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The Steady-State Theory of the Carrier Transport of Ions

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Summary. The theory for the carrier transport of ions across lipid membranes is extended to consider the potential dependence of the association-dissociation reactions between the carrier and the ions and to consider the effects of aqueous unstirred layers on the exchange of the carrier molecules between the membrane and the aqueous phases. Theoretical expressions are presented under the assumptions that the rate constants vary only with the applied potential and that any one of the following applies: (1) The association-dissociation reaction is at equilibrium; (2) There is only one form of carrier in the aqueous phase; (3) The carrier concentration in the membrane is determined by exchange with the Plateau border which surrounds a black lipid membrane; (4) The total carrier concentration in the membrane is constant; or (5) The association-dissociation reactions are independent of potential. The existing data for valinomycin, the actins, and carbonylcyanide-*m*-chlorophenylhydrazone are consistent with several of the expressions given but are inadequate to decide between them or, in the absence of additional information, to confirm the model. Within the terms of the model, the data for valinomycin and nonactin require that the association reactions depend on potential, but provide no information on the interfacial rate constants for carrier entering or leaving the membrane.

Carrier transport has been proposed to explain the action on thin lipid membranes of macrotetralides, depsipeptides, and the weak-acid uncouplers of oxidative phosphorylation. It is intended to show here that the available quantitative data for the actins, valinomycin, and one of the uncouplers carbonylcyanide-*m*-chlorophenylhydrazone (CCCP), may all be explained on the basis of approximations to one physical model where only the values of the various rate constants are allowed to change from one substance to the next. The treatments given by Ciani, Eisenman and Szabo (1969), Markin, Pastushenko, Krishtalik, Liberman and Topaly (1969), Läuger and Stark (1970), and Le Blanc (1971) are all special cases of this same physical model each derived under certain restrictive assumptions.

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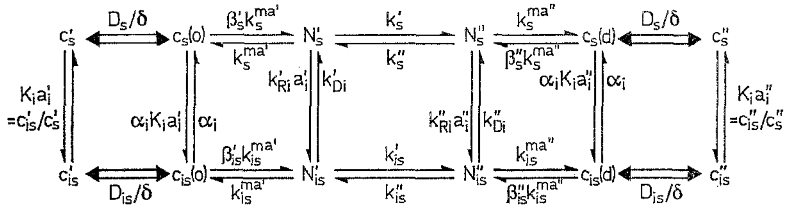

Fig. 1. The steady-state model for carrier transport. All singly primed quantities refer to the left-hand side of the membrane, doubly primed to the right, and unprimed to either side. The subscript "*s*" refers to free carrier, "*i*" to the *i*th species of carried ion, and "*is*" to the complex of the carrier with the ion of species *i*.

Rate constants

Symbols	Units	Process
k'_s, k'_{is}	$\sec^{-1}$	left-to-right across membrane interior
$k_s^{ma'}, k_{is}^{ma'}$	$\sec^{-1}$	desorption, membrane to aqueous
$\beta'_s k_s^{ma'}, \beta'_{is} k_{is}^{ma'}$	cm/sec	adsorption, aqueous to membrane

Concentrations

Symbols	Units	Location
$c_s(x), c_{is}(x)$	moles/cm^3	point x within either unstirred layer
c'_s, c'_{is}	moles/cm^3	stirred portion of left-hand aqueous phase
N'_s, N'_{is}	moles/cm^2	adsorbed to the left side

Parameters

Symbol	Units	Meaning
x	cm	position
d	cm	membrane thickness
δ	cm	unstirred layer thickness
ΔV	volt	potential

Miscellaneous

Symbols	Units	Meaning
D_s, D_{is}	cm^2/sec	aqueous diffusion coefficient
β_s, β_{is}	cm	adsorption equilibrium constants at $\Delta V=0$
$P_s=\beta_s k_s$ $P_{is}=\beta_{is} k_{is}$	cm/sec	permeability of indicated species

While the theory is presented in terms of artificial black lipid membranes (lipid bilayers), the results should apply to any thin hydrophobic permeability barrier. The principal exception is the section on lipid buffered carriers which assumes the existence of a Plateau border composed of the solution or suspension from which the artificial membrane was made. A review of the quantitative studies on the carrier mechanism has been given by Haydon and Hladky (1972).

The model to be considered is the simplest self-consistent treatment which allows the carrier to be soluble in the aqueous phases. The scheme of reactions and flows is given in Fig. 1. The initial formulation must be somewhat complicated to account correctly for the limitations imposed by the finite rate of diffusion in the aqueous phases and the finite rate constants for crossing the membrane-aqueous interfaces. However, the end result is surprisingly simple: in most cases it is possible to describe the entire effect of aqueous polarization and interfacial resistance by introducing an effective series boundary permeability for the total flux of the carrier. The treatment follows the same lines as that presented in Appendix A of Haydon and Hladky (1972) for the simpler case of lipid-soluble ions.

The General Model

A symmetrical membrane of thickness d separates two aqueous phases. These phases are assumed to be perfectly mixed except in layers of thickness δ which are adjacent to the membrane. This division of each aqueous phase into a perfectly stirred region and a completely unstirred layer is a fiction designed to account for the fact that transport perpendicular to a surface, close to that surface, occurs by diffusion alone, while transport occurs by both diffusion and convection when the distance from the surface is greater[1]. This treatment of the aqueous phases is discussed at length in Vetter (1967).

The carrier, species S, which may be neutral or charged, is present far from the membrane at concentrations $c_s' = c_s(-\delta, t)$ and $c_s'' = c_s(d+\delta, t)$ for all values of the time t. Ions of various species M_i are present in the aqueous phases at activities a_i' (left-hand side) and a_i'' (right-hand side). These activities are assumed constant right up to the membrane. The effect

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[1] The electric field in the aqueous phase is assumed to be zero, an assumption which is justified provided the membrane conductance is sufficiently low for each electrolyte concentration used. The membrane resistance must be considerably greater than that of the aqueous phase in series; i.e. for 0.1 M electrolyte, it must be greater than 10^2 Ω cm^2. There is, of course, a portion of any applied potential which occurs in the aqueous phase even in the limit of zero current, but this effect has been shown theoretically to be negligible provided the electrolyte concentration is sufficiently high (Everitt & Haydon, 1968). Thus, in the unstirred layers, movement of the charged forms of the carrier is assumed to be by diffusion alone while the current is carried partially by each species of ion and thus, since they are more numerous, primarily by the ions of the electrolyte.

of surface charge, dipole potentials, etc. on the ionic conductances is expressed in the values of the interfacial rate constants defined below.

The aqueous complex between the carrier and an ion is formed and broken by the reaction

$$a_i + c_s \underset{\alpha_i}{\overset{\alpha_i K_i}{\rightleftharpoons}} c_{is} \tag{1}$$

where K_i is the aqueous association constant and c_{is} is the concentration of the complex MS_i. The activity coefficients for MS_i and S have been included in K_i. Since c_s and c_{is} are both small, these coefficients should not depend on c_s or c_{is}, but they will, in general, vary with the a_i. The same comments apply to β_j defined below whenever j refers to a charged species. To a first approximation these variations may be eliminated if the total concentration of similar species is maintained constant as a_i is varied; e.g., by the use of an excess of a nonpermeating, nonreacting electrolyte. The total concentration of the carrier in all forms in the left phase far from the membrane is, from Eq. (1),

$$c_s^{T'} = c_s' + \sum_i K_i a_i' c_s' \tag{2}$$

and similarly on the right side.

