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Coupling between Simultaneous Movements of Carrier Substrates

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Summary. Countertransport and competitive acceleration were studied experimentally in the glucose system of the red cell membrane and theoretically by analysis of kinetics. It is shown that, although the conditions for demonstration of the two phenomena differ, they are related by a symmetric interdependence of the simultaneous movements of two substrates. The symmetry can be shown by different types of kinetic treatment. Using the phenomenological equations of nonequilibrium thermodynamics for the description, the cross-coefficients are found to be equal in accordance with Onsager's law.

Among the corollaries of the concept of carrier mediation in biological transport systems, two predictions refer to the simultaneous movement of two transport substrates: countertransport and competitive acceleration. This paper deals with the relation between these two phenomena. The system is assumed to operate with one carrier, two substrates having affinity to the same site on the carrier. Conventional conditions (defined in detail below) are assumed to prevail.

Although in principle the conditions allowing observation of both phenomena are less restrictive, in the present treatment the simultaneous movement of two substrates, R and S, will be mainly considered under the specified assumptions depicted in Fig. 1. Three situations, I, II and III, are compared: In I, substrate S moves down its concentration gradient out of a cell into the surrounding medium with a certain net rate. In II, substrate R is in equilibrium (equal concentrations inside and outside). No net movement occurs. In III, situations I and II are combined. Two changes may then, under certain conditions, ensue. The net rate of movement of S may of course decrease, due to competition. Under suitable conditions, however, it may increase ("competitive acceleration"). Furthermore, R may display a net movement into the cell ("countertransport"), against an increasing

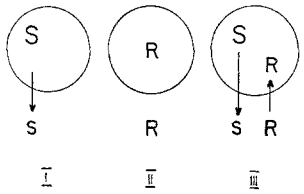

Fig. 1. Schematic situation yielding countertransport and competitive acceleration as discussed in this paper. Combination of situations I and II may, under suitable conditions, yield III. For details *see* text

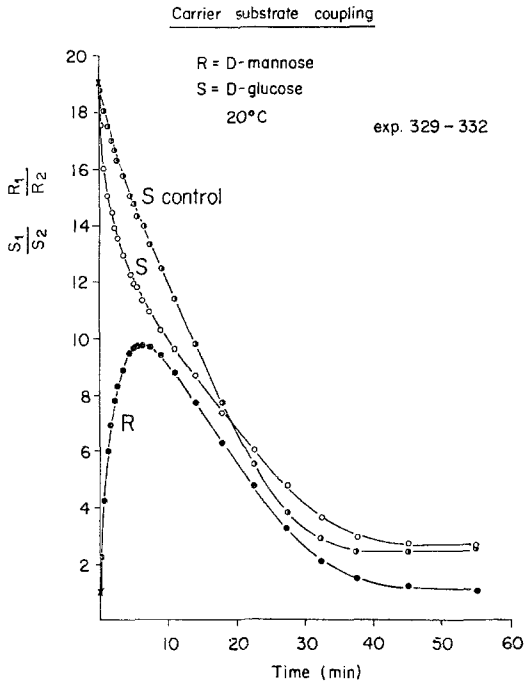

Fig. 2. Countertransport of D-mannose (initially in equilibrium), induced by exit of D-glucose, and simultaneous competitive acceleration of D-glucose in human red cells. Exit "*S* control" in the absence, "*S*" in the presence of D-mannose. Loading concentration of D-glucose 1.0 isotonic (=300 mM). Initial concentration of D-mannose 0.02 isotonic (=6 mM). *Ordinate:* concentration ratios (inside/outside) for *R* and for *S*. *Abscissa:* time. The fact that S_1/S_2 does not reach the level 1.0, is presumably due to binding of D-glucose. Means of 4 experiments. Isotope technique

concentration gradient. Fig. 2 shows experiments on sugar transport in human red cells in which both phenomena are demonstrated.

