ANALYSIS OF THE COMPONENTS OF IONIC FLUX ACROSS A MEMBRANE

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ABSTRACT The unidirectional flux of an ionic species may occur because of several mechanisms such as active transport, passive diffusion, exchange diffusion, etc. The contribution of such mechanisms to the total unidirectional flux across a membrane cannot be determined by only measuring that flux. It is shown that if the pertinent experimental data (the opposite unidirectional fluxes and the composite phenomenological resistance coefficient of the ionic species for a given electrochemical potential difference) obey a certain inequality, then the parameters of a model consisting of parallel, independent, active transport, and passive processes may be determined. Although the existence of "additional" processes including exchange diffusion, single-file pore diffusion, isotope interaction, etc. is not disproved, their existence is unnecessary if the inequality is satisfied. Two types of violations of the inequality may occur: (*a*) if the upper limit is disobeyed the presence of another substance contributing to the measured resistance and/or a constant affinity of the active transport process may be indicated; (*b*) if the lower limit is disobeyed it is necessary to postulate the existence of an additional process. For the latter type of violation, exchange diffusion is chosen as an example. Methods are given for determining the contribution of exchange diffusion, active transport, and passive diffusion to the unidirectional flux for some special cases.

I. INTRODUCTION

It is well known that some biological membranes translocate ions by an active transport mechanism. The active transport process is the result of an interaction or coupling of the flow of the solute with that of metabolism. The energy necessary for active transport is derived from a metabolic reaction. From a thermodynamic approach the phenomenological resistance coefficients must be known in order to determine the energetics of an active transport system. Any biochemical model that is proposed to explain this process must, at least, satisfy energy expenditure that can be calculated by the thermodynamics of irreversible processes.

Evidence for active transport of an ionic species has been the existence of a net movement of the test species in the absence of an electrochemical potential difference. Ussing and Zerahn (1) have attempted to determine the electromotive force of the active sodium transport (E_{Na}) of frog skin. They reasoned that the value of

E_{Na} was equivalent to the potential difference (PD) required to make the sodium influx equal to the sodium efflux. A second method used by them to calculate E_{Na} was to determine the PD required to yield a sodium flux ratio, as evaluated by Ussing's (2) flux ratio equation, equal to the flux ratio at short circuit. (A membrane is in the short-circuit condition if ΔV and ΔC [see section II] are zero.) This PD should be equal to E_{Na}. However, as pointed out by Kedem and Essig (3), these methods are correct only in the absence of both passive diffusion of sodium via a parallel pathway and isotope interaction.

A general treatment of ion translocation has been presented by Kedem and Essig (3). They considered the possibility of an ionic species being transported across a membrane via a system of two independent sets of parallel pathways, one active and the other passive. In the case of the active pathway the effect of isotope interaction was considered. The active pathway consists of a set of identical, parallel paths, as does the passive pathway. According to their treatment the flux ratio of the active path (hence, the energetics of active transport) may be evaluated only if the ratio of the phenomenological coefficients of both pathways is known. In general, knowledge of the phenomenological coefficients of the component processes is required. However, such information can not be directly obtained experimentally.

Essig (4) applied these developments to sodium transport in red blood cells. He showed that a bidirectional Na^+ "pump" and a parallel Na^+ leak path can account for the apparent disagreement between the rate of energy expenditure and the rate of supply of metabolic energy. He also indicated that it was not absolutely necessary to suggest an exchange diffusion process as described by Ussing (5) in order to satisfy the data.

Exchange diffusion (5) and single-file pore diffusion (6) have also been suggested as possible modes of translocation when the flux ratio differed from that calculated by Ussing's flux ratio equation. Thus, it is apparent that many different processes may be responsible for the translocation of an ionic species and it is often difficult to determine the components of the total translocation.

Experimentally, the usual techniques of separating the various kinds of fluxes involve the use of metabolic poisons and reducing the concentration of a particular ionic species on one side of the membrane to zero. However, it is difficult to determine to what extent these procedures influence a membrane beyond their obvious effects.

It is the purpose of this work to show how these fluxes may be separated without following the above procedures. Despite the fact that Kedem and Essig (3) indicated the necessary parameters to be known in order to determine the energetics of the transport system, they gave no indication how this could be accomplished by using accessible experimental data. Their work has been extended in this paper by incorporating the definition of a composite phenomenological resistance coefficient. Then, a derivation is presented that permits, under certain conditions, the determination of the phenomenological coefficients of the various parallel pathways which may exist in a membrane. It is shown theoretically that a quantity made up of the unidirectional fluxes, the composite phenomenological resistance coefficient, and the electrochemical potential difference of an ionic species (of a membrane model consisting of parallel, independent, active transport, and free diffusion processes) must satisfy a certain inequality which depends upon the electrochemical potential difference of the ionic species. Of the necessary experimental data that need to be known, the measurement of the composite resistance coefficient presents the greatest difficulty. Recently Essig and Caplan (7) have shown a relationship between the differential resistance and the composite resistance coefficient. Their results are discussed in terms of the model presented in this paper. The effect and determination of exchange diffusion is considered for a variety of situations.

II. PARTIAL LIST OF SYMBOLS

$-X$	Electrochemical potential difference of the test species.
z	Valence of the test species.
C	Concentration of the test species.
ΔV	Electrical potential difference.
F	Faraday constant.
$-E$	Equivalent electrochemical potential difference of the active transport process; represents coupling of the flow of metabolism to that of the ionic species.
R	Universal gas constant.
T	Absolute temperature.
ϕ	Unidirectional influx of the test species.
β	Unidirectional efflux of the test species.
J	Net influx of the test species.
r_a, r_p	Phenomenological resistance coefficients of the active and passive pathways, respectively, of the test species.
r	Composite phenomenological resistance coefficient of the test species.
$f_o(u)$	A dimensionless function defined as $(u/RT)/[\exp(u/RT) - 1]$.
$f_i(u)$	A dimensionless function defined as $f_o(u) \exp(u/RT)$.