The carrier and the complexes are assumed to adsorb to the left side of the membrane according to (written for a complex)

$$c_{is}(0) \underset{k_{is}^{m\,a'}}{\overset{\beta_{is}' k_{is}^{m\,a'}}{\rightleftharpoons}} N_{is}' \tag{3}$$

and to the right according to

$$c_{is}(d) \underset{k_{is}^{m\,a''}}{\overset{\beta_{is}'' k_{is}^{m\,a''}}{\rightleftharpoons}} N_{is}''. \tag{4}$$

In these equations $c_{is}(0)$ is an aqueous concentration adjacent to the membrane, N_{is}' is a surface concentration, and β_{is}' is a partition coefficient, all for the left side of the membrane. The length β_{is} is that thickness of the aqueous phase which contains the same quantity of species MS_i (at equilibrium) as half of the membrane.

In addition to the fluxes into and out of the membrane in Eqs. (3) and (4), ions may cross the interfaces via the heterogeneous reactions

$$a_i' + N_s' \underset{k_{Di}'}{\overset{k_{Ri}'}{\rightleftharpoons}} N_{is}' \tag{5}$$

and

$$a_i'' + N_s'' \underset{k_{Di}''}{\overset{k_{Ri}''}{\rightleftharpoons}} N_{is}'' \tag{6}$$

in which the species S and MS_i are adsorbed while M_i is not. The necessity of including these heterogeneous reactions when dealing with ion transport has been demonstrated by Le Blanc (1971) for CCCP and by Stark and Benz (1971) for monactin and valinomycin. The activity coefficients of the adsorbed species are taken as unity.

The fluxes across the membrane interior need only be specified as simple linear relations such that

$$J_s = k'_s N'_s - k''_s N''_s \tag{7}$$

and

$$J_{is} = k'_{is} N'_{is} - k''_{is} N''_{is}. \tag{8}$$

Several examples of suitable k's are given by Haydon and Hladky (1972).

The model is formally specified by the equations of conservation of mass (i.e., the equations of continuity) and the assumption of linear flows. (For a simpler example, *see* Appendix A of Haydon & Hladky, 1972.) Thus for the left-hand side

$$\frac{\partial c_s}{\partial t} = \sum_i \alpha_i (c_{is} - K_i c_s a_i) + D_s \frac{\partial^2 c_s}{\partial x^2} \tag{9}$$

$$\frac{\partial c_{is}}{\partial t} = \alpha_i (K_i a_i c_s - c_{is}) + D_{is} \frac{\partial^2 c_{is}}{\partial x^2} \tag{10}$$

$$\frac{dN'_s}{dt} = \sum_i (k'_{Di} N'_{is} - k'_{Ri} a'_i N'_s) + J'_s - J_s \tag{11}$$

and

$$\frac{dN'_{is}}{dt} = k'_{Ri} a'_i N'_s - k'_{Di} N'_{is} + J'_{is} - J_{is} \tag{12}$$

where

$$J'_s = -D_s \left(\frac{\partial c_s}{\partial x}\right)_{x=0} = k_s^{ma'} (\beta'_s c_s(0) - N'_s) \tag{13}$$

and

$$J'_{is} = -D_{is} \left(\frac{\partial c_{is}}{\partial x}\right)_{x=0} = k_{is}^{ma'} (\beta'_{is} c_{is}(0) - N'_{is}). \tag{14}$$

Eqs. (13) and (14) are the boundary conditions used to match the solutions of the preceding equations. Similar equations and boundary conditions apply to the doubly primed (right-hand) side. All of the constants are assumed to be independent of the concentrations and currents; i.e., for a given membrane they depend only on the applied potential and temperature.

In the steady state, all of the time derivatives are zero. Hence, the sum of Eqs. (9) and (10) for all species becomes

$$\frac{d^2}{dx^2} \left(D_s c_s + \sum_i D_{is} c_{is}\right) = 0. \tag{15}$$

After integration once,

$$-\frac{d}{dx}\left(D_s c_s + \sum_i D_{is} c_{is}\right) = J_s' + \sum_i J_{is}'. \tag{16}$$

Then, after a second integration,

$$J_s' + \sum_i J_{is}' = \left\{D_s c_s' + \sum_i D_{is} c_{is}' - D_s c_s(0) - \sum_i D_{is} c_{is}(0)\right\}/\delta. \tag{17}$$

Thus, in the steady state, the total net flux of carrier is the same at all levels in the unstirred layer. However, the individual net fluxes of free carrier and complexes are not independent of x.

The model equations have not been solved even in the steady state. What will be done here is to present certain special cases which correspond to the types of data available or which illustrate the basic features of carrier transport. A complete derivation will be given for the local-equilibrium approximation since this allows the simplest solution displaying the effects of aqueous polarization. A somewhat briefer statement will be made of the expressions which apply when there is negligible charged form of the carrier in the aqueous phase. It is, of course, probable that for some systems the carrier concentration in the membrane is not determined by exchange of carrier molecules with the aqueous phase. In particular, artificial black lipid membranes are surrounded by a plateau border of the membrane-forming solution and exchange of the carrier with this plateau border may determine the membrane concentration. Another solution is therefore obtained under the assumption that the neutral form of the carrier is buffered by the carrier in the border. It is also possible that there are carriers which are not soluble in either the aqueous phases or the border (or the border may be absent). In this case, the simple assumption is that the total amount of carrier in the membrane is constant. A solution is provided for this case as well. Finally, if the partition coefficients of the carrier and complex into the membrane from the aqueous phases are independent of the applied potential, and the two aqueous phases are identical, it is possible to provide a solution allowing both nonequilibrium at the interfaces and arbitrary amounts of both forms of the carrier.

Local Equilibrium

If the association-dissociation reactions are considered to be fast relative to transport, then in the aqueous phase on the left

$$c_{is}(x, t) = K_i a_i' c_s(x, t) \tag{18}$$

and at the left interface

$$N'_{is} = \frac{k'_{Ri}\, a'_i}{k'_{Di}} N'_s = \frac{\beta'_{is}}{\beta'_s} K_i\, a'_i\, N'_s. \tag{19}$$

The last equality follows since

$$\frac{N'_{is}}{N'_s} \equiv \left(\frac{N'_{is}}{c_{is}(0)}\right) \left(\frac{c_{is}(0)}{c_s(0)}\right) \left(\frac{c_s(0)}{N'_s}\right). \tag{20}$$

Two similar relations hold on the right. A steady-state solution is now merely a matter of some linear algebra. Solve first for the total net flux of carrier, both free and combined, from $-\delta$ all the way across to $d+\delta$. This flux ϕ is equal to the total net flux across the left-hand unstirred layer $J'_s + \sum_i J'_{is}$ given in Eq. (17). Under the present assumptions,

$$\phi = \left(D_s/\delta + \sum_i D_{is} K_i\, a'_i/\delta\right) [c'_s - c_s(0)]. \tag{21}$$

The flux $\phi = J'_s + \sum_i J'_{is}$ is also, of course, still equal to the total net flux across the left-hand interface,

$$\phi = k_s^{ma'} [\beta'_s\, c_s(0) - N'_s] + \sum_i k_{is}^{ma'} [\beta'_{is}\, c_{is}(0) - N'_{is}] \tag{22}$$

or, after rearrangement,

$$\phi = [\beta'_s\, k_s^{ma'} + \sum_i \beta'_{is} K_i\, a'_i\, k_{is}^{ma'}]\, [c_s(0) - N'_s/\beta'_s]. \tag{23}$$

Use either Eq. (21) or Eq. (23) to eliminate $c_s(0)$ from the other, then solve to find

$$\phi = Q' [c'_s - N'_s/\beta'_s] \tag{24}$$

where

$$Q' = \frac{\left(\frac{D_s}{\delta} + \sum_i K_i\, a'_i \frac{D_{iS}}{\delta}\right) (\beta'_s\, k_s^{ma'} + \sum_i \beta'_{is} K_i\, a'_i\, k_{is}^{ma'})}{\frac{D_s}{\delta} + \sum_i K_i\, a'_i \left(\frac{D_{is}}{\delta} + \beta'_{is}\, k_{is}^{ma'}\right) + \beta'_s\, k_s^{ma'}}. \tag{25}$$

Corresponding relations hold on the doubly primed side. The quantity Q is the "permeability" of the series combination of the unstirred layer and the interface for the carrier[2]. Since the unstirred layer is generally about 10^6 times thicker than the membrane interface, in the absence of peculiar conformation changes, it will normally be expected for a water-

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[2] The permeability defined as Q' relates the total flux of carrier in all forms to the concentration of free carrier. On aesthetic grounds it might be preferable to refer to the total concentration of carrier. However, the resulting algebra is more complicated and leads, of course, to exactly the same results.

soluble carrier that

$$Q' = D_s/\delta + \sum_i K_i a_i' D_{is}/\delta \ll \beta_s' k_s^{ma'} + \sum_i K_i a_i' \beta_{is}' k_{is}^{ma'} \tag{26}$$

and similarly for Q''. These relations are upper limits for the Q's.

