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TWO-CARRIER MODELS FOR MEDIATED TRANSPORT

I. THEORETICAL ANALYSIS OF SEVERAL TWO-CARRIER MODELS

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SUMMARY

Several possible models of two sequential and two simultaneous carriers of different affinities are theoretically analysed. Following the analysis we suggest for each model an experimental procedure capable of testing and rejecting the model.

It has become clear that the models which are required to account for membrane transport are more complex than those considered up to a few years ago [1]. To facilitate research on transport kinetics this paper treats a number of transport models in which two carriers serve to transport the substrate. We present a general analysis of transport systems composed of two types of independent carriers with different affinities. The number of the individual carriers of each type per unit membrane area is not necessarily equal and both carriers may be sequential or simultaneous. For each of the possible two-carrier models we calculated the predicted fluxes for different procedures and suggest an experimental procedure capable of testing each model.

Models of two carriers are the simplest hypothesis to be tested when more than one transport site of different affinity is detected on the same face of the membrane. An example for such kinetics is provided by the sugar transport system in red cells as shown below.

Sugar transport system in red blood cells – an example for a system with two affinity sites

The terminology used in this paper, given in Table I, follows Stein and Lieb [2]. Tables II and III summarize the kinetic parameters of glucose and galactose transport across the erythrocyte membrane. It can be seen from the Tables that two classes of K_m values can be recognized for each sugar. For glucose, K_m values between 20 and 38 mM can be considered as corresponding to a low affinity site (L), those between 1.6 and 3.0 mM as corresponding to a high affinity site (H). The respective values for galactose are 100–240 mM (L) and 11–25 mM (H). In Table IV the K_m values are classified accordingly and it can be seen that the data for glucose are consistent with

TABLE I

DEFINITIONS AND TERMINOLOGY

Type of experiment	Internal concentration	External concentration
Equilibrium exchange	Varied*	Varied
	Varied	Varied*
Zero trans		
ZT 1 → 2	Varied*	Zero
ZT 2 → 1	Zero	Varied*
Infinite trans		
IT 1 → 2	Varied*	Limitingly high
IT 2 → 1	Limitingly high	Varied*
Infinite cis		
IC 1 → 2	Limitingly high*	Varied*
IC 2 → 1	Varied*	Limitingly high*

* Labelled substrate.

TABLE II

KINETIC PARAMETERS OF GLUCOSE TRANSPORT ACROSS THE ERYTHROCYTE MEMBRANE AT 20 °C AND pH 7.4

Type of experiment	K_m (± S.E.) (mM)	V (± S.E.) (mmol/min·l cell water)	Reference
Exchange	38 ± 3	260 ± 30	Miller [3]
	32 ± 1	357 ± 10	Eilam and Stein [4]
	20 ± 1	264 ± 42	Lacko et al. [5]
	14	300	Edwards [6]
IC 1 → 2	1.7	83	Sen and Widdas [7]
	1.86	210	Harris [8]
	1.8 ± 0.3	104 ± 12	Miller [3]
ZT 1 → 2	25 ± 3	139 ± 11	Karlish et al. [9]
IC 2 → 1	2.8 ± 0.6	85 ± 26	Hankin et al. [10]
ZT 2 → 1	1.6 ± 0.2	36 ± 1.2	Lacko et al. [5]
IT 2 → 1	2.0, 1.7 ± 0.3	174 ± 3	Lacko et al. [5]

those of galactose, with the exception of the parameters for zero trans (2 → 1). Only the high affinity site was observed for glucose (Table II). However, the relevant experiments were carried out at a low range of glucose concentrations (0.1–18 mM) [5] and the low affinity site may not be detected in this range. The two affinity sites were observed in the following procedures: For the two substrates, at the inner face of the membrane, a high-affinity site was observed in the infinite cis (2 → 1) procedure (refs. 10 and 11, Tables II and III) and a low-affinity site in the zero trans (1 → 2) procedure (refs. 9 and 11, Tables II and III). On the outer face of the membrane both low and high affinity sites were observed in the zero trans (2 → 1) procedure for galactose (ref. 13, Table III).

TABLE III

KINETIC PARAMETERS OF GALACTOSE TRANSPORT ACROSS THE ERYTHROCYTE MEMBRANE AT 20 C, pH 7.4

Type of experiment	K_m (± S.E.) (mM)	V (± S.E.) (mmol/min/l cell water)	Reference
Exchange	138 ± 57	432 ± 44	Ginsburg and Ram [11]
IC 1 - 2	12		Krupka [12]
ZT 1 - 2	240 ± 57	255 ± 96	Ginsburg and Ram [11]
IC 2 - 1	21; 25* ± 17		Ginsburg and Stein [13]
ZT 2 - 1	(11** ± 6 286 ± 282	16 ± 6 21 ± 16)	Ginsburg and Stein [13]
IT 2 - 1	21 ± 2	239 ± 11	Ginsburg and Stein [13]

* Values obtained by different methods.