Subscripts a, p, and e refer to active, passive, and exchange diffusion pathways, respectively. The experimental value of a quantity is indicated by the subscript "EXP." Superscripts x and 0 indicate that the quantity is evaluated at any X and at $X = 0$, respectively. Unless otherwise stated all quantities are evaluated at any X.

It is assumed that $J^0 > 0$. If the direction of flow of ϕ^x is from bath I to bath II then $-X = RT \ln (C_{II}/C_I) + zF(V_{II} - V_I)$.

III. GENERAL SOLUTION

The equivalent circuit of the transport mechanism of a particular test species is shown in Fig. 1. Although the equations are developed on the basis of an equivalent circuit the basic equations (Equations 4 and 7) are identical with those developed by Kedem and Essig (3), provided the following assumptions are made.

(*a*) The test species is transported across the membrane via a system of two independent, parallel pathways, one active and the other passive. In only the active pathway are there coupled flows.

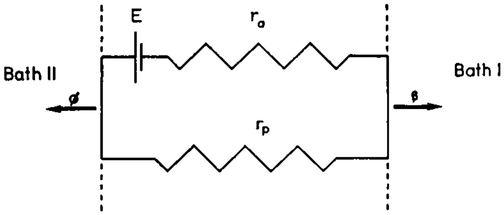

FIGURE 1 Equivalent circuit of the transport pathways of an ionic species. The dotted lines indicate the surfaces of the membrane and also the possible existence of similar equivalent circuits of other ionic species. Use of a battery in the equivalent circuit is not meant to imply that metabolism is completely coupled to transport. It is only used for conceptual reasons.

(*b*) The effect of isotope interaction is neglected.

(*c*) The passive diffusion and the pump mechanism of the active path are bidirectional processes (i.e., the total influx of the test species occurs via both pathways and the same is true for the total efflux of the test species).

(*d*) The applicability of Ussing's flux ratio equation is assumed.

According to assumption *c*

$$\phi = \phi_a + \phi_p \tag{1}$$

and

$$\beta = \beta_a + \beta_p\,. \tag{2}$$

The phenomenological resistance coefficient of the passive path is defined in the following equation

$$X = r_p(\phi_p - \beta_p). \tag{3}$$

In terms of the electrochemical potential difference Ussing's flux ratio equation when applied to the passive pathway yields

$$X = RT \ln\,(\phi_p/\beta_p). \tag{4}$$

From Equations 3 and 4

$$r_p\phi_p = RTf_i(X). \tag{5}$$

Even though the resistance of the passive path and the value of the unidirectional influx via the passive path may not be known their product is known.

Similarly, the phenomenological resistance coefficient of the active pathway is defined by the equation

$$E + X = r_a(\phi_a - \beta_a). \tag{6}$$

Notice that the above equation is equivalent to Equation 9 in reference 3. The resistance of the active path is implied to be a diffusion resistance. Therefore, the net electrochemical potential difference that is responsible for the flux via the active path is also given by

$$E + X = RT \ln (\phi_a/\beta_a). \tag{7}$$

As previously

$$r_a\phi_a = RTf_i(E + X). \tag{8}$$

The composite phenomenological resistance coefficient is related to the phenomenological resistance coefficients of the active and passive pathways in the following way

$$\frac{1}{r} = \frac{1}{r_a} + \frac{1}{r_p}. \tag{9}$$

The phenomenological resistance coefficients are defined as integral resistance coefficients. In general, therefore, the composite resistance coefficient can not be identified with the differential resistance $(\partial J/\partial \Delta V)^{-1}$. In particular, according to Essig and Caplan (7), even if the resistance coefficients r_a and r_p are constant, independent of X, the composite resistance coefficient still may not be equated to the differential resistance unless it is known that the flow of metabolism (e.g. oxygen consumption) is also constant.

This is a severe restriction to the experimental application of the results that follow. However, let us assume that the composite resistance coefficient can be established by experiment.

The net influx of the test species is defined as

$$J = \phi - \beta. \tag{10}$$

Hence, by Equations 1 and 2

$$J = (\phi_a - \beta_a) + (\phi_p - \beta_p). \tag{11}$$

Substitution of Equations 3, 6, and 9 into Equation 11 yields

$$\frac{E}{r_a} = J - \frac{X}{r}. \tag{12}$$

Define a quantity J_{vl} called "virtual level-flow influx" for a given value of X as

$$J_{vl} \equiv J - \frac{X}{r}. \tag{13}$$

It is emphasized that Equation 12 is an equality, while Equation 13 is an identity. The quantity J_{vl} is defined by Equation 13 and, in general, can not be directly measured experimentally but depends on the separate determinations of J, r, and X. The reason why such a quantity has been defined is because of its close resemblance to a level-flow flux. J_{vl}^{x} is the net influx one would expect to measure at $X = 0$ *if* the ratio of E to r_a remained constant. It may not be equal to J^0, the *experimentally* determined net influx at $X = 0$. However, if J_{vl}^{x} is equal to J^0 this only means that the equality expressed in Equation 12 has remained constant,[1] but, on the other hand, this does not imply that the composite phenomenological resistance coefficient has remained constant. This latter statement is clearly shown in Equation 13 since any value of $r > 0$ is permissible at $X = 0$. The virtual level-flow influx is an important physical quantity because it not only indicates the presence of active transport of an ionic species but also its direction. It is interesting to note that even though the virtual level-flow influx can be determined by parameters of the observable composite system it yields information about a part of the composite system.