Countertransport was predicted by Widdas [8] and independently demonstrated by Park *et al.* [6] and by Rosenberg and Wilbrandt [7] in sugar transport across the red cell membrane. It has been observed in numerous other systems [11]. Since it is predicted for carrier systems in the strict sense (binding of substrate to a mobile membrane component) but not

to otherwise comparable systems with binding to fixed membrane sites [7] it is sometimes used as a criterion for carrier mediation.

The criterion is more reliable when it is positive than when it is negative. False positive criteria may be due, in the case of ions, to electrical potential differences (*see*, e.g., [2]); false negative criteria to extreme saturation conditions. Low saturation may lead to failure because countertransport becomes vanishing in extent, high saturation because it may become exceedingly slow.

The term countertransport is, today, sometimes used in a wider sense, designating any substrate flow (or change of flow rate) induced by the movement of another substrate in the opposite direction and including systems of higher complexity than that of the one carrier-one site assumption. Our considerations do not, in general, apply to such cases of countertransport in a wider sense. One example would be the effect of the potassium gradient (better: potassium flow) on substrate movement in the ternary complex interpretation of cation dependence according to Crane, first applied to intestinal absorption of sugars [1].

Competitive acceleration was derived from the kinetic rate equations [11] and demonstrated likewise in the red cell sugar transport system [9]. Similar observations are numerous in the field of amino acid transport in tumor cells [3] and bacterial permeases [5]. There was some controversy with regard to their interpretation. A discussion relating to this controversy is planned for a later communication.

In the situation presented in Fig. 1, both countertransport and competitive acceleration can be described in terms of mutual and opposite effects on flow rates. Denoting the rate of movement as positive outward and negative inward, competitive acceleration of S constitutes an increase of flow rate of S, countertransport of R (change of zero rate to a negative rate) a decrease of flow rate of R. The interdependence might be expected to be symmetrical qualitatively, or possibly quantitatively. However, neither experimental observation nor kinetic prediction seems, at first sight, to corroborate this impression. In Fig. 3 a series of glucose exit experiments in human red cells are presented in which, again in the presence of D-mannose (in equilibrium initially), both the movement of D-glucose out of the cell and the counter movement of D-mannose is followed. While the countertransport of D-mannose appears to increase with decreasing mannose concentration, the same is not true for competitive acceleration which at the lowest mannose concentrations is least pronounced.

Likewise, calculated kinetics would not appear to predict symmetry. The kinetics derived for the simultaneous movement of two substrates

