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# TESTING AND CHARACTERIZING THE SIMPLE CARRIER

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## SUMMARY

1. We treat the simple carrier at the level of steady-state analysis, making no assumptions as to equilibrium or symmetry and taking into account specifically the effect of electric fields and unstirred layers.

2. We develop tests and rejection criteria applicable under these conditions.

3. For those transport systems which pass the tests for the simple carrier, we show how to obtain from the experimental data (i) the permeabilities of any unstirred layers which may be present at the membrane surfaces and (ii) a complete characterization of the transport system itself in terms of only four independent parameters.

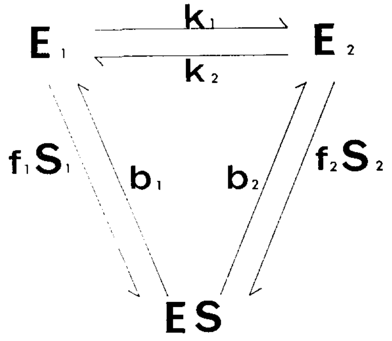
4. The interpretation of these parameters in terms of molecular rate constants is discussed.

## INTRODUCTION

Faced with the problem of analyzing the kinetic behaviour of a new transport system, one should proceed systematically from the simplest to more complex models. In the preceding paper [1], we showed how to test for and characterize the most elementary of all transport models, the simple pore. In the present paper, we present a similar treatment for perhaps the next model in order of increasing complexity, the simple carrier.

Whereas the simple pore is accessible simultaneously to substrate from both sides of the membrane, the simple carrier is defined by the property of being alternately in contact with the solutions bathing the two sides of the membrane. For both simple pore and carrier, only one substrate particle is bound at a time. One can easily show that a steady-state kinetic analysis does not allow for the distinction to be made between models in which there is only one form of the complex with substrate and models in which there are multiple forms [2, 3].

We show how to test for and characterize a simple carrier, even in the presence of unstirred layers, subject only to the assumptions listed in the introduction to the preceding paper [1], appropriately modified for carriers rather than pores.



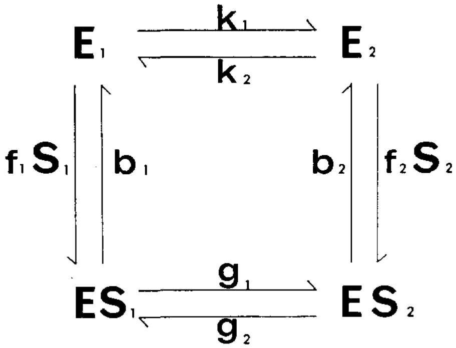


Fig. 1. One-complex form of the simple carrier.

Fig. 2. Two-complex form of the simple carrier.

## PROCEDURE AND DISCUSSION

### *Steady-state solutions for the simple carrier*

Two of the many possible forms of the simple carrier are given in Figs 1 and 2. The steady-state solutions, obtained in an earlier paper [4] but modified to take into account the presence of transmembrane voltage, are given in Table I. In both cases, the unidirectional flux of permeant from Side 1 to Side 2 is given by

$$v_{1\to 2} = \frac{K_{21}S_1 + S_1 S_2}{K_{12}K_{21}R_{00} + K_{21}R_{12}S_1 + K_{12}R_{21}S_2 + R_{ee}S_1 S_2} \qquad (1)$$

where the terms in $R$ and $K$ have different meanings for the two variants of the simple carrier model. (Symbols are as given in the Appendix.) The unidirectional flux $v_{2\to 1}$ in the opposite direction is obtained by interchanging symbols 1 and 2 in Eqn 1. Since all steady-state experiments are describable by the same equations, this means that the two variants of the simple carrier cannot be distinguished at the level of steady-state analysis. Thus the conventional representation of the simple carrier (Fig. 2) is unnecessarily complicated, and we will not use it in the subsequent discussion. In particular, it might be noted that it is meaningless to discuss such questions as whether the rate-limiting step for transport is movement of the carrier–substrate complex through the membrane or its breakdown at the interfaces, until presteady-state data [5] become available.

### *Description of the experimental procedures*

To test and characterize the simple carrier it will be useful to define (in addition to the zero–*trans*, *cis*=*trans*, and equilibrium–exchange procedures described in the preceding paper [1] the following procedures.

(*i*) *Infinite-cis procedures*. Here the concentration of substrate in the *cis* bulk solution is set at a limitingly high level, while that in the *trans* solution is varied. The net flow of substrate is measured, either chemically or electrically, as a function of the *trans* concentration. To test whether the *cis* concentration is indeed limitingly high, one must show that any further increase in this concentration has no detectable effect upon measured rates of flow. There are two infinite-*cis* procedures, according to whether Side 1 or Side 2 of the membrane is taken as the *cis* face.

(*ii*) *Infinite-trans procedures.* Here the unidirectional flux in the *cis* to *trans* direction is measured as a function of the *cis* concentration, while the *trans* concentration is kept at a limitingly high level. The concentrations of substrate are thus those of the infinite-*cis* procedures, but one measures, with tracer, the unidirectional flux occurring against the net flow. Once again, there are two infinite-*trans* procedures.

(*iii*) *Countertransport procedures.* Here one sets up an electrochemical gradient of one substrate, the driving substrate, and measures its effect upon the movements of a second, the driven, substrate.

*Analysis of the simple carrier when unstirred layers are present*

In the presence of unstirred layers, the concentrations $S_1$ and $S_2$ in the immediate vicinity of the carrier are not in general equal to those ($S_1{}^{\mathrm{B}}$ and $S_2{}^{\mathrm{B}}$, respectively) in the bulk solutions. This situation can be expressed quantitatively as

$$S_1 = S_1^{\mathrm{B}} - \frac{NET_{1\to 2}}{P_1} \tag{2}$$

and

$$S_2 = S_2^{\mathrm{B}} + \frac{NET_{1\to 2}}{P_2} \tag{3}$$

where $NET_{1\to 2}$ is the net flow of substrate from Bulk Solution 1 to Bulk Solution 2, and $P_1$ and $P_2$ are the effective permeability coefficients of the unstirred layers at Sides 1 and 2, respectively. Substituting the above expressions for $S_1$ and $S_2$ into Eqn 1 and its analogue, taking the difference and rearranging (using $K_{21} = K_{12}\,\mathrm{e}^{u}$ from Table I) yields

$$NET_{1\to 2} = v_{1\to 2} - v_{2\to 1} = \frac{S_1^{\mathrm{B}} - \mathrm{e}^{-u} S_2^{\mathrm{B}}}{\mathrm{DENOM}} \tag{4}$$

where

$$\mathrm{DENOM} = \frac{1}{P_1} + \frac{\mathrm{e}^{-u}}{P_2} + K_{12}R_{\mathrm{OO}} + R_{12}S_1^{\mathrm{B}} + \mathrm{e}^{-u}R_{21}S_2^{\mathrm{B}} + \frac{R_{\mathrm{ee}}}{K_{21}}S_1^{\mathrm{B}}S_2^{\mathrm{B}}$$
$$+ \left[\frac{\mathrm{e}^{-u}R_{21}}{P_2} - \frac{R_{12}}{P_1} + \frac{R_{\mathrm{ee}}}{K_{21}}\left(\frac{S_1^{\mathrm{B}}}{P_2} - \frac{S_2^{\mathrm{B}}}{P_1} - \frac{NET_{1\to 2}}{P_1P_2}\right)\right] NET_{1\to 2}$$

We now proceed to consider the application of these equations to the various experimental procedures.