** Two sites detected by a non-linear least-square fitting procedure.

TABLE IV

OBSERVED K_m VALUES FOR GLUCOSE AND GALACTOSE, CLASSIFIED ACCORDING TO LOW (*L*) AND HIGH (*H*) AFFINITY SITES (SEE TEXT)

Type of experiment	K_m determined on side	Observed K_m values	
		Glucose	Galactose
Equilibrium exchange		*L*	*L*
ZT 1 - 2	inner	*L*	*L*
ZT 2 - 1	outer	*H*	*H* + *L*
IC 1 - 2	outer	*H*	*H*
IC 2 - 1	inner	*H*	*H*
IT 1 - 2	inner		
IT 2 - 1	outer	*H*	*H*

In the following we present a general theoretical analysis of several possible two carrier models. The results and the analysis may be applied to the sugar transfer system in red blood cells or to any other system showing similarly two affinity sites.

Models of two carriers

In considering carrier models at all, it is necessary first to distinguish between sequential and simultaneous carriers:

A. A sequential carrier possesses one (simple or complex) transport site per carrier, available to the substrate alternately at each face of the membrane. Such a carrier has been often described as a mobile carrier, forming a complex with the substrate molecule and facilitating its diffusion across the membrane.

B. A simultaneous carrier possesses two or more transport sites per carrier, available to the substrate simultaneously at both faces of the membrane. Such a system may be, for instance, a protein spanning the membrane from face to face and composed of one or more subunits. The transport event requires a cycle of some conformational changes in the protein.

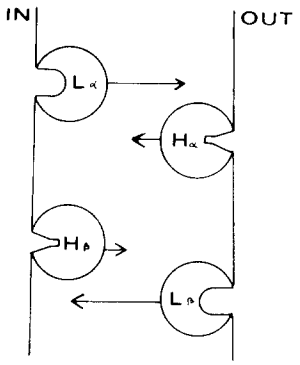

Fig. 1. A model of antiparallel sequential carriers. The carriers face alternately the inner and the outer sides of the membrane. L and H are the low- and high-affinity sites. The arrows represent differences in the maximum velocities.

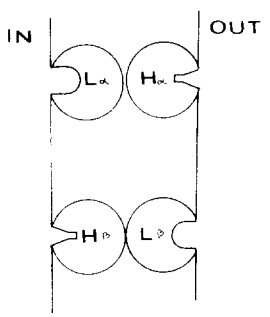

Fig. 2. One of the possible models for antiparallel simultaneous carriers. The binding sites face alternately the faces of the membrane and the internal cavity. L and H are as in Fig 1.

A two-carrier system may be obviously composed of sequential or simultaneous carriers. For all two-carrier models we denote one carrier by α and the second by β. The number of α units is defined to be equal or greater than the number of β units. One may distinguish between the following possible models:

I. Antiparallel carriers: The two carriers are each asymmetric, each having a low affinity site, of the value L, on one face of the membrane and a high affinity site, of the value H, on the other. The carriers are situated in an antiparallel fashion across the membrane. (See Fig. 1 for sequential and Fig. 2 for simultaneous antiparallel carriers. In both figures $L_\alpha = L_\beta$ and $H_\alpha = H_\beta$).

II. Different carriers: The carriers are different in all their parameters. (In Figs. 1 and 2 $L_\alpha \neq L_\beta$ and $H_\alpha \neq H_\beta$).

Model I is clearly a special case of model II.

In the following we shall analyse each of these two-carrier models and obtain the predicted flux for the different procedures (Table I), with applications to the sugar transport system in human red blood cells.

A. Sequential carriers

General considerations. To analyse the models of sequential carriers we use the

flux equation derived by Stein and Lieb [2]. The equation (Eqn 1 below) describes the unindirectional flux of the substrate from side 1 to side 2 of the membrane. The equation is based upon the King and Altman [14] and Britton [15] analyses applied to a sequential carrier model, and is free from any pre-assumptions as to the rate limiting step in transport. Thus, for a single carrier:

$$\frac{U_{12}}{T} = \frac{KS_1 + S_1 S_2}{K^2 R_{00} + KR_{12} S_1 + KR_{21} S_2 + R_{ee} S_1 S_2} \tag{1}$$

S_1 and S_2 are the substrate concentrations at side 1 (we define it as the inner face) and side 2 (outer face) respectively. K and the terms in R are each a combination of several rate constants. Stein and Lieb [2] showed that R_{ee}, R_{12} and R_{21} correspond to the resistance afforded by the membrane to a complete cycle of movement of the carrier in an equilibrium exchange, zero trans ($1 \rightarrow 2$), and zero trans ($2 \rightarrow 1$) procedure respectively, while R_{00} can be considered as the relevant resistance for the free carrier. T is a factor proportional to the number of carriers, which takes into account the unit in which the flux is measured. K corresponds to the dissociation constant for the carrier-substrate complex.