Combining Equations 1, 5, 9, 12, and 13 gives

$$\frac{1}{r} - \frac{\phi}{RTf_i(X)} = \frac{J_{\mathrm{vl}}}{E} - \frac{\phi_a}{RTf_i(X)} \, .$$

Using Equations 8, 12, and 13 the above becomes

$$\frac{1}{J_{\mathrm{vl}}}\left[\frac{1}{r} - \frac{\phi}{RTf_i(X)}\right] = \frac{1}{E} - \frac{f_i(E+X)}{Ef_i(X)} \, . \qquad (14)$$

Note that the left side of the above equation contains only observable quantities. Let

$$A = \frac{1}{J_{\mathrm{vl}}}\left[\frac{RT}{r} - \frac{\phi}{f_i(X)}\right] . \qquad (15)$$

Simplifying, Equation 14 becomes

$$f_i(E+X) = f_i(X)\left[1 - A\frac{E}{RT}\right] . \qquad (16)$$

Define a quantity s as

$$s = \frac{E+X}{RT} \, . \qquad (17)$$

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[1] This is true except for $X = 0$. Note that $J_{\mathrm{vl}}^{0} \equiv J^0$, which is the level-flow influx and, hence, the reason for identifying J_{vl} as the virtual level-flow influx.

Then Equation 16 can be written as

$$\frac{s}{1 - \exp(-s)} = f_i(X)\left[1 + \frac{AX}{RT}\right] - Af_i(X)s. \tag{18}$$

It will be more convenient to write the above equation as

$$\frac{s}{1 - \exp(-s)} = a - ms, \tag{19}$$

where

$$a = f_i(X)\left[1 + \frac{AX}{RT}\right] \tag{20}$$

and

$$m = Af_i(X). \tag{21}$$

Note that the only unknown quantity in Equation 19 is s and that the right side of it contains the experimentally determined quantities. Unfortunately, the equation is transcendental and must be solved graphically. The solution of the equation is obtained by graphing each side of the equation as an independent function of s. Their intersection(s) is (are) then solution(s) of Equation 19. This is illustrated in Fig. 2. Depending on the values of a and m it is possible to obtain two solutions for s which gives E by Equation 17. However, only one is a physical solution. Each value of s can be used to calculate the parameters of the simple model in Fig. 1. After finding E, r_a can be determined from Equation 12, then r_p can be calculated

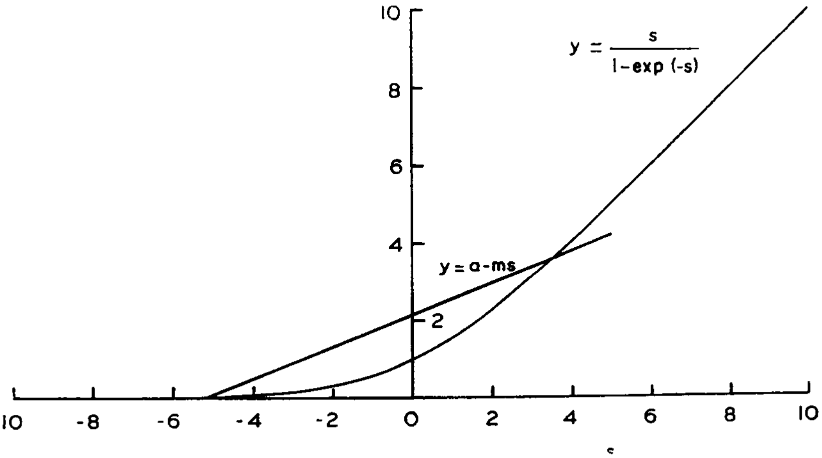

FIGURE 2 A solution of Equation 19 with $a = 2.17$ and $m = -0.42$. This should correspond to $X/RT = -2.7649$ and $A = -2.27$. The solutions of s are 3.562 and -5.092.

by Equation 9. The unidirectional influxes can thus be evaluated by Equations 5 and 8. The correct solution of Equation 19 may be ascertained by comparing the calculated parameters with their experimental counterparts. If a calculated one is not equal to the experimental value or does not satisfy a certain condition (e.g. $r_a > r$) then that value of s which was used to determine the calculated parameter is not the correct solution. The other value for s is then the correct solution.

The left side of Equation 19 is a fixed curve that is independent of the experiment. The solution is completely determined by the right side of Equation 19. Note that at a particular electrochemical potential difference and temperature the parameters a and m are determined by the single quantity A. Therefore, A completely determines the solution of Equation 19. The significance of this important quantity is discussed in more detail in a later section.

By making use of Equation 19 the influence of various pharmacological agents, transmembrane potentials and concentrations of the test species in the bathing solutions on E, r_a, and r_p may be evaluated.

IV. APPLICATION TO LEVEL FLOW

Level flow of a test species is the stationary state that occurs when $X = 0$. Accordingly, in this case Equation 19 reduces to the following:

$$\frac{s^0}{1 - \exp(-s^0)} = 1 - A^0 s^0. \qquad (22)$$

The graphical solution of the above equation is shown in Fig. 3. Note that $s^0 = 0$ is always a solution. However, it is a physical solution of the equation only when $A^0 = -0.5$ since the slope of the exponential curve is $+0.5$ at $s = 0$.[2]

The slope of the exponential curve approaches 1.0 as s approaches plus infinity. Thus -1.0 is the minimum permissible value of A^0. The active transport process must be properly oriented to correspond to the direction of the virtual level-flow influx. Thus, if the unidirectional fluxes are chosen so that J_{vl} is negative then E must also be negative. It is obvious then that there is a degree of arbitrariness in naming the unidirectional fluxes. However, as stated previously, in this paper the unidirectional fluxes are labeled in such a fashion that J^0 is positive. This automatically eliminates negative values of E^0 from our consideration. Therefore, -0.5 is the maximum value of A^0. Hence, the range of values of A^0 is

$$-1 < A^0 \leqslant -0.5. \qquad (23)$$

Once s^0 is determined the values of E^0, r_a^0, and r_p^0 can be calculated.

