UPHILL TRANSPORT INDUCED BY COUNTERFLOW

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ABSTRACT

1. In a membrane transport system containing a mobile carrier with affinities for two substrates a concentration gradient with respect to one of the substrates under certain conditions is able to induce an "uphill" transport (against the concentration gradient) of the other.
2. In a kinetic treatment quantitative conditions for such a "flow-induced uphill transport" and some of its characteristics are derived.
3. Experimentally the uphill transport of labelled glucose induced by a concentration gradient for mannose or unlabelled glucose is demonstrated in the human red cell.
4. It is shown that the flow-induced uphill transport is a feature characteristic for mobile carrier systems only and is not to be expected in systems in which the substrate is bound to a fixed membrane component ("adsorption membrane"), although such a system may yield identical transport kinetics. Also with respect to Ussing's flux ratio the two systems are different, the adsorption membrane meeting Ussing's criterion, the carrier membrane not.
5. It is concluded that the transport system in the human red cells must contain a mobile carrier, identical for glucose and mannose.

Through the epithelial cells of kidney (1) and intestine (2) sugars may be transported "actively" in the thermodynamic sense; *i.e.*, against the concentration gradients. Rosenberg (3) has discussed transports of this kind in terms of thermodynamic quantities and potentials. He showed that the mere fact of a transport occurring against the concentration gradient (hereafter termed "uphill") allows the conclusion that a "carrier" in a broader sense is involved in the transport mechanism. In addition, in the case of sugar absorption, other indirect criteria (4) likewise point to carrier mechanisms (limited capacity of the transport, competition, kinetics, effect of enzyme inhibitors).

In the erythrocyte, the transport of sugars under conventional experimental

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conditions does not occur uphill. From a number of similar indirect criteria however, in this case also a carrier mechanism has been decided upon (3, 5-7). Although this indirect evidence as a whole is fairly suggestive, none of the observations as such is strictly conclusive.

However, the assumption of a carrier transport has a corollary which can be tested experimentally. If the carrier mechanism can transport two molecular species R and S , it should be possible under certain conditions to induce an uphill transport of R by establishing a concentration gradient for S . In this paper a treatment of some aspects of this induced active transport will be given and the phenomenon will be demonstrated experimentally. Finally its conclusiveness for a carrier mechanism as compared to that of other criteria will be discussed.

Kinetics of the Induced Uphill Transport

Kinetic treatments of the supposed carrier transport as given by various authors (8-10) differ mainly in the assumptions concerning the reactions between substrate and carrier (equilibrium or steady state, enzymatic or non-enzymatic reactions). For the purpose of this discussion it appears advantageous to choose simple conditions. Thus equilibrium between substrate and carrier on both sides of the membrane will be assumed.

The following notations will be used.

R, S , substrates reacting with the carrier C .

C , free carrier.

CR, CS , carrier substrate complexes.

$(C_T) = (C)_I + (CR)_I + (CS)_I = (C)_{II} + (CR)_{II} + (CS)_{II}$ = total concentration of the carrier (free + combined).

v_R, v_S, v_C , transport velocities for R, S , and C (positive sign in the direction $I \rightarrow II$).

K_{rR}, K_{rS} , dissociation constants of the carrier substrate complexes.

$$S' = \frac{(S)}{K_{rS}}$$

$$R' = \frac{(R)}{K_{rR}}$$

D' , diffusion coefficient of C, CR , and CS in the membrane, divided by the thickness and multiplied by the surface area of the membrane.

Concentrations are denoted by symbols in parentheses.

Indices I and II refer to the two sides of the membrane.

