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TESTING THE SIMPLE CARRIER USING IRREVERSIBLE INHIBITORS

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SUMMARY

1. We analyse the kinetics of irreversible inhibition of the simple carrier. We consider how the rate of inactivation is influenced by the concentrations of permeant on the two sides of the membrane.

2. We consider various kinetic schemes for the simple carrier and show that these are all indistinguishable kinetically, using steady-state transport or inactivation data. We point out the advantages of using the simplest kinetic scheme and the possible pitfalls of using more complicated schemes, including the conventional carrier model.

3. We show that in the absence of information on the transport properties of the system, irreversible inhibition data are ambiguous. Taken together with transport data, however, inactivation data provide new tests for the applicability of the simple carrier.

4. The new tests show that for the simple carrier model to be applicable, the substrate dependencies of transport and of inactivation must be identical in comparable experimental situations. Further, the maximal rates of transport and of stimulation (or inhibition) of inactivation must obey a simple relationship, which we derive. We illustrate the use of these tests with published data on the glucose and choline transport systems of the human red blood cell.

INTRODUCTION

Substantial use has been made of irreversible inhibitors of transport systems, in an attempt to understand the molecular details of the transport process [1–13]. In particular, the fact that substrates (and competitive inhibitors) often modify the rate of inactivation by the irreversible inhibitor has led some authors to infer that conformation changes occur on binding substrate [3] and other authors to infer further a particular mechanism for transport [6, 10]. To aid the future development of such studies, we provide in this paper a very general treatment for the simple carrier, which is perhaps the simplest system that undergoes major conformation changes during the transport event. Although our analysis is general and requires none of the usual

approximations, the derived equations appear in particularly simple forms, easy to apply to experimental data.

We show that in the absence of information on the transport properties of the system, irreversible inhibition data are ambiguous. Taken together with transport data, however, the inactivation data can be most valuable in that they provide new tests for the applicability of the simple carrier.

KINETIC TREATMENT

In previous papers [14–15] we have provided a very general treatment of transport by the simple carrier, making no assumptions as to symmetry or as to which steps are rate-limiting. We have shown that steady-state transport measurements do not allow one to choose between the different formulations of the simple carrier model shown in Figs. 1 and 2. For both these formulations, the unidirectional flux of uncharged permeant from side 1 to side 2 of the membrane is given by the same expression:

$$v_{1\to 2} = \frac{KS_1 + S_1S_2}{K^2R_{oo} + KR_{12}S_1 + KR_{21}S_2 + R_{ee}S_1S_2} \tag{1}$$

where S_1 and S_2 are the concentrations of substrate at faces 1 and 2 of the membrane, and the terms in R and K are measurable transport parameters.

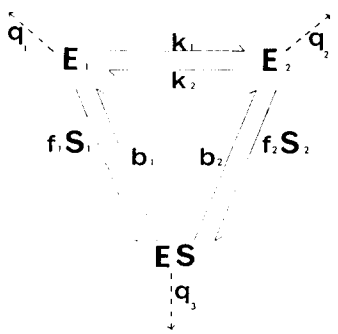

Fig. 1. One-complex formulation of the simple carrier.

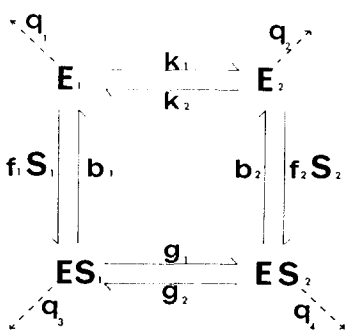

Fig. 2. Two-complex formulation of the simple carrier.

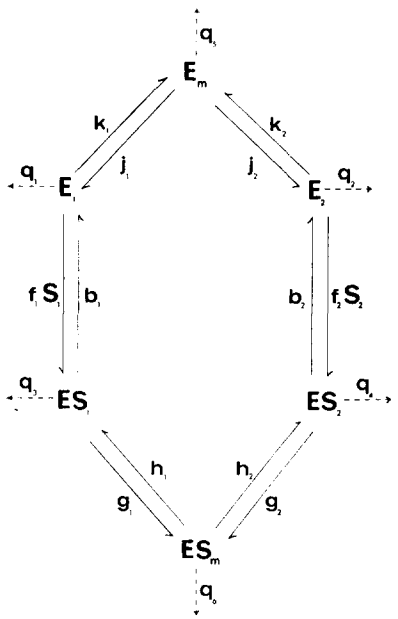

Fig. 3. Formulation of the simple carrier involving intermediate states.