The experimental value of A^0 may or may not satisfy the above requirement. If the experimental value of A^0 is greater than -0.5 the incorrect measurement of

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[2] L'Hospital's rule has been used to obtain this slope.

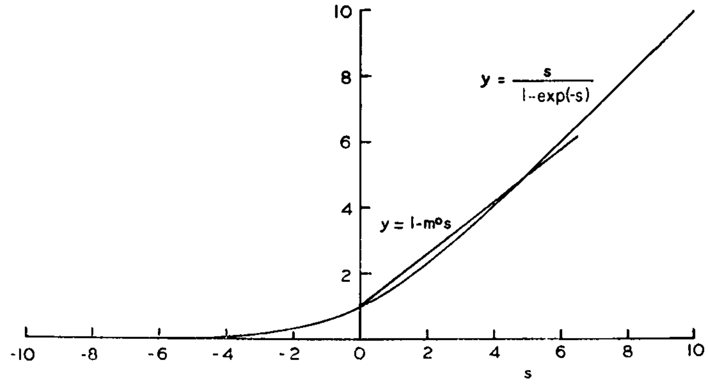

FIGURE 3 A solution of Equation 22 with $a^0 = 1.0$ and $m^0 = -0.795$. This corresponds to $A^0 = -0.795$. The solution for s is 4.661.

r^0 is indicated, assuming the unidirectional fluxes are accurately known. An implication is that the measured value of r^0 is less than its actual value. In addition to this, the experimentally measured ϕ^0 might be less than the value of ϕ^0 consistent with this simple model. This could be explained, for instance, by isotope interaction. This possibility will not be considered any further. On the other hand, if the experimental value of A^0 is less than -1.0 then the simple model is definitely not correct. This possibility is discussed in the next section.

V. ADDITION TO THE MODEL

In section IV it was mentioned that, if the experimental value of A^0 is less than -1.0, the basic model as presented could not explain the data. However, the model can be modified in order to explain the deviation in the value of A^0_{EXP}. It is assumed that r and the unidirectional fluxes are accurately known.

From the definition of A (Equation 15) A^0_{EXP} will be less than -1.0 if the experimentally measured unidirectional influx is larger than the value of ϕ^0 consistent with the model described in section III since J^0_{v1}, r^0, and $f_i(X)$ are positive quantities. One can explain this by hypothesizing isotope interaction in the active transport pathway or the existence of a third, independent, parallel pathway.

Assuming the latter possibility the simplest such pathway to consider is an exchange diffusion path. The unidirectional influx of the test species via this pathway is equal in magnitude to the unidirectional efflux via the pathway. It is assumed that this pathway does not contribute anything to the measured value of r.

Including the exchange diffusion

$$\phi_{\text{EXP}} - \phi_e = \phi_a + \phi_p\,, \qquad (24)$$

with the requirement that

$$\phi_e = \beta_e\,.$$

Using the factor $(\phi_{\mathrm{EXP}} - \phi_e)$ instead of ϕ in the development of Equation 19, Equation 19 appears again in the same form

$$\frac{s}{1 - \exp(-s)} = a - ms, \tag{25}$$

except that

$$A = \frac{1}{J_{v1}} \left[\frac{RT}{r} - \frac{\phi_{\mathrm{EXP}} - \phi_e}{f_i(X)} \right] \tag{26}$$

with the previous definitions applying to all other quantities. A as defined above can not be experimentally determined unless ϕ_e is known. Only the quantity

$$\frac{1}{J_{v1}} \left[\frac{RT}{r} - \frac{\phi_{\mathrm{EXP}}}{f_i(X)} \right] \tag{27}$$

can be determined and will be designated as A_{EXP}.

The influence of the exchange diffusion can be noted by comparing Equation 26 with Equation 27. The ratio $\phi_e/f_i(X)J_{v1}$ acts as a correction factor which when added to A_{EXP} results in a number that must satisfy the model in section III. It will be demonstrated that A as calculated by Equation 26 must satisfy the conditions given in Equation 29 for $E \geqslant 0$. It should be noted that even if A_{EXP} does meet the requirements of Equation 29 this does not mean that $\phi_e = 0$. However, in this instance, it is not necessary to invoke exchange diffusion or some other process in order to explain the data. The existence of the anomaly is clearly demonstrated by the excessive negativity of A_{EXP}.

VI. PROPERTIES AND SIGNIFICANCE OF *A*

In section IV the effect of the algebraic sign of the virtual level-flow influx on that of E was discussed. Arbitrarily designating one of the unidirectional fluxes as the unidirectional influx may yield a negative J_{v1}. For the discussion in this section and in sections IX–XI it is assumed that the unidirectional fluxes are properly labeled so that J_{v1} is positive, assuring positive values for E. If this is done the general properties of A, as defined by Equations 15 and 26, are that it is negative and has a limited range of values.

These characteristics can be shown by rearranging Equation 16 as

$$A = \frac{RT}{E} \left[1 - \frac{f_i(E + X)}{f_i(X)} \right]. \tag{28}$$

Since $f_i(E + X) > f_i(X)$ for $E > 0$, A must be negative.