Under the conditions of a steady state in the membrane ($v_R + v_S + v_C = 0$) the following equations can be derived.

$$v_R = (C_T)D' \left\{ \frac{R'_I}{R'_I + S'_I + 1} - \frac{R'_{II}}{R'_{II} + S'_{II} + 1} \right\} \quad (1)$$

$$v_S = (C_T)D' \left\{ \frac{S'_I}{R'_I + S'_I + 1} - \frac{S'_{II}}{R'_{II} + S'_{II} + 1} \right\} \quad (2)$$

Introducing

$$p = \frac{R'_I}{R'_{II}} = \frac{(R)_I}{(R)_{II}} \quad (3)$$

and

$$q = \frac{S'_I + 1}{S'_{II} + 1} = \frac{(S)_I + K_{rs}}{(S)_{II} + K_{rs}} \quad (4)$$

we may transform equation (1) to

$$v_R = (C_T)D' \frac{R'_{II}(S'_{II} + 1)(p - q)}{(R'_I + S'_I + 1)(R'_{II} + S'_{II} + 1)} \quad (5)$$

If for both R and S the concentrations at the side I are higher than at the side II, ($p > 1$, $q > 1$), equation (5) shows that R will move along its gradient from I to II ($v_R > 0$) only if $p > q$, whereas for $p < q$, v_R will be negative and consequently the transport of R will be uphill. The general condition for this event is, that p lies between 1 and q .

If $R'_I = R'_{II} = R'$ and $S'_I > S'_{II}$ the flow of S will induce a transport of R . For this case, we may also from (1) and (2) derive the relation

$$v_R = -v_S \frac{R'}{R' + 1} \quad (6)$$

yielding the limiting values

$$v_R = -v_S \quad (7)$$

and

$$v_R = -v_S R' \quad (8)$$

for $R' \gg 1$ and $R' \ll 1$ respectively.

The concentration ratio for which

$$v_R = 0 \quad (9)$$

is according to (3), (4), (5), and (9)

$$p = q = \frac{(R)_I}{(R)_{II}} = \frac{S'_I + 1}{S'_{II} + 1} \quad (10)$$

The limiting values of this ratio are $\frac{(S)_I}{(S)_{II}}$ (for $S'_I, S'_{II} \gg 1$) and 1 (for $S'_I, S'_{II} \ll 1$).

Under conditions of slowly changing values for $(R)_I$ and $(R)_{II}$ (due to accumulation or depletion of R , if the volumes at I and/or II are limited as for instance in experiments with cell suspensions) this ratio is the maximal accumulation ratio for R .

Experimental Methods and Results

The experiments were carried out with human red cells. They were equilibrated with one penetrating sugar R in a low concentration. A second penetrating sugar S

was then added in high concentration to the external solution. The movement of *R* was followed by determinations of its concentration in the external solution. In control experiments a corresponding volume of saline was added instead of *S* to produce the same degree of dilution.

The substrates used were (1) unspecifically labelled C_{14} -glucose (as *R*) and (2) mannose or unlabelled glucose (as *S*). Mannose and glucose were chosen because, according to LeFèvre and Davies (11), they have a relatively high affinity for the transport system.

FIG. 1. Experiment showing uphill transport of labelled glucose across the human red cell membrane induced by counterflow of mannose (filled circles) or unlabelled glucose (open circles). Ordinate, activity in c.p.m., in 10 μ l. of the external medium. Abscissa, time in minutes. Circle with x in center, activity before addition of 0.16 volume of unlabelled sugar (0.72 M in saline). Circle with bar, activity after addition of 0.16 volume saline. Temperature 0°C. until second arrow, then 37°C. (The temperature was raised in order to accelerate the penetration which, however, proved to be unnecessary.) The calculated maximal concentration ratio for labelled glucose is approximately 4.

It was found that the penetration of labelled glucose across the red cell membrane is very rapid. The experiments (with the exception of the equilibration phase) were therefore carried out at 0°C.