A. I. Antiparallel sequential carriers

We first consider a model of antiparallel sequential carriers (Fig. 1). Since these carriers are antiparallel, K, R_{00} and R_{ee} (parameters which do not depend on the side for which measurements are made) are each the same for both of these carriers, while R_{12} of α (R^{α}_{12}) equals R_{21} of β (R^{β}_{21}) and similarly $R^{\alpha}_{21} = R^{\beta}_{12}$. We take the case where $R_{21} > R_{12}$ and define $R_{21}/R_{12} = m$. The ratio of the number of carriers of α type to that of β type is n. When $n \neq 1$ the resulting system is asymmetric.

The undirectional flux equation for the antiparallel sequential carriers is obtained by applying the above considerations to Eq. 1 and adding the fluxes of α and of β carriers, thus:

$$\frac{U_{12}}{T} = \frac{KS_1 + S_1 S_2}{K^2 R_{00} + KR_{12} S_1 + KR_{21} S_2 + R_{ee} S_1 S_2} + \frac{KS_1 + S_1 S_2}{n(K^2 R_{00} + KR_{21} S_1 + KR_{12} S_2 + R_{ee} S_1 S_2)} \tag{2}$$

The flux in the direction 2 to 1 can be obtained by interchanging the symbols 1 and 2.

From Eq. 2 we can obtain the zero trans ($1 \rightarrow 2$) flux by setting $S_2 = 0$, and setting $m = R_{21}/R_{12}$.

$$\frac{U^{ZT}_{1\rightarrow 2}}{T} = \frac{S_1}{KR_{00} + R_{12} S_1} + \frac{S_1}{nKR_{00} + n \cdot m \cdot R_{12} S_1} \tag{3}$$

By rearranging the equation we obtain

$$\frac{U^{ZT}_{1\rightarrow 2}}{T} = \frac{\frac{1}{R_{12}} S_1}{\frac{KR_{00}}{R_{12}} + S_1} + \frac{\frac{1}{nmR_{12}} S_1}{\frac{KR_{00}}{mR_{12}} + S_1} \tag{4}$$

Eqn 4 describes the behaviour of two systems, each of which contributes to the flux according to Michaelis-Menten kinetics. The parameters are the following:

$$K^{\alpha}_{ZT\,1\to 2}\ (K_m \text{ value for the } \alpha \text{ carrier}) \qquad \frac{KR_{00}}{R_{12}} \tag{5}$$

$$K^{\beta}_{ZT\,1\to 2}\ (K_m \text{ value for the } \beta \text{ carrier}) \qquad \frac{KR_{00}}{mR_{12}} \tag{6}$$

Since by definition $m > 1$; $K^{\alpha}_{ZT\,1\to 2} > K^{\beta}_{ZT\,1\to 2}$, hence $K^{\alpha}_{ZT\,1\to 2}$ will correspond to a low affinity site and $K^{\beta}_{ZT\,1\to 2}$ to a high affinity site.

$$V^{\alpha}_{ZT\,1\to 2}\ (V \text{ value of } \alpha \text{ carrier}) \qquad \frac{T}{R_{12}} \tag{7}$$

$$V^{\beta}_{ZT\,1\to 2}\ (V \text{ value of } \beta \text{ carrier}) \qquad \frac{T}{nmR_{12}} \tag{8}$$

Zero trans ($2 \to 1$) flux is similarly obtained from Eqn 2 by setting $S_1 = 0$

$$\frac{U^{ZT}_{2\to 1}}{T} = \frac{S_2}{KR_{00}+mR_{12}S_2} + \frac{S_2}{nKR_{00}+nR_{12}S_2} \tag{9}$$

By rearranging:

$$\frac{U^{ZT}_{2\to 1}}{T} = \frac{\frac{1}{mR_{12}}S_2}{\frac{KR_{00}}{mR_{12}}+S_2} + \frac{\frac{1}{nR_{12}}S_2}{\frac{KR_{00}}{R_{12}}+S_2} \tag{10}$$

$$K^{\alpha}_{ZT\,2\to 1} = \frac{KR_{00}}{mR_{12}}; \qquad V^{\alpha}_{ZT\,2\to 1} = \frac{T}{mR_{12}} \tag{11}$$

$$K^{\beta}_{ZT\,2\to 1} = \frac{KR_{00}}{R_{12}}; \qquad V^{\beta}_{ZT\,2\to 1} = \frac{T}{nR_{12}} \tag{12}$$

The zero trans procedure in the two directions provides a means to determine m and n.

From Eqns 5 and 11:

$$m = \frac{K^{\alpha}_{ZT\,1\to 2}}{K^{\alpha}_{ZT\,2\to 1}} \tag{13}$$

From Eqns 7 and 12

$$n = \frac{V^{\alpha}_{ZT\,1\to 2}}{V^{\beta}_{ZT\,2\to 1}} \tag{14}$$

An alternative method to determine n if m is known is by measuring the total maximal rate of zero trans flux in the two directions. This method avoids having to separate the α and β components of the total flux. Thus:

$$\frac{J_{ZT\,1\to2}^{\alpha+\beta}}{J_{ZT\,2\to1}^{\alpha+\beta}} = \frac{\dfrac{1}{R_{12}} + \dfrac{1}{R_{12}nm}}{\dfrac{1}{mR_{12}} + \dfrac{1}{nR_{12}}} = \frac{nm+1}{n+m} \tag{15}$$

The value of n, being the ratio of the number of α and β carriers, is independent of the substrate. Therefore equal values of n for different substrates sharing the same transport system are predicted.

