J. Membrane Biol. 30, 45 – 63 (1976)

Influence of Molecular Variations of Ionophore and Lipid on the Selective Ion Permeability of Membranes:
II. A Theoretical Model

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Received 17 March 1976; revised 10 June 1976

Summary. The steady-state electrical properties induced by neutral carriers of ions in lipid bilayer membranes and the time dependence of the membrane current for low applied voltages are described theoretically in terms of a model which allows for a voltage dependence of the interfacial reactions, as well as for a trapezoidal shape of the internal free energy barrier for translocation of the complex. The basic features of the model are closely related to those of others presented previously (J.E. Hall, C.A. Mead & G. Szabo, 1973, *J. Membrane Biol.* **11**:75; S.B. Hladky, 1974, *Biochim. Biophys. Acta* **352**:71; S.B. Hladky, 1975, *Biochim. Biophys. Acta* **375**:327; Eisenman, Krasne & Ciani, 1975, *Ann. N.Y. Acad. Sci.* **264**:34), but the analysis of its consequences on the steady-state and nonsteady-state electrical characteristics is given here in greater detail and is extended to provide the expression for the zero-current potential in ionic gradients. It is shown that parameters, such as the width of the trapezoidal barrier, the plane of the reaction and the ratio of the rate constant of translocation across the membrane interior to the rate constant of dissociation of the complex, can be deduced from steady-state analysis, whereas the individual values of these constants and the distance between the equilibrium positions of the complexes are deducible from relaxation measurements.

We present here a theoretical model for carrier-mediated permeation with the primary purpose of deriving the expressions for the steady-state electrical properties which are used to analyze the data in the preceding paper (Krasne & Eisenman, 1976). However, in order to lay the ground for future experimental studies, in the last section we extend the treatment also to transients of currents following steps of applied voltage, and give the explicit solution for the particular case of low ionic concentrations and small potentials.

The model shares common features with that presented originally by Hall, Mead and Szabo (1973) and is basically similar to that used by

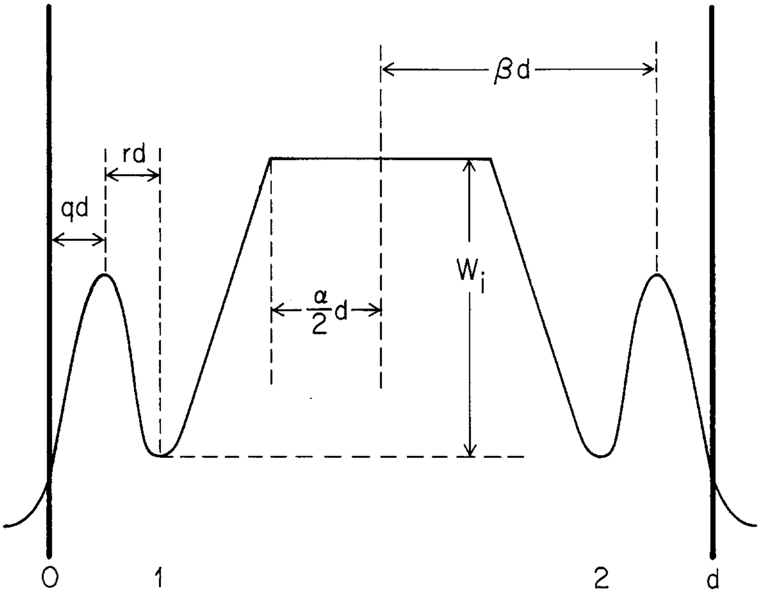

Fig. 1. Energy profile associated with the mechanism of carrier-mediated transport of ions across the membrane. The difference between the internal and the external wells is the "affinity" of the ion-carrier dissociation reaction, namely the difference between the standard chemical potential of the complex at its equilibrium position, $\mu_{is}^{0}*(1)$, and the sum of the standard chemical potentials of the ion in the aqueous phase and that of the neutral carrier at its equilibrium position in the membrane (not shown in the figure), $\mu_i^0(\text{aq.})+\mu_s^0*(1)$. The peak between these two wells represents the free energy of the activated complex. Since the distance between the ion in the aqueous phase and the complex in the membrane is assumed to be a finite fraction of the membrane thickness, the height of the interfacial barrier with respect to the wells will be modified by the membrane potential. The central trapezoid represents the profile of the energy barrier for translocation of the formed complex. α, β, r and q are fractions of the membrane thickness, and their precise meaning should be evident from inspection of the figure

Hladky (1974), except that its consequences are extended here to describe the zero-current potential in the presence of ionic mixtures, as well as to include the effect of the displacement currents in the analysis of relaxation.

Two features, schematically illustrated in Fig. 1, distinguish this model from the simpler one proposed by Läuger and Stark (1970):

1. The equilibrium position of the ion-carrier complexes is displaced from the interfaces toward the interior by some fraction of the membrane thickness. This provides a physical basis to account for a voltage dependence of the height of the activation energy barrier and therefore of the rate constants of the interfacial reaction.

2. The free energy profile of the charged ion-carrier complexes in the central portion of the membrane is approximated by a trapezoid, and

its effect on the fluxes is described by a generalized Nernst-Planck equation, in which the standard chemical potential is assumed to be a function of the distance, x, from the membrane/solution interface.

Since the use of a rather cumbersome formalism for this more elaborate model seems to be unavoidable, a definition of the various symbols is given on page 7.

Theory

The Conservation Equations at the Membrane-Solution Boundaries

Using an Eyring formalism to express the voltage dependence of the rate constants of the interfacial reactions, the net rate of formation of complexes at the two interfaces will be

$$\frac{dN_{is}^*(1)}{dt} = \bar{K}_i^F c_i' N_s^*(1) e^{q_i \phi} - \bar{K}_i^B N_{is}^*(1)_i e^{-r_i \phi} - J_{is} \qquad (i=1,2,\dots,n) \tag{1}$$

$$\frac{dN_{is}^*(2)}{dt} = \bar{K}_i^F c_i'' N_s^*(2) e^{-q_i \phi} - \bar{K}_i^B N_{is}^*(2)_i e^{r_i \phi} + J_{is} \qquad (i=1,2,\dots,n), \tag{2}$$

where J_{is} indicates the flux of complexes across the membrane interior.

