Absence of Rapid Exchange Component in a Low-Affinity Carrier Transport

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ABSTRACT A previous study showed that human red blood cells equilibrate much less rapidly with D-glucose at moderately high concentrations than with C^{14}-glucose added after the net movement is completed. This had been predicted from a simple reversible mobile-carrier mediated–transport model system suggested by the net monosaccharide transport kinetics in these cells, but is also consistent with the more complex models proposed for certain *active* transport systems to account for elevation of tracer fluxes of even low-affinity "substrates" when their *trans*-concentration is raised. The simple model predicts, however, that with any sugar showing a much lower apparent affinity for the reactive sites, such as D-ribose, this phenomenon would not be observed, and tracer equilibration should proceed at approximately the same rate as net uptake. The latter expectation was confirmed experimentally by analyses of the ribose, or radioactivity, content of washed red cells sampled serially during incubation with ribose or C^{14}-ribose in the appropriate mixtures. The tracer ribose movement showed no evidence of a relatively rapid exchange component. The relative rapidity of glucose tracer uptake into cells preloaded with ordinary glucose may therefore more readily be attributed simply to depression of tracer efflux by competition for the saturated reactive sites, than to any action of the *trans*-concentration on the influx by way of a coupled exchange process.

In an earlier report dealing with the movements of C^{14}-glucose through the human red blood cell membrane (1), it was shown that, under the specified experimental circumstances, the speed of such tracer equilibration (in the absence of net movement of the sugar) was many times greater than the speed with which the preceding chemical equilibration had occurred. This phenomenon, although incompatible with a diffusion process, had been expected on the basis of a working model (1) which had previously been developed from a variety of kinetic studies (2–4) on the transfer of monosaccharides in these cells. The essentials of this model are that (*a*) the sugars gain entry into the cell membrane phase only by forming a reversible tran-

sient association with a limited number of special reactive sites in the membrane, and (*b*) the diffusion of this sugar-"carrier" complex within the membrane constitutes the rate-limiting step in the translocation process. The resultant transfer leads only to a uniform concentration of the sugars inside and outside the cells, and need involve no energy other than the thermal kinetic energies of the molecules concerned.

The interpretation, provided by this model, of the high relative speed of tracer-glucose equilibration hinges on the postulate, experimentally supported, that the reactive sites were nearly completely saturated with the glucose passenger molecules at the sugar levels used in the experiments. Thus, shortly after the initial addition of the glucose to the cell suspensions, the inward and outward fluxes became nearly maximal. Accordingly, the *net* movement, being the difference between these two nearly maximal fluxes, amounted to only a small fraction of the fluxes reflected in the later tracer equilibration (after completion of the chemical equilibration).

The relatively high rate of tracer equilibration would thus not be expected if, instead of glucose, some sugar were used which has considerably lower affinity for the reactive sites, so that saturation would not be approached at the experimental range of concentrations. Among the common monosaccharides, D-ribose stands out as particularly suitable for the investigation of this expected contrast in behavior. At concentrations of 0.1 to 0.3 M such as used in the glucose tracer experiments (1), the two sugars equilibrate at roughly comparable speeds; but the apparent carrier-complex dissociation constant for ribose, as estimated from kinetic analyses of its net transfer (5), falls well above this range of concentrations. Its kinetics are thus not distinguishable from ordinary diffusion kinetics, and it is only by way of competition phenomena (6) that it appears certain that ribose penetration involves the monosaccharide carrier system. Consequently, the model predicts that equilibration of tracer ribose should proceed only slightly more rapidly than the net entry of ribose at such concentrations, in marked contrast with the behavior of glucose. The verification of this expectation is the subject of the present report.

Phenomena comparable to the rapid glucose tracer equilibration have been observed in certain other transport systems by Heinz and his colleagues (7–10) and have been given a basically different interpretation in these instances. Heinz suggests that a secondary coupled "exchange" process effects a considerable tracer transfer without making any contribution to net movement. The present observation of the absence of a rapid exchange component in the ribose movements appears to negate the involvement of such a coupled exchange in the red cell's monosaccharide transport system, and to favor the retention of the postulated model (1) as the basis for the observed glucose behavior.