The only difference between these two formulations of the simple carrier model (Figs. 1 and 2) is in the interpretation of the measurable transport parameters in terms of the experimentally inaccessible (to steady-state measurement) rate constants of the kinetic models (see Table I). Since it has been proposed [3–4] that the irreversible inhibition of the human red cell glucose transport by 1-fluoro-2,4-dinitrobenzene involves an attack on a conformation intermediate in the transport cycle, we have analyzed a formulation of the simple carrier (Fig. 3) in which both free and bound forms of the carrier have an additional major intermediate conformation. The results are given in Table I, where it can be seen that once again Eqn. 1 is obeyed, but that now the observable transport parameters (terms in R and K) involve more complicated expressions when expressed in terms of the experimentally inaccessible rate constants of the kinetic model of Fig. 3. It thus appears by induction that, in line with the conclusions of Cleland [16] for enzyme kinetics, the addition of any number of intermediate forms to the model of Fig. 1 must lead to the same transport equation in terms of observable parameters.

We will show that the use of irreversible inhibitors also does not allow one to distinguish among these various formulations (Figs. 1, 2 and 3) of the simple carrier. The inactivation rate under all conditions can be described by the introduction of a small number of additional measurable parameters, which once again are expressed on the different formulations as combinations of experimentally inaccessible rate constants.

We begin by analyzing the simplest formulation of the simple carrier model (Fig. 1). Here there are two conformations of the free carrier, E_1 and E_2, but only one of the loaded carrier, ES. The experimentally observed rate constant q for inactivation is given by

TABLE I

STEADY-STATE SOLUTIONS FOR VARIOUS FORMULATIONS OF THE SIMPLE CARRIER

$$v_{1\to 2} = \frac{KS_1 + S_1S_2}{K^2R_{oo} + KR_{12}S_1 + KR_{21}S_2 + R_{ee}S_1S_2} \quad \text{and}$$

where $R_{oo} = R_{12} + R_{21} + R_{ee}$ and n = total number of carriers per unit area of membrane

	Model of Fig. 1	Model of Fig. 2
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_{ee}	$\frac{1}{b_1} + \frac{1}{b_2}$	$\frac{1}{b_1} + \frac{1}{b_2} + \frac{1}{g_1}\left(\frac{b_2+g_2}{b_2}\right) + \frac{1}{g_2}\left(\frac{b_1+g_1}{b_1}\right)$
nR_{oo}	$\frac{1}{k_1} + \frac{1}{k_2}$	$\frac{1}{k_1} + \frac{1}{k_2}$
K	$\frac{k_1}{f_1} + \frac{k_2}{f_2}$	$\frac{k_1}{f_1} + \frac{k_2}{f_2} + \frac{b_1k_1}{f_1g_1}\left(= \frac{b_2k_2}{f_2g_2}\right)$
$Q_{12} \cdot nR_{12}$	$\frac{q_2}{k_2} + \frac{q_3}{b_2}$	$\frac{q_2}{k_2} + \frac{q_4}{b_2} + \frac{q_3}{g_1}\left(\frac{b_2+g_2}{b_2}\right)$
$Q_{21} \cdot nR_{21}$	$\frac{q_1}{k_1} + \frac{q_3}{b_1}$	$\frac{q_1}{k_1} + \frac{q_3}{b_1} + \frac{q_4}{g_2}\left(\frac{b_1+g_1}{b_1}\right)$
$Q_{ee} \cdot nR_{ee}$	$q_3\left(\frac{1}{b_1} + \frac{1}{b_2}\right)$	$q_3\left[\frac{1}{b_1} + \frac{1}{g_1}\left(\frac{b_2+g_2}{b_2}\right)\right] + q_4\left[\frac{1}{b_2} + \frac{1}{g_2}\left(\frac{b_1+g_1}{b_1}\right)\right]$
$Q_{oo} \cdot nR_{oo}$	$\frac{q_1}{k_1} + \frac{q_2}{k_2}$	$\frac{q_1}{k_1} + \frac{q_2}{k_2}$
Constraint:	$b_1f_2k_1 = b_2f_1k_2$	$b_1f_2g_2k_1 = b_2f_1g_1k_2$

$$q = \frac{K^2 Q_{oo} R_{oo} + K Q_{12} R_{12} S_1 + K Q_{21} R_{21} S_2 + Q_{ee} R_{ee} S_1 S_2}{K^2 R_{oo} + K R_{12} S_1 + K R_{21} S_2 + R_{ee} S_1 S_2}$$

Model of Fig. 3

$$\frac{1}{b_2} + \frac{1}{j_1} + \frac{1}{h_2}\left(\frac{b_2+g_2}{b_2}\right) + \frac{1}{k_2}\left(\frac{j_1+j_2}{j_1}\right) + \frac{1}{g_1}\left[1 + \frac{h_1}{h_2}\left(\frac{b_2+g_2}{b_2}\right)\right]$$

$$\frac{1}{b_1} + \frac{1}{j_2} + \frac{1}{h_1}\left(\frac{b_1+g_1}{b_1}\right) + \frac{1}{k_1}\left(\frac{j_1+j_2}{j_2}\right) + \frac{1}{g_2}\left[1 + \frac{h_2}{h_1}\left(\frac{b_1+g_1}{b_1}\right)\right]$$