The conservation equations for the neutral uncomplexed carriers, s, are more complicated, since the exchange of carriers between the membrane and the surrounding torus, as well as diffusion across the unstirred layers, must also be allowed for. Neglecting for a moment the effects of the unstirred layers, we will have

$$\begin{aligned}\frac{dN_s^*(1)}{dt} = {} & k_s^F c_s(0) - k_s^B N_s^*(1) + k^{TM} c_s^T - k^{MT} N_s^*(1) \\ & - A_s^* [N_s^*(1) - N_s^*(2)] \\ & + \sum_{i=1}^{n} \left\{ \bar{K}_i^B N_{is}^*(1)_i e^{-r_i \phi} - \bar{K}_i^F c_i' N_s^*(1) e^{q_i \phi} \right\}\end{aligned} \tag{3}$$

and

$$\begin{aligned}\frac{dN_s^*(2)}{dt} = {} & k_s^F c_s(d) - k_s^B N_s^*(2) + k^{TM} c_s^T - k^{MT} N_s^*(2) \\ & + A_s^* [N_s^*(1) - N_s^*(2)] \\ & + \sum_{i=1}^{n} \left\{ \bar{K}_i^B N_{is}^*(2)_i e^{r_i \phi} - \bar{K}_i^F c_i'' N_s^*(2) e^{-q_i \phi} \right\}.\end{aligned} \tag{4}$$

At steady-state, and if we neglect a direct exchange of neutral carriers between the solutions and the torus, the flux of carriers in the unstirred layers must be equal to the flux of carriers across the interfaces. This requires that

$$\frac{D_s}{\delta}\,[c_s' - c_s(0)] = k_s^F\, c_s(0) - k_s^B\, N_s^*(1) \tag{5}$$

and

$$k_s^B\, N_s^*(2) - k_s^F\, c_s(d) = \frac{D_s}{\delta}\,[c_s(d) - c_s'']. \tag{6}$$

If the bulk concentrations of neutral carriers are equal in the two solutions ($c_s' = c_s'' = c_s$), and if we impose the condition of steady-state, namely, that the derivatives on the left-hand side of Eqs. (1)–(4) vanish, we obtain from Eqs. (3), (4), (5) and (6)

$$N_s^*(1) = N_s^*(\mathrm{st.}) - B \sum_{i=1}^{n} J_{is}; \qquad N_s^*(2) = N_s^*(\mathrm{st.}) + B \sum_{i=1}^{n} J_{is} \tag{7}$$

where

$$N_s^*(\mathrm{st.}) = \frac{(1 + \delta k_s^F/D_s)\, k^{TM}\, c_s^T + k_s^F\, c_s}{(1 + \delta k_s^F/D_s)\, k^{MT} + k_s^B} \tag{8}$$

and

$$B = \frac{1}{(k_s^{MT} + 2A_s^*) + \dfrac{k_s^B}{1 + \delta k_s^F/D_s}}. \tag{9}$$

Combining Eq. (7) with (1) and (2), and recalling that

$$\sum_{i=1}^{n} J_{is} = I/zF, \tag{10}$$

we find after simple rearrangements

$$\begin{aligned} N_{is}^*(1)_i = {} & \frac{\bar{K}_i^F}{\bar{K}_i^B}\, c_i' \left[N_s^*(\mathrm{st.}) - B\, \frac{I}{zF} \right] e^{(q_i + r_i)\phi} \\ & - \frac{J_{is}}{\bar{K}_i^B}\, e^{r_i \phi} \qquad (i = 1, 2, \ldots, n) \end{aligned} \tag{11}$$

$$\begin{aligned} N_{is}^*(2)_i = {} & \frac{\bar{K}_i^F}{\bar{K}_i^B}\, c_i'' \left[N_s^*(\mathrm{st.}) + B\, \frac{I}{zF} \right] e^{-(q_i + r_i)\phi} \\ & + \frac{J_{is}}{\bar{K}_i^B}\, e^{-r_i \phi} \qquad (i = 1, 2, \ldots, n). \end{aligned} \tag{12}$$

Eqs. (11) and (12) constitute a system of $2n$ equations in the $3n$ unknowns $N_{1s}^*(1)_1$, $N_{2s}^*(1)_2$, … $N_{ns}^*(1)_n$; $N_{1s}^*(2)_1$, $N_{2s}^*(2)_2$, … $N_{ns}^*(2)_n$; J_{1s}, J_{2s}, … J_{ns}. The

n additional relationships which are required for a complete solution of the problem are provided by integration of the flux equations across the membrane interior.

Generalized Equations of Nernst-Planck

It has been shown elsewhere (Ciani, Laprade, Eisenman & Szabo, 1973*a*) that the generalized Nernst-Planck equation, extended to allow for a nonuniform barrier shape, can be written in the form

$$-\frac{J^*_{is}}{D^*_{is}}\, e^{W^*_{is}(x)} = \frac{d}{dx}\left[c^*_{is}\, e^{W^*_{is}(x)}\right]; \qquad (i=1,2,\dots,n) \tag{13}$$

where $W^*_{is}(x)$ is the sum of the standard chemical potential and of the electrostatic potential, namely,

$$W^*_{is}(x) = \frac{\mu^{0*}_{is}(x)}{RT} + \phi(x); \qquad (i=1,2,\dots,n). \tag{14}$$

A formal integration of Eq. (13) between the positions $(1)_i$ and $(2)_i$ gives

$$\frac{J_{is}}{D^*_{is}} \int_{(1)_i}^{(2)_i} e^{W^*_{is}(x)}\,dx = c^*_{is}(1)_i\, e^{W^*_{is}(1)_i} - c^*_{is}(2)_i\, e^{W^*_{is}(2)_i} \qquad (i=1,2,\dots,n). \tag{15}$$