By definition the value of m can be considered a measure of the asymmetry of each carrier, while the value of n measures the asymmetry of the whole system. The possibility of detecting two affinity sites in the zero trans procedures on the two directions depends on the relative values of m and n, thus on the asymmetry of the carriers and the system. For zero trans ($1 \to 2$)

$$\frac{J_{ZT\,1\to2}^{\alpha}}{J_{ZT\,1\to2}^{\beta}} = n \cdot m \quad \text{(from Eqns 7 and 8)}$$

Therefore, in an asymmetric carrier and/or asymmetric system (m and/or n being large) only the low affinity site of the α carrier is detectable.

However, for zero trans ($2 \to 1$)

$$\frac{J_{ZT\,2\to1}^{\alpha}}{J_{ZT\,2\to1}^{\beta}} = \frac{n}{m} \quad \text{(from eqns 11 and 12)}$$

Therefore, two sites would be detected by this procedure provided that m and n are of comparable values, regardless of their magnitudes.

Interestingly, it was shown that the galactose transport in human red blood cells displays a similar kinetics; a low affinity site was observed in zero trans ($1 \to 2$) procedure [11] and both low and high affinity sites were observed in zero trans ($2 \to 1$) procedure [13] (Table IV).

Equilibrium exchange flux is obtained by setting $S_1 = S_2 = S$ in Eqn 2 and using the equality $R_{12} + R_{21} = R_{ee} + R_{00}$ (see Stein and Lieb [2]) to simplify the equation:

$$\frac{U^{ee}}{T} = \frac{S}{KR_{00} + R_{ee}S} + \frac{S}{nKR_{00} + nR_{ee}S} = \frac{\left(\dfrac{1}{R_{ee}} + \dfrac{1}{nR_{ee}}\right)S}{\dfrac{KR_{00}}{R_{ee}} + S} \tag{16}$$

Only one K_m value and hence one V value are predicted.

$$K_{ee}^{\alpha=\beta} = \frac{KR_{00}}{R_{ee}} \tag{17}$$

$$J_{ee}^{\alpha+\beta} = \frac{(n+1)T}{nR_{ee}} \tag{18}$$

The infinite trans ($1 \to 2$) flux is obtained by setting $S_2 \to \infty$

$$\frac{U_{12}^{IT}}{T} = \frac{S_1}{KmR_{12} + R_{ee}S_1} + \frac{S_1}{nKR_{12} + nR_{ee}S_1} \tag{19}$$

$$K_{IT\,1\to2}^{\alpha} = \frac{KR_{12}m}{R_{ee}}; \qquad V_{IT\,1\to2}^{\alpha} = \frac{T}{R_{ee}} \tag{20}$$

$$K_{IT\,1\to2}^{\beta} = \frac{KR_{12}}{R_{ee}} \quad \text{and} \quad V_{IT\,1\to2}^{\beta} = \frac{T}{nR_{ee}} \tag{21}$$

Similarly, the infinite trans ($2 \to 1$) flux is obtained by setting $S_1 \to \infty$

$$U_{2\to1}^{IT} = \frac{S_2}{KR_{12} + R_{ee}S_2} + \frac{S_2}{mnKR_{12} + nR_{ee}S_2} \tag{22}$$

$$K_{IT\,2\to1}^{\alpha} = \frac{KR_{12}}{R_{ee}}; \qquad V_{IT\,2\to1}^{\alpha} = \frac{T}{R_{ee}} \tag{23}$$

$$K_{IT\,2\to1}^{\beta} = \frac{KR_{12}m}{R_{ee}}; \qquad V_{IT\,2\to1}^{\beta} = \frac{T}{nR_{ee}} \tag{24}$$

For each direction of measurement a high and a low affinity site are predicted, and the ratio between the K_m values is m. There is no reason, however, for the values of the K_m obtained in the infinite trans procedure to be identical with those obtained in the zero trans procedure, since obviously $KR_{12}m/R_{ee}$ may not equal KR_{00}/R_{12} and KR_{12}/R_{ee} should not necessarily equal KR_{00}/mR_{12}.

From Eqns 20, 21, 23 and 24

$$\frac{V_{IT\,1\to2}^{\alpha}}{V_{IT\,1\to2}^{\beta}} = \frac{V_{IT\,2\to1}^{\alpha}}{V_{IT\,2\to1}^{\beta}} = n$$

For an asymmetric system, where n is large, only the transport site on α will be readily detected, and we would expect to see a low affinity site for infinite trans ($1 \to 2$) and a high affinity site for infinite trans ($2 \to 1$). In the infinite cis ($1 \to 2$) procedure, the net flow, from side 1 with a limitingly high concentration, to side 2 with a varied concentration, is measured. The maximal net flow is obtained when the concentration at side 2 equals zero and is identical with the $V_{ZT\,1\to2}$. The K_m value (the concentration reducing the net maximal flow to a half) is determined on face 2 and is equal to that for the infinite trans ($2 \to 1$).