$$\frac{1}{b_1} + \frac{1}{b_2} + \frac{1}{h_1}\left(\frac{b_1+g_1}{b_1}\right) + \frac{1}{h_2}\left(\frac{b_2+g_2}{b_2}\right) + \frac{1}{g_1}\left[1 + \frac{h_1}{h_2}\left(\frac{b_2+g_2}{b_2}\right)\right] + \frac{1}{g_2}\left[1 + \frac{h_2}{h_1}\left(\frac{b_1+g_1}{b_1}\right)\right]$$

$$\frac{1}{k_1} + \frac{1}{k_2} + \frac{1}{j_1} + \frac{1}{j_2} + \frac{j_1}{j_2 k_1} + \frac{j_2}{j_1 k_2}$$

$$\frac{k_1}{f_1}\left(\frac{b_1+g_1}{g_1}\right)\left(\frac{j_2}{j_1+j_2}\right) + \frac{k_2}{f_2}\left(\frac{b_2+g_2}{g_2}\right)\left(\frac{j_1}{j_1+j_2}\right)$$

$$\frac{q_4}{b_2} + \frac{q_5}{j_1} + \frac{q_2}{k_2}\left(\frac{j_1+j_2}{j_1}\right) + \frac{q_6}{h_2}\left(\frac{b_2+g_2}{b_2}\right) + \frac{q_3}{g_1}\left[1 + \frac{h_1}{h_2}\left(\frac{b_2+g_2}{b_2}\right)\right]$$

$$\frac{q_3}{b_1} + \frac{q_5}{j_2} + \frac{q_1}{k_1}\left(\frac{j_1+j_2}{j_2}\right) + \frac{q_6}{h_1}\left(\frac{b_1+g_1}{b_1}\right) + \frac{q_4}{g_2}\left[1 + \frac{h_2}{h_1}\left(\frac{b_1+g_1}{b_1}\right)\right]$$

$$q_3\left[\frac{1}{b_1} + \frac{1}{g_1} + \frac{h_1}{g_1 h_2}\left(\frac{b_2+g_2}{b_2}\right)\right] + q_4\left[\frac{1}{b_2} + \frac{1}{g_2} + \frac{h_2}{g_2 h_1}\left(\frac{b_1+g_1}{b_1}\right)\right] + q_6\left[\frac{1}{h_1}\left(\frac{b_1+g_1}{b_1}\right) + \frac{1}{h_2}\left(\frac{b_2+g_2}{b_2}\right)\right]$$

$$\frac{q_1}{k_1}\left(\frac{j_1+j_2}{j_2}\right) + \frac{q_2}{k_2}\left(\frac{j_1+j_2}{j_1}\right) + q_5\left(\frac{j_1+j_2}{j_1 j_2}\right)$$

$$b_1 f_2 g_2 h_1 j_2 k_1 = b_2 f_1 g_1 h_2 j_1 k_2$$

$$q = \frac{q_1[E_1]+q_2[E_2]+q_3[ES]}{[E_1]+[E_2]+[ES]} \tag{2}$$

where q_1, q_2 and q_3 are the inactivation rate constants for the irreversible inhibitor acting on conformations E_1, E_2 and ES, respectively, and $[E_1]$, $[E_2]$ and [ES] are the number of functional carriers per unit area of membrane existing in conformations E_1, E_2 and ES, respectively.

When, as will usually be the case, q is much less than the turnover number of the transport system, the relative surface concentrations of the forms E_1, E_2 and ES will be determined only by the transport properties of the system. Thus we can use our previous result [14] that

$$\frac{[E_1]}{n} = \frac{b_1k_2+b_2k_2+b_1f_2S_2}{\Sigma} \tag{3}$$

$$\frac{[E_2]}{n} = \frac{b_2k_1+b_1k_1+b_2f_1S_1}{\Sigma} \tag{4}$$

$$\frac{[ES]}{n} = \frac{k_2f_1S_1+k_1f_2S_2+f_1f_2S_1S_2}{\Sigma} \tag{5}$$

where $n = [E_1]+[E_2]+[ES]$ is the total surface concentration of functional carrier at any time and

$$\Sigma \equiv (b_1+b_2)(k_1+k_2)+(b_2+k_2)f_1S_1+(b_1+k_1)f_2S_2+f_1f_2S_1S_2 \tag{6}$$

is the sum of all numerator terms on the right-hand sides of Eqns. 3–5.

Substituting Eqns. 3–5 into Eqn. 2 yields

$$q = \frac{q_1(b_1k_2+b_2k_2+b_1f_2S_2)+q_2(b_2k_1+b_1k_1+b_2f_1S_1)+q_3(k_2f_1S_1+k_1f_2S_2+f_1f_2S_1S_2)}{(b_1+b_2)(k_1+k_2)+(b_2+k_2)f_1S_1+(b_1+k_1)f_2S_2+f_1f_2S_1S_2} \tag{7}$$