A small formal discrepancy between the model and some of the present data is to be noted, but the resolution of this deviation is inadequate for serious evaluation.

TECHNICAL

The experiments involved addition of ribose (in buffered balanced salt medium) to thick suspensions of washed human red blood cells at 37–38°C, and removal of serial samples at timed intervals for analysis of the ribose distribution. Generally eleven samples were taken at geometrically increasing intervals from about 0.5 minute to about 60 minutes. Upon completion of this equilibration, C^{14}-ribose was then added to the mixture without significant change in the ribose concentration, and the equilibration of the tracer followed in the same manner. The isotonic medium in which all sugar solutions and cell suspensions were prepared was mixed from the several chloride solutions as previously described (1), and had the following approximate final composition: Na^+, 119 mM; K^+, 4.8 mM; Ca^{++}, 2.6 mM; Mg^{++}, 1.7 mM; tris(hydroxymethyl)aminomethane, 35 mM; pH 7.4.

Abrupt termination of the sugar movements at the desired instants was provided by transfer to a large volume of ice cold salt solution containing $HgCl_2$ (1, 11), followed at once by cold centrifugation to separate the supernatant mixture and the partially packed cells. Allowance for the ribose or radioactivity content trapped between the cells was made by analyses for the non-penetrant, raffinose, which had been included in the cell suspensions. Previous reports dealing with experiments of similar design (1, 5) provide full details of the above procedures, as well as the handling of the blood, the preparatory separation and washing of the erythrocytes, and the chemical analytic methods.

The tracer ribose used was D-ribose-1-C^{14} purchased from Calbiochem, while the ordinary D-ribose was obtained in part from Nutritional Biochemicals Corporation and in part from Matheson, Coleman & Bell. Counting of the radioactivity was carried out in the Tracerlab LSC-10B system, employing the following liquid scintillation mixture in each vial: 500 μl aqueous sample containing about 1 to 10 mμc of the C^{14}-ribose, 7 ml absolute ethanol, and 12.5 ml toluene containing the scintillator diphenyloxazole at 4 gm/liter. All the analyses (for ribose, raffinose, and radioactivity) were performed at least in duplicate, on the preparation from each supernatant mixture and each packed cell mass. Completion of the entire procedure for each experiment required about 2 weeks; throughout this period the various preparations were kept frozen at about −18°C except briefly during the actual sampling operations.

THEORETICAL BASIS

The data were analyzed in the framework of the model transport system characterized above. If, as postulated, the association-dissociation reactions between the sugar and the special sites are so much more rapid than the

diffusion step as to be always nearly at equilibrium, the fraction of carrier associated with the passenger sugar at each interface will be equal to $P/(P + K)$, where P is the penetrant's concentration in the adjacent aqueous phase, and K is the dissociation constant of the carrier-passenger complex. The net movement, based on the diffusion of this complex along its gradient through the membrane, should be approximately defined by the relation:

$$\text{Rate of transfer}_{(cis \to trans)} = k\left[\frac{P_{cis}}{P_{cis} + K} - \frac{P_{trans}}{P_{trans} + K}\right], \tag{1}$$

where k is a maximal flux determined by the diffusivity of the complex in the membrane, the fixed concentration of carrier sites present, and the membrane's dimensions.

For the present application, it is convenient to reexpress this relation in conformity with terms used in previous studies of this sort, which reflect the actual experimental parameters:

$$\frac{dS}{dt} = kWI\left[\frac{\frac{Q - S}{W - V}}{\frac{Q - S}{W - V} + K} - \frac{S/V}{S/V + K}\right], \tag{2}$$

where W = total volume of water in the entire mixture (in any convenient unit):
V = that part of W which is intracellular;
Q = total sugar in the entire mixture, in units constituting an isosmotic solution in the unit volume of water;
S = that part of Q which is intracellular;
and I = fraction of total water which is intracellular at isotonicity.
(Thus K is expressed in isosmotic concentration units, 1 *iso*smolal unit being taken as equal to 300 milliosmolal; and k is expressed as isosmolal units per minute.)