A third equation for ϕ is provided by the relation for the total flux across the membrane. Thus,

$$\phi = J_s + \sum_i J_{is} \tag{27}$$

which from Eqs. (7), (8) and (19) becomes

$$\phi = \left(k_s' + \sum_i \frac{\beta_{is}'}{\beta_s'} K_i a_i' k_{is}'\right) N_s' - \left(k_s'' + \sum_i \frac{\beta_{is}''}{\beta_s''} K_i a_i'' k_{is}''\right) N_s'' \tag{28}$$

$$= (N_s'/\beta_s')\,[P_s' + \Pi_s'] - (N_s''/\beta_s'')\,[P_s'' + \Pi_s''] \tag{29}$$

where

$$P_s' = \beta_s' k_s', \quad P_{is}' = \beta_{is}' k_{is}', \quad \Pi_s' = \sum_i P_{is}' K_i a_i', \quad \text{etc.} \tag{30}$$

The limits $P_s' = P_s'' = P_s$ and $P_{is}' = P_{is}'' = P_{is}$ which hold for the applied potential ΔV equal to 0 are the (interior) permeability coefficients for the species S and MS_i, respectively. A solution for ϕ in terms of c_s' and c_s'' may now be written down by eliminating N_s' and N_s'' from Eq. (24), the comparable doubly primed equation for the right-hand side, and Eq. (29). Then

$$\phi = \frac{(P_s' + \Pi_s')\,c_s' - (P_s'' + \Pi_s'')\,c_s''}{1 + \dfrac{P_s' + \Pi_s'}{Q'} + \dfrac{P_s'' + \Pi_s''}{Q''}}. \tag{31}$$

This expression is then inserted back into Eq. (24) and its parallel to yield N_s' and N_s''. From these it is possible to write down immediately that from Eqs. (7) and (8)

$$J_s = \frac{P_s' c_s' - P_s'' c_s'' + \left[\dfrac{c_s'}{Q''} + \dfrac{c_s''}{Q'}\right] [P_s' \Pi_s'' - P_s'' \Pi_s']}{1 + \dfrac{P_s' + \Pi_s'}{Q'} + \dfrac{P_s'' + \Pi_s''}{Q''}} \tag{32}$$

and

$$J_{is} = \frac{P_{is}' K_i a_i' c_s' - P_{is}'' K_i a_i'' c_s'' + \left[\dfrac{c_s'}{Q''} + \dfrac{c_s''}{Q'}\right] \cdot [(P_s'' + \Pi_s'') P_{is}' K_i a_i' - (P_s' + \Pi_s') P_{is}'' K_i a_i'']}{1 + \dfrac{P_s' + \Pi_s'}{Q'} + \dfrac{P_s'' + \Pi_s''}{Q''}}. \tag{33}$$

These two equations are the predictions of the local equilibrium approximation. They have been written in such a way as to emphasize the two routes by which transport is occurring. In each case the first two terms of the numerator represent transport all the way across from the stirred phase on one side to the stirred phase on the other. Thus $P_s' c_s' - P_s'' c_s''$ would be the transport of free carrier across the membrane in the absence of aqueous polarization and interfacial resistance, and $K_i[P_{is}' a_i' c_s' - P_{is}'' a_i'' c_s'']$ has the same meaning for the ith complex.

The third, more elaborate term in each equation represents the net circulation of the carrier. Thus $P_s' \Pi_s''$ is the rate expression for the process in which free carrier crosses from left to right while complex returns from right to left. The difference, $P_s' \Pi_s'' - P_s'' \Pi_s'$, is thus proportional to the net movement of free carrier across the membrane which is balanced by a movement of complex in the opposite direction.

The denominator scales these two types of fluxes to make the total equal to the actual flux in the presence of the boundary resistance. As the resistance of the boundary increases, i.e. as Q decreases, the denominator increases and the straight-through flux decreases. The circulation, however, remains independent of Q provided that

$$2(P_s + \Pi_s)/Q \gg 1. \tag{34}$$

Thus, whenever this inequality is satisfied Eqs. (31)–(33) imply that

$$J_s \simeq -\sum_i J_{is}. \tag{35}$$

To compare these predictions with quantitative experimental data, it is necessary to specify the charges of the carrier and the complex. The two cases to be considered are a charged carrier with a neutral complex and a neutral carrier of ions. Other cases analogous to those considered by Markin, Pastushenko *et al.* (1969) may be reduced from Eqs. (32) and (33) in the same general manner. Since there is at present no data available for a characterized neutral carrier of neutral solutes, the case has not been considered.

Negative Carrier, Neutral Complex

There are two experimental arrangements which are particularly convenient. In the first, the solutions are different and the zero current potential is determined. In the second, the solutions on the two sides are the same and the current-voltage relation is measured. In both arrangements the symmetry of the membrane requires that $k_{is}' = k_{is}''$ and $\beta_{is}' = \beta_{is}''$ since MS_i is a neutral species.

The zero current condition is always the solution to Eq. (32) when $J_s=0$. To express P_s'/P_s'' in terms of ΔV consider the limiting case for which all $a_i=0$. Then the zero current condition is simply

$$0=P_s' c_s' - P_s'' c_s''. \tag{36}$$

Since now only one species is permeable, zero current implies that all net fluxes are equal to zero. Thus, when $a_i=0$ the zero current potential is the equilibrium potential and is therefore given by

$$e^{-z_s F\Delta V/RT}=c_s''/c_s'. \tag{37}$$

Eq. (36) thus requires that

$$P_s'/P_s''=e^{-z_s F\Delta V/RT}. \tag{38}$$

Since P_s'/P_s'' is a constant independent of a_i, c_{is}, etc., this relation must hold in general.

The relation for the zero-current potential may now be written down for arbitrary a_i and c_s, from Eq. (32) with $J_s=0$

$$\frac{P_s'}{P_s''}=\frac{Q'Q''c_s''+(Q'c_s'+Q''c_s'')\left(\sum_i P_{is}K_i a_i'\right)}{Q'Q''c_s'+(Q'c_s'+Q''c_s'')\left(\sum_i P_{is}K_i a_i''\right)}. \tag{39}$$

For $\sum_i P_{is}K_i a_i \ll Q$, the potential is determined by the gradient of the carrier, while for the reverse inequality, it is determined by the various cations.