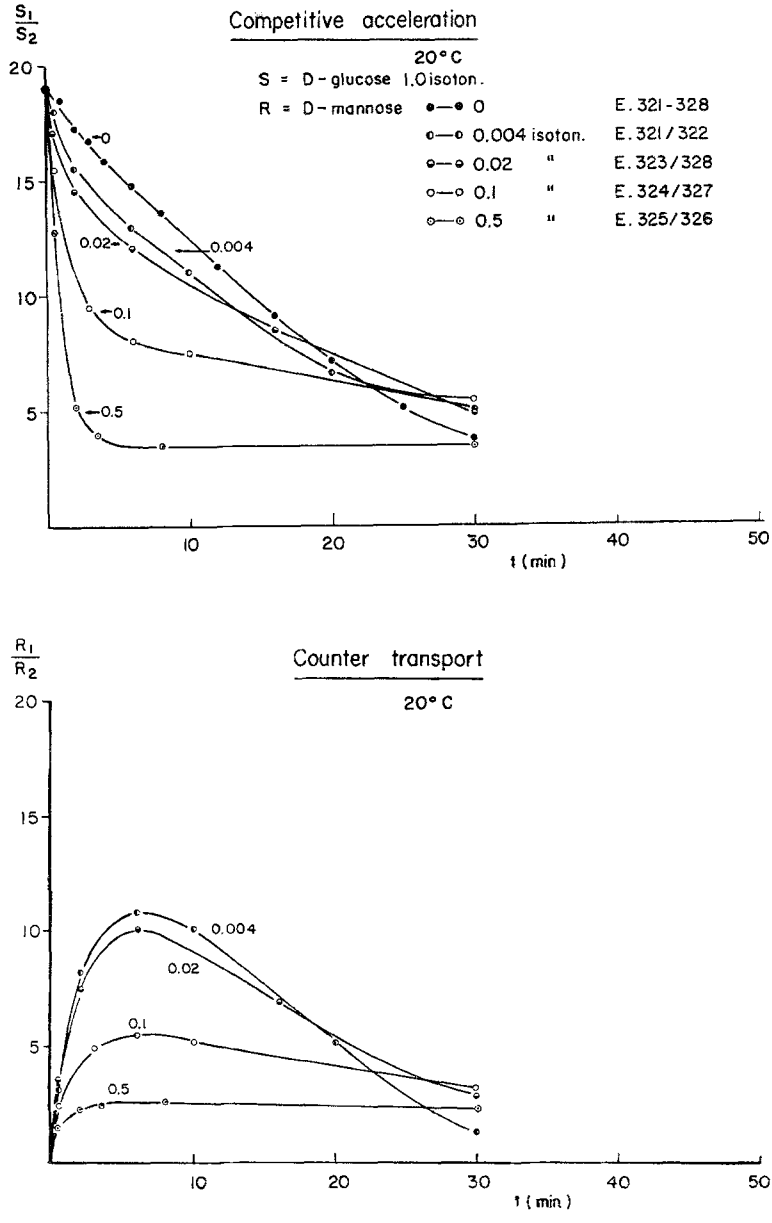

Fig. 3. Competitive acceleration of D-glucose exit and countertransport of D-mannose (initially in equilibrium) in human red cells for four initial concentrations of D-mannose (0.004, 0.02, 0.1 and 0.5 isotonic = 1.2, 6, 30, 150 mM, respectively). *Ordinate:* concentration ratios of *S* and *R* (inside/outside). *Abscissa:* time. Means of 2 experiments. Isotope technique

with affinity to the same site on the same carrier under conventional conditions (rate-limiting movement of the complex, approximate equilibrium

between substrate and carrier, equal mobilities of free and loaded carrier) yield the following relations for the transport rates in the steady state:

$$v_S = V\left(\frac{S_1'}{R_1'+S_1'+1} - \frac{S_2'}{R_2'+S_2'+1}\right)$$

and (1)

$$v_R = V\left(\frac{R_1'}{R_1'+S_1'+1} - \frac{R_2'}{R_2'+S_2'+1}\right).$$

In these equations, v denotes the rate of movement (amount passing per unit time), V the maximum rate (assumed to be equal for the two substrates), S' and R' relative concentrations of the two substrates (absolute concentrations divided by the Michaelis constant for the respective substrate) and indices 1 and 2 refer to the two sides of the membrane.

From Eq. (1), under the assumption that $R_1 = R_2 = R$ the effect of varying concentrations of R on v_S can readily be evaluated. Increasing R lowers both Michaelis terms. The net effect of v_S will be accelerating if the lowering of the second Michaelis term is larger than that of the first. The dependence of the effect on concentration and saturation of S and R can be represented graphically (Fig. 4) or numerically.

The calculated curves in Fig. 4 show essentially two features. First, that an accelerating effect of R (i.e. $Q > 1$) is limited to conditions of high saturation of the carrier with respect to S. At low saturation only inhibition is predicted. Second, even under conditions of high saturation with respect to S, only in a limited range of concentrations of R does acceleration ensue. At higher concentrations of R the acceleration turns into inhibition.

Calculation from Eq. (1) yields the result that, again under the condition $R_1 = R_2 = R$ competitive acceleration is to be expected as long as

$$R' + 1 < S_1' S_2'. \tag{2}$$

This relation implies the two features just mentioned. If $S_1' S_2' < 1$, obviously no positive concentration of R can induce competitive acceleration. This is the first feature discussed above. On the other hand, even if $S_1' S_2' > 1$, only within the range between $R' = 0$ and $R' = S_1' S_2' - 1$, R can accelerate, as is also evident from Fig. 4.

