Biochimica et Biophysica Acta, 689 (1982) 444–450
Elsevier Biomedical Press

BBA 71257

GENERAL RATE EQUATIONS AND REJECTION CRITERIA FOR THE RAPID EQUILIBRIUM CARRIER MODEL OF COTRANSPORT

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(Received January 13th, 1982)

Key words: Coupled transport; Transport kinetics; Carrier model; Rejection criteria

It is demonstrated that under fixed activator conditions the general flux equation for the rapid equilibrium carrier model of cotransport can be written entirely in terms of five independent kinetic constants. Thus the kinetic parameters from any experiment carried out under the same activator conditions must necessarily be expressible in terms of these five constants. These predicted relationships between experimental kinetic parameters provide rejection criteria for the model, a number of which are derived here. Generalization of the treatment to the case where a competitive substrate is present on both sides of the membrane is also given.

Introduction

In the kinetic analysis of a transport model one can distinguish between two basically distinct sets of predictions which can be used as tests or equivalently as rejection criteria for the model. The first set arises from the mathematical form of the kinetic equations. These equations describe the predicted dependence of flux on substrate concentrations (*cis* and *trans*), inhibitor concentrations, and so on. Thus, for example, a certain model might require that under zero *trans* conditions the substrate flux shows a Michaelis-Menten-type dependence on substrate concentration. Because predictions of this type concern only the functional form of the flux equations they may well be common to more than one transport mechanism.

The second set of predictions concerns relationships between the experimentally determined parameters of the model. For example, for the simple carrier model to hold the $V_{\max}$ of the transporter measured under equilibrium exchange conditions must be equal to the $V_{\max}$ measured under infinite *trans* conditions [1,2]. In general one expects predictions of the latter type to provide more stringent tests of the model since these essentially involve internal consistency checks on the details of the transport mechanism.

In an earlier publication [3] the kinetic properties of a family of contransport models of the rapid equilibrium* carrier type were studied in some detail. In particular, it was demonstrated that under zero *trans*, equilibrium exchange and infinite *trans* conditions, as usually defined (see below), the kinetic parameters K_m and $V_{\max}$ could be expressed as simple functions of the activator concentration and a minimal set of model-dependent constants with well defined kinetic interpretations. In this analysis, however, few simple rela-

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* The term 'rapid equilibrium' [4] refers to the assumption made here that the translocation of the transporter binding site from one membrane surface to the other is the rate-limiting step in the transport process. The significance of this assumption has been discussed previously [3].

tionships between the experimentally determined parameters (i.e., rejection criteria of the second type discussed above) were obvious. In the present paper it is demonstrated that such a set of relationships can indeed be derived, but for slightly different experimental conditions. The rejection criteria which result are similar to those previously derived for the simple carrier model [1,2].

As a basis for the present analysis it is demonstrated that under fixed activator conditions the general flux equation for the rapid equilibrium carrier model of cotransport can be written entirely in terms of five independent kinetic constants. Thus the kinetic parameters from any experiment carried out under the same activator conditions must necessarily be expressible in terms of these five constants. These predicted relationships between experimental kinetic parameters provide the desired rejection criteria. Additional rejection criteria from kinetic measurements carried out in the presence of competing substrates are also discussed.

Theoretical derivations

The model

The cotransport model treated in this paper is shown in Fig. 1. The two cotransported species are referred to as the substrate, S, and the activator, A. The 'free carrier' on side *n* of the membrane is represented by C_n, the carrier plus bound substrate by CS_n, and so on. The rate constants for the translocation of the various carrier species are designated k_{12}, k_{21}, f_{12}, etc. The dissociation constants K_{A_n}, K_{AS_n}, etc. which characterize the binding events at the two membrane faces are defined below. The binding of A and S to the transporter has been assumed to occur by a random process, however, the results derived here apply equally well to ordered models of the carrier type since these may be regarded as limiting cases of the random model [3].