For a finite value of X the greatest value of A is that value at $E = 0$ and the lowest value is at $E = \infty$. It can be shown that for $\infty > E \geqslant 0$ the range of values of A^x is[3,4]

$$-\frac{1}{f_i(X)} < A^x \leqslant \frac{RT}{X}\,(f_o(X) - 1). \tag{29}$$

The same considerations apply to the limits of A^x as those which were discussed in section IV about the limits of A^0. The upper limit of A^x is representative of a purely passive behavior; while the lower limit is that of a process of an infinite amount of energy.

Equation 28 indicates very clearly that A is intimately related to E. An interesting form for A can be obtained by substituting Equations 5, 9, and 24 into Equation 26

$$A = \frac{1}{J_{\text{vl}}}\left[\frac{RT}{r_a} - \frac{\phi_a}{f_i(X)}\right]. \tag{30}$$

The above expression as well as Equation 28 shows that the parameter A is strictly a property of the active transport pathways. Parallel "leak" pathways and exchange diffusion paths have absolutely no effect upon it.

The parameter A indicates deviation of the transport process of the ionic species from purely passive behavior. To see this, Equation 26 is better written as

$$A = \frac{1}{f_i(X)}\,\frac{\left(\dfrac{RTf_i(X)}{r} - \phi\right)}{\left(J - \dfrac{X}{r}\right)}, \tag{31}$$

where

$$\phi = \phi_a + \phi_p\,.$$

Both the numerator and denominator of the above indicate that the translocation of the solute differs from that of free diffusion. The numerator represents the difference between the sum of the unidirectional influxes via the active and passive pathways and the equivalent unidirectional influx of a passive transport process having a resistance coefficient r. The denominator has a similar meaning with respect to the net influx.

VII. MODEL WITH CONSTANT E AND CONSTANT EXCHANGE DIFFUSION

In the previous sections it was demonstrated that transport of an ionic species via a third independent pathway might possibly be suggested by the data. If considera-

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[3] The limits of A as $E \to 0$ and $E \to \infty$ can be determined by applying L'Hospital's rule.

[4] Actually, even if the unidirectional fluxes are improperly designated so that J_{vl} is negative A^x will still have a limited range of values which is not the same as in Equation 29. In this case recall that E will be negative.

tion of data is limited to a single value of X only limits of the amount of the anomaly can be determined.

In this section it will be shown that the amount of exchange diffusion may be calculated if it is assumed that the unidirectional exchange diffusion flux and E are constant at two electrochemical potential differences, zero and some other value.

In this section the direction of ϕ^x_{EXP} is the same as ϕ^0_{EXP}. Since the direction of ϕ^0_{EXP} has been chosen to yield a positive level-flow influx only positive values of E are considered.

For this development Equation 25 is better written in terms of experimentally determined quantities

$$\frac{s^x}{1-\exp(-s^x)} = (a^x_{\mathrm{EXP}} - m^x_{\mathrm{EXP}}s^x) - \left(s^x - \frac{X}{RT}\right)\frac{\phi_e}{J^x_{\mathrm{vl}}}, \qquad (32)$$

where

$$a^x_{\mathrm{EXP}} = f_i(X)\left[1 + \frac{A_{\mathrm{EXP}}X}{RT}\right]$$

and

$$m^x_{\mathrm{EXP}} = A_{\mathrm{EXP}}f_i(X).$$

Since it has been assumed that E is constant,

$$s^x = s^0 + \frac{X}{RT}, \qquad (33)$$

where

$$s^0 = \frac{E}{RT}.$$

Hence, Equation 32 becomes

$$\frac{s^x}{1-\exp(-s^x)} = (a^x_{\mathrm{EXP}} - m^x_{\mathrm{EXP}}s^x) - \frac{s^0\phi_e}{J^x_{\mathrm{vl}}}. \qquad (34)$$

Application of the above equation at $X = 0$ yields

$$s^0\phi_e = J^0\left[1 - m^0_{\mathrm{EXP}}s^0 - \frac{s^0}{1-\exp(-s^0)}\right]. \qquad (35)$$

Introducing Equations 33 and 35 into Equation 34

$$f_i(E+X) = a^x_{\mathrm{EXP}} - m^x_{\mathrm{EXP}}\left(s^0 + \frac{E}{RT}\right) - \frac{J^0}{J^x_{\mathrm{vl}}}[1 - m^0_{\mathrm{EXP}}s^0 - f_i(E)]. \qquad (36)$$

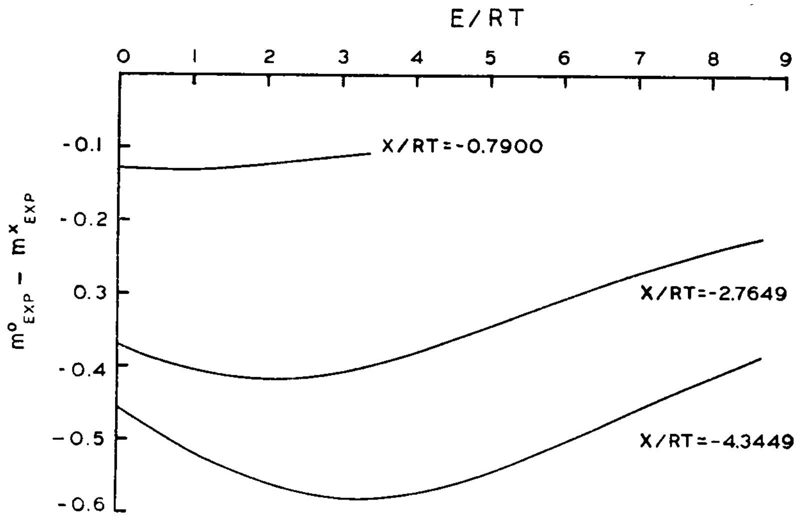

FIGURE 4 Solutions of Equation 37 for various values of X/RT and $J^0/J^x_{v1} = 1$. These curves were obtained by solving Equation 37 for $(m^0_{\text{EXP}} - m^x_{\text{EXP}})$ as a function of E/RT and X/RT. For positive J^x_{v1} and $RT/F = 25.317$ mv; from top to bottom, the curves represent values of $V_{\text{II}} - V_{\text{I}} = 20$ mv, 70 mv, and 110 mv, respectively.