(*i*) *Infinite-cis procedures.* When $S_1{}^{\mathrm{B}}$ is limitingly large, Eqn 4 reduces to

$$\frac{1}{NET_{1\to 2}^{\mathrm{ic}}} = R_{12} + \frac{R_{\mathrm{ee}}}{K_{21}}\left(S_2^{\mathrm{B}} + \frac{NET_{1\to 2}^{\mathrm{ic}}}{P_2}\right) \tag{5}$$

The result for limitingly large $S_2{}^{\mathrm{B}}$ is obtained by interchanging symbols 1 and 2.

(*ii*) *Zero-trans procedures for special case of perfectly stirred trans face.* When $S_2{}^{\mathrm{B}} = 0$ and $1/P_2 = 0$, Eqn 4 reduces to

$$\frac{S_1^{\mathrm{B}}}{NET_{1\to 2}^{\mathrm{zt}}} = K_{12}R_{\mathrm{OO}} + R_{12}S_1^{\mathrm{B}} + \frac{1}{P_1}\left(1 - R_{12}\,NET_{1\to 2}^{\mathrm{zt}}\right) \tag{6}$$

TABLE I

STEADY-STATE SOLUTIONS FOR ONE- AND TWO-COMPLEX FORMS OF SIMPLE CARRIER

$$v_{1\to 2} = \frac{K_{21}S_1 + S_1S_2}{K_{12}K_{21}R_{00} + K_{21}R_{12}S_1 + K_{12}R_{21}S_2 + R_{ee}S_1S_2}$$

where $K_{21} = K_{12}\mathrm{e}^{u}$, $R_{ee} = R_{12} + R_{21} - R_{00}$, and:

| | One form of complex | Two forms of complex |
|---|---|---|
| $nR_{12} =$ | $\frac{1}{b_2} + \frac{1}{k_2}$ | $\frac{1}{b_2} + \frac{1}{k_2} + \frac{1}{g_1}\left(\frac{b_2+g_2}{b_2}\right)$ |
| $nR_{21} =$ | $\frac{1}{b_1} + \frac{1}{k_1}$ | $\frac{1}{b_1} + \frac{1}{k_1} + \frac{1}{g_2}\left(\frac{b_1+g_1}{b_1}\right)$ |
| $nR_{00} =$ | $\frac{1}{k_1} + \frac{1}{k_2}$ | $\frac{1}{k_1} + \frac{1}{k_2}$ |
| $nR_{ee} =$ | $\frac{1}{b_1} + \frac{1}{b_2}$ | $\frac{1}{b_1} + \frac{1}{b_2} + \frac{1}{g_1}\left(\frac{b_2+g_2}{b_2}\right) + \frac{1}{g_2}\left(\frac{b_1+g_1}{b_1}\right)$ |
| $K_{12} =$ | $\frac{b_1k_1}{f_1}\left(\frac{1}{b_1} + \frac{1}{b_2}\right)$ | $\frac{b_1k_1}{f_1}\left[\frac{1}{b_1} + \frac{1}{g_1}\left(\frac{b_2+g_2}{b_2}\right)\right]$ |
| $K_{21} =$ | $\frac{b_2k_2}{f_2}\left(\frac{1}{b_1} + \frac{1}{b_2}\right)$ | $\frac{b_2k_2}{f_2}\left[\frac{1}{b_2} + \frac{1}{g_2}\left(\frac{b_1+g_1}{b_1}\right)\right]$ |
| Constraint: | $b_1f_2k_1\mathrm{e}^{u} = b_2f_1k_2$ | $b_1f_2g_2k_1\mathrm{e}^{u} = b_2f_1g_1k_2$ |

The corresponding result is obtained for the 2 to 1 direction.

We turn now to the unidirectional fluxes. We must consider separately quantities for the total and tracer substrates, the latter being denoted here by asterisks. In general, one adds tracer to one bulk solution only; if this is solution 1, then corresponding to Eqns 2 and 3 we have

$$^{*}S_1 = {^{*}S_1^{\mathrm{B}}} - \frac{^{*}NET_{1\to 2}}{P_1} \tag{7}$$

and

$$^{*}S_2 = \frac{^{*}NET_{1\to 2}}{P_2} \tag{8}$$

Continuity requires that the net flow of tracer from bulk solution to bulk solution, $^{*}NET_{1\to 2}$, be equal to the net flow of tracer through the carrier system. This latter flow is equal to the difference between two flows, each being the product of the unidirectional flow of total substrate (given by Eqn 1 and its analogue) and the specific activity of tracer at the relevant membrane face. Thus

$$*NET_{1\rightarrow 2} = \frac{\frac{*S_1}{S_1}(K_{21}S_1+S_1S_2)-\frac{*S_2}{S_2}(K_{12}S_2+S_1S_2)}{K_{12}K_{21}R_{00}+K_{21}R_{12}S_1+K_{12}R_{21}S_2+R_{ee}S_1S_2} \qquad (9)$$

Finally, the unidirectional flux $v_{1\rightarrow 2}$ of total substrate from Bulk Solution 1 to Bulk Solution 2 is equal to $*NET_{1\rightarrow 2}$ divided by the specific activity in Bulk Solution 1, or

$$v_{1\rightarrow 2} = \frac{S_1^B}{*S_1^B}\,*NET_{1\rightarrow 2} \qquad (10)$$

Using this result and Eqns 7–9 one obtains after rearranging

$$v_{1\rightarrow 2} = \frac{S_1^B(K_{21}+S_2)}{K_{12}K_{21}R_{00}+\frac{K_{21}}{P_1}+\frac{K_{12}}{P_2}+\left(K_{21}R_{12}+\frac{1}{P_2}\right)S_1+\left(K_{12}R_{21}+\frac{1}{P_1}\right)S_2+R_{ee}S_1S_2} \qquad (11)$$

We now consider two experimental procedures where unidirectional fluxes are measured.