Thus, competitive acceleration is limited to restrictive conditions both with respect to carrier saturation with R and with S. The same restrictions, however, do not hold for the countertransport of R. This transport is characterized by the rate equation

$$v_R = V\frac{R'(S_2' - S_1')}{(R_1'+S_1'+1)(R_2'+S_2'+1)}. \tag{3}$$

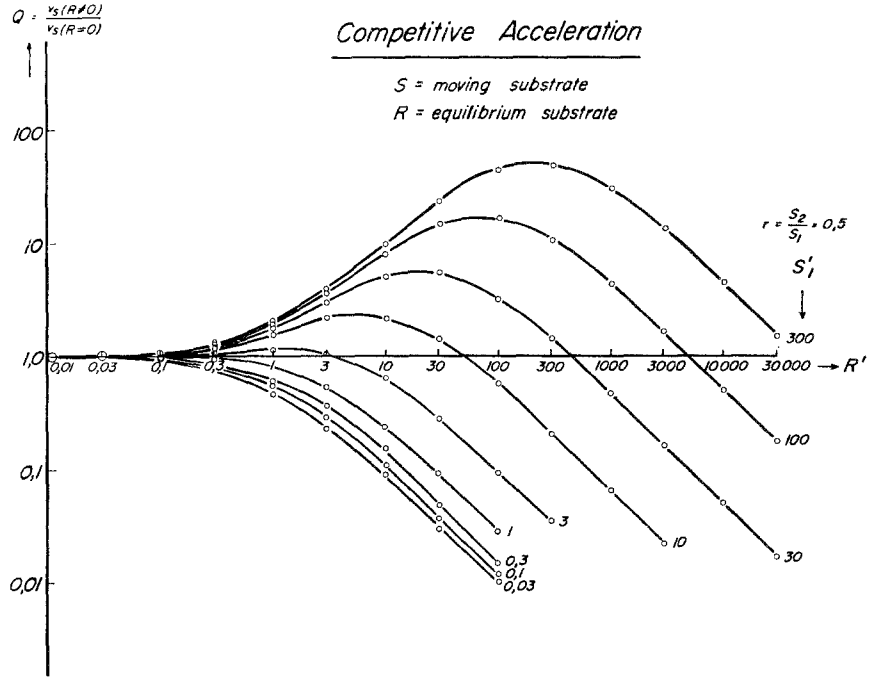

Fig. 4. Theoretical curves, calculated from Eq. (1), for competitive acceleration and inhibition of movement of substrate S by the presence of R (in equilibrium), as a function of the relative concentrations of R and of S. ($R' = R/K_{CR}$, $S' = S/K_{CS}$)

Countertransport, therefore, depends only on the condition that $S_1 > S_2$. It may, as pointed out above, be slight or slow under conditions of low or of high carrier saturation with R, but qualitatively it will always be expected, as long as $S_1 > S_2$.

Thus, the two phenomena would appear to follow different laws and, therefore, not be symmetrical. However, a closer examination reveals that the effect on carrier substrate movement of a second substrate can be decomposed into two components, one of which does show symmetry while the other does not.

Eq. (1) may be written [10] in the form

$$\begin{aligned} v_S &= V\frac{S'_1 - S'_2}{(R'_1+S'_1-1)(R'_2+S'_2+1)} + V\frac{S'_1R'_2 - S'_2R'_1}{(R'_1+S'_1+1)(R'_2+S'_2+1)}, \\ v_R &= V\frac{R'_1 - R'_2}{(R'_1+S'_1+1)(R'_2+S'_2+1)} + V\frac{R'_1S'_2 - R'_2S'_1}{(R'_1+S'_1+1)(R'_2+S'_2+1)}. \end{aligned} \tag{4}$$

In this form, the flow rates appear to be controlled by two terms A and B. The second term B is identical for the two substrates, but with opposite

sign. Thus, with respect to B there is a kind of symmetry. In both terms the numerator may be considered as a driving force, the denominator as a resistance. The driving force in A is conjugate, in B it is a coupling force. The osmotic force disappears in equilibrium ($R_1 = R_2$ or $S_1 = S_2$), for instance in the present experiments for R at time zero. The movement of R, then, is purely coupling-controlled.