We now define 4 new parameters, which will turn out to be conveniently measurable experimentally:

$$Q_{12} \equiv \frac{\frac{q_2}{k_2}+\frac{q_3}{b_2}}{nR_{12}} \tag{8}$$

$$Q_{21} \equiv \frac{\frac{q_1}{k_1}+\frac{q_3}{b_1}}{nR_{21}} \tag{9}$$

$$Q_{ee} \equiv q_3 \tag{10}$$

$$Q_{oo} \equiv \frac{\frac{q_1}{k_1}+\frac{q_2}{k_2}}{nR_{oo}} \tag{11}$$

With these definitions and those of K and terms in nR given in Table I for the model of Fig. 1, it can be verified that Eqn. 7 is identical with

$$q = \frac{K^2Q_{oo}R_{oo} + KQ_{12}R_{12}S_1 + KQ_{21}R_{21}S_2 + Q_{ee}R_{ee}S_1S_2}{K^2R_{oo} + KR_{12}S_1 + KR_{21}S_2 + R_{ee}S_1S_2} \tag{12}$$

From the definitions in Eqns. 8–11 and the relationship $nR_{ee} = (1/b_1 + 1/b_2)$ from Table I, it immediately follows that

$$Q_{12}R_{12} + Q_{21}R_{21} = Q_{ee}R_{ee} + Q_{oo}R_{oo} \tag{13}$$

Also, from Table I:

$$R_{12} + R_{21} = R_{ee} + R_{oo} \tag{14}$$

Thus q in Eqn. 12, which describes inactivation rates under all possible substrate concentrations, involves four independent measurable transport parameters (K and three of the four terms in R) and three independent measurable inactivation parameters (three of the four terms in Q).

Turning now to the model of Fig. 2, one can write the experimentally observed inactivation rate constant q in terms of the intrinsic inactivation rate constants (now q_1, q_2, q_3 and q_4) as:

$$q = \frac{q_1[E_1] + q_2[E_2] + q_3[ES_1] + q_4[ES_2]}{[E_1] + [E_2] + [ES_1] + [ES_2]} \tag{15}$$

When one solves [14–15, 17] for $[E_1]$, $[E_2]$, $[ES_1]$ and $[ES_2]$ in terms of S_1, S_2, n, and the rate constants of Fig. 2 and then uses the definitions of K and the R and Q parameters as given in the middle column of Table I, it can be verified that for this model too, Eqns. 12–13 are appropriate.

Finally, for the model of Fig. 3, where

$$q = \frac{q_1[E_1] + q_2[E_2] + q_3[ES_1] + q_4[ES_2] + q_5[E_m] + q_6[ES_m]}{[E_1] + [E_2] + [ES_1] + [ES_2] + [E_m] + [ES_m]} \tag{16}$$

entirely analogous considerations show that Eqns. 12–13 are yet again appropriate, with the definitions of K and the R and Q parameters now being as given in the last column of Table I.

In summary, we have shown that the same Eqns. 12–13 are obeyed by all three formulations of the simple carrier model depicted in Figs. 1–3 (and hence, by induction, to more complex formulations of this type). Only in the experimentally inaccessible molecular rate constants do the models differ.

HOW TO DETERMINE DIRECTLY THE INACTIVATION PARAMETERS Q

In the preceding section, we showed that the measured rate of inactivation of all formulations of the simple carrier model can be expressed by

$$q = \frac{K^2Q_{oo}R_{oo} + KQ_{12}R_{12}S_1 + KQ_{21}R_{21}S_2 + Q_{ee}R_{ee}S_1S_2}{K^2R_{oo} + KR_{12}S_1 + KR_{21}S_2 + R_{ee}S_1S_2} \tag{12}$$

On looking at this equation, it is obvious that the four Q parameters can in principle be determined directly in the following four experimental situations:

(i) when $S_1 = S_2 = 0$, $q = Q_{oo}$

(ii) when $S_1 \to \infty$, $S_2 = 0$, $q = Q_{12}$

(iii) when $S_1 = 0$, $S_2 \to \infty$, $q = Q_{21}$

(iv) when $S_1 = S_2 \to \infty$, $q = Q_{ee}$

Using the terminology of our previous papers [14–15], these situations are simply (i) the substrate-free system, (ii) the zero-trans procedure at limitingly high concentration at side 1, (iii) the zero-trans procedure at limitingly high concentration at side 2, and (iv) the equilibrium-exchange procedure with limitingly high concentrations at both sides.

REJECTION CRITERIA USING IRREVERSIBLE INHIBITION DATA

In the absence of transport data, it is impossible to use irreversible inhibition data to either test the applicability of the simple carrier model to a given transport system or to characterize the details of the transport process. This is because the experimentally accessible inactivation parameters Q involve combinations of both molecular transport and inactivation rate constants (see Table I). Thus, there is a basic asymmetry between transport and inactivation data, since we have shown [15] that the transport data alone can be used both to test and to characterize the simple carrier. This basic asymmetry is emphasized by comparing Eqns. 13 and 14, which show that although the measurable transport parameters R can be used alone to form a consistency test, the measurable inactivation parameters Q require the R parameters to form another such test.

