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Transport across Biological Membranes: A Rigorous Test for the Carrier Hypothesis

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Summary. This paper presents several equations derived from an asymmetrical nonconcentrative carrier mechanism for biological transport. These equations inter-relate the kinetic constants derived from different types of kinetic experiments, and act as criteria for the validity of the mechanism. Because they require a precise numeral relationship between rate constants, these equations can act as efficient discriminators for transport systems which are not mediated by a simple carrier mechanism, while they can provide presumptive evidence for a carrier mechanism when the observed kinetic constants do fulfill the predictions.

Transport across biological membranes has frequently been interpreted by postulating a membrane-bound carrier capable of transporting the substrate across the membrane. It is not necessary to propose a molecular mechanism for this carrier to account for the kinetics of biological transport processes. The most searching test of this carrier hypothesis has been its application to the nonconcentrative transport of glucose and amino-acids across the membranes of mammalian erythrocytes (reviewed by Stein, 1967; Miller, 1969; and Lieb & Stein, 1972). The original mechanisms were simplified by a number of assumptions before they were used to interpret kinetic data, but more recently there has been interest in the practical applications of more general versions of the carrier hypothesis, in which the carrier reorientation rate is affected by bound substrate (Levine, Oxender & Stein, 1965; Levine & Stein, 1966; Hoare, 1972*a*) and in which the kinetic properties of the carrier are not identical for inward and outward fluxes across the membrane (Geck, 1971; Miller, 1971; Schultz, 1971; Hoare, 1972*a*; Kalsow & Doyle, 1972; Leib & Stein, 1972).

Two questions must be answered about the generalized version of this carrier mechanism. Is it consistent with the available kinetic data, and, to what extent does the kinetic data provide a proof of the validity of the car-

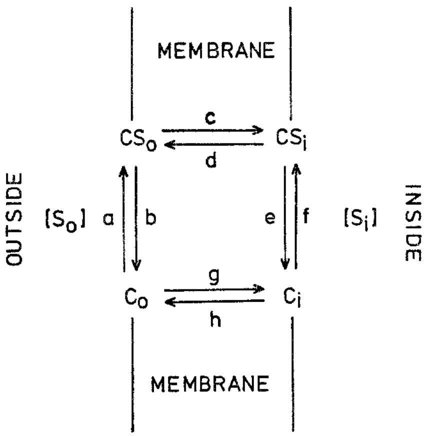

Fig. 1. The general carrier mechanism considered in this paper. The carrier is assumed to be nonconcentrative and the substrate uncharged, so that $aceh = bdfg$ (Britton, 1966)

rier mechanism? These questions have been tackled by Lieb and Stein, who have summarized their work in a recent review (Lieb & Stein, 1972). They developed the original kinetic analyses of Regen and Morgan (1964), Jacquez (1964) and Britton (1966) and derive two algebraic expressions, composed of measurable kinetic constants, whose values cannot both exceed two if the carrier mechanism is correct. They have also presented kinetic data on glucose transport in human erythrocytes, which shows, on the basis of this criterion, that glucose transport in human erythrocytes cannot be mediated by a simple carrier mechanism (as defined in Fig. 1).

This paper presents a series of equations inter-relating the kinetic constants derived from various types of kinetic experiments; they are based on the same analyses by Regen and Morgan (1964), Jacquez (1964) and Britton (1966) and use some of the same expressions derived by Lieb and Stein (1972). These equations provide a clear picture of the kinetic behavior of a carrier system, and allow the kinetic constants of some types of experiments to be predicted from those of others. Because of this predictive element, these equations provide several additional rigorous criteria with which the kinetics of any carrier-mediated transport must conform, and these may prove to be very valuable in transport systems other than glucose in human erythrocytes, where the Lieb and Stein criterion alone may not be adequate[1].