The second term disappears when $S_1/S_2 = R_1/R_2$. Thus, coupling depends on a difference in concentration ratios (or in chemical potential differences).

The apparent lack of symmetry between countertransport and competitive acceleration, then, rests on the fact that the introduction of a second substrate has a double effect: it increases the resistence and simultaneously it creates coupling forces. Only with respect to coupling can symmetry be expected.

Therefore, to see the symmetry, the effect of the second substrate on the resistance must be eliminated. For v_S this can be done by comparing $v_{S(R'\neq 0)}$ not with $v_{S(R=0)}$ but with the A component of v_S; i.e., with

$$V\frac{S_1'-S_2'}{(R_1'+S_1'+1)(R_2'+S_2'+1)};$$

in other words, with a rate that is already affected by the second substrate in the resistence, but not in the driving forces. Thus, symmetry should become apparent if

$$v_R-\frac{R_1'-R_2'}{(R_1'+S_1'+1)(R_2'+S_2'+1)}\equiv \Delta'_{v_R}$$

is compared with (4a)

$$v_S-\frac{S_1'-S_2'}{(R_1'+S_1'+1)(R_2'+S_2'+1)}\equiv \Delta'_{v_S}.$$

The result of this comparison, obtained from the data in Fig. 2 is shown in Fig. 5 by a plot of Δ'_{v_R} against Δ'_{v_S}. As expected, the points lie close to a straight line with slope -1.

Thus, it appears that there is actually symmetry between the two phenomena considered here, but it is in general obscured by superimposed effects on the resistance. Therefore, in competitive inhibition, there is in general (unless $S_1/S_2 = R_1/R_2$) a hidden coupling component, which may increase or decrease the observed overall inhibitory effect depending on whether

$$\frac{S_1}{S_2}>\frac{R_1}{R_2} \quad \text{or} \quad \frac{S_1}{S_2}<\frac{R_1}{R_2}.$$

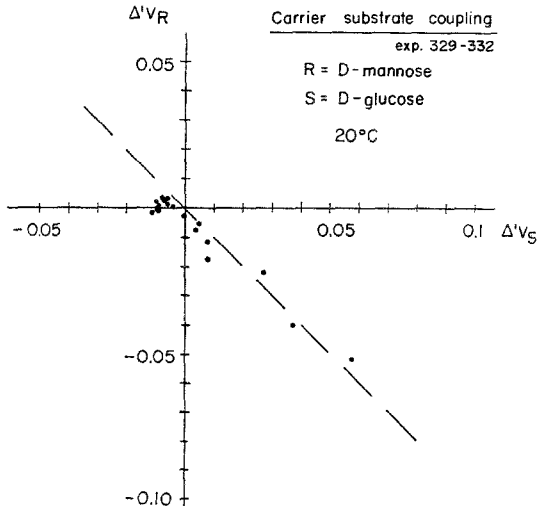

Fig. 5. The data from the experiments shown in Fig. 2, plotted according to Eqs. (4) and (4a)

It can be shown that the symmetry predicted in Eq. (4) and demonstrated in Fig. 5 is closely related to Onsager's law. This can be done by re-writing Eq. (4) in the form of the phenomenological equations in irreversible thermodynamics (4) and by demonstrating that, then, the cross-coefficients become identical.

The phenomenological equation for the flows of two substances R and S are

$$\begin{aligned} J_R &= X_R L_{RR} + X_S L_{RS} \\ J_S &= X_R L_{SR} + X_S L_{SS} \end{aligned} \tag{5}$$

with J=flow rate, X=driving force and L=coefficients (L_{RR} and L_{SS}= "straight" coefficients; L_{RS} and L_{SR}=cross-coefficients). Onsager's law states that L_{RS} and L_{SR} are equal (*see* Ref. [4]).

Eq. (4) can first be re-arranged to read

$$\begin{aligned} v_R &= \frac{V}{D_1 D_2} R_1'(1+S_1') \frac{R_1 - R_2}{R_1} + \frac{V}{D_1 D_2} R_1' S_1' \frac{S_1 - S_2}{S_1} \\ v_S &= \frac{V}{D_1 D_2} S_1'(1+R_1') \frac{S_1 - S_2}{S_1} + \frac{V}{D_1 D_2} R_1' S_1' \frac{R_1 - R_2}{R_1} \end{aligned} \tag{6}$$

with $D_1 = R_1' + S_1' + 1$ and $D_2 = R_2' + S_2' + 1$.