It is often convenient to look at the difference between the inactivation rate in the presence and absence of substrate. We write this difference as

$$\Delta q = q - Q_{oo} \tag{17}$$

If we also define

$$\Delta Q_{12} = Q_{12} - Q_{oo} \tag{18}$$

$$\Delta Q_{21} = Q_{21} - Q_{oo} \tag{19}$$

and

$$\Delta Q_{ee} = Q_{ee} - Q_{oo} \tag{20}$$

then Eqn. 12 can be written as

$$\Delta q = \frac{K(\Delta Q_{12})R_{12}S_1 + K(\Delta Q_{21})R_{21}S_2 + (\Delta Q_{ee})R_{ee}S_1S_2}{K^2R_{oo} + KR_{12}S_1 + KR_{21}S_2 + R_{ee}S_1S_2} \tag{21}$$

and Eqn. 13 as

$$(\Delta Q_{12})R_{12} + (\Delta Q_{21})R_{21} = (\Delta Q_{ee})R_{ee} \tag{22}$$

TABLE II

INTERPRETATION OF EXPERIMENTAL TRANSPORT DATA IN TERMS OF BASIC MEASURABLE TRANSPORT PARAMETERS

Procedure	Direction of measured flux	Maximal velocity	Half-saturation concentration
Zero trans	$1 \rightarrow 2$	$V^{zt}_{1\rightarrow 2} = \frac{1}{R_{12}}$	$K^{zt}_{1\rightarrow 2} = K\frac{R_{oo}}{R_{12}}$
Zero trans	$2 \rightarrow 1$	$V^{zt}_{2\rightarrow 1} = \frac{1}{R_{21}}$	$K^{zt}_{2\rightarrow 1} = K\frac{R_{oo}}{R_{21}}$
Equilibrium-exchange	either	$V^{ee} = \frac{1}{R_{ee}}$	$K^{ee} = K\frac{R_{oo}}{R_{ee}}$

In Table II, there are listed the relationships previously derived [15] between maximal velocities and half-saturation concentrations in the zero-trans and equilibrium exchange procedures and the measurable transport parameters K and R, using the same nomenclature (see the Appendix for a list of symbols). Using the relationships between maximal velocities V and transport parameters R listed in Table II, it is possible to rewrite Eqn. 22 in the form of a rejection criterion for the simple carrier:

$$\frac{\Delta Q_{12}}{V^{zt}_{1\rightarrow 2}} + \frac{\Delta Q_{21}}{V^{zt}_{2\rightarrow 1}} = \frac{\Delta Q_{ee}}{V^{ee}} \tag{23}$$

The power of this rejection criterion lies in the fact that the ΔQ and V parameters of each of the three terms are obtained under identical conditions.

We can derive additional rejection criteria by considering the substrate dependence of both inactivation and transport. If we consider the zero-trans procedure with side 2 being the trans side, then Eqn. 21 reduces to

$$\Delta q^{zt}_{1\rightarrow 2} = \frac{(\Delta Q_{12})R_{12}S_1}{KR_{oo} + R_{12}S_1} = \frac{(\Delta Q_{12})S_1}{(KR_{oo}/R_{12}) + S_1} \tag{24}$$

This is a Michaelis-Menten form with half-saturation concentration given by ${}^{\Delta}K^{zt}_{1\rightarrow 2} = KR_{oo}/R_{12}$. But we see from Table II that this is precisely the half-saturation concentration found by transport measurements under exactly the same conditions. Thus, for a system which behaves as a simple carrier, these two half-saturation concentrations must be identical, a result which provides a rejection criterion for this model. In an analogous fashion, we find a similar rejection criterion for the zero-trans procedure performed in the opposite direction.

For the equilibrium-exchange procedure ($S_1 = S_2 \equiv S$), Eqn. 21 becomes

$$\Delta q^{ee} = \frac{K[(\Delta Q_{12})R_{12} + (\Delta Q_{21})R_{21}]S + (\Delta Q_{ee})R_{ee}S^2}{K^2R_{oo} + K(R_{12} + R_{21})S + R_{ee}S^2} \tag{25}$$

Using now Eqns. 14 and 22 to substitute for $(R_{12} + R_{21})$ and $[(\Delta Q_{12})R_{12} + (\Delta Q_{21})R_{21}]$, respectively, we obtain:

TABLE III

SOME REJECTION CRITERIA FOR THE SIMPLE CARRIER USING INACTIVATION AND TRANSPORT MEASUREMENTS

$$\frac{\Delta Q_{12}}{V^{zt}_{1\to 2}} + \frac{\Delta Q_{21}}{V^{zt}_{2\to 1}} = \frac{\Delta Q_{ee}}{V^{ee}}$$

$${}^{\Delta}K^{zt}_{1\to 2} = K^{zt}_{1\to 2}$$

$${}^{\Delta}K^{zt}_{2\to 1} = K^{zt}_{2\to 1}$$

$${}^{\Delta}K^{ee} = K^{ee}$$

$$\Delta q^{ee} = \frac{(\Delta Q_{ee})R_{ee}S(K+S)}{(KR_{oo}+R_{ee}S)(K+S)} = \frac{\Delta Q_{ee}S}{(KR_{oo}/R_{ee})+S} \tag{26}$$

Once again, this is a Michaelis-Menten form, now having a half-saturation concentration ${}^{\Delta}K^{ee} = KR_{oo}/R_{ee}$, which is the same as the transport half-saturation concentration K^{ee} (see Table II).