(*iii*) *Infinite-trans procedures.* When $S_2{}^B$ is limitingly large, so is $S_2$, since in Eqn 3 the term $NET_{1\rightarrow 2}/P_2$ is finite. Thus Eqn 11 reduces to

$$v^{it}_{1\rightarrow 2} = \frac{S_1^B}{K_{12}R_{21}+\frac{1}{P_1}+R_{ee}S_1} \qquad (12)$$

Using Eqn 2 and rearranging gives

$$\frac{S_1^B}{v^{it}_{1\rightarrow 2}} = K_{12}R_{21}+\frac{1}{P_1}+R_{ee}\left(S_1^B+\frac{NET^{ic}_{2\rightarrow 1}}{P_1}\right) \qquad (13)$$

with a similar result in the opposite direction.

(*iv*) *Equilibrium exchange procedure.* Since the system is in electrochemical equilibrium, there is no net flow of total substrate, so that

$$S_1 = S_1^B = e^{-u}S_2^B = e^{-u}S_2 \qquad (14)$$

Using these relations, with the facts (see Table 1) that $R_{00}+R_{ee} = R_{12}+R_{21}$ and $K_{12} = e^{-u}K_{21}$, and noticing that the term $(K_{21}+e^{u}S_1{}^B)$ is a factor of both the resulting numerator and denominator of Eqn 11, one obtains

$$v^{ee} = \frac{S_1^B}{K_{12}R_{00}+\frac{1}{P_1}+\frac{e^{-u}}{P_2}+R_{ee}S_1^B} \qquad (15)$$

In contrast to the three preceding procedures, here the flux depends on the bulk concentration in a simple Michaelis–Menten fashion. Clearly the maximum velocity is unaffected by the presence of unstirred layers, although the half saturation concentration is increased. Eqn 15 can be rearranged to give

$$\frac{S_1^{\mathrm{B}}}{v^{\mathrm{ee}}} = K_{12}R_{00} + \frac{1}{P_1} + \frac{\mathrm{e}^{-u}}{P_2} + R_{\mathrm{ee}}S_1^{\mathrm{B}} \tag{16}$$

*Testing and characterizing the simple carrier when unstirred layers may be present*

We are now in a position to apply the results of the preceding analysis to the interpretation of experimental data obtained from a given transport system. There are several approaches which one might take, and we shall illustrate only some of these in this section.

First, we consider the problem of determining whether or not unstirred layers are present at either face of the membrane. If the transport system does behave as a simple carrier, estimates of $P_1$ and $P_2$ can be obtained directly from the two infinite-*cis* procedures, as follows. For the case when ${S_1}^{\mathrm{B}}$ is limitingly large, Eqn 5 applies:

$$\frac{1}{NET^{\mathrm{ic}}_{1\to 2}} = R_{12} + \frac{R_{\mathrm{ee}}}{K_{21}}\left(S_2^{\mathrm{B}} + \frac{NET^{\mathrm{ic}}_{1\to 2}}{P_2}\right) \tag{5}$$

Since $NET^{\mathrm{ic}}_{1\to 2} = V^{\mathrm{ic}}_{1\to 2}$ when ${S_2}^{\mathrm{B}} = 0$, we have that

$$\frac{1}{V^{\mathrm{ic}}_{1\to 2}} = R_{12} + \frac{R_{\mathrm{ee}}V^{\mathrm{ic}}_{1\to 2}}{K_{21}P_2} \tag{17}$$

Subtracting Eqn 17 from Eqn 5 and rearranging gives

$$\frac{1}{NET^{\mathrm{ic}}_{1\to 2}} = \frac{R_{\mathrm{ee}}V^{\mathrm{ic}}_{1\to 2}}{K_{21}}\left(\frac{S_2^{\mathrm{B}}}{V^{\mathrm{ic}}_{1\to 2} - NET^{\mathrm{ic}}_{1\to 2}} - \frac{1}{P_2}\right) \tag{18}$$

If for clarity we write $v = NET^{\mathrm{ic}}_{1\to 2}$ and $V = V^{\mathrm{ic}}_{1\to 2}$, then Eqn 18 can be expressed as

$$\frac{1}{v} = \frac{R_{\mathrm{ee}}V}{K_{21}}\left(\frac{S_2^{\mathrm{B}}}{V-v} - \frac{1}{P_2}\right) \tag{19}$$

Now $V$ is simply the measured value of $v$ when ${S_2}^{\mathrm{B}} = 0$, so that one can plot $1/v$ versus ${S_2}^{\mathrm{B}}/(V-v)$ and obtain the value of $1/P_2$ as the $x$-intercept of the extrapolated straight line, as is shown in Fig. 3. One obtains $1/P_1$ in an entirely analogous manner by performing the infinite-*cis* procedure in the opposite direction.

If, when one plots the experimental points as in Fig. 3, a straight line is not obtained, then the transport system does not behave as a simple carrier and one must consider other models. If, however, a straight line is obtained, then one can proceed to test and characterize the system in terms of a simple carrier. If $1/P_1$ and $1/P_2$ are found to be not significantly different from zero, then unstirred layers need not be considered and the methods of the next section, in addition to those of the present section, can be employed. But if unstirred layers are found to be important, then one might proceed as follows.

If one considers Eqns 5, 6, 13, 16, and their analogues, it can be seen that all of them may be written in the form

$$y = MS^{\mathrm{B}} + Y + f(\mathrm{NET}) \tag{20}$$

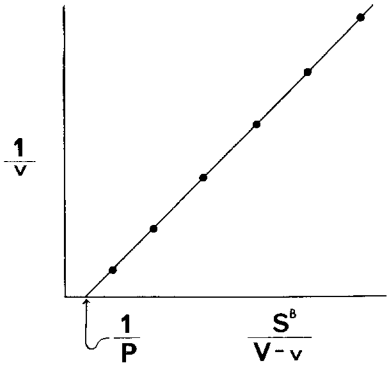


Fig. 3. Experimental determination of unstirred layer permeabilities. See text for explanation.