For the infinite cis ($2 \to 1$) flow the symbols 1 and 2 in the above paragraph should be interchanged. From Eqns 20–24 and 7, 8 and 12

$$K_{IC\,1\to2}^{\alpha} = \frac{KR_{12}}{R_{ee}}; \qquad V_{IC\,1\to2}^{\alpha} = \frac{T}{R_{12}} \tag{25}$$

$$K_{IC\,1\to2}^{\beta} = \frac{KR_{12}m}{R_{ee}}; \qquad V_{IC\,1\to2}^{\beta} = \frac{T}{nmR_{12}} \tag{26}$$

$$K_{IC\,2\to1}^{\alpha} = \frac{KR_{12}m}{R_{ee}}; \qquad V_{IC\,2\to1}^{\alpha} = \frac{T}{mR_{12}} \tag{27}$$

$$K_{IC\,2\to1}^{\beta} = \frac{KR_{12}}{R_{ee}}; \qquad V_{IC\,2\to1}^{\beta} = \frac{T}{nR_{12}} \tag{28}$$

In a similar fashion to that used above for the zero trans experiments, it will be seen that for a transport system, where m and n are fairly large, a high affinity site is predicted for infinite cis ($1 \rightarrow 2$). Both a high and a low affinity site are predicted for infinite cis ($2 \rightarrow 1$), when m and n are of fairly similar values.

The antiparallel sequential carriers model can be tested, as shown in the following: (1) By measuring the kinetics of equilibrium exchange. It was shown in Eqn 17 that Michaelis-Menten type kinetics are predicted for the equilibrium exchange procedure. Any different results would reject the antiparallel sequential model. (2) When equilibrium exchange data do not reject the antiparallel sequential carriers model it may be tested further by comparing the ratio of the parameters V/K_m obtained by an equilibrium exchange procedure, and in the zero trans procedures in the two directions. The ratio of the parameters of the equilibrium exchange is obtained from Eqns 17 and 18.

$$\frac{V_{ee}}{K_{ee}} = \frac{(n+1)}{\dfrac{nR_{ee}}{KR_{00}}} = \frac{(n+1)}{nKR_{00}} \tag{29}$$
$$R_{ee}$$

The ratio of the parameters of the zero trans ($1 \rightarrow 2$) is obtained from Eqns 5, 6, 7 and 8.

$$\frac{V^{\alpha}_{ZT\,1\rightarrow 2}}{K^{\alpha}_{ZT\,1\rightarrow 2}} = \frac{T}{\dfrac{R_{12}}{KR_{00}}} = \frac{T}{KR_{00}} \tag{30}$$
$$R_{12}$$

$$\frac{V^{\beta}_{ZT\,1\rightarrow 2}}{K^{\beta}_{ZT\,1\rightarrow 2}} = \frac{1}{\dfrac{mnR_{12}}{KR_{00}}} = \frac{1}{nKR_{00}} \tag{31}$$
$$mR_{12}$$

Adding Eqns 30 and 31 we obtain

$$\frac{V^{\alpha}_{ZT\,1\rightarrow 2}}{K^{\alpha}_{ZT\,1\rightarrow 2}} + \frac{V^{\beta}_{ZT\,1\rightarrow 2}}{K^{\beta}_{ZT\,1\rightarrow 2}} = \frac{V^{\alpha}_{ZT\,1\rightarrow 2}}{K^{\alpha}_{ZT\,1\rightarrow 2}}\left(1+\frac{1}{n}\right) = \frac{(n+1)}{nKR_{00}} = \frac{V_{ee}}{K_{ee}} \tag{32}$$

In a similar fashion, the parameters for zero trans ($2 \rightarrow 1$) can be obtained from Eqns 11 and 12 and compared to Eqn 29.

$$\frac{V^{\alpha}_{ZT\,2\rightarrow 1}}{K^{\alpha}_{ZT\,2\rightarrow 1}} + \frac{V^{\beta}_{ZT\,2\rightarrow 1}}{K^{\beta}_{ZT\,2\rightarrow 1}} = \frac{V_{ee}}{K_{ee}} \tag{33}$$

Thus it is required by the model that in the zero trans procedures, the sum of the V/K_m of the α and β carriers must be equal to the V/K_m of the equilibrium exchange procedure. Since the site on β is sometimes undetectable in the zero trans ($1 \rightarrow 2$) procedure, Eqn 32 can be used to test the model, provided the value of n has been previously calculated.

A II. Different sequential carriers

We consider now a system of two different carriers, where K and the terms in R refer to the α carriers and Q and the terms in Z refer to the β carriers, respectively. We define that $K \neq Q$, $R_{00} \neq Z_{00}$ and $R_{ee} \neq Z_{ee}$. Although the expressions for the zero trans and infinite trans are difficult to interpret, the equilibrium exchange predictions provide a simple way to distinguish between the "antiparallel" and "different" two-carrier models.