Both S and V vary with time until equilibrium is reached. However, since water movement is so much more rapid than sugar movement in this system, the mixture's osmotic pressure may be considered to be homogeneous throughout the aqueous compartments, so that numerically $V = (WI + S)/C$, where C = the total isosmolality of the mixture (given numerically by $1 + Q/W$). Elimination of V and Q by substitution of these identities in equation (2) reduces the expression to a function of the single variable S, the transport constants k and K, and the experimentally fixed constants W, I, and C. Integration (setting $t = 0$ when $S = 0$ at the instant of addition

of Q) then gives the relation:

$$kKt = \frac{(K+C)^2}{2ICW^2}S^2 - \frac{C-I(C+1)}{ICW}(K+C)\left[K+\frac{C(C-1)(1-I)}{C-I(C+1)}\right]S - C(1-I)(K+C-1)^2\ln(1-S/S_e), \quad (3)$$

where $S_e = IQ = IW(C-1)$ = the value of S at equilibrium ($t \to \infty$). Equation (3) defines the course of the equilibration of the cells with added sugar; the half-time for this, at which $S = S_e/2$, is thus given by the relation:

$$kKt_{1/2} = 0.0682C(C-1)^2(2.834-I) + (C-1)\left[\frac{I(C+1)}{4} + 0.386(1-I)(C+1.294)\right]K + \left[\frac{I(C-1)(C+3)}{8C} + 0.1932(1-I)(C+2.59)\right]K^2. \quad (4)$$

Correspondingly, if the labeled sugar is presumed to be thoroughly mixed within each of the compartments so that it acts as a true tracer of the corresponding sugar pools, its transport should follow the general relation:

$$\frac{dS^*}{dt} = kWI\left[\frac{\frac{Q^*-S^*}{W-V}}{\frac{Q-S}{W-V}+K} - \frac{S^*/V}{S/V+K}\right], \quad (5)$$

where Q^* and S^* respectively denote the total label in Q and S (in any consistent units). In the present application, chemical equilibration has already occurred prior to addition of the tracer, so that $(Q-S)/(W-V) = S/V = C-1$, the isosmolality of the sugar throughout the water of the entire mixture; and $V = WI$. Hence

$$\frac{dS^*}{dt} = \frac{kI(IQ^*-S^*)}{(C+K-1)(1-I)}. \quad (6)$$

Integration ($S^* = 0$ when $t = 0$) gives the relation:

$$S^*/Q^* = I[1 - e^{-kt/(C+K-1)(1-I)}]; \quad (7)$$

and, for the tracer equilibration half-time:

$$kt^*_{1/2} = 0.693\,(C+K-1)(1-I). \quad (8)$$

Since I and C are measurable[1] constants, the half-time given by each phase of the experiment defines a simple numerical relation between k and K. For the chemical equilibration phase of each experiment, the cell ribose content was plotted as a function of time (logarithmic) since addition of the sugar. The tracer equilibration data were similarly plotted in terms of radioactivity against time. From the curves drawn by eye through these two sets of experimental points, the respective half-equilibration times were estimated, and appropriate comparisons drawn by application of equations (4) and (8). The fit of the entire time course of the equilibrations with the half-time figures was then examined by application of equations (3) and (7).

RESULTS

In four experiments, a single preparation of washed cells was used for the entire procedure, chemical equilibration with ordinary D-ribose being followed by equilibration with tracer ribose in the absence of further net movement. In addition, several net uptake runs carried out in connection with unrelated ribose studies also conformed to the same patterns for this phase of the experiments. The crucial finding was that the tracer exchange did not proceed notably more rapidly than had the preceding net ribose entry process; this was in striking contrast to the behavior of glucose in the identical experimental situation (1). For one of the experiments, the complete course of the cells' uptake of ribose, and then of labeled ribose, is given in Fig. 1; Table I presents the half-times estimated from such figures for each of the four full experiments. It is clear from Table I that the tracer equilibration half-times are only 5 to 25 per cent shorter than the corresponding figures for the chemical equilibration, instead of one or two orders of magnitude smaller as was found with glucose (1).