7.5 ml. defibrinated blood were equilibrated with 0.2 ml. labelled glucose solution, 5.6 mM with an activity of 1 μ c./ml. The mixture was stored in the cold for 6½ hours. During this time several samples were centrifuged and the activity of the supernatant was counted. The hematocrit value was found to be 0.48 (extrapolated to infinite time of spinning at 3500 R.P.M.). The blood (6.7 ml.) was then centrifuged 9 minutes at 3500 R.P.M., 2.5 ml. supernatant were siphoned off, and the remaining 4.2 ml. were thoroughly mixed. The (calculated) hematocrit reading was now 0.77. Three additional samples were taken within 2 hours for determination of the activity in the external medium.

Three samples of 0.9 ml. blood each were then pipetted into ice cold centrifuge tubes and 144 μ l. of one of the following (ice cold) solutions added: (1) mannose 2.4 isotonic (0.72 M) in isotonic saline; (2) glucose 2.4 isotonic (0.72 M) in isotonic saline; (3) isotonic saline. The samples were thoroughly mixed, kept at 0°C., and aliquots taken at definite time intervals for the determination of the activity in the external medium.

The activities were counted with a gas flow counter. 10 μ l. of external medium were used for each determination. The self-absorption was determined separately and corrected for.

Fig. 1 shows the results obtained. The activity of the external solution is plotted against time. Before the addition of S it is approximately constant. After addition of NaCl it attains a new level which corresponds closely to that calculated for the degree of dilution brought about by the added volume ("dilution level").

In the experiment with mannose or glucose the activity of the external solution first rises to a higher level, where it remains for some time. This means that *R* moves from the cells into the external solution against a concentration gradient, the maximum value of which can be calculated to be about 4:1.

Later the external activity falls and finally reaches the dilution level, as is to be expected, if the sugars penetrate into the cells.

The penetration was followed in a separate experiment at 0°C. under identical conditions using conventional osmotic methods. It was found that the penetration of mannose is somewhat faster (equilibrium after about 30 to 35 minutes) than that of glucose (equilibrium after about 40 minutes).

The observations thus show the occurrence of an induced uphill transport of *R* and are in qualitative agreement with predictions derived from the penetration characteristics of mannose and glucose.

DISCUSSION

The experiments reported here clearly demonstrate the occurrence of induced uphill transports of glucose across the red cell membrane. Similar observations on phosphate and arsenate transports in bacterial cells have been reported by Mitchell (12).

In the treatment given for the induced active transport the type chosen for the reaction-diffusion system (non-enzymatic reactions and equilibrium between carrier and substrate on both sides) is a special (and the simplest) case.

The question arises therefore whether the results of the treatment have more general validity and how far they are influenced by the simplifying assumptions used. In general, those results which also would follow from thermodynamic considerations, especially the maximal possible values for the concentration ratio of *R* and for the ratio v_R/v_S , will be independent of special assumptions concerning the reactions and will be generally valid for carrier systems of the type treated here. On the other hand conclusions with respect to velocities, e.g. equation (5) will depend on the characteristics of the reactions involved.

The induced uphill transport as observed in the red cell has been derived from the transport kinetics of a simple carrier system. Rosenberg (3) has shown

by thermodynamic arguments that a mobile carrier is indeed an indispensable condition for such a transport. This may be illustrated by a comparison of the system treated here and a model membrane consisting of two adsorption layers. These two models which show a close resemblance in their transport kinetics differ in the essential point, that the substrate is bound to a mobile membrane component in one case, to a fixed component in the other.

It will first be shown that the steady state kinetics of the two systems are formally identical. However, as will be derived later only the carrier model is able to induce an uphill transport. Another characteristic difference in the two systems which will finally be discussed is the flux ratio introduced by Ussing (13).