In the second experimental arrangement, $c_s^{T'}=c_s^{T''}$, $a_i'=a_i''$, $c_s'=c_s''$, $\Pi_s'=\Pi_s''$, and $I=z_s J_s F$. Thus, the prediction for the current is, from Eq. (32)

$$\frac{I}{z_s F}=J_s=\left(\frac{c_s^T}{1+\sum_i K_i a_i}\right)\frac{P_s'-P_s''}{1+\dfrac{Q''P_s'+Q'P_s''}{Q'Q''+(Q'+Q'')\left(\sum_i P_{is}K_i a_i\right)}}. \tag{40}$$

For small applied potentials $P_s'\simeq P_s''$ and $Q'\simeq Q''$. In addition, Eq. (38) requires

$$P_s'/P_s''\simeq 1-z_s F\Delta V/RT$$

and

$$P_s'-P_s''\simeq -P_s z_s F\Delta V/RT. \tag{41}$$

The conductance in the limit of zero potential difference is therefore

$$G(0)\equiv \mathop{\mathscr{L}}_{\Delta V\to 0}\frac{-I}{\Delta V}=\frac{z_s^2F^2}{RT}\left(\frac{c_s^T}{1+\sum_i K_i a_i}\right)\frac{P_s}{1+\dfrac{2P_s}{Q+2\sum_i P_{is}K_i a_i}}. \tag{42}$$

The Q's in Eqs. (39), (40) and (42) will, in general, depend on the a_i. However, only when the a_i are so small that

$$2\sum_i P_{is} K_i a_i \lesssim Q \tag{43}$$

or, for a single ionic species,

$$a_i \lesssim Q/(2P_{is} K_i) \tag{44}$$

is a value for Q required. Consider first the value of Q when Eq. (26) applies; then Eq. (44) becomes

$$a_i \lesssim \frac{D_s/\delta}{K_i(2P_{is} - D_{is}/\delta)}. \tag{45}$$

For all carrier systems which have been investigated, the permeability of the neutral form, here MS_i, has obeyed[3] $2P_{is} \gg D_{is}/\delta \sim D_s/\delta$ and hence only for

$$a_i \lesssim \frac{D_s}{2K_i P_{is} \delta} \ll \frac{1}{K_i} \tag{46}$$

is Q important. If Eq. (26) applies then Eq. (46) with Eq. (26) yields $Q = D_s/\delta$. If Eq. (26) does not apply then no simple expression is available for Q. However, the inequality in Eq. (46) still applies since Eq. (26) gives an upper limit for Q.

In the instances where Eqs. (26) and (46) do apply, from Eq. (39),

$$e^{-z_s FAV/RT} = \frac{(D_s/\delta)\, c_s'' + (c_s'' + c_s')\,(\sum_i P_{is} K_i a_i')}{(D_s/\delta)\, c_s' + (c_s'' + c_s')\,(\sum_i P_{is} K_i a_i'')}. \tag{47}$$

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[3] This inequality is likely to apply to all carriers which are only sparingly soluble in the aqueous phase. To compare P_{is} and D_{is}/δ it is necessary to separate two effects, the partition of the neutral form into the membrane, and the purely kinetic factors of rate of movement and distance to be moved for each particle. Thus, for this comparison, P_{is} is conveniently written as $P_{is} = K_p D^m_{is}/d$. The correspondence of this way of writing P_{is} with the product used in the remainder of this paper is discussed in Haydon and Hladky (1972). The partition coefficient K_p refers to partition into the membrane interior, while β_{is} refers to the adsorption layer. The diffusion constant in the membrane D^m_{is} will probably be less than D_{is}, but only by a few orders of magnitude such that for liquid membranes it should be safe to contend that $D^m_{is} > 10^{-5}\, D_{is}$. The thicknesses of the layers are such that $(1/d)/(1/\delta) \sim 2 \times 10^4$. The partition coefficient K_p will vary widely from one substance to the next. However, for any efficient carrier of ions, $K_p \gg 1$ since the substance must have an abundance of hydrophobic groups to be able to solubilize ions in the hydrocarbon interior of the membrane (*see* discussion at the end of the section of carrier transport in Haydon & Hladky, 1972). In any case, $K_p \gg 1$ for all the carriers reported to date. Thus, $P_{is} \gg D_{is}/\delta$. (Of course when the carrier is neutral the relation becomes $P_s \gg D_s/\delta$.)

Similarly, from Eqs. (39) and (26),

$$G(0)=\frac{z_s^2 F^2}{RT}\left(\frac{c_s^T}{1+\sum_i K_i a_i}\right)\frac{P_s}{1+\dfrac{2P_s}{D_s/\delta+\sum_i K_i a_i(2P_{is}+D_{is}/\delta)}}. \tag{48}$$

Eq. (48) is the relation derived by Le Blanc (1971).

To rewrite Eq. (40) in terms of the applied potential, some assumption must be made about the potential dependence of P_s'. Eq. (38) then determines P_s''. There is no one theory which is obviously better *a priori* than the others. If it is assumed that

$$P_s'=P_s\, e^{-z_s F\Delta V/(2RT)} \tag{49}$$

and Eq. (26) applies, then

$$J_s=\left(\frac{c_s}{1+\sum_i K_i a_i}\right)\frac{(-2)P_s \sinh[z_s F\Delta V/(2RT)]}{1+\dfrac{2P_s \cosh[zF\Delta V/(2RT)]}{D_s/\delta+\sum_i K_i a_i(2P_{is}+D_{is}/\delta)}}. \tag{50}$$

Alternative versions are discussed in Haydon and Hladky (1972). Whatever assumption is made, it should be checked by measuring the current-voltage relation under conditions where

$$\sum_i P_{is} K_i a_i \gg P_s. \tag{51}$$

This procedure is not, however, foolproof since it depends on the correctness of the local equilibrium assumption.

A lipid-soluble ion is the limiting case of a carrier for which $K_i=0$ for all i. Eq. (22) then reduces to

$$Q'=\frac{\beta_s' k_s^{ma'} D_s/\delta}{D_s/\delta+\beta_s' k_s^{ma'}} \tag{52}$$

and similarly for Q''. The equation for the current [Eq. (40)] becomes

$$\frac{I}{z_s F}=J_s=c_s^T\,\frac{P_s'-P_s''}{1+\dfrac{P_s'}{Q'}+\dfrac{P_s''}{Q''}} \tag{53}$$

and finally

$$e^{+z_s F\Delta V/RT}=c_s^{T'}/c_s^{T''}. \tag{54}$$

These are the results derived directly in Appendix A of Haydon and Hladky (1972).

Neutral Carrier, Charged Complex

For the neutral, free form of the carrier, symmetry requires $k'_s = k''_s$ and $\beta'_s = \beta''_s$ in precisely the same manner as before. The observable flux [Eq. (33)] in the presence of a single ionic species becomes

$$J_{is} = \left(\frac{c_s^T K_i a_i}{1+K_i a_i}\right) \frac{P'_{is} - P''_{is}}{1 + \dfrac{K_i a_i [Q'' P'_{is} + Q' P''_{is}]}{Q' Q'' + (Q' + Q'') P_s}} \tag{55}$$

and thus the limiting conductance is

$$G(0) = \frac{z_{is}^2 F^2}{RT} \left(\frac{c_s^T K_i a_i}{1+K_i a_i}\right) \frac{P_{is}}{1 + \dfrac{2P_{is} K_i a_i}{Q + 2P_s}}. \tag{56}$$

An explicit form of the current-voltage relation still depends on the assumption for the potential dependence of P'_{is}. If Eqs. (49) and (26) are both assumed to apply, then

$$J_{is} = \left(\frac{c_s^T K_i a_i}{1+K_i a_i}\right) \frac{(-2) P_{is} \sinh[z_{is} F \Delta V/(2RT)]}{1 + \dfrac{2K_i a_i P_{is} \cosh[z_{is} F \Delta V/(2RT)]}{2P_s + D_s/\delta + K_i a_i D_{is}/\delta}}. \tag{57}$$

The zero current condition for a single ionic species becomes, from Eq. (33) with $J_{is} = 0$,

$$\frac{P'_{is}}{P''_{is}} = e^{-z_{is} F \Delta V/RT} = \left(\frac{a''_i}{a'_i}\right) \frac{Q' Q'' c''_s + P_s [Q' c'_s + Q'' c''_s]}{Q' Q'' c'_s + P_s [Q' c'_s + Q'' c''_s]}. \tag{58}$$

Whenever Q' and Q'' may be written as $Q' = Q_0(1 + K_i a'_i)$ and $Q'' = Q_0(1 + K_i a''_i)$ for some Q_0, e.g. D_s/δ, then Eq. (58) simplifies to

$$e^{-z_{is} F \Delta V/RT} = \left(\frac{a''_i}{a'_i}\right) \frac{c_s^{T'} + c_s^{T''} + \dfrac{Q_0}{P_s} c_s^{T''} (1 + K_i a'_i)}{c_s^{T'} + c_s^{T''} + \dfrac{Q_0}{P_s} c_s^{T'} (1 + K_i a''_i)}. \tag{59}$$

For $P_s \gg Q_0$, the result is strikingly simple:

$$e^{-z_{is} F \Delta V/RT} = a''_i / a'_i \tag{60}$$

as found by Szabo, Eisenman and Ciani (1970).