The four rejection criteria which we have derived, which are only some of the many possible ones, are listed for convenience in Table III.

SPECIAL CASES

It is convenient at this point to discuss two special cases. The first case, which is essentially trivial, is when all forms of the carrier are inactivated at the same rate. Then, since all of the rate constants q are equal, inspection of Table I for all three formulations of the carrier shows that $q = Q_{oo} = Q_{12} = Q_{21} = Q_{ee}$. Thus, as expected, the inactivation rate is independent of substrate concentration.

A more interesting case, which has indeed been proposed [6, 9–10, 12] as the basis for the mechanism of action of certain irreversible inhibitors, is where the inhibitor reacts at one rate with all inward-facing forms of the carrier and at another rate with outward-facing forms, irrespective of whether or not the carrier has bound substrate. The influence of substrate on the inactivation rate then resides merely in its effect upon redistributing carrier between the two faces of the membrane. The formulation of Fig. 2 is the relevant kinetic scheme for this case. It can be seen that $q_1 = q_3$ and $q_2 = q_4$ are the required constraints on the molecular inactivation rate constants. Using these constraints, it is possible to derive a useful rejection criterion for this restricted mechanism. We can assume that one of the two molecular inactivation rate constants is larger than the other (since, otherwise, substrate would have no effect on the observed inactivation rates); for convenience, let this be q_1. The maximum possible rate of inactivation would occur if all carriers were at face 1 of the membrane and would then be q_1. Thus the maximum observed rate of inactivation q_{max} cannot be larger than q_1, or

$$q_{max} \leqq q_1 \tag{27}$$

In the complete absence of substrate, the observed rate of inactivation is simply Q_{oo}, for which (see Table I)

$$Q_{oo} = q_1 \frac{k_2}{k_1+k_2} + q_2 \frac{k_1}{k_1+k_2} \tag{28}$$

so that

$$Q_{oo} > q_1 \frac{k_2}{k_1+k_2} \geqslant q_{max} \frac{k_2}{k_1+k_2} \tag{29}$$

But using Table I again we have that

$$1 - \frac{R_{12}}{R_{oo}} = \frac{k_2}{k_1+k_2} - \frac{\frac{1}{b_2} + \frac{1}{g_1}\left(\frac{b_2+g_2}{b_2}\right)}{\frac{1}{k_1} + \frac{1}{k_2}} \tag{30}$$

so that

$$\frac{k_2}{k_1+k_2} > 1 - \frac{R_{12}}{R_{oo}} \tag{31}$$

From Eqns. 29 and 31 it follows that

$$Q_{oo} > q_{max}\left(1 - \frac{R_{12}}{R_{oo}}\right) \tag{32}$$

Also, from Table IV of ref. 15:

$$\frac{R_{12}}{R_{oo}} = \frac{K^{ic}_{1\to 2}}{K^{ee}} = \frac{K^{it}_{2\to 1}}{K^{ee}} \tag{33}$$

where $K^{ic}_{1\to 2}$ is the transport half-saturation concentration in the infinite-cis procedure when the cis face is face 1, and $K^{it}_{2\to 1}$ is the transport half-saturation concentration in the infinite-trans procedure when the trans face is face 1. Finally, then, from Eqns. 32 and 33, we have that

$$Q_{oo} > q_{max}\left(1 - \frac{K^{ic}_{1\to 2}}{K^{ee}}\right) = q_{max}\left(1 - \frac{K^{it}_{2\to 1}}{K^{ee}}\right) \tag{34}$$

When K^{ee} is found experimentally to be greater than $K^{ic}_{1\to 2} = K^{it}_{2\to 1}$, then Eqn. 34 sets a limit to the possible degree of stimulation by substrate of the inactivation process.

DISCUSSION

The availability of a very general kinetic treatment of the simple carrier has enabled us to derive a similarly general kinetic analysis for irreversible inhibition. Our analysis is general in that we make no assumptions as to the symmetry of the carrier nor do we assume that any form of the carrier is in equilibrium with substrate. In their pioneering studies, carried out before a general treatment was available, both Krupka [3] and Edwards [10] had to make either one or both of these assumptions.