In the system discussed here, if $(S)_I = (S)_{II} = 0$, equation (1) may be written

$$v_R = D'(C_T) \left\{ \frac{R'_I}{R'_I + 1} - \frac{R'_{II}}{R'_{II} + 1} \right\} \quad (11a)$$

or

$$v_R = D'(C_T) \frac{R'_I - R'_{II}}{(R'_I + 1)(R'_{II} + 1)} \quad (11b)$$

It can be shown that an equation closely resembling equation (11b) is obtained for the "adsorption membrane." The transfer from layer I to layer II is assumed to be the rate-limiting step (velocity factor = a). Using Langmuir's adsorption equation (equilibrium constant = K_L and $R' = \frac{(R)}{K_L}$), we then obtain

$$v_R = an^2 \frac{R'_I - R'_{II}}{(R'_I + 1)(R'_{II} + 1)} \quad (12)$$

in which n is the total number of adsorption places in each layer. Equations (11b) and (12) are formally identical. Near saturation both equations yield the special kinetic type which has been termed E , characterized by

$$v_R = K_E \left(\frac{1}{(R)_{II}} - \frac{1}{(R)_I} \right) \quad (13)$$

and which actually was observed for transports of sugars in erythrocytes (6, 7, 14). Thus a differentiation of the two mechanisms by a kinetic analysis of the transport of a single substance appears impossible as was pointed out previously (15).

With respect to the flow-induced uphill transport however, the two mechanisms differ. For the carrier system containing both R and S the flow-induced active transport emerged from equation (5). The corresponding equation for the adsorption membrane is

$$v_R = an^2 R'_{II} \frac{p - 1}{(R'_I + S'_I + 1)(R'_{II} + S'_{II} + 1)} \quad (14)$$

Since here v_R always has the same sign as $(p - 1)$, the transport always occurs along the gradient.

The essential point in this discussion is whether or not the membrane component combining with the substrate is mobile. For example a transfer by combination with a rotating protein or enzyme molecule, as occasionally suggested for active transport (*e.g.* reference 16), would not only follow kinetics similar to those of the aforementioned cases but would also be able to perform an induced uphill transport in the same manner as the carrier system. On the other hand, the "carrier" assumed by LeFèvre for sugar transports (5), is actually a fixed membrane component and consequently would not be able to perform flow-induced uphill transport.

Another characteristic difference between the two types of membrane components emerges from the application of Ussing's flux ratio criterion (13) for passive diffusion:

$$\frac{m_I}{m_{II}} = - \frac{a_I}{a_{II}} \quad (15)$$

(m = flux; a = activity).

It was pointed out (15) that as a general condition for the validity of equation (15) the two diffusion streams which are called flux, must be independent of each other. This is certainly not true for the carrier system, since here both fluxes are interdependent by mediation of the movement of the carrier, and the effect of this movement on the two fluxes is opposite. In the adsorption system, however, although there also is an interdependence by mediation of the number of free places, the role of this number is that of a resistance which reduces the two fluxes by the same fraction so that their ratio remains unchanged.

In accordance with this argument the flux ratio in a transport through an adsorption membrane following equation (12) is (activity coefficients being neglected):

$$\frac{m_I}{m_{II}} = - \frac{(R)_I}{(R)_{II}} \quad (16)$$

thus meeting the above-mentioned criterion for passive diffusion. For the carrier membrane equation (11 *a*) yields for the flux ratio:

$$\frac{m_I}{m_{II}} = - \frac{(R)_I}{(R)_{II}} \frac{(R'_{II} + 1)}{(R'_I + 1)} \quad (17)$$

not obeying Ussing's criterion. Actually the experiments reported with glucose as S show that Ussing's criterion cannot hold for the transport of glucose.

The conclusion from these considerations and from the experimental demonstration of the flow-induced uphill transport of glucose in red cells is that here a mobile carrier is involved in the transport of mannose and glucose. It might be argued that more than one system may be involved in sugar transfer

across the red cell membrane. The induced uphill transport demonstrated here might be due to a system which can only perform exchange. For the net transfer system involved in osmotic experiments, then, a mobile carrier would not necessarily be required, although the kinetics and other criteria exclude free diffusion.

With respect to quantitative details the experiments have preliminary value. It is hoped that a study of the quantitative relationships between the transfer of labelled sugar and the net transport will shed more light on this question.

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