With such paired determinations of the half-times for the two phases of the experiment, an explicit evaluation of the two constants, k and K, should be given by the simultaneous solution of equations (4) and (8), as was done in the glucose study (1). However, meaningful application of this procedure is not feasible when the affinity of the sugar is exceedingly low so that K becomes substantially larger than the experimental sugar concentration, as in the present experiments with ribose. In this circumstance, the resolution of the value of the high K (and consequently of k) becomes very poor, since a small uncertainty in the ratio $t_{1/2}/t^*_{1/2}$ leads to a very large uncertainty in the

[1] The parameters C and I, which remain fixed for each phase of each experiment but are not precisely predetermined, were estimated by means of the algebraic relations previously developed (1), from osmometric and hematocrit determinations (using the Fiske osmometer and the International MB microcapillary centrifuge).

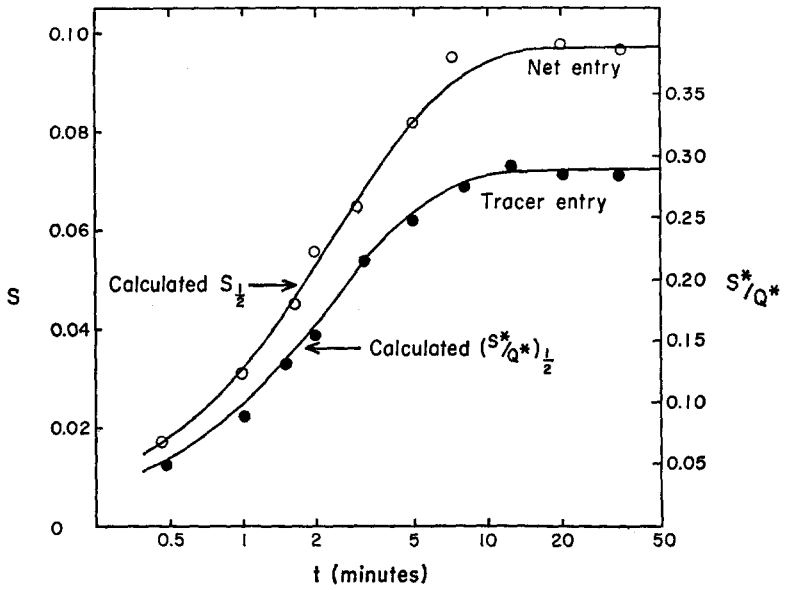

FIGURE 1. Chemical equilibration and tracer equilibration of D-ribose with human red blood cells at body temperature. Open circles mark experimental readings of cell ribose content, S, on left-hand scale (in units which would constitute an isosmotic solution in the total suspension volume of water) *vs.* time t since addition of ribose to sugar-free suspension. Solid circles mark experimental readings of cell radioactivity S^* as fraction of total suspension radioactivity Q^*, on right-hand scale, *vs.* time t since addition of C^{14}-ribose to cell suspension preequilibrated with ordinary ribose at same concentration. Curves are those given theoretically by equations (3) and (7), using the figures: $K = 8$ isosmolal units; $k = 2.5$ isosmolal units/min., chosen so as best to fit the net uptake half-time.

TABLE I

RIBOSE EQUILIBRATION WITH HUMAN RED BLOOD CELLS

Each line gives figures from a single complete experiment on one batch of cells; units for C, I, K, and k are as described in text.

Net entry				Tracer entry			
Fixed parameters		Estimated‡	Resultant k	Fixed parameters		Estimated‡	$t^*_{1/2}$ calculated
C	I	$t_{1/2}$	$(K = 8)$	C	I	$t^*_{1/2}$	from net entry
		min.				*min.*	
1.813	0.263	2.7	2.4	1.820	0.226	2.15	2.0
1.787	0.275	3.2	2.0	1.798	0.222	2.3	2.4
1.791	0.319	2.85	2.1	1.791	0.248	2.5	2.1
1.316	0.373	1.75	2.5	1.321	0.289	1.65	1.62

‡ Half-times are given to nearest 0.05 min. as estimated from graphs, but must be considered uncertain by perhaps ±5 per cent.

resultant value calculated for K. In application to the present data, this is especially evident in the experiments given in lines 1 and 3 of Table I, in that substitution of the observed half-time figures for direct simultaneous solution of equations (4) and (8) will not yield any real values for the constants in these instances. The nature of the discrepancy is that the tracer