Since the shape of the trapezoidal barrier is symmetrical, so that $\mu^{0*}_{is}(1)_i = \mu^{0*}_{is}(2)_i$, Eq. (15) can be rewritten in the form

$$J_{is} = A^*_{is}(\phi)\left[N^*_{is}(1)_i\, e^{\frac{\phi(1)_i-\phi(2)_i}{2}} - N^*_{is}(2)_i\, e^{-\frac{\phi(1)_i-\phi(2)_i}{2}}\right]; \quad (i=1,2,\dots,n) \tag{16}$$

where the surface concentrations $N^*_{is}(1)_i$ and $N^*_{is}(2)_i$ are related to the volume concentrations $c^*_{is}(1)_i$ and $c^*_{is}(2)_i$ by

$$N^*_{is}(1)_i = \frac{d}{2}\, c^*_{is}(1)_i \quad \text{and} \quad N^*_{is}(2)_i = \frac{d}{2}\, c^*_{is}(2)_i \qquad (i=1,2,\dots,n) \tag{17}$$

and

$$A^*_{is}(\phi) = \frac{2D^*_{is}}{d}\, \frac{\exp\left[\frac{\mu^{0*}_{is}(1)_i}{RT} + \frac{\phi(1)_i+\phi(2)_i}{2}\right]}{\int_{(1)_i}^{(2)_i} e^{W^*_{is}(x)}\,dx}; \qquad (i=1,2,\dots,n). \tag{18}$$

It would be easy to show that when the standard chemical potential is a constant between the planes $(1)_i$ and $(2)_i$, Eq. (16) reduces to the well-known, integrated expression for the flux deducible from the classic Nernst-Plank equation.

Also the Eyring expression for the flux across a single barrier can be obtained from Eqs. (16) and (18) when the standard chemical potential $\mu_{is}^{0}(x)$ has very high values in a narrow region centered around the middle of the membrane. If δ^* denotes the thickness of this narrow region, the integral in the denominator of Eq. (18) is given approximately by

$$\int_{(1)_i}^{(2)_i} e^{W_{is}^*(x)}\,dx \approx \delta^* \exp\left[\frac{\mu_{is}^{0*}(d/2)}{RT} + \frac{\phi(1)_i+\phi(2)_i}{2}\right] \qquad (i=1,2,\ldots,n). \qquad (19)$$

Inserting Eq. (19) into Eq. (18) and (16) we find

$$\begin{aligned} J_{is} = \frac{2D_{is}^*}{\delta^* d} \exp\left[\frac{\mu_{is}^{0*}(1)_i - \mu_{is}^{0*}(d/2)}{RT}\right] \Big\{ N_{is}^*(1)_i\, e^{\frac{\phi(1)_i-\phi(2)_i}{2}} & \\ - N_{is}^*(2)_i\, e^{-\frac{\phi(1)_i-\phi(2)_i}{2}} \Big\} \qquad (i=1,2,\ldots,n), & \end{aligned} \qquad (20)$$

which is formally equivalent to the Eyring expression for the flux.

Trapezoidal barrier. When the barrier has a trapezoidal shape similar to that illustrated in Fig. (1), and the applied field is assumed constant, the integral in the denominator of Eq. (18) can be evaluated analytically. However, for simplicity we will assume that the height and the width of the barrier are sufficiently big that the total integral between $(1)_i$ and $(2)_i$ can be approximated by the portion of the integral along the flat top of the barrier. If so, one finds

$$\int_{(1)_i}^{(2)_i} e^{W_{is}^*(x)}\,dx \simeq 2d \exp\left[\frac{\mu_{is}^{0*}(1)_i + W_i}{RT} + \phi(0) - \frac{\phi}{2}\right] \frac{\sinh\left[\frac{\alpha_i}{2}\phi\right]}{\phi} \qquad (21)$$
$$(i=1,2,\ldots,n)$$

where α_i is the width of the top of the trapezoid. Since

$$\phi(0) - \tfrac{1}{2}\phi = \frac{\phi(0)+\phi(d)}{2} = \frac{\phi(1)_i+\phi(2)_i}{2} \qquad (i=1,2,\ldots,n) \qquad (22)$$

Eq. (18) becomes

$$A_{is}^*(\phi) = \tilde{A}_{is}^* \frac{\phi}{\sinh\left[\frac{\alpha_i}{2}\phi\right]} \qquad (i=1,2,\ldots,n) \qquad (23)$$

where $\tilde{A}_{is}^*$ is a constant defined as

$$\tilde{A}_{is}^* = \frac{D_{is}^*}{d^2} \exp\left[-\frac{W_i}{RT}\right] \qquad (i=1,2,\ldots,n). \qquad (24)$$

Steady-State Voltage-Current Relationships

Combining Eq. (16) with Eqs. (11) and (12) and solving for the flux J_{is}, we find

$$J_{is}=L_i(\phi)\left\{N_s^*(\text{st.})\,(c_i' e^{\phi/2}-c_i'' e^{-\phi/2})-\frac{I}{zF}\,B(c_i' e^{\phi/2}+c_i'' e^{-\phi/2})\right\} \qquad (25)$$

$$(i=1,2,\ldots,n)$$

where

$$L_i(\phi)=\frac{\bar{K}_i A_{is}^*(\phi)}{1+2\dfrac{A_{is}^*(\phi)}{\bar{K}_i^B}\cosh\,[\beta_i\phi]} \qquad (i=1,2,\ldots,n) \qquad (26)$$

and

$$\beta_i\phi=\frac{\phi(1)_i-\phi(2)_i}{2}+r_i\phi. \qquad (27)$$

Note that $\beta_i\phi$ represents the potential drop between the middle of the membrane and the peak of the interfacial barrier. If this peak coincided with the membrane interface, β_i would be 0.5.