TABLE II

THE SIMPLE CARRIER: INTERPRETATION OF EXPERIMENTAL DATA IN TERMS OF BASIC PARAMETERS IN THE PRESENCE OF UNSTIRRED LAYERS

| Procedure | | | |
|---|---|---|---|
| Infinite-*cis* | $M^{ic}_{1\to2} = \frac{R_{ee}}{K_{21}}$ | $Y^{ic}_{1\to2} = R_{12}$ | $\Delta^{ic}_{1\to2} = \frac{R_{ee}V^{ic}_{1\to2}}{K_{21}P_2}$ |
| | $M^{ic}_{2\to1} = \frac{R_{ee}}{K_{12}}$ | $Y^{ic}_{2\to1} = R_{21}$ | $\Delta^{ic}_{2\to1} = \frac{R_{ee}V^{ic}_{2\to1}}{K_{12}P_1}$ |
| Infinite-*trans* | $M^{it}_{1\to2} = R_{ee}$ | $Y^{it}_{1\to2} = K_{12}R_{21} + \frac{1}{P_1}$ | $\Delta^{it}_{1\to2} = \frac{R_{ee}V^{ic}_{2\to1}}{P_1}$ |
| | $M^{it}_{2\to1} = R_{ee}$ | $Y^{it}_{2\to1} = K_{21}R_{12} + \frac{1}{P_2}$ | $\Delta^{it}_{2\to1} = \frac{R_{ee}V^{ic}_{1\to2}}{P_2}$ |
| Equilibrium-exchange | $M^{ee} = R_{ee}$ | $Y^{ee}_1 = K_{12}R_{00} + \frac{1}{P_1} + \frac{e^{-u}}{P_2}$ | $\Delta^{ee}_1 = 0$ |
| | | $Y^{ee}_2 = K_{21}R_{00} + \frac{e^{u}}{P_1} + \frac{1}{P_2}$ | $\Delta^{ee}_2 = 0$ |
| Zero-*trans* with no *trans* unstirred layer | $M^{zt}_{1\to2} = R_{12}$ | $Y^{zt}_{1\to2} = K_{12}R_{00}$ | $\Delta^{zt}_{1\to2} = \frac{1}{P_1}$ |
| | $M^{zt}_{2\to1} = R_{21}$ | $Y^{zt}_{2\to1} = K_{21}R_{00}$ | $\Delta^{zt}_{2\to1} = \frac{1}{P_2}$ |

where $y$ is $1/NET^{\text{ic}}$ for Eqn 5, $S^{\text{B}}/NET^{\text{zt}}$ for Eqn 6, $S^{\text{B}}/v^{\text{it}}$ for Eqn 13, and $S^{\text{B}}/v^{\text{ee}}$ for Eqn 16; $M$ and $Y$ are constants, values of which are given in Table II; and $f(\text{NET})$ is a function of the appropriate net flux with the properties:

$$\lim_{S^{\text{B}} \to \infty} f(\text{NET}) = 0 \tag{21a}$$

and

$$\lim_{S^{\text{B}} \to 0} f(\text{NET}) = \Delta \tag{21b}$$

where the values of $\Delta$ are also given in Table II. Eqn 21a follows since in Eqn 5 $NET^{\text{ic}}_{1\to 2} \to 0$ as $S_2{}^{\text{B}} \to \infty$, in Eqn 6 $NET^{\text{zt}}_{1\to 2} \to 1/R_{12}$ as $S_1{}^{\text{B}} \to \infty$, in Eqn 13 $NET^{\text{ic}}_{2\to 1} \to 0$ as $S_1{}^{\text{B}} \to \infty$, and in Eqn 18 $f(\text{NET})$ is identically zero at all concentrations.

A graphical method of representing Eqn 20 is shown in Fig. 4. At large values of $S^{\text{B}}$ the plot approaches a straight line (since $f(\text{NET}) \to 0$) with a slope $M$ and a $y$-intercept $Y$. As the concentration $S^{\text{B}}$ becomes smaller the plot deviates upwards from the straight line, and the limiting value at zero concentration on the $y$-axis is $Y+\Delta$. Thus $M$, $Y$, and $\Delta$ can be determined.

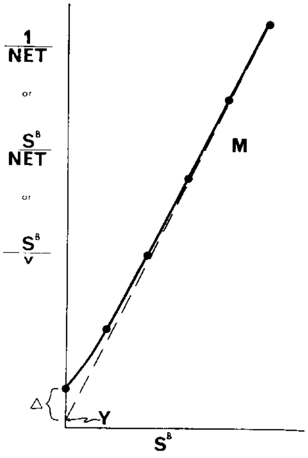


Fig. 4. Experimental determination of the basic parameters $M$, $Y$, and $\Delta$ when unstirred layers may be present. See text for details.

Now there are only six independent basic parameters which characterize the steady-state behaviour of the simple carrier with unstirred layers; these are one of the two $K$ parameters, three of the four $R$ parameters, and $P_1$ and $P_2$. However, there are many more than six different parameters which can be measured experimentally. Thus if the system behaves as a simple carrier, it is overdetermined; this forms the basis for the construction of rejection criteria. For example, from Table II it can be seen that $K_{12}$ can be determined in a number of different ways, including the following:

$$K_{12} = \frac{M^{\text{it}}_{1\to 2}}{M^{\text{ic}}_{2\to 1}} \tag{22a}$$

or

$$K_{12} = \frac{M^{\mathrm{ic}}_{2\to 1}\,Y^{\mathrm{it}}_{1\to 2} - \Delta^{\mathrm{ic}}_{2\to 1}/V^{\mathrm{ic}}_{2\to 1}}{M^{\mathrm{ic}}_{2\to 1}\,Y^{\mathrm{ic}}_{2\to 1}} \tag{22b}$$

or

$$K_{12} = \frac{Y^{\mathrm{it}}_{1\to 2} + \mathrm{e}^{-u}Y^{\mathrm{it}}_{2\to 1} - Y^{\mathrm{ee}}_{1}}{M^{\mathrm{ee}}} \tag{22c}$$

or

$$K_{12} = \frac{\Delta^{\mathrm{it}}_{1\to 2}}{\Delta^{\mathrm{ic}}_{2\to 1}} \tag{22d}$$

If the transport system is behaving as a simple carrier, then all of these methods of obtaining $K_{12}$ must yield the same answer. Alternatively, rejection criteria involving measured parameters only can be obtained by equating such equivalent expressions. For example, equating the right-hand side of Eqn 22a with that of Eqn 22d yields the rejection criterion

$$M^{\mathrm{it}}_{1\to 2}\,\Delta^{\mathrm{ic}}_{2\to 1} = M^{\mathrm{ic}}_{2\to 1}\,\Delta^{\mathrm{it}}_{1\to 2} \tag{23}$$

whereas Eqns 22a and 22c yield the rejection criterion

$$\frac{M^{\mathrm{it}}_{1\to 2}}{M^{\mathrm{ic}}_{2\to 1}}\,M^{\mathrm{ee}} = Y^{\mathrm{it}}_{1\to 2} + \mathrm{e}^{-u}Y^{\mathrm{it}}_{2\to 1} - Y^{\mathrm{ee}}_{1} \tag{24}$$

Clearly, one can derive numerous additional rejection criteria in this fashion. All we have tried to do is to illustrate the method.