In the derivations which follow the effects of an additional transported species, T, which competes with S for its binding site on the carrier have also been included. The presence of T has not been shown in Fig. 1 in order to preserve clarity. The rate constants for the translocation of CT_n and CAT_n are designated f'_{nm} and h'_{nm}, respectively.

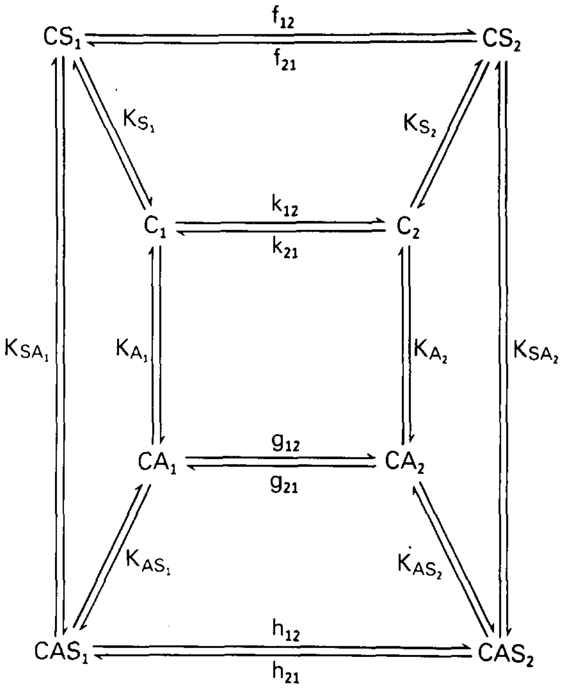

Fig. 1. Schematic representation of the rapid equilibrium carrier model of cotransport (see text for details).

Assumptions

The usual assumptions associated with the rapid equilibrium carrier model are made here [3,5], namely that:

1. The transporter is a rapid equilibrium system *. Thus the carriers are in equilibrium with the ligands S, T and A at the membrane surface.
2. The total number of transporters, free and loaded, is constant and equal to C_0.
3. There is no net movement of transporter binding sites from one face of the membrane to the other; in other words, a steady state exists at the time of measurement.
4. The transporter itself is not primary active.

Solution of the model

The solution of the model shown in Fig. 1 has been given previously [3,5]. The incorporation of a second competing substrate, T, is quite straightforward. A brief description of the solution follows.

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* See footnote on p. 444.

From assumption 1 above we have that on side n of the membrane

$$K_{S_n} = [C_n][S_n]/[CS_n]$$

$$K_{SA_n} = [CS_n][A_n]/[CAS_n]$$

$$K_{T_n} = [C_n][T_n]/[CT_n]$$

$$K_{TA_n} = [CT_n][A_n]/[CAT_n]$$

$$K_{A_n} = [C_n][A_n]/[CA_n]$$

$$K_{AS_n} = [CA_n][S_n]/[CAS_n]$$

$$K_{AT_n} = [CA_n][T_n]/[CAT_n]$$

It follows directly from the above equations that

$$K_{S_n}K_{SA_n} = K_{A_n}K_{AS_n}$$

$$K_{T_n}K_{TA_n} = K_{A_n}K_{AT_n}$$

The unidirectional flux of S from side 1 to side 2 of the membrane is given by

$$J_S^{1\to 2} = f_{12}[CS_1] + h_{12}[CAS_1] \tag{1}$$

Using assumptions 2 and 3 we find that

$$[CS_1] = [S_1]Q_{12}/K_{S_1} \tag{2a}$$

and

$$[CAS_1] = [S_1][A_1]Q_{12}/K_{S_1}K_{SA_1} \tag{2b}$$

where

$$Q_{12} = C_0F_{21}/(\alpha_1F_{21} + \alpha_2F_{12}) \tag{2c}$$

with F_{nm} and α_n given by

$$\begin{aligned} F_{nm} = k_{nm} &+ g_{nm}([A_n]/K_{A_n}) + f_{nm}([S_n]/K_{S_n}) \\ &+ h_{nm}([A_n][S_n]/K_{A_n}K_{AS_n}) + f'_{nm}([T_n]/K_{T_n}) \\ &+ h'_{nm}([A_n][T_n]/K_{A_n}K_{AT_n}) \end{aligned}$$