Summating both sides of Eq. (25) with respect to i, and recalling that $zF\sum_{i=1}^{n} J_{is}=I$, one finds

$$\frac{I}{zF}=N_s^*(\text{st.})\frac{\sum_{i=1}^{n}\{L_i(\phi)[c_i' e^{\phi/2}-c_i'' e^{-\phi/2}]\}}{1+B\sum_{i=1}^{n}\{L_i(\phi)[c_i' e^{\phi/2}+c_i'' e^{-\phi/2}]\}}. \qquad (28)$$

Note that Eq. (28) gives the current-voltage relationship for membranes separating ionic mixtures, and such an expression may be written as an explicit function of the potential ϕ in the case of a trapezoidal barrier, using Eqs. (26) and (23).

Some general considerations concerning the behavior of Eq. (28) at high voltages ($|\phi|\to\infty$) can be made with the help of Eqs. (26) and (23). Recalling that $\beta_i>\alpha_i/2$, it should become apparent from a simple inspection of such equations that

$$\lim_{\phi\to+\infty} L_i(\phi)e^{\phi/2}=\begin{cases}+\infty, & \text{if } \beta_i<\frac{1}{2}\\ \frac{1}{2}\bar{K}_i\bar{K}_i^B=\dfrac{\bar{K}_i^F}{2}, & \text{if } \beta_i=\frac{1}{2}.\end{cases} \qquad (29)$$

Consequently, in the limit of high positive voltages, Eq. (28) gives

$$\lim_{\phi\to+\infty}\frac{I}{zF}=\frac{N_s^*(\text{st.})}{B}, \quad \text{if } \beta_i<\tfrac{1}{2} \qquad (30)$$

and

$$\lim_{\phi\to+\infty}\frac{I}{zF}=\tfrac{1}{2}N_s^*(\mathrm{st.})\frac{\sum\limits_{i=1}^{n}\bar{K}_i^F c_i'}{1+\frac{B}{2}\sum\limits_{i=1}^{n}\bar{K}_i^F c_i'}, \qquad \text{if } \beta_i=\tfrac{1}{2}. \tag{31}$$

Note that in either case ($\beta_i<\frac{1}{2}$ or $\beta_i=\frac{1}{2}$) Eq. (28) predicts a finite limiting current. However, when $\beta_i<\frac{1}{2}$, the limiting current given by Eq. (30) depends solely on the properties of the neutral carriers and is independent of the bound cations; whereas this is not necessarily so when $\beta_i=\frac{1}{2}$, unless the condition $\sum\limits_{i=1}^{n}\bar{K}_i^F c_i'\gg 2/B$ is satisfied.

Explicit I – V Relationship for the Case of Identical Solutions and a Single Permeant Ion

When $c_i'=c_i''\equiv c_i$, and i is the only permeant ion, Eq. (28) becomes

$$\frac{I}{zF}=N_s^*(\mathrm{st.})\frac{2L_i(\phi)c_i\sinh\left[\frac{\phi}{2}\right]}{1+2BL_i(\phi)c_i\cosh\left[\frac{\phi}{2}\right]}. \tag{32}$$

If the ion concentration and the potential are sufficiently low that

$$2BL_i(\phi)c_i\cosh\left[\frac{\phi}{2}\right]\ll 1, \tag{33}$$

Eq. (32) simplifies to

$$\frac{I}{zF}=2N_s^*(\mathrm{st.})L_i(\phi)c_i\sinh\left[\frac{\phi}{2}\right]. \tag{34}$$

Using Eqs. (26) and (23), defining

$$\tilde{w}_i=\tilde{A}_{is}^*/\bar{K}_i^B \tag{35}$$

and recalling that $\phi=zFV/RT$, Eq. (34) can be written in the form

$$G(\phi)=\frac{I}{V}=\frac{z^2F^2}{RT}\bar{K}_i\tilde{A}_{is}^*N_s^*(\mathrm{st.})c_i\frac{2\sinh\left[\frac{\phi}{2}\right]}{\sinh\left[\frac{\alpha_i}{2}\phi\right]+2\tilde{w}_i\phi\cosh[\beta_i\phi]}. \tag{36}$$

Near zero-voltage Eq. (36) becomes

$$G(0)=\frac{z^2F^2}{RT}\bar{K}_i\tilde{A}^*_{is}N^*_s(\text{st.})c_i\frac{2}{\alpha_i+4\tilde{w}_i} \tag{37}$$

so that the ratio $G(\phi)/G(0)$ is given by

$$\frac{G(\phi)}{G(0)}=\frac{(\alpha_i+4\tilde{w}_i)\sinh\left[\frac{\phi}{2}\right]}{\sinh\left[\frac{\alpha_i}{2}\phi\right]+2\tilde{w}_i\phi\cosh[\beta_i\phi]}. \tag{38}$$

Eq. (38) is the expression used in the previous paper (Krasne & Eisenman, 1976; Eq. (2)) to fit the conductance-voltage data. When $\tilde{w}_i$ is negligible compared to unity, Eq. (38) becomes

$$\frac{G(\phi)}{G(0)}=\alpha_i\frac{\sinh[\phi/2]}{\sinh\left[\frac{\alpha_i\phi}{2}\right]} \tag{39}$$

which shows that the conductance ratio depends solely on the width of the barrier.

In the opposite alternative case ($\tilde{w}_i>1$), Eq. (38) becomes approximately

$$\frac{G(\phi)}{G(0)}=2\frac{\sinh[\phi/2]}{\phi\cosh[\beta_i\phi]} \tag{40}$$

in which case the conductance ratio depends only on β_i. The limiting Eqs. (39) and (40) are particularly useful for the evaluation of the parameters α_i and β_i when a series of ion-carrier complexes with $\tilde{w}_i$'s ranging from values smaller than one to values greater than one is studied, and when the assumption is made that the width of the trapezoidal barriers, α_i, and the planes of reaction, β_i, are the same for all of them.