Some points should be noted in connection with the use of Table II. (1) If one estimates the various $M$, $Y$, and $\Delta$ parameters from a plot like that of Fig. 4, if one does not use high enough concentrations $S^{\mathrm{B}}$ there will be a tendency to slightly overestimate $Y$ and to underestimate $\Delta$ and $M$ (except for the equilibrium–exchange procedure, where the estimates are not dependent on the concentration range used). This tendency should be taken into account when testing the system. (2) The three parameters $M^{\mathrm{it}}_{1\to 2}$, $M^{\mathrm{it}}_{2\to 1}$, and $M^{\mathrm{ee}}$ are trivially identical, since they involve essentially the same experimental situation. Thus one must find them to be equivalent, on any model. (3) The relations in the bottom two rows of Table II can only be used if it has first been shown that no unstirred layer is present at the *trans* face, for example by showing that $\Delta^{\mathrm{ic}}_{1\to 2}$ or $\Delta^{\mathrm{ic}}_{2\to 1}$ is indistinguishable from zero, or that the $x$-intercept in Fig. 3 is not significantly different from zero for one of the two infinite-*cis* procedures.

In the process of testing the transport system as above, one will have obtained estimates for the basic $K$, $R$, and $P$ parameters, if indeed the tests were passed. If one is concerned with obtaining particularly accurate estimates of these basic parameters, it is convenient to plot the experimental data in a manner which utilizes efficiently all of the data. Thus the plot of Fig. 3 allows one to obtain very accurate estimates of $P_1$ and $P_2$, using all of the data from the two infinite-*cis* procedures. Having obtained $P_1$ and $P_2$, one can then use the same data but now plot $1/NET^{\mathrm{ic}}$ versus $(S^{\mathrm{B}} + NET^{\mathrm{ic}}/P)$ according to Eqn 5 and its analogue; the $y$-intercepts of such plots give directly estimates of $R_{12}$ and $R_{21}$ while the slopes give estimates of $R_{\mathrm{ee}}/K_{21}$ and $R_{\mathrm{ee}}/K_{12}$. But $R_{\mathrm{ee}}$ can be estimated very accurately since (see Eqn 15) it is the reciprocal of the maximum velocity of the equilibrium–exchange procedure. In this way one would have obtained estimates of all the basic measurable parameters: $P_1$, $P_2$, $K_{12}$, $K_{21} = e^u K_{12}$, $R_{12}$, $R_{21}$, $R_{ee}$, and $R_{00} = R_{12}+R_{21}-R_{ee}$. Once again, there are many other ways of obtaining accurate estimates of the basic measurable parameters; we have only tried to illustrate the general approach. In fact, as with the approach based on Table II, one can actually test the transport system for simple carrier behaviour by obtaining accurate estimates of the same parameter in two or more independent ways and checking that the estimates are not significantly different from each other.

Using any of the above approaches, it is possible to test the validity of the simple carrier as a model for a given transport system, even in the presence of unstirred layers. If the data fail to satisfy any one of these tests, then of course the simple carrier model must be rejected in favour of a more complex model. However, if all the tests are satisfied, then there is no reason to assume a more complicated model and we can proceed to characterize the transport system in terms of the basic parameters of the simple carrier model. Furthermore, we are then in a position to make certain statements concerning the molecular rate constants of the most elementary form of the simple carrier, the one-complex form of Fig. 1.

We recall that for the simple pore it was possible to obtain exact values for all possible ratios of the rate constants of the one-complex form, or absolute values for these rate constants if the number of pores were known [1]. For the simple carrier it is only possible to obtain bounds for the ratios of certain molecular constants [6]. The reason for this difference is that whereas for the simple pore there are three independent measurable basic parameters and three independent molecular rate constants, for the simple carrier there are only four independent measurable parameters but five independent molecular rate constants.

To obtain bounds for the simple carrier, we express ratios of the $R$ parameters in terms of the molecular rate constants of the one-complex form. Thus from the first two columns of Table I we have that

$$\frac{R_{12}}{R_{ee}} = \frac{\dfrac{1}{b_2}+\dfrac{1}{k_2}}{\dfrac{1}{b_1}+\dfrac{1}{b_2}} \tag{25}$$

from which it follows that both

$$\frac{b_1}{b_1+b_2} < \frac{R_{12}}{R_{ee}} \quad \text{and} \quad \frac{k_2}{b_2+k_2} < \frac{R_{ee}}{R_{12}} \tag{26}$$

Although both of these inequalities are true, only one of them will be useful. This limitation arises because the left-hand sides of both inequalities are of necessity less than unity, so that new information is only obtained when the right-hand side is less than unity. This can be the case for only one of the two inequalities. In a similar fashion, one can easily derive the other pairs of inequalities listed in Table III.

Under favourable circumstances one can derive both an upper and a lower bound for a particular ratio of rate constants. For example, if both $R_{12}/R_{ee}$ and $R_{21}/R_{ee}$ are found to be less than unity, then it follows from Table III that

TABLE III

USEFUL BOUNDS FOR MOLECULAR RATE CONSTANTS OF ONE-COMPLEX FORM OF SIMPLE CARRIER

| | | |
|---|---|---|
| $\frac{b_1}{b_1+b_2} < \frac{R_{12}}{R_{ee}}$ | or | $\frac{k_2}{b_2+k_2} < \frac{R_{ee}}{R_{12}}$ |
| $\frac{b_2}{b_1+b_2} < \frac{R_{21}}{R_{ee}}$ | or | $\frac{k_1}{b_1+k_1} < \frac{R_{ee}}{R_{21}}$ |
| $\frac{k_1}{k_1+k_2} < \frac{R_{12}}{R_{00}}$ | or | $\frac{b_2}{b_2+k_2} < \frac{R_{00}}{R_{12}}$ |
| $\frac{k_2}{k_1+k_2} < \frac{R_{21}}{R_{00}}$ | or | $\frac{b_1}{b_1+k_1} < \frac{R_{00}}{R_{21}}$ |

$$\frac{R_{ee}-R_{21}}{R_{21}} < \frac{b_1}{b_2} < \frac{R_{12}}{R_{ee}-R_{12}}$$

However, had $R_{21}/R_{ee}$ been greater than unity, only the upper bound in this inequality would be useful. It might happen that both $R_{12}/R_{ee}$ and $R_{21}/R_{ee}$ are found to be greater than unity; in this circumstance no bounds can be set for the ratio $b_1/b_2$. The ratios $k_1/k_2$, $b_1/k_1$, and $b_2/k_2$ can be handled in a similar manner.

### *Analysis of the simple carrier when unstirred layers are absent*

For many of the commonly encountered transport systems, unstirred layers are probably of little significance. (Whether or not they are significant can be discovered using the methods outlined in the preceding section.) For these systems, additional ways of testing and characterizing the system become available, as we will show in the next section.

In the absence of unstirred layers, the concentrations $S_1$ and $S_2$ in the immediate vicinity of the carrier are equal to the concentrations $S_1{}^{B}$ and $S_2{}^{B}$, respectively, in the bulk solutions. Thus Eqn 1 and its analogue can be used directly to predict the results of the various procedures, as follows.