Negligible Aqueous Association

The complexity of a solution to the model equations results primarily from the coupling of the fluxes of S and MS_i in the aqueous phase. Whenever these aqueous fluxes may be ignored or approximated, a solution becomes comparatively simple. If the total concentration of the complexes in the aqueous phase is assumed to be negligible, then it is possible to set $J'_{is}=J''_{is}=0$ and thus obtain a steady-state solution in a manageable closed form without assuming that the association-dissociation reactions are at equilibrium. A comparable expression for a negative carrier is presented in the section on lipid-buffered carriers. For simplicity the results are presented assuming a single ionic species M_i.

The combination of the interfacial and diffusion terms proceeds as before,

$$\begin{aligned} J'_s &= (D_s/\delta)\,[c'_s - c_s(0)] \\ &= k_s^{ma}\,[\beta_s c_s(0) - N'_s] \\ &= Q\,[c'_s - N'_s/\beta_s] \end{aligned} \tag{61}$$

where now

$$Q = \frac{(D_s/\delta)\,\beta_s k_s^{ma}}{D_s/\delta + \beta_s k_s^{ma}}. \tag{62}$$

Thus, the steady-state solution for a single species of ion is obtained from the following set of equations which replace Eqs. (7), (8), (11) and (12):

$$-(k'_{Ri}a'_i + Q/\beta_s)\,N'_s + k'_{Di}N'_{is} - J_s = -Q\,c'_s \tag{63}$$

$$k'_{Ri}a'_i N'_s - k'_{Di}N'_{is} - J_{is} = 0 \tag{64}$$

$$-(k''_{Ri}a''_i + Q/\beta_s)\,N''_s + k''_{Di}N''_{is} + J_s = -Q\,c''_s \tag{65}$$

$$k''_{Ri}a''_i N''_s - k''_{Di}N''_{is} + J_{is} = 0 \tag{66}$$

$$J_{is} = k'_{is}N'_{is} - k''_{is}N''_{is} \tag{67}$$

$$J_s = k_s(N'_s - N''_s). \tag{68}$$

The algebra of the solution is tedious but straight-forward (e.g., by the method of determinants, *see* Thomas, 1953, p. 435). The solution for J_{is} is

$$J_{is} = \beta_s \cdot \left\{ \frac{\dfrac{k'_{Ri}a'_i}{k'_{Di}}\,k'_{is}\left[\dfrac{Q c'_s + \beta_s k_s (c'_s + c''_s)}{Q + 2\beta_s k_s}\right] - \dfrac{k''_{Ri}a''_i}{k''_{Di}}\,k''_{is}\left[\dfrac{Q c''_s + \beta_s k_s (c'_s + c''_s)}{Q + 2\beta_s k_s}\right]}{1 + \dfrac{k'_{is}}{k'_{Di}} + \dfrac{k''_{is}}{k''_{Di}} + \dfrac{k'_{Ri}a'_i}{k'_{Di}}\left(\dfrac{\beta_s k'_{is}}{Q + 2\beta_s k_s}\right) + \dfrac{k''_{Ri}a''_i}{k''_{Di}}\left(\dfrac{\beta_s k''_{is}}{Q + 2\beta_s k_s}\right)} \right\}. \tag{69}$$

If, as expected, $P_s = \beta_s k_s \gg Q$, then

$$J_{is} = \beta_s \frac{c'_s + c''_s}{2} \left\{ \frac{\dfrac{k'_{Ri}a'_i}{k'_{Di}}\,k'_{is} - \dfrac{k''_{Ri}a''_i}{k''_{Di}}\,k''_{is}}{1 + \dfrac{k'_{is}}{k'_{Di}} + \dfrac{k''_{is}}{k''_{Di}} + \dfrac{k'_{Ri}a'_i k'_{is}}{k'_{Di}2k_s} + \dfrac{k''_{Ri}a''_i k''_{is}}{k''_{Di}2k_s}} \right\}. \tag{70}$$

The expression for the limiting conductance between identical solutions is

$$G(0)=\frac{z_{is}^2 F^2}{RT}\beta_s c_s \frac{\left(\dfrac{k_{Ri}a_i}{k_{Di}+2k_{is}}\right)k_{is}}{1+\left(\dfrac{k_{Ri}a_i}{k_{Di}+2k_{is}}\right)\left(\dfrac{2\beta_s k_{is}}{Q+2\beta_s k_s}\right)} \tag{71}$$

which is only slightly different from Eq. (56). The current-voltage relation is, however, much more complicated. It is still true that

$$\frac{k'_{Ri}k'_{is}}{k'_{Di}}\Big/\frac{k''_{Ri}k''_{is}}{k''_{Di}}=e^{-z_{is}F\Delta V/RT} \tag{72}$$

but now the potential dependence of k'_{Ri} and k'_{is}/k'_{Di} must be specified separately. Thus, removing the assumption of local equilibrium introduces an additional arbitrary function. The available current-voltage data is neither precise enough nor sufficiently extensive to make a fit to the experimental curves in itself a convincing proof of the model.

Lipid-Buffered Carrier

In the two approximations discussed above, it has been assumed that the total carrier concentration in the aqueous phase c_s^T is known and constant. Thus, the expressions for the membrane fluxes have been derived under the assumption that, when the net fluxes are zero, the concentrations in the membrane are at equilibrium with those in the aqueous phase. However, in experiments with artificial black lipid membranes, there is a hydrocarbon-lipid Plateau border around the edge of the membrane. Both Stark and Benz (1971) and Hladky (1971, 1972) have pointed out that, in experiments with the neutral carriers, reproducible, stable conductances are much easier to achieve when the carrier is added to the hydrocarbon phase. Thus, carrier in the membrane appears to be closer to equilibrium with carrier in the Plateau border than with that in the aqueous phase. This inference might seem surprising since the exchange of carrier with the Plateau border must occur through the walls of a very short cylinder; i.e., an area $2\pi\rho d$ where ρ is the radius of the membrane, while exchange with the aqueous phase occurs across the ends of the cylinder with area $2\pi\rho^2$. For $\rho=500$ μ and $d=50$ Å, the latter area is 10^5 times larger! However, exchange with the Plateau border occurs by diffusion within the membrane where the concentration of a neutral form of the carrier may well be 10^4 to 10^5 times higher than the concentration of the neutral form in the aqueous phase.