Zero-Current Conductance

When the condition in Eq. (33) is not satisfied, the explicit dependence of the zero-current conductance on the concentration of the permeant ion can be easily deduced from Eq. (32). For ϕ sufficiently small that $\sinh\phi/2\approx\phi/2$ and $\cosh\phi/2\approx 1$, we find

$$G(0)=\lim_{V\to 0}\frac{I}{V}=\frac{z^2F^2}{RT}N^*_s(\text{st.})\frac{L_i(0)c_i}{1+2BL_i(0)c_i} \tag{41}$$

As expected, this extended model also predicts saturation of the conductance at sufficiently high ion concentration.

When the carrier is added to the lipid solution rather than to the aqueous phase, so that the aqueous concentration of carriers is negligible, Eq. (8) gives

$$N_s^*(\text{st.}) = \frac{k^{TM} c_s^T}{k^{MT} + k_s^B/(1+\delta k_s^F/D_s)} \tag{42}$$

and Eq. (41) can be more concisely rewritten as

$$G(0) = \frac{z^2 F^2}{RT} \Omega_i \frac{c_s^T c_i}{1+N_i c_i} \tag{43}$$

when

$$\Omega_i = L_i(0) \frac{k^{TM}}{k^{MT} + k_s^B/(1+\delta k_s^F/D_s)} \tag{44}$$

and

$$N_i = 2BL_i(0). \tag{45}$$

Note that, because of the exchange of neutral carriers between the membrane and the torus, the value of N_i depends here on more parameters than in simpler models presented previously (e.g., Läuger & Stark, 1970; Ciani *et al.*, 1973 *a*, *b*), and is not necessarily determined by the rate constant A_s^* for the movement of carriers across the membrane thickness. For instance, if this rate constant were small (which would have implied large values for N_i in previous models), in the present model N_i could still be small if the rate constant of exchange between the membrane and the torus, k_s^{MT}, is sufficiently large. This can be readily seen from Eqs. (45) and (9), and indicates that highly resistive pathways for the movement of free carriers across the membrane thickness can be short-circuited by pathways via the torus.

Zero-Current Membrane Potential in the Presence of Ionic Mixtures

At zero-current Eq. (28) gives

$$\phi_0 = \frac{zF}{RT} V_0 = \ln \frac{\sum_{i=1}^{n} L_i(\phi_0) c_i''}{\sum_{i=1}^{n} L_i(\phi_0) c_i'}. \tag{46}$$

This expression is only formally similar to the equation of Goldman-Hodgkin-Katz, since the quantities $L_i(\phi_0)$ are dependent on voltage. When only two ionic species, i and j are present, Eq. (46) can be written in the form

$$\phi_0 = \ln \frac{c_i'' + \left(\frac{P_j}{P_i}\right)_{\text{app.}} c_j''}{c_i' \left(\frac{P_j}{P_i}\right)_{\text{app.}} c_j'} \tag{47}$$

where the apparent permeability ratios are

$$\left(\frac{P_j}{P_i}\right)_{\text{app.}} = \frac{L_j(\phi_0)}{L_i(\phi_0)}. \tag{48}$$

With the help of Eqs. (26), (23) and (35) and defining

$$\left(\frac{P_j}{P_i}\right)_{\text{Eq.}} = \frac{\bar{K}_j \tilde{A}_{js}^*}{\bar{K}_i \tilde{A}_{is}^*}, \tag{49}$$

Eq. (48) becomes

$$\left(\frac{P_j}{P_i}\right)_{\text{app.}} = \left(\frac{P_j}{P_i}\right)_{\text{Eq.}} \frac{1 + 2\tilde{w}_i \phi_0 \dfrac{\cosh[\beta_i \phi_0]}{\sinh\left[\frac{\alpha_i}{2}\phi_0\right]}}{1 + 2\tilde{w}_j \phi_0 \dfrac{\cosh[\beta_j \phi_0]}{\sinh\left[\frac{\alpha_j}{2}\phi_0\right]}}. \tag{50}$$

Equations of a similar form, and referring to the cases in which translocation across the membrane interior was described either by the classical Nernst-Planck or by the Eyring formalisms, have been derived previously (Ciani, Eisenman, Laprade, Szabo, 1973*b*). Eq. (50) has also been reported previously in a preliminary study on the effects of methylation on the nonactin and valinomycin types of carriers (Eisenman, Krasne, Ciani, 1975).

A notable consequence of Eq. (50) is that the permeability ratio is in general a function of voltage, unless the following conditions are satisfied:

$$\alpha_i = \alpha_j = \alpha; \qquad \beta_i = \beta_j = \beta; \tag{51}$$

and

$$\tilde{w}_i = \tilde{w}_j; \quad \text{or} \quad \tilde{w}_i \gg 1 \quad \text{and} \quad \tilde{w}_j \gg 1. \tag{52}$$

Whether the apparent permeability ratio $(P_j/P_i)_{\text{app.}}$ increases or decreases with potential is dependent solely on the relative values of the parameters $\tilde{w}_i$ and $\tilde{w}_j$. Thus, an increase of ϕ_0 will cause an increase or a decrease of $(P_j/P_i)_{\text{app.}}$, depending on whether $\tilde{w}_i$ is larger or smaller than $\tilde{w}_j$, respectively.