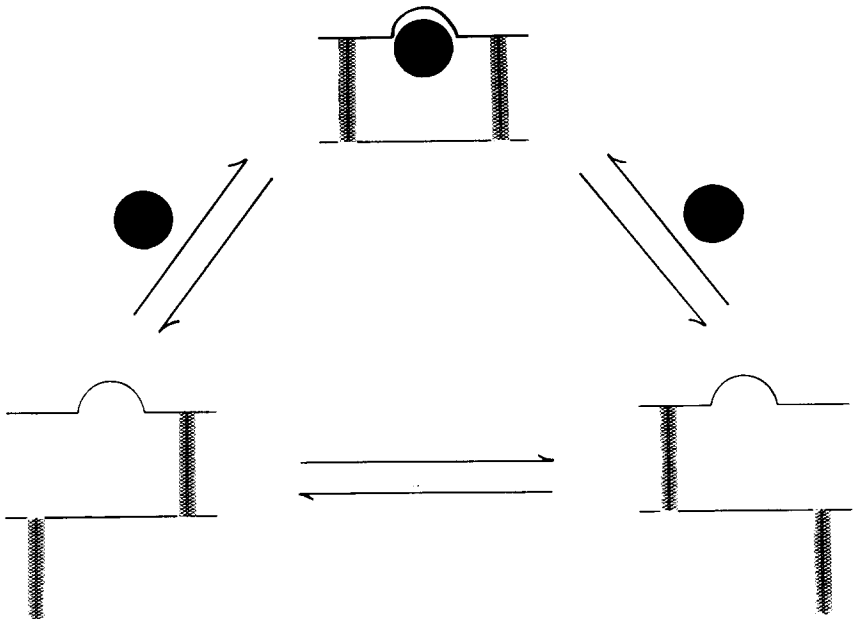

Fig. 4. Simplified "molecular" representation of a transport system corresponding to the kinetic scheme of Fig. 1.

We find that both for transport and irreversible inhibition, it is impossible on the basis of steady-state data to distinguish among the models of Figs. 1–3. Each of these kinetic schemes shows the dominant conformational states of the carrier for that particular model. Since the kinetic scheme of Fig. 1 is so simple to handle analytically, yet accounts for all of the steady-state behaviour of the simple carrier, it may be worthwhile to try to develop an intuitive perception of it. To this end we show in Fig. 4 a possible molecular example of a transport system the kinetics of which are formally represented in Fig. 1. Here there is a single stationary binding site, access to which is controlled by the positions of two gates. In the process of the substrate molecule binding to the site, the open gate closes behind the substrate. During its transit through the membrane, the substrate molecule spends most of its time in this resultant occluded state. Fig. 2 is simply the conventional carrier model, while Fig. 3 is a special formulation of the conventional carrier in which allowance is made for the fact that intermediate forms (in transit from one membrane face to the other) of the carrier may exist for substantial fractions of the transport cycle.

Of course to a certain extent any of these kinetic schemes (Figs. 1–3) are approximations, since a real transport event must involve in addition a very large number of short-lived intermediate states. But we have shown that the presence of any number of intermediate states will not affect the observable steady-state behavior of the system towards transport or inactivation. With this in mind, it would seem to make sense to refrain from using kinetic schemes more complicated than that of Fig. 1 until pre-steady-state data become available, for two reasons: (1) the kinetic scheme of Fig. 1 is simple to analyze mathematically with no approximations, and the measurable transport and inactivation parameters have particularly simple forms (see Table I).

(2) One is not tempted to ask questions which cannot be answered with steady-state data, such as (i) Does the loaded carrier move at a different rate than the unloaded carrier? (ii) Do the inward- and outward-facing forms of the carrier react with irreversible inhibitors at different rates? (iii) Are intermediate forms inactivated at rates different from other forms?

We have shown previously [15] that steady-state transport data provide a substantial number of consistency tests or rejection criteria for the applicability of the simple carrier model to any given transport system. We have seen in the present paper that although inactivation data by themselves cannot provide such tests, in conjunction with transport data certain additional tests are possible all the same. Of course, existing data in the literature have not been obtained with these particular tests in mind, so that one cannot now apply the tests with the rigour that one might wish. Yet we can illustrate the approach by using what data is available.

Probably the most complete set of inactivation data in a situation where the transport data is also available comes from a valuable study by Edwards [9]. Edwards measured the rates of inactivation by 1-fluoro-2,4-dinitrobenzene of the glucose transport system of the human red cell at 25 °C under many different concentrations of glucose. From Table I of ref. 9, taking face 1 as the inner face of the membrane and using our notation, he found that $\Delta Q_{12} = -0.50 Q_{oo}$, $\Delta Q_{21} = 0.26 Q_{oo}$, and $\Delta Q_{ce} = -0.04 Q_{oo}$. Written in the form of ratios of Q and V values, the rejection criterion of Eqn. 23 becomes

$$\frac{\Delta Q_{12}}{\Delta Q_{ee}} \frac{V^{ee}}{V_{1\to 2}^{zt}} + \frac{\Delta Q_{21}}{\Delta Q_{ee}} \frac{V^{ee}}{V_{2\to 1}^{zt}} = 1 \tag{35}$$

Now at 25 °C the value of $V^{ee}/V_{1\to 2}{}^{zt}$ has been found [18] to be about 2, while for $V^{ee}/V_{2\to 1}{}^{zt}$ we have a value [19] of about 7 at 20 °C, which we must take as the best estimate. (A reviewer has pointed out that the ΔQ values are from experiments performed in the presence of 12.3 % ethanol, while the V values were obtained in the absence of ethanol.) Because of these difficulties, it is not possible to place much reliance on the result of an analysis of these data nor to perform a statistical analysis. But for what it is worth and to illustrate the approach, one notes that the terms on the left-hand side of Eqn. 35, which should add to unity if the test holds, in fact add up to some −20. One might be tempted to use these data to reject the simple carrier model for the glucose transport system of the human red blood cell, but clearly experiments designed with this rejection criterion in mind should be performed.