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[1] I am much indebted to the referees, who pointed out that the relationships derived in this paper are valid for the general carrier mechanism in Fig. 1, without any restrictions on the relative magnitudes of the rate constants. My original derivation had been for a carrier system for which the carrier reorientation steps are rate-determining.

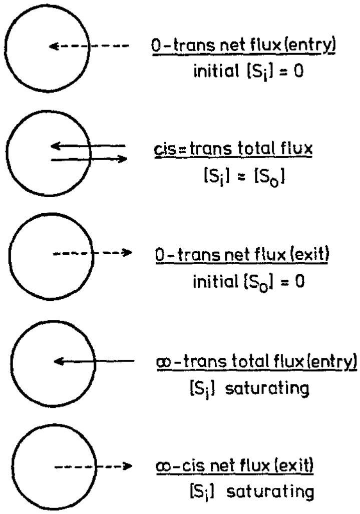

Fig. 2. The types of kinetic experiments discussed in this paper. The arrows give the direction of measured fluxes (solid arrows — total flux; broken arrows — net flux). Entry and exit fluxes are equal in the "*cis* = *trans* total flux" experiment

A preliminary account of part of this work was communicated to the Biochemical Society (Hoare, 1972*c*). The equation in that reference is incorrectly printed; the correct version is Eq. (8) of this paper.

Nomenclature

Rate constants: a, b, c ... h. The nomenclature used by Britton (1966) and Lieb and Stein (1972), defined in Fig. 1.

Types of experiments: The nomenclature is intended to be descriptive and is based on that used by Lieb and Stein (1972). The measured flux is directed *cis* → *trans*; exit or entry define the flux direction with respect to the cell. 0-*trans*, ∞-*cis*, *cis* = *trans* give information about substrate concentrations at the start of the experiment. The list below relates these terms to those used by other authors. The experiments are illustrated in Fig. 2.

0-*trans* net flux (exit): "net loss" (Miller, 1971); "exit" (Hoare, 1972*a*)

0-*trans* net flux (entry): "entry" (Hoare, 1972*a*)

cis = *trans* total flux (entry): "type IV", "exchange" (Miller, 1968; Hoare, 1972*a*)

∞-*cis* net flux (exit): "Sen-Widdas" (Sen & Widdas, 1962); "type I" (Miller, 1968)

∞-*trans* total flux (entry): "counter transport", "type V" (Miller, 1968).

Miscellaneous: Subscripts i and o = in and out, respectively; S = substrate; $\{C\}$ = total concentration of carrier in membrane; V and K = are constants from the Michaelis-Menten equation, $flux = V.\,[S]/(K+[S])$.

Kinetic Deriviations

An important feature of kinetic studies of nonelectrolyte transport in erythrocytes is that a wide range of different types of experiments are possible. They can differ in the type of flux measured (net or total), the direction of the flux (entry to or exit from the erythrocyte), and in the presence of zero or (effectively) infinite substrate concentrations at the *cis* or *trans* surfaces of the membrane. (The measured flux is defined as going from the *cis* to the *trans* surface.) Fig. 2 illustrates the types of experiments discussed in this paper, and the nomenclature.

Britton (1966) has derived a general equation for carrier-mediated flux across a membrane [Eq. (1) in his paper] from which Eqs. (1) and (2) giving total and net flux can be derived. (Eq. (2) corresponds to Eq. (A.1) of Lieb & Stein, 1972.) Eq. (2) is valid only for a nonconcentrative carrier system, but this restriction does not apply to Eq. (1).

$$\text{Total inward flux} = \frac{\{h(e(b+c)+bd)+bdf[S_i]\}\left\{\dfrac{ace}{e(b+c)+bd}\right\}[S_o]\,\{C\}}{F}. \tag{1}$$

$$\text{Net inward flux} = \frac{aceh([S_o]-[S_i])\,\{C\}}{F} \tag{2}$$

where

$$\begin{aligned} F=&(h+g)(e(b+c)+bd)+a(h(d+e)+c(e+h))[S_o]\\ &+f(g(b+c)+d(b+g))[S_i]+af(c+d)[S_o][S_i]. \end{aligned}$$