(*i*) *Zero-trans procedures.* When $S_2 = 0$, Eqn 1 reduces to

$$v^{zt}_{1\to 2} = \frac{S_1}{K_{12}R_{00}+R_{12}S_1} \tag{27}$$

Interchanging symbols 1 and 2 yields the corresponding result for the zero-*trans* procedure in the other direction. It is useful to introduce the concept of the limiting permeability $\Pi$, defined to be the limit of the flux divided by concentration as the concentration approaches zero. The half-saturation concentrations, the maximal velocities, and the limiting permeabilities for the two zero-*trans* procedures are then as listed in Table IV.

(*ii*) *Cis-trans procedure.* Here $S_1 = S_2 = S$, so that from Eqn 1 and its analogue we have that

$$NET^{c=t}_{1\to2} = -NET^{c=t}_{2\to1} = v^{c=t}_{1\to2}-v^{c=t}_{2\to1} = \frac{(e^u-1)S}{e^uK_{12}R_{00}+(e^uR_{12}+R_{21})S+(R_{ee}/K_{12})S^2} \tag{28}$$

In contrast to the simple pore where the net flow reaches its maximal value only at limitingly high concentrations [1], here the net flow is zero at both zero and limitingly high concentrations, passing through a maximum value $V^{c=t}$ at a finite concentration $C^{c=t}$. To solve for $C^{c=t}$ and then $V^{c=t}$, one differentiates $NET^{c=t}_{1\to2}$ in Eqn 28 with respect to $S$ and sets the derivative equal to zero; this yields the solutions given in Table IV.

The limiting ohmic conductance is then

$$\begin{aligned}\lambda_0 &= \lim_{(\psi_1-\psi_2)\to0}\frac{zF\,NET^{c=t}_{1\to2}}{(\psi_1-\psi_2)} = \frac{z^2F^2}{N_AkT}\cdot\lim_{u\to0}\frac{NET^{c=t}_{1\to2}}{u}\\ &= \frac{z^2F^2}{N_AkT}\cdot\frac{S}{K_0R_{00}+(R_{12}+R_{21})S+(R_{ee}/K_0)S^2}\end{aligned} \tag{29}$$

Thus $\lambda_0$ has a maximum value $\Lambda_0$ at the finite concentration $C^{\lambda_0}$, where these parameters have the values given in Table IV.

The limiting permeability is

$$\Pi^{c=t} = \lim_{S\to0}\frac{NET^{c=t}_{1\to2}}{S} = \frac{1-e^{-u}}{K_{12}R_{00}} = \frac{e^u-1}{K_{21}R_{00}} \tag{30}$$

and the equivalent conductance in the limit of infinite dilution and zero voltage is

$$\Phi = \lim_{\substack{S\to0\\(\psi_1-\psi_2)\to0}}\frac{zF\,NET^{c=t}_{1\to2}}{S(\psi_1-\psi_2)} = \lim_{S\to0}\frac{\lambda_0}{S} = \frac{z^2F^2}{N_AkT}\cdot\lim_{u\to0}\frac{\Pi^{c=t}}{u} = \frac{z^2F^2}{N_AkT}\,\frac{1}{K_0R_{00}} \tag{31}$$

(*iii*) *Infinite-cis procedures*. When $S_1$ is limitingly large, it follows from Eqn 1 and its analogue that

$$NET^{ic}_{1\to2} = \frac{K_{21}}{K_{21}R_{12}+R_{ee}S_2} \tag{32}$$

The maximal velocity is the same as that in the corresponding zero-*trans* procedure, while that *trans* concentration $K^{ic}_{1\to2}$ at which the velocity is half of this is as given in Table IV. The corresponding results for the procedure in the other direction are obtained as usual.

(*iv*) *Infinite-trans procedures*. When $S_2$ is limitingly large, Eqn 1 becomes

$$v^{it}_{1\to2} = \frac{S_1}{K_{12}R_{21}+R_{ee}S_1} \tag{33}$$

The maximal velocities, half-saturation concentrations, and limiting permeabilities for this procedure in both directions are given in Table IV.

(*v*) *Equilibrium-exchange procedure*. Here $S_2 = e^uS_1$ so that using Eqn 1 and the relationship (see Table I) $R_{ee} = R_{12}+R_{21}-R_{00}$ we have that

TABLE IV

THE SIMPLE CARRIER: INTERPRETATION OF EXPERIMENTAL DATA IN TERMS OF BASIC PARAMETERS IN THE ABSENCE OF UNSTIRRED LAYERS

| Procedure | | | |
|---|---|---|---|
| *Net flows** | | | |
| Zero-*trans* | $V^{zt}_{1\to 2} = \dfrac{1}{R_{12}}$ | $K^{zt}_{1\to 2} = K_{12}\dfrac{R_{00}}{R_{12}}$ | $\Pi^{zt}_{1\to 2} = \dfrac{1}{K_{12}R_{00}}$ |
| | $V^{zt}_{2\to 1} = \dfrac{1}{R_{21}}$ | $K^{zt}_{2\to 1} = K_{21}\dfrac{R_{00}}{R_{21}}$ | $\Pi^{zt}_{2\to 1} = \dfrac{1}{K_{21}R_{00}}$ |
| Infinite-*cis* | $V^{ic}_{1\to 2} = \dfrac{1}{R_{12}}$ | $K^{ic}_{1\to 2} = K_{21}\dfrac{R_{12}}{R_{ee}}$ | |
| | $V^{ic}_{2\to 1} = \dfrac{1}{R_{21}}$ | $K^{ic}_{2\to 1} = K_{12}\dfrac{R_{21}}{R_{ee}}$ | |
| *cis*=*trans* | $V^{c=t} = \dfrac{2\sinh(u/2)}{R_{12}e^{u/2}+R_{21}e^{-u/2}+2\sqrt{R_{00}R_{ee}}}$ | $C^{c=t} = K_{12}e^{u/2}\sqrt{\dfrac{R_{00}}{R_{ee}}}$ | $\Pi^{c=t} = \dfrac{e^{u}-1}{K_{21}R_{00}}$ |
| *cis*=*trans* when $u\to 0$ | $\Lambda_0 = \dfrac{z^2F^2}{N_A\boldsymbol{k}T}\,\dfrac{1}{(\sqrt{R_{00}}+\sqrt{R_{ee}})^2}$ | $C^{\lambda o} = K_0\sqrt{\dfrac{R_{00}}{R_{ee}}}$ | $\Phi = \dfrac{z^2F^2}{N_A\boldsymbol{k}T}\,\dfrac{1}{K_0R_{00}}$ |