Note that at values of the potential sufficiently low that $\cosh \beta_{i(j)} \phi_0 \approx 1$ and $\sinh \alpha_{i(j)} \phi_0/2 \approx \alpha_{i(j)} \phi_0/2$, Eq. (50) becomes

$$\left(\frac{P_j}{P_i}\right)_{\text{app.}} = \left(\frac{P_j}{P_i}\right)_{V\to 0} = \left(\frac{P_j}{P_i}\right)_{\text{Eq.}} \frac{1+4\tilde{w}_i/\alpha_i}{1+4\tilde{w}_j/\alpha_j}. \tag{53}$$

Interpretation of the Permeability Ratios in Terms of the Energy Profile of Fig. 1

In the treatment of ion permeation through channels, Bezanilla and Armstrong (1972), as well as Hille (1975), have pointed out that, within the framework of Eyring's picture of the diffusion pathways as sequences of activation energy barriers, permeabilities and permeability ratios depend on the peaks of the activation energy barriers, but are independent of the wells, under fairly general conditions. A conclusion of the same type can be drawn for ion permeation mediated by carriers. As a matter of fact, none of the quantities which appear in Eq. (50) depends on the free energy of the two wells of Fig. (1). Let us consider, for instance, the parameter $\tilde{w}_i$, defined in Eqs. (35) and (24)

$$\tilde{w}_i = \tilde{A}_{is}^*/\bar{K}_i^B. \tag{35'}$$

$\tilde{A}_{is}^*$ is proportional to the exponential of the free energy difference between the internal wells and the peak of the central barrier. According to the absolute rate reaction theory, $\bar{K}_i^B$ is proportional to the exponential of the free energy difference between the same wells and the interfacial peaks (which corresponds to the free energy of the activated complex). The ratio between these two quantities, which defines $\tilde{w}_i$, contains the energy difference between the two peaks, while the free energy of the well cancels out. It can be seen that the same cancellation of the free energy of the internal wells occurs also in the products $\bar{K}_i \tilde{A}_{is}^*$ and $\bar{K}_j \tilde{A}_{js}^*$, which appear in the definition of the equilibrium permeability ratios given in Eq. (49): Denoting by $\mu_i^0(\text{aq})$ the standard chemical potentials of the ion i in the aqueous phase, and by $\mu_s^{0*}(1)$ that of the neutral carrier, s, at its equilibrium position in the membrane (not shown in Fig. 1), the product

$\bar{K}_i\tilde{A}^*_{is}$ is given by

$$\begin{aligned}\bar{K}_i\tilde{A}^*_{is}=\bar{v}&\left[\exp\left(\frac{\mu_i^0(\mathrm{aq})+\mu_s^{0*}(1)-\mu_{is}^{0*}(1)}{RT}\right)\right]\\ &\cdot\frac{D^*_{is}}{d^2}\left[\exp\left(\frac{\mu_{is}^{0*}(1)-\mu_{is}^{0*}(\text{top of barrier})}{RT}\right)\right],\end{aligned}\tag{54}$$

$$\bar{K}_i\tilde{A}^*_{is}=\bar{v}\frac{D^*_{is}}{d^2}\exp\left[\frac{\mu_i^0(\mathrm{aq})+\mu_s^{0*}(1)-\mu_{is}^{0*}(\text{top of barrier})}{RT}\right]\tag{55}$$

where $\bar{v}$ is the mean partial molar volume in the aqueous solution. From the above expression it is clear that the free energy in the wells, corresponding to the equilibrium position of the complexes, $\mu_{is}^{0*}(1)$, cancels out in the product, as we wanted to demonstrate.

Outline of a Theory for Transients of Currents Compatible with the Extended Model

Consistent with the model shown in Fig. 1, and approximating the aqueous solutions as ideal conductors, having the charges of the diffuse double layers distributed uniformly over the interfaces, four surfaces of charge distribution can be identified, as is shown schematically in Fig. 2. Let σ' and σ'' denote the surface densities of the charges of the diffuse double layers, and σ_1 and σ_2 the densities of the positive charges of the ion-carrier complexes at their equilibrium positions in the membrane. Since we consider the case of only one ionic species, we will have[1]

$$\sigma_1=zFN^*_{is}(1);\qquad \sigma_2=zFN^*_{is}(2).\tag{56}$$

The electroneutrality condition requires that

$$\sigma'+\sigma_1+\sigma_2+\sigma''=0.\tag{57}$$

If a voltage step, V, is applied across the membrane, a time dependent current will arise and decay to its steady-state value. Since in the transient state the flux of complexes from the left interface, (′) to (1), J_A, is different from the flux of complexes between (1) and (2), J_B, and also different from the flux between (2) and (″), J_C, *none of these fluxes is identifiable with the total measured electric current.* Such total current is given, instead, by the sum of the ionic and the displacement currents, and must be

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[1] Since in this paragraph we restrict considerations to the case of only one permeant ion, we will omit the subscript, i, from $(1)_i$, $(2)_i$, q_i, r_i, α_i, β_i, γ_i.

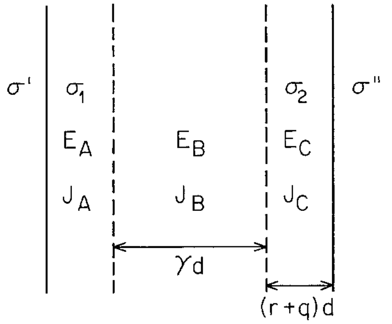

Fig. 2. Schematic diagram of the membrane, showing the planes where the space charge is most concentrated. σ' and σ'' denote the charge densities of the diffuse double layers, σ_1 and σ_2 those of the ion-carrier complexes at their equilibrium positions. γ is the distance between the equilibrium positions of the complexes in membrane thickness units

continuous in all the three compartments; namely,

$$I = zFJ_A + \varepsilon\frac{dE_A}{dt} = zFJ_B + \varepsilon\frac{dE_B}{dt} = zFJ_C + \varepsilon\frac{dE_C}{dt} \tag{58}$$

where ε is the dielectric constant which is assumed to be uniform, and E_A, E_B and E_C are the electric fields in the three compartments into which the membrane is subdivided (*see* Fig. 2). From simple considerations of electrostatics, and with the help of Eq. (57), the electric fields in these three regions are found to be given by

$$\begin{gathered} E_A = \frac{V}{d} - \frac{(1+\gamma)}{2\varepsilon}\sigma_1 - \frac{(1-\gamma)}{2\varepsilon}\sigma_2; \quad E_B = \frac{V}{d} + \frac{1-\gamma}{2\varepsilon}(\sigma_1 - \sigma_2); \\ E_C = \frac{V}{d} + \frac{(1-\gamma)}{2\varepsilon}\sigma_1 + \frac{(1+\gamma)}{2\varepsilon}\sigma_2 \end{gathered} \tag{59}$$

where γd is the distance between the equilibrium positions of the complexes. Note from Fig. 1 that

$$\gamma + 2(r+q) = 1. \tag{60}$$

Substituting (59) into (58) and recalling that

$$\frac{d\sigma_1}{dt} = zF(J_A - J_B); \quad \frac{d\sigma_2}{dt} = zF(J_B - J_C), \tag{61}$$

it can be shown that the three expressions for the total current density given in Eq. (58) are identical, as they must be, in order to satisfy the basic requirement of continuity of the electric current. Choosing to express the current in terms of the flux of complexes and the electric field in the middle compartment, we deduce from Eqs. (58) and (59)

$$\frac{I}{zF}=J_B+\frac{1-\gamma}{2zF}\frac{d}{dt}(\sigma_1-\sigma_2). \tag{62}$$

