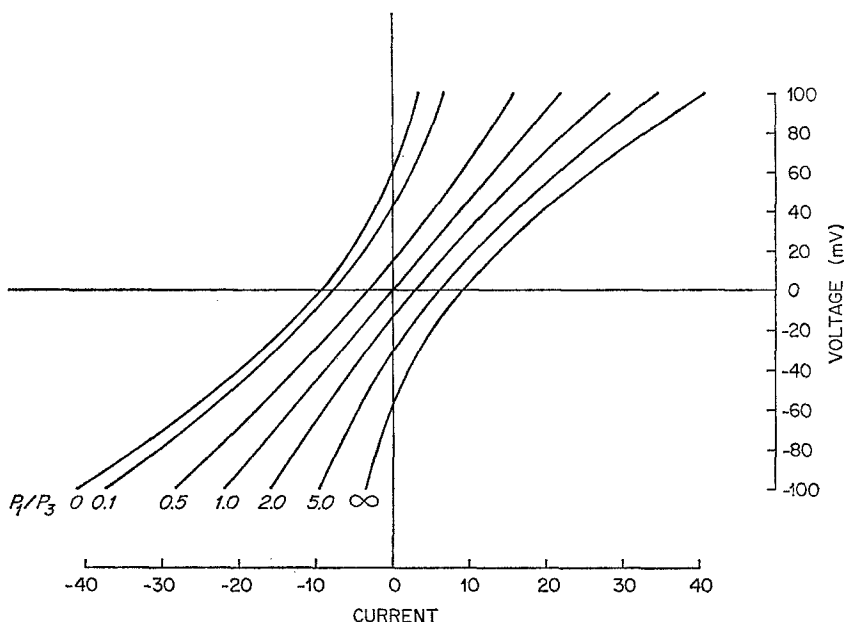


Fig. 8. Current-voltage relation for a thin membrane with fixed neutral sites in asymmetrical single-salt solutions, calculated from Eq. (100), as a function of the cation-to-anion permeability ratio $P_1/P_3 \equiv u_1(K_1)^{1/n}/u_3(K_3)^{1/n}$. The activity of one bathing solution (a') was taken as 100 mM, that of the other (a'') as 1000 mM. The potential is that of side '' with respect to side '. Note that if the anion is more permeant than the cation ($P_1/P_3 < 1$), the slope of the curves (the resistance) is higher for applied voltages making the concentrated solution positive than for voltages making it negative, that the reverse is true if $P_1/P_3 > 1$, and that the relation is linear if $P_1 = P_3$

From Eq. (101) and the definition of conductance ($G = -I/E^*$),

$$\begin{aligned}
 G &= \frac{F^2 a^{1/n}}{d \gamma^{1/n}} (u_1 K_1^{1/n} + u_3 K_3^{1/n}) \\
 &= \frac{F^2}{d} \left(\frac{\gamma_w}{\gamma} \right)^{1/n} [C(w)]^{1/n} (u_1 K_1^{1/n} + u_3 K_3^{1/n})
 \end{aligned} \tag{102}$$

where again $C(w)$ is the salt concentration and γ_w the activity coefficient in the aqueous phase.

Eq. (102) shows that the conductance-concentration relation is linear or nearly linear for n equal to or close to 1. Eq. (102) also shows that the partial cation conductance $(F^2/d)(\gamma_w/\gamma)^{1/n}[C(w)]^{1/n}u_1K_1^{1/n}$ and the partial anion conductance $(F^2/d)(\gamma_w/\gamma)^{1/n}[C(w)]^{1/n}u_3K_3^{1/n}$ are independent of each other, in contrast to the result for a thick membrane [Eq. (46), p. 306]. Finally, cation permeabilities are proportional to $u_iK_i^{1/n}$, whether determined by biionic potentials [Eq. (99)], dilution potentials [Eq. (99)], or conductances [Eq. (102)]. Thus, these three types of measurements yield the

same set of permeability ratios, in contrast to the result for a thick membrane (p. 306).

Comparison of Theory with Experiments on Nystatin-Treated Membranes

Thin lipid membranes treated with the antibiotics nystatin and amphotericin B undergo a large increase in conductance, principally to anions (Finkelstein & Cass, 1968; Cass, Finkelstein & Krespi, 1970). Such membranes behave as if these antibiotics induce the formation of pores through which ions, water, and small nonelectrolytes can permeate (Finkelstein & Cass 1968; Andreoli, Dennis & Weigl, 1969; Holz & Finkelstein, 1970). The hydroxyl groups of these antibiotics apparently serve as dipolar sites producing anion selectivity. Thus, nystatin-treated membranes probably exemplify thin membranes with fixed neutral sites, and the experimental results of Cass *et al.* (1970) may therefore be summarized for comparison with model 3.