Since

$$a^x_{\text{EXP}} = f_i(X) + \frac{X}{RT} m^x_{\text{EXP}} ,$$

Equation 36 becomes

$$f_i(E + X) - \frac{J^0}{J^x_{v1}} f_i(E) = \left(f_i(X) - \frac{J^0}{J^x_{v1}}\right) + \left(m^0_{\text{EXP}} \frac{J^0}{J^x_{v1}} - m^x_{\text{EXP}}\right) \frac{E}{RT} . \quad (37)$$

The only unknown quantity in Equation 37 is E. It can be graphically determined as before. There will be a family of curves that are dependent on the electrochemical potential difference and the ratio J^0/J^x_{v1}. Solutions of Equation 37 for the special case of linear r_a (i.e., $J^x_{v1} = J^0$) are shown in Fig. 4. In general, there will be two possible values of E for a given set of experimental data. The correct solution can be determined in a similar manner as previously discussed. After solving for E, ϕ_e may be calculated by using Equation 35 or by some other convenient means such as determining the excess unidirectional influx.

Except for the assumptions of constant E and independence of ϕ_e on X the linearity or nonlinearity of r, r_a, or r_p has not been assumed in order to arrive at the result expressed in Equation 37.

VIII. VARIABLE *E* AND CONSTANT EXCHANGE DIFFUSION

It has been reported that sodium transport and oxygen consumption in frog skin are linearly related to the transmembrane PD (8) (chemical composition of the bathing solutions was maintained constant). This suggests that the affinity of the metabolic reaction is constant (7). It also suggests that the phenomenological resistance coefficients are constant (7). In the present terminology these results indicate that the equivalent electrochemical potential difference of the active transport process is a function of X and that r_a and r_p remain constant.

It is shown below that if either r_a or r_p is independent of X then the energetics of the active transport process may be evaluated even if it should depend on the electrochemical potential difference.

In order to compare the data for two values of the electrochemical potential difference ϕ^x_{EXP} and ϕ^0_{EXP} should be in the same direction. As noted earlier in sections IV and VI the direction of flow that has been chosen for ϕ^x_{EXP} may result in a negative J^x_{vl} $(X \neq 0)$ even though J^0 is positive. Hence, the directional variability of E must be considered as a possibility.

A. Constant r_a

In general, if the equivalent electrochemical potential difference of the active transport process, E^x, evaluated at any $X \neq 0$, is expressed in terms of E^0 the parameters of the composite transport system at both values of X can be calculated. This can be readily accomplished by requiring that r_a be constant, independent of X. Thus by Equations 12 and 13

$$E^x = E^0(J^x_{\mathrm{vl}}/J^0). \tag{38}$$

Equation 32 is a general expression, applicable at any given value of X. Using the above equation for E^x, Equation 32 can be written as

$$f_i\left(E^0\frac{J^x_{\mathrm{vl}}}{J^0} + X\right) = a^x_{\mathrm{EXP}} - m^x_{\mathrm{EXP}}\left(\frac{E^0}{RT}\frac{J^x_{\mathrm{vl}}}{J^0} + \frac{X}{RT}\right) - \frac{E^0}{RT}\frac{\phi_e}{J^0}. \tag{39}$$

Assuming that exchange diffusion is constant, the exchange diffusion term can be eliminated by using Equation 32 for $X = 0$. The resulting equation is

$$f_i\left(E^0\frac{J^x_{\mathrm{vl}}}{J^0} + X\right) - f_i(E^0) = (f_i(X) - 1) + \left(m^0_{\mathrm{EXP}} - m^x_{\mathrm{EXP}}\frac{J^x_{\mathrm{vl}}}{J^0}\right)\frac{E^0}{RT}. \tag{40}$$

The solution of the above equation for E^0 can be obtained in a manner similar to that of Equations 19 or 37.

B. Constant r_p

Requiring that r_p be constant, independent of X, and by the definition of the composite phenomenological resistance coefficient

$$\frac{r^x_a}{r^0_a} = 1 + r^x_a\left(\frac{1}{r^0} - \frac{1}{r^x}\right). \tag{41}$$

Substitution of Equations 12 and 13 into the above yields

$$\frac{E^x}{E^0}\frac{J^0}{J^x_{\text{vl}}} = 1 + \frac{E^x}{J^x_{\text{vl}}}\left(\frac{1}{r^0} - \frac{1}{r^x}\right).$$

The above can be simplified to the following

$$E^x = \frac{J^x_{\text{vl}}E^0}{J^0 - \left(\frac{1}{r^0} - \frac{1}{r^x}\right)E^0}. \tag{42}$$

As before, Equation 42 can be substituted into Equation 32. Assuming that it is constant, exchange diffusion can be eliminated by again evaluating Equation 32 at $X = 0$. After simplification the final equation is

$$(1 - \sigma s^0)f_i(E^x + X) - f_i(E^0) = (f_i(X) - 1) + (m^0_{\text{EXP}} - jm^x_{\text{EXP}} - \sigma f_i(X))s^0, \tag{43}$$

where

$$\sigma = \frac{RT}{J^0}\left(\frac{1}{r^0} - \frac{1}{r^x}\right), \; j = \frac{J^x_{\text{vl}}}{J^0}$$

and E^x is given by Equation 42. Equation 43 can be solved for E^0 by the graphical procedure described previously.