Note that only when $\gamma=1$, and therefore only when the reaction occurs at the interfaces, is the total current expressed by the flux of complexes, as was assumed in the original treatment by Stark, Ketterer, Beny and Läuger (1971).

Expressing σ_1 and σ_2 in terms of the surface density of the complexes, $N_{is}^*(1)$ and $N_{is}^*(2)$, using Eq. (16), and observing that

$$\phi(1)-\phi(2)=\gamma\phi \tag{63}$$

we find

$$\frac{I}{zF}=A_{is}^*(\phi)[N_{is}^*(1)\,e^{\gamma\phi/2}-N_{is}^*(2)\,e^{-\gamma\phi/2}]+\frac{1-\gamma}{2}\frac{d}{dt}[N_{is}^*(1)-N_{is}^*(2)]. \tag{64}$$

The explicit calculation for the current requires the integration of the conservation equations (1 to 4), which is a laborious and cumbersome task even in the approximation of constant field. However, we will restrict considerations to, and will solve the problem for, the particular case in which the concentration of the permeant ion and the applied potential are sufficiently low that the following approximations can be made: (a) the surface densities of the neutral carriers, $N_s^*(1)$ and $N_s^*(2)$, are much larger than those of the complexes, $N_{is}^*(1)$ and $N_{is}^*(2)$, and are practically unperturbed by the applied potential; (b) the perturbations of the surface densities of the complexes are equal and opposite in the two wells; namely,

$$\delta N_{is}^*(1)=-\delta N_{is}^*(2). \tag{65}$$

We shall also assume that the electric field in all the three compartments of Fig. 2 can be approximated by V/d, so that the additional terms of Eq. (59) can be neglected in the exponentials, $e^{q_i\phi}$ and $e^{-r_i\phi}$, of Eq. (1) as well as in the evaluation of the flux of complexes, J_{is}. (Note, however, that this approximation does not imply that the displacement currents in Eq. (58) are negligible compared to the ionic currents, J_A, J_B and J_C.) Since we consider small potentials ($\phi<1$), the exponentials $e^{q\phi}$, $e^{r\phi}$ and $e^{\gamma\phi}$ can be approximated by

$$e^{q\phi}=(1+q\phi);\qquad e^{r\phi}=(1+r\phi);\qquad e^{\gamma\phi}=(1+\gamma\phi). \tag{66}$$

With these simplifications, Eqs. (1) and (2) are identical in the first order approximation, so that the problem reduces to that of integrating only

Eq. (1). As a consequence of assumption (a) and of the approximations (65) and (66), using Eq. (16) and (63), and approximating Eq. (23) with the low-voltage limit,

$$A_{is}^*(\phi) = 2\tilde{A}_{is}^*/\alpha, \tag{67}$$

Eq. (1) can be written in the form

$$\begin{aligned} \frac{d[\delta N_{is}^*(1)]}{dt} = {} & \bar{K}_i^F\, c_i\, N_s^*(1+q\phi) - \bar{K}_i^B[N_{is}^*(\text{Eq.}) + \delta N_{is}^*(1)](1-r\phi) \\ & -2\,\frac{\tilde{A}_{is}^*}{\alpha}\,[(N_{is}^*(\text{Eq.}) + \delta N_{is}^*(1))(1+\gamma\phi/2) \\ & -(N_{is}^*(\text{Eq.}) - \delta N_{is}^*(1))(1-\gamma\phi/2)]. \end{aligned} \tag{68}$$

Considering that $\bar{K}_i^F\, c_i\, N_s^* \equiv \bar{K}_i^B\, N_{is}^*(\text{Eq.})$, and neglecting the terms which contain the products $\phi\,\delta N_{is}^*(1)$ as infinitesimals of the second order, Eq. (68) becomes

$$\begin{aligned} \frac{d[\delta N_{is}(1)]}{dt} = {} & -\delta N_{is}^*(1)\left[\bar{K}_i^B + \frac{4}{\alpha}\,\tilde{A}_{is}^*\right] \\ & + N_{is}^*(\text{Eq.})\left[\frac{1-\gamma}{2}\,\bar{K}_i^B - 2\gamma\,\frac{\tilde{A}_{is}^*}{\alpha}\right]\phi \end{aligned} \tag{69}$$

where use has been made of Eq. (60) and of the identity

$$\bar{K}_i^F\, c_i\, N_s^* = \bar{K}_i^B\, \bar{K}_i\, c_i\, N_s^* = \bar{K}_i^B\, N_{is}^*(\text{Eq.}). \tag{70}$$

Using the same type of approximations, Eq. (64) becomes

$$\frac{I}{zF} = \frac{2\tilde{A}_{is}^*}{\alpha}\,[\gamma\phi\, N_{is}^*(\text{Eq.}) + 2\delta\, N_{is}^*(1)] + (1-\gamma)\,\frac{d}{dt}\,[\delta N_{is}(1)]. \tag{71}$$

Integrating Eq. (69) and substituting the result into Eq. (71) we find

$$I = I(\infty)[1 + \Gamma e^{-t/\tau}] \tag{72}$$

where

$$I(\infty) = 2zF\, N_{is}^*(\text{Eq.})\,\frac{\tilde{A}_{is}^*}{\alpha + 4\tilde{w}_i}\,\phi, \tag{73}$$

$$\Gamma = \frac{\alpha}{4\tilde{w}_i}\left[1 - \gamma\left(1 + \frac{4}{\alpha}\,\tilde{w}_i\right)\right]^2 \tag{74}$$

and

$$\tau = \frac{1}{\bar{K}_i^B\left(1 + \dfrac{4\tilde{w}_i}{\alpha}\right)}. \tag{75}$$

Since the parameters α and $\tilde{w}_i$ can be deduced from the steady-state analysis of the conductance, the experimental determination of the relaxation time and amplitude will allow us to calculate the individual rate constants, $\bar{K}_i^B$, $\tilde{A}_{is}^*$, as well as γ, which defines the distance between the internal wells.