(a) The current-voltage relation in symmetrical NaCl solutions is approximately linear (Cass *et al.*, 1970, Fig. 5 and p. 108), as predicted by Eq. (101).

(b) As predicted by Eq. (100) for an anion-selective membrane, the current-voltage relation in asymmetrical NaCl solutions is nonlinear, the conductance being lower for currents tending to make the concentrated solution positive. The experimental curves (Cass *et al.*, 1970, Fig. 5) are quite similar to the theoretical ones displayed in Fig. 7 of the present paper.

(c) Conductance in symmetrical NaCl solutions increases approximately linearly as the 1.4th power of the salt concentration (Cass *et al.*, Fig. 3), which could be interpreted to mean that n is $1/1.4 = 0.7$ [Eq. (102)].

(d) At first sight, the biionic potentials and conductance measurements of Cass *et al.* (1970) suggest a considerably greater permeability difference between chloride and isethionate than do their dilution potential measurements, contrary to prediction. However, the measured potential differences include junction potentials, correction for which would increase the permeability ratio based on dilution potentials, and estimates of these junction potentials indicate that they could be large enough to remove the apparent discrepancy⁴.

⁴ Cass *et al.* (1970) used saturated KCl bridges to record potential differences and neglected junction potentials. The signs of the expected junction potentials are such that correction for them would increase the dilution potential slope for NaCl ($u_{Cl} > u_{Na}$) but decrease the slope for Na isethionate ($u_{Na} > u_{isethionate}$) and increase the NaCl–Na isethionate biionic potential ($u_{Cl} > u_{isethionate}$). From a modified Henderson equation, we estimate that these junction potentials are somewhere between 2–3 mV and 11–12 mV, depending on the exact history of the KCl bridges (Barry & Diamond, 1970, pp. 97–101).

(e) At salt concentrations above 0.1 M, the conductance-concentration relation (Cass *et al.*, 1970, Fig. 3) and the dilution-potential:concentration-gradient relation (Cass *et al.*, Fig. 4) begin to deviate from linearity, an effect that may be due either to buildup of significant space charge (see p. 319, and Neumcke & Läuger, 1970) or to effects of surface charge (McLaughlin, Szabo, Eisenman & Ciani, 1970*b*).

Model 4. Thin Membrane with Shunt

This model is the same as model 3 (thin membrane without shunt), except that, in parallel with and insulated from the thin membrane containing fixed neutral sites, is a shunt in which ion mobilities (symbolized by $v_1, v_2,$ and v_3) stand in the same ratios as in free solution. As in model 2, the effective area, the activity coefficient, and ion mobilities are symbolized by $\rho_v, \gamma_v,$ and u_i in the neutral-site channel and by $\rho_s, \gamma_s,$ and v_i in the shunt.

The excess cation flux over anion flux in the shunt, $J^* \equiv J_1^* + J_2^* - J_3^*$, has been shown previously to be

$$J^* = \frac{\rho_s RT}{\gamma_s d} \left(\frac{a' - a''}{\ln \frac{a''}{a'}} \right) \left\{ \frac{[\xi(v_1 a_1'' + v_2 a_2'') - (v_1 a_1' + v_2 a_2')] \left[\ln \left(\frac{\xi a''}{a'} \right) \right]}{\xi a'' - a'} - v_3 \ln \left(\frac{a''}{\xi a'} \right) \right\} \tag{50}$$

where it is assumed that $a_1 + a_2 = a_3 = a$.

The excess cation flux over anion flux in the neutral-site channel is obtained from Eq. (98), by multiplying by ρ_v/F and substituting $\ln \xi_n = EF/nRT$ [Eq. (23)]:

$$J = \frac{EF \rho_v}{d \gamma_v^{1/n} (\xi_n - 1)} \{ [u_1 (K_1 a_1')^{1/n} + u_2 (K_2 a_2')^{1/n} + u_3 (K_3 a_3')^{1/n}] - \xi_n [u_1 (K_1 a_1'')^{1/n} + u_2 (K_2 a_2'')^{1/n} + u_3 (K_3 a_3'')^{1/n}] \} \tag{103}$$

Adding Eqs. (50) and (103) and multiplying by F gives the total current $I = F(J + J^*)$:

$$I = \frac{E \rho_v F^2}{d \gamma_v^{1/n} (\xi_n - 1)} \{ [u_1 (K_1 a_1')^{1/n} + u_2 (K_2 a_2')^{1/n} + u_3 (K_3 a_3')^{1/n}] - \xi_n [u_1 (K_1 a_1'')^{1/n} + u_2 (K_2 a_2'')^{1/n} + u_3 (K_3 a_3'')^{1/n}] \} + \frac{E \rho_v F^2}{d \gamma_v^{1/n}} \frac{\lambda_s (a' - a'')}{\ln(\xi a''/a')} \left\{ \left[\frac{\ln(\xi a''/a')}{(\xi a'' - a')} \right] [\xi(v_1 a_1'' + v_2 a_2'') - (v_1 a_1' + v_2 a_2')] - v_3 \ln(a''/\xi a') \right\} \tag{104}$$

where

$$\lambda_s \equiv \rho_s \gamma_v^{1/n} / \rho_v \gamma_s \quad (56)$$

is a measure of the area of the shunt relative to the area of the neutral-site channel.