These equations may be further simplified for each particular experimental situation by substituting zero or infinite substrate concentrations at the cis or trans surface, as appropriate. The simplified equations show that the carrier-mediated flux is related to the experimentally varied substrate concentration by a simple Michaelis-Menten relationship ($flux=V\cdot[S]/(K+[S])$), and they yield expressions showing how the experimentally accessible kinetic constants, V and K, are related to the rate constants of the carrier. Lieb and Stein (1972) give a table (Table 2 of their paper) giving these expressions for a number of important types of experiments. The only additional expressions required are for the "∞-*trans* total flux (entry)" experiment ("counter-transport"); simplification of Eq. (1) yields

$$V=\frac{bcde\{C\}}{(c+d)(e(b+c)+bd)} \tag{3}$$

$$K=\frac{g(b+c)+d(b+g)}{a(c+d)}. \tag{4}$$

The equations derived above are only valid for a nonconcentrative carrier. This condition is implicit in the relationship $aceh = bdfg$ which was used in deriving Eq. (2) (Britton, 1966), and must therefore apply to any relationship derived from Eq. (2). This means that the *cis = trans* total flux experiments are made at thermodynamic equilibrium; the direction of the flux need not be specified since entry and exit fluxes must be equal.

∞-*cis and* ∞-*trans Experiments*

In 1962, Sen and Widdas introduced the "∞-*cis* net flux (exit)" experiment. They measured, by a photometric technique, the net efflux of glucose from erythrocytes preloaded with a saturating concentration of glucose, and noted that it was inhibited by extracellular glucose, so that *measured flux* = $V/(K+[S_0])$. It is the particularly small value of K observed in this experiment which has been the major, and perhaps fatal, challenge to the simple carrier theory for glucose transport.

An "∞-*trans* total flux (entry)" experiment ("counter flux") involves measurement of the total inward flux under exactly the same limiting conditions as Sen and Widdas' experiments. Simplification of Eq. (1) for these conditions gives the expressions for V and K given in Eqs. (3) and (4). Comparison of these expressions with those derived by Lieb and Stein (1972) show that the carrier mechanism predicts that

$$\underset{\text{"counter transport"}}{V[\infty\text{-}\mathit{trans}\ \text{total flux (entry)}]} = \underset{\text{"exchange"}}{V[\mathit{cis}=\mathit{trans}\ \text{total flux}]} \tag{5}$$

$$\underset{\text{"counter transport"}}{K[\infty\text{-}\mathit{trans}\ \text{total flux(entry)}]} = \underset{\text{"Sen-Widdas"}}{K[\infty\text{-}\mathit{cis}\ \text{net flux (exit)}]}. \tag{6}$$

A second equation can be derived from Eq. (5) or (6) by substituting "entry" for "exit" and vice versa.

Although the time course of counter-transport experiments have been followed for glucose in human erythrocytes (Miller, 1968), the data is not sufficient as yet to yield values of V or K, and so Eqs. (5) and (6) cannot yet be tested for this system.

Carrier-Mediated Transport at Very Low Substrate Concentrations

At very low substrate concentrations ($[S] \ll K$), the Michaelis-Menten relationship between flux and substrate concentration reduces to *flux* = $V \cdot [S]/K$ and so, if the model in Fig. 1 is valid, the ratio V/K can be obtained from the flux at low substrate concentrations. Using the expressions for V

and K given by Lieb and Stein (1972), one can readily show that

$$\begin{aligned} V/K[0\text{-}trans \text{ net flux (entry)}] &= V/K[0\text{-}trans \text{ net flux (exit)}] \\ &= V/K[cis = trans \text{ total flux}]. \end{aligned} \tag{7}$$