If the Plateau border and the aqueous phase are not themselves in equilibrium, the dependence of G on a_i will depend on the factors governing the carrier concentration in the membrane. For equilibrium with the aqueous phase, i.e. an aqueous-buffered carrier, and a single species of cation,

$$N_s = \beta_s \frac{c_s^T}{1 + K_i a_i} \tag{73}$$

and

$$N_{is} = \beta_{is} \frac{c_s^T K_i a_i}{1 + K_i a_i} = \frac{k_{Ri} a_i}{k_{Di}} \left(\frac{\beta_s c_s^T}{1 + K_i a_i} \right). \tag{74}$$

For equilibrium with the Plateau border, i.e. a lipid-buffered carrier,

$$N_s = \zeta_s c_s^L \tag{75}$$

and

$$N_{is} = \frac{k_{Ri} a_i}{k_{Di}} \zeta_s c_s^L. \tag{76}$$

Thus, in the first instance for constant c_s^T, $N_s + N_{is}$ first increases with a_i then reaches a plateau at $\beta_{is} c_s^T$. In the second instance, at constant c_s^L, $N_s + N_{is}$ continues to rise indefinitely while N_s remains constant. Of course, the distribution of carrier between the bulk hydrocarbon and aqueous phases also depends on a_i in such a manner,

$$c_s^L \propto c_s = c_s^T / (1 + K_i a_i), \tag{77}$$

that at true equilibrium there is no ambiguity about the dependence of the membrane concentrations on the total quantity of carrier and the concentration of ions.

For lipid-buffered carriers, solutions are available to the model equations under less restrictive assumptions than for aqueous buffering. It is now possible to ignore completely the complications of aqueous polarization and thus the terms involving Q. Setting $Q = 0$ in Eqs. (63) and (65) is not equivalent to denying the aqueous solubility of the carrier or to requiring an infinite resistance at the boundary. What it does require is merely that membrane fluxes, i.e. J_s and J_{is}, be much larger than the fluxes across the unstirred layers; that is, it requires Q to be small in the sense of Eq. (34). In the local equilibrium and negligible aqueous association approximations, the Q's had to be carried through the calculations to determine the effect of the fluxes on $N_s' + N_s''$. By definition in the present approximation, they are no longer needed for this purpose.

Neutral Carrier

If the partition of a neutral, lipid-buffered carrier from the Plateau border into the membrane is assumed to be independent of applied potential, then

$$N_s' + N_s'' = 2\zeta_s c_s^L \tag{78}$$

for all a_i and ΔV. If there is only one species of ion, and, as suggested in the previous paragraph, it is assumed that

$$J_{is} = -J_s \tag{79}$$

then Eqs. (63)–(68) with $Q=0$, and Eq. (78) may be solved simply by the use of the same device for J_{is} which led to Eq. (31) for ϕ_s. The solution for J_{is} is

$$J_{is} = \zeta_s c_s^L \left\{ \frac{\dfrac{k_{Ri}' a_i'}{k_{Di}'} k_{is}' - \dfrac{k_{Ri}'' a_i''}{k_{Di}''} k_{is}''}{1 + \dfrac{k_{is}'}{k_{Di}'} + \dfrac{k_{is}''}{k_{Di}''} + \dfrac{k_{Ri}' a_i'}{k_{Di}'} \dfrac{k_{is}'}{2k_s} + \dfrac{k_{Ri}'' a_i''}{k_{Di}''} \dfrac{k_{is}''}{2k_s}} \right\} \tag{80}$$

which has exactly the same form as Eq. (70) derived under the assumption of negligible aqueous association. Indeed, the leading factor in both cases is just the constant $(N_s' + N_s'')/2$. There are, however, two important differences. Here, $N_s' + N_s''$ is presumed constant for all a_i so long as the carrier is still lipid-buffered. In Eq. (70), constant $N_s' + N_s''$ depends on $K_i a_i \ll 1$. The second difference is in the interpretation of k_{Ri} and k_{Di}. For use in Eq. (80) these are defined as those constants which make Eqs. (63) through (68) with $Q=0$ an accurate description. If the process

$$MS_i' \rightarrow S' + M_i(0) \tag{81}$$

occurs solely via the intermediate steps of desorption and adsorption,

$$MS_i' \rightarrow MS_i(0) \rightarrow S(0) + M_i(0) \rightarrow S' + M_i(0), \tag{82}$$

then this is the process described by k_{Di}. The use of k_{Di} assumes, not the route of the dissociation, but that the association-dissociation reactions, depend on N_{is}' and N_s' and not on $c_s(-\delta)$ and $c_{is}(-\delta)$. If $\beta_{is}' k_{is}^{ma'} \gg D_{is}/\delta$, D_s/δ; this assumption applies to dissociation occurring in the aqueous phase immediately adjacent to the membrane. As mentioned in connection with Eqs. (5) and (6), Stark and Benz (1971) and Le Blanc (1971) have shown that the magnitudes of k_{Ri} and k_{Di} indicate that for valinomycin, the actins and CCCP the dissociation-association reactions occur without the necessity of the intermediate steps in Eq. (82). However, contrary to the statement in Stark and Benz (1971), terms of the form

$$\frac{k_{Ri}a_i}{k_{Di}}\,\frac{k_{is}}{2k_s} \tag{83}$$

will occur regardless in the $G-a_i$ relation; e.g. Eq. (56), or that reduced from Eq. (80).

Since Eqs. (70) and (80) have the same form, it cannot be shown from an I–V curve with $K_i a_i \ll 1$ whether a neutral carrier is lipid- or aqueous-buffered. In general, the answer depends on the relative rates of exchange of carrier between the membrane and the aqueous phase and Plateau border, respectively, which depend in different ways upon ρ. Thus, for a neutral carrier with $K_i a_i > 1$, if the $G-a_i$ curve is determined by merely changing the aqueous phase, the data may well depend on membrane area. This difficulty may be avoided by pre-equilibrating the hydrocarbon and aqueous phases at each ion concentration thus allowing c_s^L and c_s^T to vary according to the restrictions imposed by Eq. (77) and the known total amount of carrier.

Neutral Complex

For a charged carrier with a neutral complex, where the complex is lipid-buffered, the expression for the current is

$$\frac{I}{z_s F} = J_s = \zeta_{is}\,c_{is}^L \left\{ \frac{\dfrac{k'_{Di}}{k'_{Ri}a'_i}\,k'_s - \dfrac{k''_{Di}}{k''_{Ri}a''_i}\,k''_s}{1+\dfrac{k'_s}{k'_{Ri}a'_i}+\dfrac{k''_s}{k''_{Ri}a''_i}+\dfrac{k'_{Di}}{k'_{Ri}a'_i}\,\dfrac{k'_s}{2k_{is}}+\dfrac{k''_{Di}}{k''_{Ri}a''_i}\,\dfrac{k''_s}{2k_{is}}} \right\}. \tag{84}$$

This equation corresponds to the relation derivable under the negligible aqueous dissociation approximation where $\zeta_s c_s^L$ would be replaced by $\beta_{is}(c'_{is}+c''_{is})/2$. For $a'_i=a''_i$, $k'_{Ri}=k''_{Ri}$, and $k'_{Di}=k''_{Di}$, Eq. (84) becomes

$$J_s = \zeta_{is}\,c_{is}^L \left\{ \frac{k'_s - k''_s}{\dfrac{k_{Ri}a_i}{k_{Di}} + (k'_s + k''_s)\left(\dfrac{1}{k_{Di}}+\dfrac{1}{2k_{is}}\right)} \right\} \tag{85}$$

which may be compared with Eq. (40) calculated on the basis of the local equilibrium assumption.