Note that the amplitude of relaxation, given by Eq. (74), reduces to that originally deduced by Stark *et al.* (1971) (for the case of low potentials and low ion concentrations) only when the equilibrium position of the complexes is right at the interfaces ($\gamma = 1$). In such a case, Eq. (74) yields

$$\Gamma = \frac{4 w_i}{\alpha} \tag{76}$$

and the instantaneous current ($t = 0$), becomes with the aid of Eqs. (72), (73), and (76)

$$I(0) = 2 z F \, N_{is}^*(\text{Eq.}) \, \tilde{A}_{is}^* \frac{\phi}{\alpha} . \tag{77}$$

Eq. (77) shows that, when $\gamma = 1$, the instantaneous current depends on the rate constant for translocation of the complex across the internal barrier, but is independent of the kinetic parameter, $\tilde{w}_i$. By contrast, when $\gamma < 1$ (equilibrium position of the complexes inside the membrane), the instantaneous current, deducible from Eqs. (72), (73) and (74), is *not* independent of the kinetic parameter, $\tilde{w}_i$. Moreover, the amplitude of relaxation, Eq. (74), and the instantaneous current corresponding to the case of $\gamma < 1$ are smaller than those given by Eqs. (76) and (77), which refer to the case $\gamma = 1$, for comparable values of the other parameters $\tilde{A}_{is}^*$, $\tilde{w}_i$, α, and N_{is}^*(Eq.). Even though the treatment given here is approximate and refers to a very particular case, it indicates that relaxation measurements might have to be reinterpreted for a more precise evaluation of the parameters.

This work was supported by NSF GB 30835 and U.S. Public Health Service Grant NS 09931.

Definition of the Symbols

A_s^*	rate constant for translocation of the neutral carrier across the membrane interior.
$A_{is}^*(\phi)$	defined by Eq. (18).
$\tilde{A}_{is}^*$	defined by Eq. (24).
B	defined by Eq. (9).
c_i', c_i''	aqueous concentrations of the ionic species i in the two bulk solutions.
c_s, c_s^T, $c_s(0)$, $c_s(d)$	concentrations of the neutral carrier in the bulk aqueous phases, in the membrane-surrounding torus, and at the ends of the unstirred layers near the membrane-solution interfaces.

d	membrane thickness.
D_s	diffusion coefficient of the carrier in the aqueous phase.
D_{is}^*	diffusion coefficient of the complex in the membrane.
E_A, E_B, E_C	electric fields in the compartments shown in Fig. 2.
$G(0)$	conductance near zero voltage.
$G(\phi)$	conductance at the normalized voltage ϕ.
I	electric current density.
J_{is}	flux of complexes across the membrane interior.
k_s^F, k_s^B	rate constants for the transfer of neutral carriers across the interfaces.
k_s^{TM}, k_s^{MT}	rate constants for the transfer of carriers from the torus into the membrane and vice versa.
$\bar{K}_i^F, \bar{K}_i^B$	rate constants of the heterogeneous reaction describing the formation and the dissociation of the ion-carrier complexes.
$\bar{K}_i$	$\bar{K}_i^F/\bar{K}_i^B$.
$L_i(\phi)$	defined by Eq. (26).
N_i	defined in Eq. (45).
$N_s^*(1), N_s^*(2)$	surface densities of the neutral carrier at their equilibrium positions inside the membrane; note that the equilibrium positions for the neutral carrier, (1) and (2), do not coincide necessarily with the equilibrium positions, $(1)_i$ and $(2)_i$, of the complex is.
$N_s^*(\text{st.})$	defined by Eq. (8).
$N_{is}^*(1)_i, N_{is}^*(2)_i$	surface densities of the ion-carrier complexes at their equilibrium positions inside the membrane.
q, r	fractions of membrane thickness defined in Fig. 1.
V, V_0	transmembrane potential and potential at zero-current, respectively.
$\tilde{w}_i$	defined by Eq. (35).
$W_{is}^*(x)$	defined by Eq. (14).
W_i	free energy difference between the base and the top of the trapezoid in Fig. 1.
α_i	width of the flat top of the energy barrier, measured in membrane thickness units.
β_i	distance of the interfacial peaks from the middle of the membrane, measured in membrane thickness units.
γ	distance between the two internal free energy wells for the complexes, measured in membrane thickness units (*see* Fig. 2).
Γ	relaxation amplitude.
δ	thickness of the unstirred layers.
ε	dielectric constant of the membrane phase.
$\mu_{is}^{0*}(x)$	standard chemical potential of the ion-carrier complex inside the membrane.
ϕ	transmembrane potential in RT/zF units, namely $zFV/RT = zF(V' - V'')/RT$.
$\phi(1)_i, \phi(2)_i$	electric potential at the positions $(1)_i$ and $(2)_i$, respectively.
ϕ_0	membrane potential at zero current.
σ', σ''	net charge of the diffuse double layers per unit membrane area. For small Debye lengths this charge can be viewed as distributed at the membrane-solution interfaces.
σ_1, σ_2	surface charge due to the complexes located at their equilibrium positions.
τ	relaxation time.
Ω_i	defined in Eq. (44).

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