and

$$\begin{aligned} \alpha_n = 1 &+ ([A_n]/K_{A_n}) + ([S_n]/K_{S_n}) \\ &+ ([A_n][S_n]/K_{A_n}K_{AS_n}) + ([T_n]/K_{T_n}) \\ &+ ([A_n][T_n]/K_{A_n}K_{AT_n}) \end{aligned}$$

The corresponding unidirectional flux from side 2 to side 1 of the membrane may be simply obtained by exchanging the roles of the subscripts 1 and 2 in Eqns. 1 and 2. Likewise, the unidirectional flux of T, $J_T^{1\to 2}$, is obtained by substituting T for S in Eqns. 1, 2a and 2b and replacing f_{12} and h_{12} by f'_{12} and h'_{12} in Eqn. 1.

Assumption 4 above imposes the following thermodynamic constraints on the parameters of the model [3,5],

$$K_{S_2}f_{12}k_{21}e^{z_S u} = K_{S_1}f_{21}k_{12} \tag{3a}$$

$$K_{A_2}g_{12}k_{21}e^{z_A u} = K_{A_1}g_{21}k_{12} \tag{3b}$$

$$K_{AS_2}h_{12}g_{21}e^{z_S u} = K_{AS_1}h_{21}g_{12} \tag{3c}$$

$$K_{T_2}f'_{12}k_{21}e^{z_T u} = K_{T_1}f'_{21}k_{12} \tag{3d}$$

$$K_{AT_2}h'_{12}g_{21}e^{z_T u} = K_{AT_1}h'_{21}g_{12} \tag{3e}$$

where

$$u = F\Delta\psi/RT$$

Here z_S, z_T and z_A are the electrical charges on S, T and A, respectively, F/RT has the usual thermodynamic definition and $\Delta\psi = \psi_2 - \psi_1$ is the electrical potential difference between side 1 and side 2 of the membrane.

The kinetics of substrate flux

Experimental kinetic constants and nomenclature

Four types of experimental conditions which are of interest here are defined below. In each case, the unidirectional flux, $J_S^{1\to 2}$, can be shown to have Michaelis-Menten form, i.e.,

$$J_S^{1\to 2} = V_{S_1}^{ex}[S_1]/(K_{S_1}^{ex} + [S_1])$$

where $V_{S_1}^{ex}$, the maximal velocity, and $K_{S_1}^{ex}$, the Michaelis constant, are independent of $[S_1]$. Here the superscript 'ex' refers to the particular experimental condition (see below). It is also convenient to define the limiting permeability [1], $P_{S_1}^{ex}$, by

$$P_{S_1}^{ex} = \lim_{[S_1]\to 0} J_S^{1\to 2}/[S_1] = V_{S_1}^{ex}/K_{S_1}^{ex}$$

Under some experimental circumstances it may be more convenient to evaluate $P_{S_1}^{ex}$ than $V_{S_1}^{ex}$ and/or $K_{S_1}^{ex}$ [3].

The experimental conditions which are of interest here are defined below for $J_S^{1\to 2}$. The corresponding nomenclature for kinetic constants is given in parentheses.