IX. LIMITS OF EXCHANGE DIFFUSION

It is necessary to know the exchange diffusion so that the energetics of the active transport system can be determined. But usually the exchange diffusion flux is not known. Nevertheless, it is still possible to obtain some information about its magnitude if it is suspected to exist.

As has been shown before if $A_{\text{EXP}} > -1/f_i(X)$ the minimum value of exchange diffusion is zero. But if $A_{\text{EXP}} < -1/f_i(X)$ what is the minimum value of ϕ_e so that $A = -1/f_i(X)$? From the definition of A as given in Equation 26 this minimum value of ϕ_e is

$$(\phi_e)_{\min} = -J_{\text{vl}}(1 + f_o(X)A_{\text{EXP}}). \tag{44}$$

Now what is the maximum value of ϕ_e so that $A < (RT/X)(f_o(X) - 1)$? The largest possible value of E can be calculated by assuming the minimum exchange diffusion flux. This is because A is a minimum for minimum ϕ_e. Also, by Equation 12 r_a would be a maximum and, therefore, r_p a minimum. By Equation 26 as ϕ_e is increased, A is increased. The exchange diffusion flux can be increased until $r_a \equiv r$. Thus, it is seen that if $J_{\text{vl}} \neq 0$ and r is finite exchange diffusion is a maximum when ϕ_p is zero. Then, when ϕ_e is a maximum, by Equation 8 the unidirectional influx via the active transport pathway is

$$(\phi_a)_{(\phi_e)_{\max}} = \frac{RT}{r} f_i(rJ). \tag{45}$$

So the maximum exchange diffusion is

$$(\phi_e)_{\max} = \phi_{\mathrm{EXP}} - \frac{RT}{r} f_i(rJ). \tag{46}$$

X. DISCUSSION

One of the objectives of this work is to state the condition (Equation 29) which must be satisfied in order for active transport and passive diffusion in the free state of an ionic species to coexist independently of each other. If this condition is met, then it is postulated that in the absence of any other evidence the simple model of Fig. 1 will explain the experimental data completely. That is, the incorporation of exchange diffusion, single-file pore diffusion, isotope interaction, or any other type of mechanism is unnecessary.

It has been recognized that the experimental results may not conform with this simple model and have been interpreted depending upon the type of violation of the condition expressed in Equation 29. If $A_{\mathrm{EXP}} > RT/X(f_0(X) - 1)$ the measured value of r is apparently less than the actual value, assuming that X and the unidirectional fluxes are accurately known. There are two possible reasons for this. If r is evaluated from the electrical measurement $(\partial I/\partial \Delta V)^{-1}_{\Delta C}$, it is possible that the existence of another path due to the movement of a different substance is included in the measurement. The effective resistance of such a combination is obviously less than that due to only the test species. The second reason is associated with the computation of r from the isotopic measurement of the net flux. The differential resistance $(\partial J/\partial \Delta V)^{-1}_{\Delta C}$ can be equated with r only by making severely restrictive assumptions. Essig and Caplan (7) have shown that for constant resistance coefficients it is possible to attribute two meanings to the differential resistance, $(\partial J/\partial \Delta V)^{-1}_{\Delta C}$. They find that

$$(\partial J/\partial \Delta V)^{-1}_{\Delta C, J_r} = r,$$

where J_r is the reaction rate of the metabolic reaction (flow of metabolism) that supplies the energy to the active transport process, or

$$(\partial J/\partial \Delta V)^{-1}_{\Delta C, \mathrm{A}} = r(1 - q^2),$$

where A is the chemical affinity of the above mentioned metabolic reaction and q is the degree of coupling (9) of the metabolic reaction to the transport of the ionic species. If the mechanism of the metabolic reaction is such that the chemical affinity

A is maintained constant but the flow of metabolism is dependent on X the differential resistance will be less than r since $q^2 \leqslant 1$. Similar conclusions may be expected even if the resistance coefficients are not constants. Thus, a variable metabolic flow and constant chemical affinity of the driving reaction is also a possible explanation of such a value of A_{EXP}.

At the other extreme, when $A_{\text{EXP}} < -1/f_i(X)$ the simple two-pathway model can not explain the data. Assuming that r is accurately known the "error" seems to be due to an overestimation of ϕ. Since ϕ, as used in Equation 15 is the total unidirectional influx via active and passive pathways, an overestimation of ϕ would indicate, for example, the existence of isotope interaction in the active transport pathway or of a third, independent, parallel pathway. Assuming an additional pathway, its exact nature is not revealed by the level-flow data. The simplest process to consider is exchange diffusion. Anything else might have an effect on the phenomenological resistance coefficients and the net influx as used in the basic equations.

In view of the above, results of this paper might prove useful as a criterion for the presence of "abnormal" processes which are independent of active transport and passive diffusion processes. Unfortunately there is insufficient data in the literature in order to apply these equations.

One of the most critical quantities that needs to be known is the composite phenomenological resistance coefficient. In the development of the previous sections it was assumed that somehow the resistance coefficient was known or could be determined. A small error in the value of this quantity will yield very erroneous results. Most likely it will be underestimated by experiment which will allow a lower limit of E to be established.