Membrane-Bound Carrier

The presence of a hydrocarbon-lipid Plateau border is a peculiarity of artificial black lipid membranes. The only possible reservoir of carrier in most membrane systems will be the aqueous phase. Thus, either the carrier concentration in the membrane is determined by exchange with the aqueous phase or else it is constant. The membrane-bound carrier assumption requires that

$$N_T = N_s' + N_s'' + \sum_i (N_{is}' + N_{is}'') \tag{86}$$

be independent of a_i and ΔV. By definition in this case, charge transfer occurs by the association-dissociation reactions. The equations to be solved for a single carried species are Eqs. (63)–(68) with $Q=0$, and Eq. (86). The algebra proceeds in the same manner as for local equilibrium, but with rather more involved expressions, leading to

$$\begin{aligned} J_{is} = N_T (k_s'' k_{is}' K_i^{m'} - k_s' k_{is}'' K_i^{m''}) \bigg/ \bigg\{ & [k_s'(1+K_i^{m''}) + k_s''(1+K_i^{m'})] \\ & \cdot \left[1 + \frac{k_{is}'}{k_{Di}'} + \frac{k_{is}''}{k_{Di}''}\right] + k_{is}' K_i^{m'}(1+K_i^{m''}) + k_{is}'' K_i^{m''}(1+K_i^{m'}) \\ & + \left(\frac{k_{Di}' - k_{Di}''}{k_{Di}' k_{Di}''}\right) [k_{is}' k_s'' K_i^{m'} - k_{is}'' k_s' K_i^{m''}] \bigg\} \end{aligned} \tag{87}$$

where

$$K_i^{m'} = k_{Ri}' a_i' / k_{Di}' \quad \text{and} \quad K_i^{m''} = k_{Ri}'' a_i'' / k_{Di}''. \tag{88}$$

In the special case of a neutral carrier ($k_s' = k_s''$) for which $K_i^{m'} = K_i^{m''}$ and $k_{Di}' = k_{Di}''$, Eq. (87) simplifies considerably to become

$$J_{is} = \left(\frac{\dfrac{k_{Ri} a_i}{k_{Di}} \dfrac{N_T}{2}}{1 + \dfrac{k_{Ri} a_i}{k_{Di}}} \right) \frac{k_{is}' - k_{is}''}{1 + (k_{is}' + k_{is}'') \left(\dfrac{1}{k_{Di}} + \dfrac{k_{Ri} a_i}{2 k_{Di} k_s} \right)}. \tag{89}$$

None of the carrier substances which has been isolated to date is sufficiently insoluble in the aqueous phase to allow the *a priori* assumption of constant N_T independent of a_j and ΔV to apply.

Interfacial Constants Independent of Potential

Markin, Krishtalik, Liberman and Topaly (1969) and Markin, Pastushenko *et al.* (1969) have presented another approximation for carrier transport which treats carriers and complexes of arbitrary charges but on the assumption of a constant potential energy gradient for an ion crossing the membrane (*see* Haydon & Hladky, 1972, for a discussion of the different potential assumptions). Läuger and Stark (1970) have given alternative expressions based on a rate-theory approach which is, however, restricted to neutral carriers. The formal analysis in the two cases is the same. Both treatments allow a finite rate of exchange of carrier and complex across the aqueous-membrane interface but completely ignore diffusion polarization in the aqueous phases; e.g., they assume $c_s(0)=c'_s$. Thus, the expressions as derived are limited to the range of k_s^{ma} and k_{is}^{ma} such that Q is determined at the interface and hence is much less than $D_s/\delta+\sum_i K_i a_i D_{is}/\delta$. Läuger and Stark's (1970) equation for a neutral carrier, which assumes $k'_{Ri}a'_i=k''_{Ri}a''_i$, $k'_{Di}=k''_{Di}$, $k_{is}^{ma'}=k_{is}^{ma''}$, $k_s^{ma'}=k_s^{ma''}$, $c_s^{T'}=c_s^{T''}$, and a single electrolyte is

$$J_{is}=c_s^T\left(\frac{K_i a_i\beta_{is}}{1+K_i a_i}\right)\frac{(k'_{is}-k''_{is})\left[1+\frac{k_{is}^{ma}}{k_{Di}}\left(1+\frac{k_{Ri}a_i}{2k_s+k_s^{ma}}\right)\right]}{\left(1+\frac{k_{Ri}a_i}{2k_s+k_s^{ma}}\right)\left(1+\frac{k_{is}^{ma}}{k_{Di}}+\frac{k'_{is}+k''_{is}}{k_{Di}}\right)-\frac{k_{Ri}a_i}{2k_s+k_s^{ma}}}. \tag{90}$$

In Eq. (90) only if

$$k_{is}^{ma}>k_{Di} \tag{91}$$

can k_{is}^{ma} be detected and only if both

$$k_s^{ma}>2k_s \quad \text{and} \quad k_{Ri}a_i/k_s^{ma}>1 \tag{92}$$

can k_s^{ma} be detected. However, unless

$$\beta_{is}k_{is}^{ma},\ \beta_s k_s^{ma}\ll D_s/\delta,\ D_{is}/\delta, \tag{93}$$

neither interfacial process can be detected in series with the aqueous phase and Eq. (90) is invalid. No results are known for which these inequalities appear to be satisfied. Indeed, if

$$P_s=\beta_s k_s\gg D_s/\delta, \tag{94}$$

then either Eq. (92) or Eq. (93) must be violated. In the limit where Eqs. (91), (93) and (94) apply,

$$J_{is}=c_s^T\left(\frac{K_i a_i\beta_{is}}{1+K_i a_i}\right)\frac{k'_{is}-k''_{is}}{1+k'_{is}/k_{is}^{ma}+k''_{is}/k_{is}^{ma}}. \tag{95}$$

In the other limiting case where

$$k_{is}^{ma}/k_{Di}\ll 1 \tag{96}$$

and

$$k_s^{ma}\ll 2k_s, \tag{97}$$

Eq. (90) simplifies to

$$J_{is}=c_s^T\left(\frac{K_i a_i\beta_{is}}{1+K_i a_i}\right)\frac{k'_{is}-k''_{is}}{1+(k'_{is}+k''_{is})\left(\frac{1}{k_{Di}}+\frac{k_{Ri}a_i}{2k_{Di}k_s}\right)} \tag{98}$$

which is the equation used by Stark and Benz (1971) to analyze their data for valinomycin and monactin. It is interesting that Eq. (98) is of the same form as Eq. (89). For $k'_{Ri}a'_i=k''_{Ri}a''_i$, $k'_{Di}=k''_{Di}$, $k_{is}^{ma'}=k_{is}^{ma''}$, $k_s^{ma'}=k_s^{ma''}$, and $c_s^{T'} = c_s^{T''}$, the concentration of carrier in the membrane becomes independent of applied potential regardless of the values of k_s^{ma} and k_{is}^{ma}. This is most easily seen from Eqs. (7)–(14) by means of an example. Consider the combination of Eqs. (11) and (13) written for a single ionic species M_i,

$$J_s = k_{Di} N'_{is} - k_{Ri} a_i N'_s + k_s^{ma}[\beta_s c_s(0) - N'_s], \tag{99}$$

and the doubly primed equivalent,

$$J_s = k_{Ri} a_i N''_s - k_{Di} N''_{is} + k_s^{ma}[N''_s - \beta_s c_s(d)]. \tag{100}$$

Suppose the solution to Eq. (99) is known; i.e., the values of N'_{is}, N'_s, $c_s(0)$ and J_s are available. Let a bar denote the value at $\Delta V = 0$. Then

$$\begin{aligned} N''_s &= 2\bar{N}_s - N'_s, \\ N''_{is} &= 2\bar{N}_{is} - N'_{is}, \\ c_s(d) &= 2\bar{c}_s - c_s(0), \end{aligned} \tag{101}$$

and J_s will constitute a solution of Eq. (100) since $k_{Ri} a_i \bar{N}_s = k_{Di} \bar{N}_{is}$, etc. The same applies to all of Eqs. (7)–(14), thus the relations in Eq. (101) are a property of the solution.

Since $N'_s + N''_s$ and $N'_{is} + N''_{is}$ are now constants at a given a_i, either a simplified version of the lipid-buffered carrier or membrane-bound carrier approximations may be used to calculate the flux provided only that a suitable form can be found to replace $\zeta_s c_s^L$ or N_T, respectively. These are found by calculating $N'_s + N''_s$ or $N'_s + N''_s + N'_{is} + N''_{is}$ at $\Delta V = 0$. The necessary expressions for the carrier concentrations are just Eqs. (73) and (74). Thus, from either Eq. (80) or Eq. (89)

$$J_{is} = c_s^T \left(\frac{K_i a_i \beta_{is}}{1 + K_i a_i}\right) \frac{k'_{is} - k''_{is}}{1 + (k'_{is} + k''_{is}) \left(\dfrac{1}{k_{Di}} + \dfrac{k_{Ri} a_i}{2 k_{Di} k_s}\right)} \tag{102}$$

which is the same as Eq. (98). The comments following Eq. (80) apply to k_{Ri} and k_{Di} as they are used in Eq. (102).