Edwards [10] has also studied the irreversible inhibition of the human red blood cell choline transport system by *N*-ethylmaleimide. Here, it was found that the half-saturation concentrations ${}^{A}K_{1\to 2}{}^{zt}$ and ${}^{A}K_{2\to 1}{}^{zt}$ for inactivation were not significantly different from the corresponding half-saturation concentrations $K_{1\to 2}{}^{zt}$ and $K_{2\to 1}{}^{zt}$, respectively, for transport. Thus, the choline transport system passes two of the rejection criteria listed in Table III. Since ΔQ_{ee} and K^{ee} are not available, it is not possible to apply the other rejection criteria. The present data do not require the rejection of the simple carrier as a model for the choline transport system.

A theory based on the simple redistribution of carrier forms between two interfaces using the model of Fig. 2 has been proposed [10] to explain the data on both fluorodinitrobenzene inhibition of glucose transport and *N*-ethylmaleimide inhibition

of choline transport in human red cells. This theory postulated that only inward-facing forms of the carrier react with irreversible inhibitor and that loaded and unloaded forms react at the same rate. Rejection criteria for a slightly more general form of this theory were developed in the preceding section. For glucose transport at 20 °C, we need estimates of the ratio $K_{1\to 2}^{ic}/K^{ee}$, where face 1 is the inner face of the membrane, in order to apply the rejection criterion of Eqn. 34. Estimates [20–22] for K^{ee} at 20 °C range from 9–38 mM, while those [22–24] for $K_{1\to 2}^{ic}$ range from 1.7–1.9 mM, so that the ratio $K_{1\to 2}^{ic}/K^{ee}$ ranges from 0.04–0.21. Putting these values into Eqn. 34 gives the prediction on the simple carrier model that $q_{max} < 1.3Q_{oo}$. Yet Jung [11] at 21 °C found that $q_{max} > 8Q_{oo}$ for the inhibition of the glucose transport system by fluorodinitrobenzene. The published data on the choline transport system are not sufficiently extensive to allow one to apply this test. Thus it seems unlikely that Edwards' theory applies to the glucose transport system, although it may be true for the choline transport system.

We have shown that inactivation data, when coupled with transport data, can be very useful in providing additional tests for the simple carrier model. However, the use of irreversible inhibitors has clear limitations, in that in the absence of transport data no such tests are possible and even together with the transport data no further characterization of the transport kinetic properties can be obtained. Further, the information from irreversible inhibition studies, like that from transport studies, does not allow one to distinguish between the various formulations of the simple carrier model.

APPENDIX

List of symbols

b_1, b_2	effective interfacial molecular rate constants for breakdown of ES to E and S.
E	free carrier.
ES	carrier-substrate complex.
f_1, f_2	effective interfacial molecular rate constants for formation of ES from E and S.
g_1, g_2, h_1, h_2	molecular rate constants for interconversion of forms ES.
j_1, j_2, k_1, k_2	molecular rate constants for interconversion of forms E.
K	(without subscripts or superscripts) basic measurable membrane transport parameter.
K	(otherwise) that substrate concentration at which half of the maximum rate of transport occurs.
^{A}K	that substrate concentration at which half of the maximum substrate-dependent rate of inactivation occurs.
n	total number of functional carriers per unit area of membrane.
$R_{oo}, R_{12}, R_{21}, R_{ee}$	additional basic measurable membrane transport parameters.
q	observed inactivation rate constant.
q_{max}	maximum value of q.
$q_1, q_2, q_3, q_4, q_5, q_6$	molecular inactivation rate constants as defined in kinetic schemes of Figs. 1–3.
$Q_{oo}, Q_{12}, Q_{21}, Q_{ee}$	basic measurable inactivation parameters.

S	substrate concentration at membrane face.
v	rate of unidirectional transport per unit area of membrane.
V	maximum value of v.
[E], [ES]	surface concentration (number per unit area of membrane) of functional carrier in forms E and ES, respectively.
Δq	$= q - Q_{oo}$.
ΔQ	$= Q - Q_{oo}$.
Σ	sum of all numerator terms on the right-hand sides of Eqns. 3–5.

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

Right superscripts	
ee	equilibrium-exchange procedure.
ic	infinite-cis procedure.
it	infinite-trans procedure.
zt	zero-trans procedure.
Subscripts	
1	value or form at face 1 of membrane.
2	value or form at face 2 of membrane.
m	form intermediate between above two forms.
$1 \rightarrow 2$	value in an experiment in which transport is measured from face 1 to face 2 of membrane.
$2 \rightarrow 1$	value in an experiment in which transport is measured from face 2 to face 1 of the membrane.

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