|  |  |  |  |
|---|---|---|---|
| *Unidirectional fluxes* |  |  |  |
| Equilibrium–exchange | $V^{ee} = V^{ee}_{1\to 2} = V^{ee}_{2\to 1}$ | $K^{ee}_1 = K_{12}\dfrac{R_{00}}{R_{ee}}$ | $\Pi^{ee}_1 = \dfrac{1}{K_{12}R_{00}}$ |
|  | $= \dfrac{1}{R_{ee}}$ | $K^{ee}_2 = K_{21}\dfrac{R_{00}}{R_{ee}}$ | $\Pi^{ee}_2 = \dfrac{1}{K_{21}R_{00}}$ |
| Infinite-*trans* | $V^{it} = V^{it}_{1\to 2} = V^{it}_{2\to 1}$ | $K^{it}_{1\to 2} = K_{12}\dfrac{R_{21}}{R_{ee}}$ | $\Pi^{it}_{1\to 2} = \dfrac{1}{K_{12}R_{21}}$ |
|  | $= \dfrac{1}{R_{ee}}$ | $K^{it}_{2\to 1} = K_{21}\dfrac{R_{12}}{R_{ee}}$ | $\Pi^{it}_{2\to 1} = \dfrac{1}{K_{21}R_{12}}$ |
| Counter-transport |  | see text |  |

---

* To convert net chemical flows $V$ to electrical currents $J$ multiply by $zF$.

$$v^{ee} = v^{ee}_{1\to 2} = v^{ee}_{2\to 1} = \frac{S_1}{R_{00}K_{12}+R_{ee}S_1} = \frac{S_2}{R_{00}K_{21}+R_{ee}S_2} \tag{34}$$

The half-saturation concentration and the limiting permeability can be defined with reference to either Solution 1 ($K^{ee}_1$ and $\Pi^{ee}_{1\to 2}$) or Solution 2 ($K^{ee}_2$ and $\Pi^{ee}_{2\to 1}$), although of course $K^{ee}_2 = e^u K^{ee}_1$ and $\Pi^{ee}_{1\to 2} = e^u \Pi^{ee}_{2\to 1}$. The results for the maximal velocity, the half-saturation concentrations, and the limiting permeabilities will be found in Table IV.

(*vi*) *Countertransport experiment.* The simplest countertransport procedure is where one sets up an electrochemical gradient of total substrate and measures its effect upon the movement of labeled substrate of the same species. If we denote labeled quantities with an asterisk and reserve unstarred symbols for total quantities, we can write the unidirectional flux of labeled substrate from Solution 1 to 2 as

$$^{*}v_{1\to 2} = \frac{^{*}S_1}{S_1}\, v_{1\to 2} = \frac{K_{21}\,^{*}S_1 + {}^{*}S_1 S_2}{K_{12}K_{21}R_{00}+K_{21}R_{12}S_1+K_{12}R_{21}S_2+R_{ee}S_1S_2} \tag{35}$$

The net flow of labeled substrate in this direction is thus

$$^{*}NET_{1\to 2} = \frac{K_{12}(^{*}S_1 e^u - {}^{*}S_2) + {}^{*}S_1 S_2 - S_1\,^{*}S_2}{K_{12}K_{21}R_{00}+K_{21}R_{12}S_1+K_{12}R_{21}S_2+R_{ee}S_1S_2} \tag{36}$$

This equation illustrates the well-known result that there can be a net flow of labeled substrate even though this is at electrochemical equilibrium ($^{*}S_1 e^u = {}^{*}S_2$). This is not true for the simple pore [1], the reason being that there is no $S_1S_2$ term in the numerator of the general expression for unidirectional flux (Eqn 3 of [1]). Thus the presence of such "coupled" transport can be thought of as another rejection criterion for the pore.

The net flow of labeled substrate vanishes when the numerator of Eqn 11 is equal to zero. The concentration ratio for labeled substrate is then

$$\frac{^{*}S_1}{^{*}S_2} = \frac{K_{12}+S_1}{K_{21}+S_2} \tag{37}$$

This is the maximum ratio of driven substrate for a given electrochemical gradient of the total, driving, substrate, and is greatest for $S_1$ large and $S_2$ small compared with $K_{12}$ and $K_{21}$, respectively.

### *Testing and characterizing the simple carrier in the absence of unstirred layers*

In addition to the methods already discussed for the general case, there are other convenient methods available if no unstirred layers are present. For example, in the absence of unstirred layers the parameters $K_{12}$ and $K_{21} = e^u K_{12}$ can be obtained simply and directly from a single countertransport experiment by measuring the total substrate concentrations $S_1$ and $S_2$ and the ratio $r = {}^{*}S_1/{}^{*}S_2$ of tracer substrate concentration at the point where the net tracer flow vanishes, and then calculating (see Eqn 37)

$$K_{12} = \frac{S_1 - rS_2}{re^u - 1} \tag{38}$$

TABLE V

SOME REJECTION CRITERIA FOR THE SIMPLE CARRIER IN THE ABSENCE OF UNSTIRRED LAYERS

I. Using electrical or chemical* measurements

A. For $u \neq 0$

$$\frac{\dfrac{zF}{J^{c=t}} + \dfrac{zF}{J^{zt}_{2\to 1}} - \dfrac{2}{C^{c=t}\Pi^{c=t}}}{\dfrac{zF}{J^{c=t}} - \dfrac{zF}{J^{zt}_{1\to 2}} - \dfrac{2}{C^{c=t}\Pi^{c=t}}} = e^{u}$$

$$C^{c=t} = \sqrt{K^{zt}_{1\to 2}K^{ic}_{1\to 2}} = \sqrt{K^{zt}_{2\to 1}K^{ic}_{2\to 1}}$$

$$\frac{J^{zt}_{1\to 2}}{J^{zt}_{2\to 1}} + 1 \geqq 2e^{u/2}\sqrt{\frac{K^{zt}_{1\to 2}}{K^{ic}_{1\to 2}}}$$

B. For $u = 0$

$$\frac{1}{\Lambda_0} = \frac{N_A kT}{zF}\left(\frac{1}{J^{zt}_{1\to 2}} + \frac{1}{J^{zt}_{2\to 1}}\right) + \frac{2}{C^{\lambda_0}\Phi}$$

$$C^{\lambda_0} = \sqrt{K^{zt}_{1\to 2}K^{ic}_{1\to 2}} = \sqrt{K^{zt}_{2\to 1}K^{ic}_{2\to 1}}$$

II. Using chemical measurements only

For all values of $u$:

$$\frac{V^{zt}_{1\to 2}}{V^{zt}_{2\to 1}} + 1 = \frac{K^{ee}_{2}}{K^{ic}_{1\to 2}} + \frac{K^{zt}_{1\to 2}}{K^{ee}_{1}}$$

$$K^{ic}_{1\to 2} = K^{it}_{2\to 1} \quad \text{and} \quad K^{ic}_{2\to 1} = K^{it}_{1\to 2}$$

* For chemical measurements, substitute $zFV$ for $J$.