(i) zero *trans* conditions ($V_{S_1}^{zt}$, $K_{S_1}^{zt}$, $P_{S_1}^{zt}$):

$$[S_2] = [T_1] = [T_2] = 0.$$

(ii) infinite *trans* conditions ($V_{S_1}^{it}$, $K_{S_1}^{it}$, $P_{S_1}^{it}$):

$$[T_1] = [T_2] = 0, [S_2] \to \infty.$$

(iii) hetero-infinite *trans* conditions ($V_{S_1}^{it,T}$, $K_{S_1}^{it,T}$, $P_{S_1}^{it,T}$):

$$[T_1] = [S_2] = 0, [T_2] \to \infty.$$

(iv) equilibrium exchange conditions ($\bar{V}_S^{ee}$, $\bar{K}_S^{ee}$, $\bar{P}_S^{ee}$):

$$[T_1] = [T_2] = 0$$

and

$$[S_1] = [S_2]e^{z_S u}, [A_1] = [A_2]e^{z_A u}$$

The latter conditions above are equivalent to the requirement that the transmembrane electrochemical potential differences for S and A are zero, i.e., that $\Delta\mu_S = \Delta\mu_A = 0$. Note that due to Assumption 4 above one necessarily has that $\bar{V}_{S_1}^{ee} = \bar{V}_{S_2}^{ee} = \bar{V}_S^{ee}$ and $\bar{K}_{S_1}^{ee} = \bar{K}_{S_2}^{ee} = \bar{K}_S^{ee}$.

Expressions for the kinetic constants defined above are given in Table I.

TABLE I

EXPRESSIONS FOR KINETIC CONSTANTS DEFINED IN THE TEXT FOR THE RAPID EQUILIBRIUM CARRIER MODEL OF COTRANSPORT (Fig. 1)

The following 'relative concentrations' have been used for compactness: $a_n = [A_n]/K_{A_n}$, $a_{sn} = [A_n]/K_{SA_n}$, $a_{tn} = [A_n]/K_{TA_n}$.

Experimental kinetic constant	Expression in terms of parameters of the model
$V_{S_1}^{zt}$	$\dfrac{C_0(f_{12}+a_{s1}h_{12})(k_{21}+a_2g_{21})}{(1+a_{s1})(k_{21}+a_2g_{21})+(1+a_2)(f_{12}+a_{s1}h_{12})}$
$K_{S_1}^{zt}$	$K_{S_1}\dfrac{(1+a_1)(k_{21}+a_2g_{21})+(1+a_2)(k_{12}+a_1g_{12})}{(1+a_{s1})(k_{21}+a_2g_{21})+(1+a_2)(f_{12}+a_{s1}h_{12})}$
$V_{S_1}^{it}$	$\dfrac{C_0(f_{12}+a_{s1}h_{12})(f_{21}+a_{s2}h_{21})}{(1+a_{s1})(f_{21}+a_{s2}h_{21})+(1+a_{s2})(f_{12}+a_{s1}h_{12})}$
$K_{S_1}^{it}$	$K_{S_1}\dfrac{(1+a_1)(f_{21}+h_{21}a_{s2})+(1+a_{s2})(k_{12}+a_1g_{12})}{(1+a_{s1})(f_{21}+a_{s2}h_{21})+(1+a_{s2})(f_{12}+a_{s1}h_{12})}$
$\bar{V}_S^{ee}$	$\dfrac{C_0f_{21}(f_{12}+a_{s1}h_{12})}{f_{21}(1+a_{s1})+f_{12}(1+a_{s2})}$
$\bar{K}_S^{ee}$	$(K_{S_1}f_{12}/k_{21})\dfrac{k_{21}(1+a_1)+k_{12}(1+a_2)}{f_{21}(1+a_{s1})+f_{12}(1+a_{s2})}$
$V_{S_1}^{it,T}$	$\dfrac{C_0(f_{12}+a_{s1}h_{12})(f'_{21}+a_{t2}h'_{21})}{(1+a_{s1})(f'_{21}+a_{t2}h'_{21})+(1+a_{t2})(f_{12}+a_{s1}h_{12})}$
$K_{S_1}^{it,T}$	$K_{S_1}\dfrac{(1+a_1)(f'_{21}+a_{t2}h'_{21})+(1+a_{t2})(k_{12}+a_1g_{12})}{(1+a_{s1})(f'_{21}+a_{t2}h'_{21})+(1+a_{t2})(f_{12}+a_{s1}h_{12})}$