Case 1. Conductance and the Current-Voltage Relation in Single-Salt Solutions. If there is only a single salt, then $a'_2 = a''_2 = 0$, $a'_1 = a'_3 \equiv a'$, $a'_1' = a'_3' \equiv a''$, and Eq. (104) simplifies to:

$$\begin{aligned} I = & -\lambda_s v_3 a' E \Omega_s \left\{ \frac{B_1 (a')^{(1-n)/n}}{1 - \xi_n} \left[1 - \xi_n \left(\frac{a''}{a'} \right)^{1/n} \right] \right. \\ & \left. + \frac{B_3 (a')^{(1-n)/n}}{1 - \xi_n} \left[\left(\frac{a''}{a'} \right)^{1/n} - \xi_n \right] + \frac{\left(\frac{v_1}{v_3} + 1 \right) \left(\frac{a''}{a'} - 1 \right)}{\ln \frac{a''}{a'}} \right\} \\ & - \lambda_s v_3 a' \Omega_s (RT/F) \left(\frac{v_1}{v_3} - 1 \right) \left(\frac{a''}{a'} - 1 \right) \end{aligned} \quad (105)$$

where

$$\Omega_s \equiv \rho_v F^2 / d \gamma_v^{1/n}, \quad (106)$$

$$B_1 \equiv u_1 K_1^{1/n} / \lambda_s v_3, \quad (107)$$

$$B_3 \equiv u_3 K_3^{1/n} / \lambda_s v_3. \quad (108)$$

Since the factor multiplying E on the right-hand side of Eq. (105) contains terms in $E(\xi_n)$, the current-voltage relation in asymmetrical solutions is nonlinear. An example of a calculated curve will be illustrated in paper III, Fig. 11.

In symmetrical solutions ($a' = a'' \equiv a$), however, Eq. (105) reduces, after substitution of Eqs. (107) and (108), to:

$$I = -\Omega_s a E \{ [u_1 (K_1)^{1/n} + u_3 (K_3)^{1/n}] a^{(1-n)/n} + \lambda_s (v_1 + v_3) \}. \quad (109)$$

The current-voltage relation is now linear, and conductance ($-I/E$) varies approximately linearly with concentration if n is close to 1. In Eq. (109), the terms $u_1 (K_1)^{1/n}$ and $u_3 (K_3)^{1/n}$ have the significance of relative cation and anion permeabilities, respectively, in the neutral-site channel, whereas $\lambda_s v_1$ and $\lambda_s v_3$ have a corresponding significance in the shunt channel.

Case 2. Dilution Potentials. When $I=0$, Eq. (105) reduces to:

$$\begin{aligned} & \frac{u_1 K_1^{1/n} (a')^{1/n} S_n}{\lambda_s v_3} + \frac{u_3 K_3^{1/n} (a')^{1/n} B_n}{\lambda_s v_3} + \frac{\left(\frac{v_1}{v_3} + 1 \right) (a'' - a')}{\ln(a''/a')} \\ & = -\frac{RT}{E_0 F} \left(\frac{v_1}{v_3} - 1 \right) (a'' - a') \end{aligned} \quad (110)$$

where

$$S_n \equiv \frac{1 - \xi_n (a''/a')^{1/n}}{1 - \xi_n} \tag{111}$$

and

$$B_n \equiv \frac{(a''/a')^{1/n} - \xi_n}{1 - \xi_n}. \tag{112}$$

Case 3. General Expression for the Potential at Zero Current, E₀. Setting I=0 in Eq. (104), multiplying through by (ξ_n - 1)/u₁K₁^{1/n}, and rearranging gives:

$$\frac{v_2 K_2^{1/n}}{u_1 K_1^{1/n}} = \frac{\lambda_s v_3}{n u_1 K_1^{1/n}} \left(\frac{\xi_n - 1}{\ln \xi_n} \right) \left(\frac{a'' - a'}{\ln \frac{a''}{a'}} \right) \left[\frac{1}{(a_2')^{1/n} - \xi_n (a_2'')^{1/n}} \right] \cdot \left\{ \frac{\left[\xi \left(\frac{v_1 a_1''}{v_3} + \frac{v_2 a_2''}{v_3} \right) - \left(\frac{v_1 a_1'}{v_3} + \frac{v_2 a_2'}{v_3} \right) \right] \left(\ln \frac{\xi a''}{a'} \right)}{\xi a'' - a'} - \ln \frac{a''}{\xi a'} \right\} \tag{113}$$

$$= \frac{[(a_1')^{1/n} - \xi_n (a_1'')^{1/n}] - \frac{u_3 K_3^{1/n}}{u_1 K_1^{1/n}} [(a_3')^{1/n} - \xi_n (a_3'')^{1/n}]}{(a_2')^{1/n} - \xi_n (a_2'')^{1/n}}.$$

u₂K₂^{1/n}/u₁K₁^{1/n} has the significance of the cation permeability ratio in the neutral-site channel.

Case 3a. Bionic Potential. If a₂' = a₁' = 0, a₁' = a₂' = a, and n = 1, Eq. (113) simplifies, remembering that ln ξ = EF/RT = n ln ξ_n, to:

$$E_0 = \frac{-RT}{F} \ln \frac{(u_2 K_2 + \lambda_s v_2) + (u_3 K_3 + \lambda_s v_3)}{(u_1 K_1 + \lambda_s v_1) + (u_3 K_3 + \lambda_s v_3)}. \tag{114}$$

As in Eq. (109), each term u_iK_i in the logarithm of Eq. (114) has the significance of a permeability coefficient for the ith ion in the neutral-site channel, whereas λ_sv_i has a corresponding significance in the shunt channel.

Discussion: How to Recognize a Membrane with Fixed Neutral Sites

Given a biological membrane or model membrane of unknown structure, how can one most easily decide from black-box measurements alone if permeation is occurring via fixed neutral sites?

Neutral vs. Charged Sites. The distinguishing characteristic of neutral-site membranes generally, whether thick or thin, and whether with fixed or

mobile sites, is a nearly linear conductance-concentration relation in symmetrical salt solutions⁵. In contrast, the conductance of an ion exchange is virtually independent of concentration until the bathing-solution salt concentration approaches the concentration of the sites themselves. However, this distinction between the behavior of neutral sites and ion exchanger may break down in membranes thinner than the Debye length, where the conductance-concentration relation may also tend towards linearity for an ion-exchanger.

Thick vs. Thin Membranes. Three methods can be suggested for distinguishing a thick membrane from a thin membrane with fixed neutral sites (1) The current-voltage relation in asymmetrical solutions of a single salt is linear for a thick membrane [Eq. (45)] and nonlinear for a thin membrane [Eq. (100)]. (2) The partial conductances of cation and of anion are independent of each other in a thin membrane [Eq. (102)] and mutually dependent in a thick membrane [Eq. (46)]. Thus, if the anion flux in symmetrical solutions is measured by radioactive tracers, the flux should be the same whatever cation is used if the membrane is thin, but not if it is thick (provided that there is little or no exchange diffusion). (3) Measurements of conductance, dilution potentials, and biionic potentials should yield the same set of permeability ratios among ions of like sign if the membrane is thin, but should yield three different sets if the membrane is thick. This test has to be qualified by the statement that if a thick membrane has a shunt and has zero anion mobility in the neutral-site channel (as appears to be true for the gallbladder: see paper IV), then nearly the same cation permeability ratios should be obtained from conductances and dilution potentials, and possibly from biionic potentials.

Mobile vs. Fixed Neutral Sites. If a membrane is thick and contains neutral sites, then measurements of conductance, dilution potentials, and biionic potentials will yield the same set of permeability ratios if the sites are mobile, different sets if the sites are fixed. If a membrane is thin and contains neutral sites, it may prove difficult to decide whether these sites are mobile (e.g., similar to the carriers monactin and valinomycin) or fixed since most black-box properties would be the same in either case. One possible clue is that the current-voltage relation for mobile sites becomes:

⁵ Strictly speaking, G varies as C^n , where n need not equal 1; and the relation for a thin membrane becomes sublinear at concentrations sufficiently high that the space charge is no longer negligible. Effects of surface charge (Szabo *et al.*, 1969; McLaughlin *et al.*, 1970b) may obscure the linearity if the membrane is thin, but this complication can be avoided by keeping ionic strength constant with an impermeant salt.

supralinear above about 40–60 mV (Szabo *et al.*, 1969, Fig. 1), whereas the relation is approximately linear for fixed sites (Cass *et al.*, 1970, p. 108), but the linearity in the latter case has apparently not been tested beyond 75 mV.

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