It was shown previously that the antiparallel carrier model predicts one K_m value for equilibrium exchange, $K_{ee} = KR_{00}/R_{ee}$. The different carriers model predicts two-site kinetics for equilibrium exchange as follows: Since $K \neq Q$, $R_{00} \neq R_{ee}$ and $Z_{00} \neq Z_{ee}$, the relevant values for K_m for the equilibrium exchange procedure, namely KR_{00}/R_{ee} and QZ_{00}/Z_{ee} are unequal, unless accidentally

$$\frac{K}{Q} = \frac{Z_{00}}{R_{00}} \cdot \frac{R_{ee}}{Z_{ee}} \tag{34}$$

It is clear that if one-site equilibrium-exchange kinetics are found for one substrate, it does not follow that similar kinetics will be found for another substrate sharing the same transport system. Indeed, Eqn 34 may be valid for one substrate but not for another. On the other hand, if more than one substrate shows one-site equilibrium-exchange kinetics it is probable, though not certain that the different-carriers model can be rejected.

If two-site equilibrium exchange kinetics are found even for a single substrate, the antiparallel carriers model should be rejected for that transfer system.

In the accompanying paper we report the results of equilibrium exchange experiments with glucose and galactose in red blood cells, which enable us to decide between these models.

B. Simultaneous carriers

General considerations. A scheme representing a simultaneous carrier is shown in Fig. 3. S_1 and S_2 are the substrate concentrations at faces 1 and 2 of the membrane. The terms K_1, K_2, K_{21} and K_{12} are the relevant dissociation constants. k_3 and k_4 are the rates of transfer of S_1 to the other side of the membrane, when bound to the carrier. k_3 refers to that rate when S_1 is bound as a single molecule and k_4 refers to that rate when a second molecule, S_2, is bound to the same carrier at the other face of the membrane. A general analysis of the model depicted in Fig. 3 is very difficult, we simplify it by using the "equilibrium" assumption, that is, the

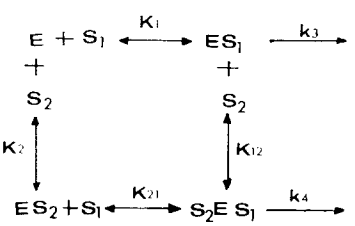

Fig. 3. A schematic representation of a simultaneous carrier. E represents the free carrier, S_1 and S_2 are the substrate concentration at side 1 and 2, respectively. See text for more details.

reactions are rapid in comparison with k_3 and k_4, and obtain the following equation for the undirectional flux.

$$\frac{U_{12}}{T} = \frac{k_3 \frac{S_1}{K_1} + k_4 \frac{S_2 S_1}{K_2 K_{21}}}{1 + \frac{S_1}{K_1} + \frac{S_2}{K_2} + \frac{S_1 S_2}{K_2 K_{21}}} \tag{35}$$

By rearranging:

$$\frac{U_{12}}{T} = \frac{S_1 K_2 K_{21} k_3 + S_1 S_2 k_4 K_1}{K_1 K_2 K_{21} + S_1 K_2 K_{21} + S_2 K_1 K_{21} + S_1} \tag{36}$$

Several models for simultaneous carriers assume that the transport units are dimers, with transport sites facing alternately the faces of the membrane and an internal cavity [16] (Fig. 2). In the following we shall compare the rate-equations derived from the dimer model with the equations obtained for the general simultaneous carrier (36).

The rate equation for a dimer with an internal cavity is derived as follows: Two components are added; one is the rate when the site on the opposite face of the membrane is empty, and one is the rate when that site is full. We assume that L is the dissociation constant of the site on face 1 and H, on face 2. To obtain the first component we multiply the probability of the site on face 1 to be full ($S_1/(S_1 + L)$); by the probability of the site on the opposite face to be empty ($H/(S_2 + H)$); by the probability that the molecule from side 1, while in the internal cavity, will bind to the site from side 2 ($L/(L + H)$); by the rate of a dimer-transition-cycle (proportional to 1).

The second component is obtained by multiplying the probability of the sites on both faces of the membrane to be full; by the probability of the molecule from side 1, while in the internal cavity together with a molecule from side 2, to bind to the site 2 ($\frac{1}{2}$); by the rate of a dimer-transition cycle (now proportional to W).

Thus, the rate equations are given by:

$$\frac{U_{12}}{T} = \frac{S_1}{L + S_1}\left(\frac{H}{H + S_2} \cdot \frac{L}{L + H} + \frac{S_2}{H + S_2} \cdot \frac{1}{2} W\right) \tag{37}$$

$$\frac{U_{21}}{T} = \frac{S_2}{H + S_2}\left(\frac{L}{L + S_1} \cdot \frac{H}{L + H} + \frac{S_1}{L + S_1} \cdot \frac{1}{2} W\right) \tag{38}$$

T is a factor proportional to the number of units.