It should be emphasized that Eqs. (98), (102) and the result that N_T is independent of ΔV for finite k_s^{ma} and k_{is}^{ma} all require the interfacial rate constants to be independent of potential. In this case, the derivation of Eq. (102) shows that the result does not depend on Läuger and Stark's (1970) assumptions about the interfacial rate constants, Eqs. (93), (96) and (97), but rather on the much weaker condition that $P_s \gg Q$.

Experimental Data

CCCP

Le Blanc (1971) has reported on the $G-a_H$ and ΔV behavior for CCCP, a postulated negative carrier of protons. He shows that the predictions of Eqs. (47) and (48) are in agreement with the experimental data with the following values for the constants: $P_s = 2\times 10^{-3}$ cm/sec, $P_{is} = 11$ cm/sec, $D_s/\delta = 3.6\times 10^{-4}$ cm/sec. The results are discussed at length in his paper in terms of the local equilibrium approximation Eq. (48). At high pH, neither Eq. (84) nor Eq. (88) fits the experimental data. However, at low pH these predictions become indistinguishable from Eq. (48). Indeed for low pH, Eq. (84) in the version resulting from the negligible aqueous dissociation approximation may be useful for interpreting current-voltage relations.

Actins and Valinomycin

Szabo, Eisenman and Ciani (1969) have shown for the actins, and Stark and Benz (1971) have shown for valinomycin that on neutral or weakly charged phospholipid + n-decane membranes Eq. (56) or Eq. (71) is obeyed but in the degenerate form where $K_i a_i \ll 1$ and $2P_{is}K_i a_i \ll Q+2P_s$. Szabo *et al.* (1970) have looked for a potential in a gradient of the actins and find that there is none observable. This result implies that $P_s/Q \gg 1$. These authors also noted that their experimental data for ΔV in a gradient of ions is in agreement with Eq. (60).

Stark and Benz (1971) have reported data for valinomycin with 1 M KCl as the electrolyte and a highly negatively charged phosphatidyl inositol + n-decane membrane. In this case, Eq. (71) in terms of c_i rather than a_i is nondegenerate. They point out that $K_i c_i$ is independent of the membrane, and hence that $P_{is}K_i/P_s \sim 7$. They have also determined $I-V$ relations for monactin and valinomycin. At least for the latter case, they show that the coefficient of $k'_{is}+k''_{is}$ in Eq. (70) is independent of c_i for low c_i. Thus, for this system Eq. (55) is not obeyed. The same nonproportionality is observed for the nonactin K^+ fluxes across glyceryl monoleate + n-hexadecane membranes (Hladky, 1972).

Stark and Benz endeavored to fit their experimental I–V curves using Eq. (98) with the additional assumption that

$$k'_{is} = k_{is}\, e^{-z_{is}F\Delta V/2RT}. \qquad (103)$$

These authors quite properly displayed their data as $G(\Delta V)/G(0)$ *vs.* ΔV where

$$G(\Delta V) \equiv -\frac{I}{\Delta V} \tag{104}$$

since this plot eliminates scale factors and at the same time displays discrepancies between theory and experiment much more clearly than the I–V plot itself. In their results, the saturation behavior is peculiar since the value of $k_{Ri}k_{is}/(k_{Di}k_s)$ for valinomycin is about seven from the $G-c_i$ curve and about one from the I–V curve. A similar discrepancy occurs in their data for monactin. These discrepancies may be caused by an interaction between the high negative surface charge on their phosphatidyl-inositol membranes and a strong adsorption of positive carrier complexes (Stark, Ketterer, Benz & Läuger, 1971). In any case, until the effect is understood the data at high concentrations of K^+ will be difficult to interpret on a quantitative basis.

For low K^+ concentrations the possible test of the model is its ability to predict the current-voltage relation. The only sensitive fit to Eq. (98) for valinomycin was obtained using erythrocyte lipids in decane. The fit is not good. For monactin a test is possible using phosphatidyl inositol; again the fit is poor. The experimental current continues to rise as ΔV is increased while the theoretically predicted current fit for low values of ΔV reaches a saturation limit.

For $a_i' = a_i''$ sufficiently low that no concentration dependence of $G(\Delta V)/G(0)$ is observed, Eq. (70) becomes

$$J_{is} = \beta_s \frac{c_s' + c_s''}{2} a_i \left\{ \frac{k_{Ri}'\left(\dfrac{k_{is}'}{k_{Di}'}\right) - k_{Ri}''\left(\dfrac{k_{is}''}{k_{Di}''}\right)}{1 + \dfrac{k_{is}'}{k_{Di}'} + \dfrac{k_{is}''}{k_{Di}''}} \right\}. \tag{105}$$

For nonactin in glyceryl monoleate $+n$-hexadecane membranes it is possible to show that (Hladky, 1972)

$$k_{Ri}'k_{is}'/k_D' \simeq (k_{Ri}k_{is}/k_{Di})\, e^{-z_{is}F\Delta V/(2RT)} \quad \text{for } M_i = \text{Na}. \tag{106}$$

Assume that this relation also holds for the monactin-K^+ flux across phosphatidyl inositol membranes (it has in any case been assumed by Stark and Benz, 1971). Then their data implies that $k_{is}'/k_{Di}' \gg 1$ for large, negative ΔV and hence that

$$J_{is} \simeq \beta_s a_i \frac{c_s' + c_s''}{2} k_{Ri}'. \tag{107}$$

The continued increase in J_{is} thus suggests a potential dependence for k'_{Ri} but contrary to the suggestion in Stark and Benz (1971), it says nothing about k'_{Di} at high potentials except that it is less than k'_{is}. The use of Eq. (70) to interpret the experimental results for the neutral carrier nonactin will be presented in another paper (Hladky, 1972). The principal result is discussed briefly in Haydon and Hladky (1972).

Note Added in Proof: Markin and Liberman (*C. R. Acad. Sci. U. R. S. S.* **201**:975, 1971) have discussed the effect of unstirred layers on the carrier conductance of thin lipid membranes and Markin (*C. R. Acad. Sci. U. R. S. S.* **202**:703, 1972) has considered the current-voltage relation. There is, however, no mention of the rate of the association-dissociation reactions in the aqueous phase. The conductance equations therefore apparently assume that these reactions are at equilibrium; i.e., they assume the equality in Eq. (18) but not that in Eq. (19). The current-voltage relation presented by Markin is the extension of Eqs. (85) and (98) to allow for arbitrary charge of the free carrier and complex.

Ciani, Eisenman, Laprade and Szabo (*In:* Membranes-A Series of Advances, Vol. 2., Ch. 2. G. Eisenmann, editor. Marcel Dekker, New York, 1972) have derived conductance-concentration, current-voltage, and zero current-voltage relations for neutral carriers of ions under the assumptions that (1) k_{Ri} and k_{Di} are independent of potential, and (2) either the interface limits the fluxes of free carrier and complex from the membrane to the aqueous phase or the flux of carrier into and out of the membrane is entirely in the free form. The second option is another instance of treating the aqueous association as negligible. Ciani *et al.* use a "general formalism" which is equivalent to allowing the potential dependence of k'_{is} to be somewhat arbitrary (as done here) and, for high concentrations, allowing k_{is} to depend on the amount of complex in the membrane. The potential dependence of k'_{is} was discussed by Haydon and Hladky (1972).

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