In contrast to earlier work [3] the activator conditions for zero *trans* and infinite *trans* experiments have not been specified*. The reason for this will become clear in what follows. In the special case where $\Delta\mu_A = 0$, $V^{ex}_{S_1}$ and $K^{ex}_{S_1}$ will be denoted $\bar{V}^{ex}_{S_1}$ and $\bar{K}^{ex}_{S_1}$ (cf. equilibrium exchange conditions above).

Expressions for the general flux equation in terms of experimental kinetic constants

Case I: The general flux equation in the absence of competing substrate. Setting $[T_1] = [T_2] = 0$ it can be shown by straightforward algebra that Eqn. 1 may be rewritten as

$$J_S^{1\to 2} = \left\{P^{zt}_{S_1}[S_1] + \left(P^{it}_{S_1}[S_1][S_2]/K^{zt}_{S_2}\right)\right\}$$
$$\times\left\{1 + \left([S_1]/K^{zt}_{S_1}\right) + \left([S_2]/K^{zt}_{S_2}\right)\right.$$
$$\left. + \left([S_1][S_1]/K^{zt}_{S_1}K^{it}_{S_2}\right)\right\}^{-1} \tag{4}$$

It can be seen from Eqn. 4 that under fixed activator conditions the eight experimental kinetic constants defined by zero *trans* and infinite *trans* procedures are sufficient to predict the flux rate in either direction under any substrate conditions. In fact, only five of these constants are independent of one another since the relationships referred to as Eqns. R1, R2 and R3 in Table II can be proven directly from Table I. If in addition one assumes that $\Delta\mu_A = 0$ then Eqn. R4 in Table II also holds and one is left with only four independent kinetic constants.

The kinetic constants for any other experimental substrate conditions must necessarily be expressible in terms of zero *trans* and infinite *trans* parameters. The derivation of these relationships is usually quite straightforward. For example, under infinite *cis* conditions ($[S_1] \to \infty$) Eqn. 4 reduces to

$$J_S^{1\to 2} = \left(V^{zt}_{S_1}K^{it}_{S_2} + [S_2]V^{it}_{S_1}\right)/\left(K^{it}_{S_2} + [S_2]\right)$$

Deriving the expressions for the equilibrium exchange kinetic constants is, however, somewhat more complex. These relationships are given as Eqns. R5 and R6 in Table II.

TABLE II

RELATIONSHIPS AMONG EXPERIMENTAL KINETIC CONSTANTS WHICH CAN BE USED AS REJECTION CRITERIA FOR THE RAPID EQUILIBRIUM CARRIER MODEL OF CONTRANSPORT

A. General relationships for one substrate

$$V^{it}_{S_1} = V^{it}_{S_2} \tag{R1}$$

$$K^{zt}_{S_1}K^{it}_{S_2} = K^{zt}_{S_2}K^{it}_{S_1} \tag{R2}$$

$$P^{it}_{S_1}/P^{zt}_{S_1} = 1 + (V^{zt}_{S_2}/V^{zt}_{S_1}) - (V^{zt}_{S_2}/V^{it}_{S_1}) \tag{R3}$$