Considering that the osmotic driving forces for R and S [X_R and X_S in Eq. (5)] are the differences of chemical potential, $\mu R_1 - \mu R_2 = \Delta \mu_R$ and

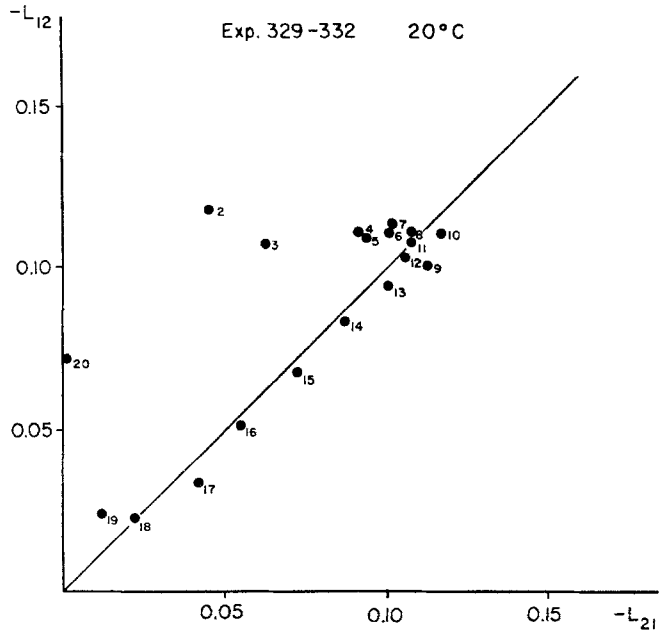

Fig. 6. The data from the experiments shown in Fig. 2, plotted according to Eqs. (8) and (8a) after correction for bound glucose (*see* legend of Fig. 2). The points are numbered according to the time sequence of the measurements. For the early measurements, equality of $L_{21}(=L_{SR})$ and $L_{12}(=L_{RS})$ cannot be expected because the system is far from equilibrium and the conditions for the use of the approximative Eq. (7) (*cf.* text there) are not fulfilled

$\mu_{S_1}-\mu_{S_2}=\Delta\mu_S$, respectively, and that near equilibrium the approximations

$$\begin{aligned}\Delta\mu_R&=\frac{R_1-R_2}{R_1}RT\\ \Delta\mu_S&=\frac{S_1-S_2}{S_1}RT\end{aligned}\tag{7}$$

can be used, the terms $\frac{R_1-R_2}{R_1}$ and $\frac{S_1-S_2}{S_1}$ can be replaced by $\frac{X_R}{RT}$ and $\frac{X_S}{RT}$, respectively.

Eq. (6) then becomes

$$\begin{aligned}v_R&=\frac{V}{RT}\frac{R_1'(1+S_1')}{D_1D_2}X_R+\frac{V}{RT}\frac{R_1'S_1'}{D_1D_2}X_S\\ v_S&=\frac{V}{RT}\frac{S_1'(1+R_1')}{D_1D_2}X_S+\frac{V}{RT}\frac{R_1'S_1'}{D_1D_2}X_R.\end{aligned}\tag{8}$$

The expressions for L_{RR} and L_{SS}, then, are $\frac{V}{RT}\frac{R_1'(1+S_1')}{D_1 D_2}$ and $\frac{V}{RT}\frac{S_1'(1+R_1')}{D_1 D_2}$, respectively. Those for L_{RS} and L_{SR} are, indeed, identical and equal to

$$-\frac{V}{RT}\frac{S_1' R_1'}{D_1 D_2}. \tag{8a}$$

Fig. 6 shows the experimental evidence for the equality of L_{RS} (denoted as L_{12}) and L_{SR} (denoted as L_{21}), from the experiments of Fig. 2.

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