Miller's (1971) data on glucose transport in human erythrocytes shows that this relationship does not hold in this system, since V/K for "0-*trans* net flux (exit)" and "*cis* = *trans* total flux" are $15.2 \pm 1.8 \text{ min}^{-1}$ and $6.8 \pm 0.5 \text{ min}^{-1}$, respectively. Karlish, Lieb, Ram and Stein's data (1972) yield a value of 5.6 min^{-1} for the "0-*trans* net flux (exit)" experiment. It is therefore not clear as yet whether glucose transport conforms to Eq. (7) or not. However, Hoare (1972*b*) has shown that L-leucine transport in human erythrocytes conforms to Eq. (7) over a wide temperature range for "0-*trans* net flux (exit)", "0-*trans* net flux (entry)" and "*cis* = *trans* total flux" experiments.

∞-*cis*, 0-*trans* and *cis* = *trans* Experiments

A further useful relationship can be derived between the parameters for "∞-*cis* net flux" (Sen-Widdas), "0-*trans* net flux" and "*cis* = *trans* total flux" (exchange) experiments. Using Lieb and Stein's (1972) expressions for V and K, one can readily show that

$$\begin{aligned} \frac{K[cis = trans \text{ total flux}]}{K[\infty\text{-}cis \text{ net flux (exit)}]} &= 1 + \frac{V[0\text{-}trans \text{ net flux (exit)}]}{V[0\text{-}trans \text{ net flux (entry)}]} \\ &\quad - \frac{V[0\text{-}trans \text{ net flux (exit)}]}{V[cis = trans \text{ total flux}]}. \end{aligned} \tag{8}$$

An equivalent equation can be made by substituting "entry" for "exit" and vice versa throughout.

There is as yet insufficient published data to test the validity of Eq. (8) for glucose or leucine transport in human erythrocytes, but Lieb and Stein's (1972) review promises that the necessary data on glucose transport will soon be available.

Discussion

The equations inter-relating the kinetic constants for carrier-mediated transport fall into two classes: those which must be true for any transport mechanism, and those which are specific predictions of the carrier mechanism in Fig. 1.

Under certain limiting conditions, various types of experiments become identical, and their fluxes should also be identical. For example, as $[S] \to \infty$, the "∞-*trans* total flux" and the "*cis* = *trans* total flux" experiments become identical, and therefore V for these two experiments should be identical [Eq. (5)]. Similarly, as $[S] \rightarrow 0$, the "0-*trans* net flux (entry)" and the "*cis* = *trans* total flux" experiments become identical, and similarly for the "0-*trans* net flux (exit)", so that the fluxes for these three types of experiments should be equal and, on the basis of the model in Fig. 1, should be given by Eq. (7). It is reassuring that the transport of L-leucine in human erythrocytes conforms to Eq. (7) (Hoare, 1972*b*), even through some disagreement remains about glucose transport (Miller, 1971; Karlish *et al.*, 1972).

Eqs. (6), (7) and (8) are specific predictions of the carrier mechanism in Fig. 1, and certainly need not be true for any carrier mechanism. It is unfortunate that as yet there is not sufficient experimental data to test these three equations, but work is progressing on the transport of glucose (Lieb & Stein, 1972) and leucine (Hoare, *unpublished results*) which will make this possible.

Eq. (5) provides a useful check on the validity of kinetic data and analysis, since any transport process should conform to these equations.

Eqs. (6), (7) and (8), and Lieb and Stein's (1972) criterion, are direct predictions which can be used to check the validity of the carrier mechanism. A transport process which does not fulfill these predictions cannot be mediated solely by a single carrier mechanism as shown in Fig. 1. If the kinetic data does fulfill all these predictions, it still remains to devise rejection criteria for other possible mechanisms, and one can never exhaust all other possible mechanisms. However, if a transport process meets the predictions of the carrier model, then the more detailed and explicit these predictions are, the stronger the presumptive case for the carrier model becomes. For this reason, Eqs. (6), (7) and (8) should be useful tools in further investigations of carrier mechanisms.

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