By rearranging Eqn 37 we obtain

$$\frac{U_{12}}{T} = \frac{S_1 H L \frac{L}{L + H} + S_1 S_2 \frac{W}{2} L}{L^2 H + L^2 S_2 + S_1 H L + S_1 S_2 L} \tag{39}$$

It can be seen that Eqns 36 and 39 are identical, when we set $L = K_1 = K_{21}$, $H = K_2$, $L/(L + H) = k_3$ and $W/2 = k_4$.

Therefore it is impossible to distinguish between a model of a dimer with an

internal cavity and any other form of a simultaneous carrier, unless an occluded state of the substrate is shown biochemically.

In the following we examine a model of two antiparallel simultaneous carriers. To simplify the treatment we use the rate equation derived from the dimer model (37) and (38), which was shown to be identical to the equation derived for a general simultaneous carrier model.

B I. Simultaneous antiparallel carriers

For a model of two simultaneous antiparallel carriers we assume two units, one unit having a low affinity site at the inner face and a high affinity site at the outer face (α unit) while the sites at the second are of the same K_m values as the first, but situated in an antiparallel fashion in the membrane (β unit) (Fig. 2).

There are more α units than β units, their numerical ratio n. Due to the inequality in the number of α and β units the resulting system is asymmetric.

From Eqns 37 and 38 we derive the flux equation for the antiparallel simultaneous carriers model.

$$\frac{U_{12}}{T} = \frac{S_1 n}{L+S_1}\left(\frac{H}{H+S_2}\frac{L}{L+H} + \frac{S_2}{H+S_2}\frac{W}{2}\right) + \frac{S_1}{H+L_1}\left(\frac{L}{L+S_2}\frac{H}{L+H} + \frac{S_2}{L+S_2}\frac{W}{2}\right) \tag{40}$$

$$\frac{U_{21}}{T} = \frac{S_2 n}{H+S_2}\left(\frac{L}{L+S_1}\frac{H}{L+H} + \frac{S_1}{L+S_1}\frac{W}{2}\right) + \frac{S_2}{L+S_2}\left(\frac{H}{H+S_1}\frac{L}{L+H} + \frac{S_1}{H+S_1}\frac{W}{2}\right) \tag{41}$$

T is a factor proportional to the number of the carriers so that

$$T = V^{\beta}_{ZT\,1\to2} + V^{\beta}_{ZT\,2\to1} \quad \text{and} \quad nT = V^{\alpha}_{ZT\,1\to2} + V^{\alpha}_{ZT\,2\to1}$$

Zero trans fluxes are obtained from Eqns 37 and 38 by setting S_2 or S_1 0

$$\frac{U^{ZT}_{1\to2}}{T} = \frac{S_1}{S_1+L}\frac{Ln}{L+H} + \frac{S_1}{S_1+H}\frac{H}{H+L} \tag{42}$$

It is clear that

$$K^{\alpha}_{ZT\,1\to2} = L, \qquad K^{\beta}_{ZT\,1\to2} = H \tag{43}$$

$$V^{\alpha}_{ZT\,1\to2} = Tn\frac{L}{L+H} \quad \text{and} \quad V^{\beta}_{ZT\,1\to2} = T\frac{H}{H+L} \tag{44}$$

$$\frac{U^{ZT}_{2\to1}}{T} = \frac{S_2}{S_2+H}\frac{nH}{H+L} + \frac{S_2}{S_2+L}\frac{L}{L+H} \tag{45}$$

$$K^{\alpha}_{ZT\,2\to1} = H, \qquad K^{\beta}_{ZT\,2\to1} = L \tag{46}$$

$$V^{\alpha}_{ZT\,2\to1} = Tn\frac{H}{H+L}, \qquad V^{\beta}_{ZT\,2\to1} = T\frac{L}{L+H} \tag{47}$$

From Eqns 43, 44, 46 and 47

$$\frac{V^{\alpha+\beta}_{ZT\,1\to2}}{V^{\alpha+\beta}_{ZT\,2\to1}} = \frac{nL+H}{nH+L} = \frac{1+nm}{n+m} \tag{48}$$

A result perfectly analogous to that obtained for the antiparallel sequential model on putting $m = L/H$.

Similarly:

$$\frac{V^{\alpha}_{ZT\,1\to2}}{V^{\beta}_{ZT\,1\to2}} = nm \quad \text{and} \quad \frac{V^{\alpha}_{ZT\,2\to1}}{V^{\beta}_{ZT\,2\to1}} = \frac{n}{m} \tag{49}$$

It is concluded therefore that the simultaneous and the sequential antiparallel models predict identical kinetics for the zero trans fluxes.