B. Additional relationships for one substrate when $\Delta\mu_A = 0$ [a]

$$\bar{P}^{zt}_{S_2} = \bar{P}^{zt}_{S_1}e^{z_S u} \tag{R4}$$

$$\bar{V}^{ee}_{S} = \bar{V}^{it}_{S_1} = \bar{V}^{it}_{S_2} \tag{R5}$$

$$\bar{P}^{ee}_{S} = \bar{P}^{zt}_{S_1} \tag{R6}$$

C. General relationships for two substrates

$$V^{it,S}_{T_1} = V^{it,T}_{S_2} \tag{R7}$$

$$V^{it,S}_{T_2} = V^{it,T}_{S_1} \tag{R8}$$

$$K^{it,S}_{T_1}/K^{it,T}_{S_2} = K^{zt}_{T_1}/K^{zt}_{S_2} \tag{R9}$$

$$K^{it,T}_{S_1}/K^{it,S}_{T_2} = K^{zt}_{S_1}/K^{zt}_{T_2} \tag{R10}$$

$$\frac{P^{it,T}_{S_1}P^{zt}_{S_2}}{P^{it,T}_{S_2}P^{zt}_{S_1}} = \frac{V^{zt}_{T_2}}{V^{zt}_{T_1}} \tag{R11}$$

$$\frac{P^{it,S}_{T_1}P^{zt}_{T_2}}{P^{it,S}_{T_2}P^{zt}_{T_1}} = \frac{V^{zt}_{S_2}}{V^{zt}_{S_1}} \tag{R12}$$

[a] When $\Delta\mu_A = 0$ it can be shown from Eqns. 3 that

$$\left(f_{12} + \left(h_{12}[A_1]/K_{SA_1}\right)\right)/\left(f_{21} + \left(h_{21}[A_2]/K_{SA_2}\right)\right) = f_{12}/f_{21}$$

and

$$\left(k_{12} + \left(g_{12}[A_1]/K_{A_1}\right)\right)/\left(k_{21} + \left(g_{21}[A_2]/K_{A_2}\right)\right) = k_{12}/k_{21}$$

These relationships have been used in deriving Eqns. R4, R5 and R6.

Case II. The general flux equation in the presence of a competing substrate, T.

In this case Eqn. 1 may be written as

$$J_S^{1\to 2} = \left\{P^{zt}_{S_1}[S_1] + \left(P^{it}_{S_1}[S_1][S_2]/K^{zt}_{S_2}\right)\right.$$
$$\left. + \left(P^{it,T}_{S_1}[S_1][T_2]/K^{zt}_{T_2}\right)\right\}$$
$$\times\left\{1 + \left([S_1]/K^{zt}_{S_1}\right) + \left([S_2]/K^{zt}_{S_2}\right)\right.$$

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* In reference [3] zero *trans* conditions required that $[S_2] = 0$ and $[A_2] = 0$, while infinite *trans* conditions required that $[S_2] \to \infty$ and $[A_2] \to \infty$.

$$+\left([\mathrm{T}_1]/K^{\mathrm{zt}}_{\mathrm{T}_1}\right)+\left([\mathrm{T}_2]/K^{\mathrm{zt}}_{\mathrm{T}_2}\right)$$

$$+\left([\mathrm{S}_1][\mathrm{S}_2]/K^{\mathrm{zt}}_{\mathrm{S}_1}K^{\mathrm{it}}_{\mathrm{S}_2}\right)$$

$$+\left([\mathrm{T}_1][\mathrm{T}_2]/K^{\mathrm{zt}}_{\mathrm{T}_1}K^{\mathrm{it}}_{\mathrm{T}_2}\right)$$

$$+\left([\mathrm{S}_1][\mathrm{T}_2]/K^{\mathrm{it,T}}_{\mathrm{S}_1}K^{\mathrm{zt}}_{\mathrm{T}_2}\right)$$

$$+\left([\mathrm{T}_1][\mathrm{S}_2]/K^{\mathrm{it,S}}_{\mathrm{T}_1}K^{\mathrm{zt}}_{\mathrm{S}_2}\right)\Big\}^{-1} \tag{5}$$

Including a second substrate introduces 16 new kinetic constants into the general flux equations. Eight of these are zero *trans* and infinite *trans* constants for T of the type already discussed above. The remaining eight are hetero-infinite *trans* constants. In general only three of these are independent since relations R7–R11 of Table II can be proven directly from Table I (Eqn. R12 can be derived from Eqns. R7–R11). Thus the general flux equations for S and T in the presence of one another can be written in terms of 13 independent kinetic constants.