To test the validity of the simple carrier as a model for a given transport system one can proceed as in the general case to estimate the same basic measurable parameter in a number of independent ways and see if the same result is obtained. Similarly, to obtain rejection criteria one can simply equate different theoretical expressions for the same set of basic parameters. For example, it can be seen from Table IV that both $K^{ic}_{1\to 2}$ and $K^{it}_{2\to 1}$ equal $K_{21}R_{12}/R_{ee}$ for the simple carrier, so that a simple rejection criterion is $K^{ic}_{1\to 2} = K^{it}_{2\to 1}$. It is possible to obtain a very large number of rejection criteria in this fashion; a few of the more convenient of these are listed in Table V. For those transport systems which pass all of the tests, a complete steady-state characterization in terms of the simple carrier can be obtained as discussed in preceding sections.

### *Suggested steps for the testing and characterizing of the simple carrier*

The transport system being studied should have first been analyzed according to the methods of the preceding paper [1] and shown not to behave as a simple pore. To test whether the system is a simple carrier, there are a number of ways in which one might proceed. A convenient route is as follows:

(1) To confirm that carrier-type models rather than pore-type models need to be considered, it is useful to establish that uphill flow driven by countertransport can occur.

(2) To show that carrier models more complex than the simple carrier need not yet be considered, the equilibrium–exchange procedure might be performed, this being the only procedure which gives simple Michaelis–Menten kinetics for the simple carrier regardless of the presence of unstirred layers.

(3) The permeability of each unstirred layer, if present, can now be measured using the infinite-*cis* procedures.

(4) Testing and then characterizing the transport system in terms of the simple carrier can be performed using the methods outlined for the general situation with unstirred layers present, including those based upon Table II. If unstirred layers are absent, then in addition one can use methods based upon Tables IV and V.

(5) If the experimental data satisfy all of the tests, there is no need to postulate a model more complex than the one-complex form of the simple carrier (Fig. 1). Useful bounds on the ratios of molecular rate constants for this model can be obtained using Table III.

(6) If the data fail to pass any one of the tests, then a model more complex than the simple carrier must be sought.

## APPENDIX

### *List of symbols*

| | |
|---|---|
| $b_1, b_2$ | effective interfacial rate constants for breakdown of *ES* to *E* and *S* |
| $C^{\mathrm{c}=\mathrm{t}}$ | that substrate concentration in the *cis* = *trans* procedure at which the net flow is maximal |
| $C^{\lambda_0}$ | that substrate concentration in the *cis* = *trans* procedure at which the limiting ohmic conductance is maximal |
| $E$ | free carrier |
| $ES$ | carrier–substrate complex |
| $f_1, f_2$ | effective interfacial rate constants for formation of *ES* from *E* and *S* |
| $f(\mathrm{NET})$ | a function of the appropriate net flux, as defined in Eqn 20, with the limiting values given by Eqn 21 |
| $F$ | Faraday constant |
| $g_1, g_2$ | rate constants for interconversion of $ES_1$ and $ES_2$ for two-complex version of simple carrier |
| $j$ | net electrical current per unit area of membrane |
| $J$ | maximum value of $j$ |
| $k$ | Boltzman constant |
| $k_1, k_2$ | rate constants for interconversion of $E_1$ and $E_2$ |
| $K$ | that substrate concentration at which half of the maximum rate of transport occurs |
| $K_{12}, K_{21}$ | basic measurable membrane transport parameters |

| | |
|---|---|
| $K_0$ | value of $K_{12}$ and $K_{21}$ when $u = 0$ |
| $M$ | slope of extrapolated straight line in Fig. 4 plot |
| $n$ | total number of carriers per unit area of membrane |
| $N_A$ | Avogadro constant |
| $NET$ | rate of net transport per unit area of membrane |
| $P$ | effective permeability coefficient of unstirred layer on one side of membrane, per unit area of membrane |
| $r$ | ratio $^*S_1/^*S_2$ of labeled substrate in countertransport experiment when net flow of tracer vanishes |
| $R_{00}$, $R_{12}$, $R_{21}$, $R_{ee}$ | additional basic measurable membrane transport parameters |
| $S$ | substrate concentration in immediate vicinity of carrier |
| $S^B$ | substrate concentration in bulk solution |
| $T$ | absolute temperature |
| $u$ | reduced transmembrane electrical potential difference, $(zF/N_A kT)(\psi_1 - \psi_2)$ |
| $v$ | rate of unidirectional transport per unit area of membrane (except for Eqn 19 and Fig. 3, where $v \equiv NET$) |
| $V$ | maximum value of either $NET$ or $v$ |
| $y$ | ordinate (independent) variable in Fig. 4 plot, equal to $1/NET$ or $S^B/NET$ or $S^B/v$ |
| $Y$ | $y$-intercept of extrapolated straight line in Fig. 4 plot |
| $z$ | electrical valence of substrate |
| $\Delta$ | limit of f(NET) as $S^B$ approaches zero |
| $\lambda_0$ | limiting ohmic conductance per unit area of membrane as $u \to 0$ in *cis*=*trans* procedure |
| $\Lambda_0$ | maximum value of $\lambda_0$ |
| $\Pi$ | limiting permeability per unit area of membrane, equal to the limit of $v/S$ or $NET/S$ as $S \to 0$ |
| $\Phi$ | equivalent conductance per unit area of membrane in the limit of infinite dilution and zero voltage in the *cis*=*trans* procedure |
| $\psi$ | electrical potential |

Except where explicitly specified otherwise in the above list, the superscript and subscript conventions are as follows:

*Right superscripts*

| | |
|---|---|
| c=t | *cis* = *trans* procedure |
| ee | equilibrium-exchange procedure |
| ic | infinite-*cis* procedure |
| it | infinite-*trans* procedure |
| zt | zero-*trans* procedure |

*Left superscript*

| | |
|---|---|
| * | labeled (tracer) substrate |

*Subscripts*

| | |
|---|---|
| 1 | value at Side 1 |

| | |
|---|---|
| 2 | value at Side 2 |
| 1 → 2 | value in an experiment in which transport is measured from Side 1 to Side 2 |
| 2 → 1 | value in an experiment in which transport is measured from Side 2 to Side 1 |

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## REFERENCES

1 Lieb, W. R. and Stein, W. D. (1974) Biochim. Biophys. Acta 373, 165–177
2 Cleland, W. W. (1963) Biochim. Biophys. Acta 67, 104–137
3 Britton, H. G. (1966) Arch. Biochem. Biophys. 117, 167–183
4 Stein, W. D. and Lieb, W. R. (1973) Isr. J. Chem. 11, 325–339
5 Läuger, P. (1972) Science 178, 24–30
6 Britton, H. G. (1973) Biochem. J. 133, 255–261