Infinite trans fluxes are obtained from Eqns 37 and 38 by setting S_2 or $S_1 \to \infty$.

$$\frac{U^{IT}_{1\to2}}{T} = \frac{S_1}{L+S_1}\frac{nW}{2} + \frac{S_1}{S_1+H}\frac{W}{2} \tag{50}$$

$$\frac{U^{IT}_{2\to1}}{T} = \frac{S_2}{H+S_2}\frac{nW}{2} + \frac{S_2}{S_2+L}\frac{W}{2} \tag{51}$$

$$K^{\alpha}_{IT\,1\to2} = L; \quad K^{\beta}_{IT\,1\to2} = H; \quad V^{\alpha}_{IT\,1\to2} = \frac{TnW}{2}; \quad V^{\beta}_{IT\,1\to2} = \frac{TW}{2} \tag{52}$$

$$K^{\alpha}_{IT\,2\to1} = H; \quad K^{\beta}_{IT\,2\to1} = L; \quad V^{\alpha}_{IT\,2\to1} = \frac{TnW}{2}; \quad V^{\beta}_{IT\,2\to1} = \frac{TW}{2} \tag{53}$$

Similarly to the antiparallel sequential carriers, when n is large, only the sites on α are detectable, since the maximum velocity on α unit in n fold that on β.

The parameters of the infinite cis procedure are related to the parameters of the infinite trans and zero trans procedures in a similar fashion as shown for the antiparallel sequential carriers.

Therefore:

$$K_{IC\,2\to1} = K_{IT\,1\to2}; \qquad K_{IC\,1\to2} = K_{IT\,2\to1} \tag{54}$$

$$V_{IC\,1\to2} = V_{ZT\,1\to2}; \qquad V_{IC\,2\to1} = V_{ZT\,2\to1} \tag{55}$$

The ratios between the maximum velocities of the zero trans procedure on α and on β units are identical for the sequential and simultaneous models, therefore the sites which are detectable by the infinite cis procedure in both models are also analogous. However, the simultaneous model predicts that the K_m values obtained in the infinite trans procedure, infinite cis procedure and zero trans procedure are identical; from the Eqns 43, 46, 52, 53 and 54

$$K^{\alpha}_{IT\,1\to2} = K^{\alpha}_{IC\,2\to1} = K^{\alpha}_{ZT\,1\to2} = K^{\beta}_{ZT\,2\to1} = L$$

$$K^{\alpha}_{IT\,2\to1} = K^{\alpha}_{IC\,1\to2} = K^{\alpha}_{ZT\,2\to1} = H$$

Equilibrium exchange flux is obtained by setting $S_1 = S_2 = S$

$$\frac{U^{cc}}{T} = \frac{Sn}{S+L}\left(\frac{L}{H+L}\frac{H}{L+H} + \frac{S}{S+H}\frac{W}{2}\right) + \frac{S}{S+H}\left(\frac{L}{L+S}\frac{H}{L+H} + \frac{S}{S+L}\frac{W}{2}\right)$$

$$= \frac{SLH(n+1)}{(S+H)(L+S)(L+H)} + \frac{S^2W(n+1)}{(S+L)(S+H)2} \tag{56}$$

The equation is not of the Michaelis-Menten type, but approaches it for $S \gg H$. The flux is then given by

$$\frac{U^{cc}_{S\gg H}}{T} = \frac{S}{S+L}\frac{W(n+1)}{2} \tag{57}$$

Fig. 4 shows the equilibrium exchange rates (S/v against S) computed according to Eqn 56. At low concentrations, the curve deviates upward from the straight line. This is expected from the S^2 term in the equation.

The equilibrium exchange procedure can be considered therefore a key experiment to decide between the different two-carrier models. The following paper describes experiments designed with this aim in view.

B II. Two different simultaneous carriers

It is very difficult to obtain any conclusive predictions for the two different simultaneous carriers model. Since the parameters of the two carriers are not related, the different combinations of possible values would yield a range of possible predictions. For the equilibrium exchange procedure we expect that the S/v against S plot would show an upward deviation due to the S^2 term in the equation as before, and a downward deviation due to the two-site kinetics. This combination may even accidentally yield a straight line, but the probability that this will happen for more than one substrate and at more than one temperature is fairly small. The model can be tested by measuring the kinetics of equilibrium exchange of several substrates sharing the same transport system at different temperatures.

The goals of the present paper were to analyse some models for a transport-

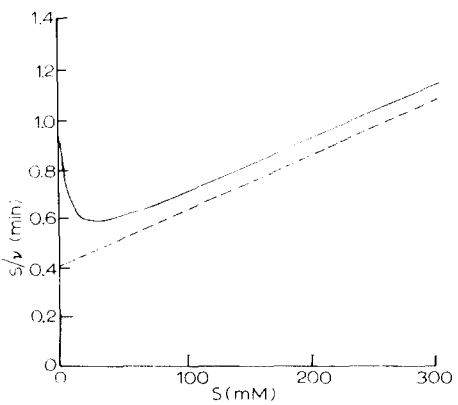

Fig. 4. The predicted values for equilibrium exchange according to the antiparallel simultaneous carriers model. The value used for computation were L = 200, H = 10, W = 4, n = 10, proportionality factor = 20. The broken line was derived by linear regression from galactose data (see the following paper).

system having two independent sites at each face of the membrane, and to point out an experimental procedure to test these models.

Several problems, as the possibility of an unstirred layer within the cell, have not been considered and will be dealt with in a forthcoming paper from our group.

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