Evaluation of kinetic constants for unlabelled substrates

It can be shown directly from Eqn. 5 that for zero *trans*, infinite *trans* and hetero-infinite *trans* conditions as defined earlier but with $[\mathrm{T}_1]\neq 0$, $J_{\mathrm{S}}^{1\to 2}$ can be written as

$$J_{\mathrm{S}}^{1\to 2}=V^{\mathrm{ex}}_{\mathrm{S}_1}[\mathrm{S}_1]/\left(K^{\mathrm{ex}*}_{\mathrm{S}_1}+[\mathrm{S}_1]\right) \tag{6a}$$

where

$$K^{\mathrm{ex}*}_{\mathrm{S}_1}=K^{\mathrm{ex}}_{\mathrm{S}_1}\left(1+\left([\mathrm{T}_1]/K^{\mathrm{ex}}_{\mathrm{T}_1}\right)\right) \tag{6b}$$

and 'ex' again denotes the particular experimental condition under consideration. Eqn. 6 also holds for equilibrium exchange conditions as defined earlier but with $[\mathrm{T}_1]=[\mathrm{T}_2]e^{z_{\mathrm{T}}u}\neq 0$. This can be proven from Eqn. 5 and Table II, however, it is somewhat simpler to derive directly from Eqns. 1 and 2 using the relation $k_{12}F_{21}=k_{21}F_{12}$ which can be shown to hold when $\Delta\mu_{\mathrm{A}}=\Delta\mu_{\mathrm{S}}=\Delta\mu_{\mathrm{T}}=0$. Using Eqn. 6 the Michaelis constants for the competing substrate T can be evaluated directly from flux measurements on S. In some cases maximal velocities or ratios of maximal velocities for T may also be derived in a similar way (cf. Eqns. R7, R8, R11 and R12 of Table II). These relationships can be used as further tests of the model or alternatively a means of studying the behaviour of substrates which are not readily available in labelled form.

Discussion

It has been demonstrated here that the general flux equation for the rapid equilibrium carrier model of cotransport and its submodels (cf. Ref. 3) can be written entirely in terms of zero *trans* and infinite *trans* experimental kinetic constants. In addition, relationships between these constants have been derived. These results provide stringent tests (or equivalently rejection criteria) for the model. For example, if zero *trans*, infinite *trans* and equilibrium exchange experiments are carried out in the absence of competing substrates and with $\Delta\mu_{\mathrm{A}}=0$, a total of ten experimental kinetic constants are determined. According to the model, however, only four of these constants are independent of one another. Thus the remaining six constants may be used along with the results given in Table II as tests of the model. Kinetic measurements in the presence of competing substrates yield additional relationships which may also be used as rejection criteria (cf. Table II). Alternatively, these relationships can be employed to determine kinetic constants for competitive substrates not available in labelled form.

Results similar to those presented here have been derived for the simple carrier model [1,2]. In the case of coupled transport systems, however, one has the added complications of the presence of cotransported activator and the possibility of two modes of translocation (via $[\mathrm{CS}_n]$ or $[\mathrm{CAS}_n]$). Thus the generalization of these earlier studies to cotransport models is far from obvious. In fact the kinetic constants for zero *trans* and infinite *trans* experiments as previously defined* for cotransport studies do not yield simple expressions of the type derived here for the general flux equations or for rejection criteria. These previous definitions do, however, result in significantly simpler expressions for $V_{\max}$ and K_{m} as functions of activator

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* See footnote on p. 448.

concentration (cf. Table I and Ref. 3). Thus they provide a much more convenient basis for kinetic tests of the type discussed in Ref. 3.

Acknowledgements

I would like to thank Drs. Clifford Patlak and Maurice Burg for reading and commenting on the manuscript.

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