If it should happen that the composite resistance coefficient of a solute is nonlinear over the entire range of flows and forces of interest, as has recently been suggested by Candia (10), it may not be possible to determine the resistance coefficient, to the best of our knowledge. However, it may be that the composite resistance coefficient is or is practically a constant within certain intervals of the forces and flows of the ionic species. If so then it may be possible to estimate the resistance coefficient from the differential resistance. A special case of this has been discussed by Essig and Caplan (7), as previously mentioned. The differential resistance can be taken as a trial value of the composite resistance coefficient and a value of A can be computed. If this value of A satisfies Equation 29 then it will be known that a value of the composite resistance coefficient greater than the differential resistance but less than a certain maximum, which depends upon the experimental unidirectional fluxes, will satisfy the conditions necessary for the simple model shown in Fig. 1. Any finite value of the composite resistance coefficient greater than this maximum will indicate that some additional contribution to the unidirectional fluxes must be considered. On the other hand, if this trial A is greater than the allowable upper limit a minimum value of the composite resistance coefficient can be established. It should be remarked that two compensating errors, such as an overestimation of ϕ and the underestimation of r could result in a value of A consistent with the simple model of Fig. 1.

XI. SUMMARY

(*a*) The virtual level-flow influx indicates the presence of active transport of an ionic species and specifies its direction.

(*b*) Assuming that the four postulates in section III hold true the energetics of the active transport process and the phenomenological resistance coefficients of a test species can be determined exactly if the unidirectional fluxes, electrochemical potential difference and the composite phenomenological resistance coefficient of the test species are known and if

$$-\frac{1}{f_i(X)} < A^x_{\text{EXP}} \leqslant \frac{RT}{X}\,[f_o(X) - 1].$$

(*c*) If $A^0_{\text{EXP}} > (RT/X)(f_0(X) - 1)$ the presence of another substance contributing to the measured resistance and/or a constant affinity of the active transport process may be indicated.

(*d*) If $A^0_{\text{EXP}} < -1/f_i(X)$ the presence of an abnormal process, such as exchange diffusion, may be indicated.

APPENDIX

This section will be devoted to illustrating how the results of the previous sections might be useful in making an interpretation of some experimental data. The objective is only to show a step-by-step procedure in the application of these results.

Assume the following set of data for a positive univalent ion: $r^0 = 1.27$ kΩ-cm², $\phi^0_{\text{EXP}} = 55.09$ μa/cm², $J^0 = 50.0$ μa/cm², $\Delta V = V_{\text{II}} - V_{\text{I}} = 50$ mv, $r^{50} = 2.35$ kΩ-cm², $\phi^{50}_{\text{EXP}} = 23.81$ μa/cm², and $J^{50} = 17.79$ μa/cm².

The following transformations have been used: RT has been replaced by $RT/F = 25.317$ mv; X by $X/F = -50$ mv.

The first step in the analysis is to calculate J^{50}_{vl}. By its definition in Equation 13, $J^{50}_{\text{vl}} = 39.07$ μa/cm². Thus, according to Equation 27 $A^0_{\text{EXP}} = -0.703$ and $A^{50}_{\text{EXP}} = -1.640$. Then by Equation 21 $m^0_{\text{EXP}} = -0.703$ and $m^{50}_{\text{EXP}} = -0.522$.

Assume that the equivalent electrochemical potential difference of the active transport process is constant. Using the above calculations

$$m^0_{\text{EXP}}\frac{J^0}{J^{50}_{\text{vl}}} - m^{50}_{\text{EXP}} = -0.378 \text{ and } \frac{J^0}{J^{50}_{\text{vl}}} = 1.280.$$

The solution of Equation 37 for E is approximately 260 mv. However, is this a physical solution? In order to find this out it must be determined whether or not all the parameters calculated with this value of E (fluxes and resistance coefficients) are consistent with the experimental data. By Equations 12 and 9 $r^0_a = 5.2$ kΩ-cm² and $r^0_p = 1.68$ kΩ-cm². Then by Equations 5 and 8 $\phi^0_p = 15.06$ μa/cm² and $\phi^0_a = 50$ μa/cm². However, $\phi^0 = \phi^0_a + \phi^0_p = 65.06$ μa/cm² which is greater than the experimental unidirectional influx. This would imply negative exchange diffusion which is not allowed. So the possibility of constant E can be eliminated.

The next possibility is variable E. First assume that r_a is constant. From the experimental data $j = 0.781$ and $\sigma = 0.183$. Then $m^0_{\text{EXP}} - jm^{50}_{\text{EXP}} = -0.295$. As it turns out the solution of Equation 40 (i.e., E^0) is in excess of 400 mv. This situation is the same as before since the passive leak will be even greater. Thus, r_a is not constant.

Now assume r_p is constant and r_a variable. Then $m^0_{\text{EXP}} - jm^{50}_{\text{EXP}} - \sigma f_o(\Delta V) = -0.354$. The solution of Equation 43 is $E^0 = 68.97$ mv. As before it can be calculated that $r^0_a = 1.38$ kΩ-cm², $r^0_p = 16.01$ kΩ-cm² and $\phi^0_a + \phi^0_p = 55.09$ μa/cm². Comparison of this result with the experimental data indicates that $\phi_e = 0$. According to Equation 42 $E^{50} = 107.60$ mv. Similarly, it can be shown that $r^{50}_a = 2.75$ kΩ-cm² and $r^{50}_p = 16.01$ kΩ-cm². Thus the requirement that the passive resistance coefficient be constant has been satisfied. Also $\phi^{50}_a + \phi^{50}_p = 23.81$ μa/cm² which shows that $\phi_e = 0$, satisfying the requirement of constant exchange diffusion.

Another example is illustrated in reference 11.

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