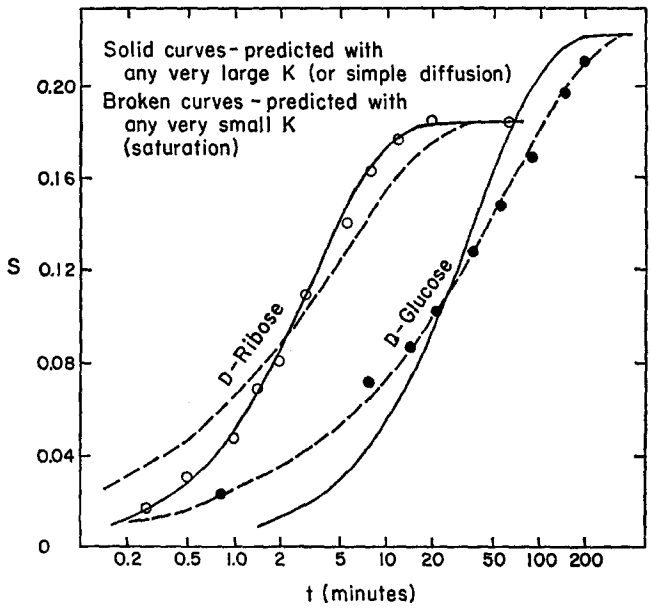

FIGURE 2. Contrast in the temporal course of uptake of D-ribose and D-glucose by human erythrocytes. Cell sugar content, S, is plotted against time on a logarithmic scale. Open circles are experimental points for ribose experiment at body temperature; $W = 0.819$; $I = 0.399$; and $C = 1.565$. Closed circles are experimental points for glucose experiment at 20°C (from Fig. 3 of reference 1); $W = 0.781$; $I = 0.469$; and C, $= 1.608$. Curves are those given theoretically by equation (3), as K approaches either zero or infinity, as indicated in the figure. (Any values for K substantially lower or higher than the sugar levels used, about 0.6 isosmotic, would yield curves not perceptibly different from these.) In each case, k is taken so as to fit with the data at the half-time; value of k has no bearing on the *shape* of the curves because of logarithmic time scale.

half-time is slightly too large relative to the net uptake half-time, even for the limiting case, $K \rightarrow \infty$;[2] thus the contrast between ribose and glucose goes even beyond that predicted by the model system. For this reason, another means of illustrating the comparison of the data with the model is adopted in Table I: the approximate average figure for K which has been estimated (5)

[2] Although the degree of uncertainty in the half-time estimation is such that the reality of these discrepancies is questionable, the possible aberration is noted here because its definitive demonstration would require at least a minor modification in the current working model system.

for ribose by means of kinetic analyses (2 to 3 M, or about 8 isosmolal units) has been substituted in equation (4) to give the figures for the transport rate constant k. These figures (reasonably consistent with each other and with previous estimates of k at body temperature) are listed in the fourth column of Table I, and were then substituted in equation (8) to yield the predicted figures for $t^*_{1/2}$ which are listed in the last column of the table. Comparison with the experimentally observed figures in the preceding column shows only one instance of a possibly significant discrepancy, and this deviation is not a serious one.

A marked contrast between ribose and glucose behavior is evident even without reference to the tracer sugar movements, in regard to the form of the time course of the net uptakes. This is shown in Fig. 2, which incorporates the analogous Fig. 3 of the earlier report (1) on glucose behavior in similar experiments. Glucose uptake always followed a pattern corresponding to high saturation of the reactive sites (like the broken lines in Fig. 2), whereas with ribose at these concentrations, the temporal form of the net entry was never distinguishable from that of a simple diffusion (like the solid lines in Fig. 2, considerably steeper on a logarithmic time base). This contrast in the shape of the uptake curves is in keeping with the large difference between the apparent K's of the two sugars, given by the kinetic studies (1, 5).

DISCUSSION

The present findings taken together with the earlier report (1) show a substantial difference between ribose and glucose behavior in the same experimental situation. Ribose did not show the extreme rapidity of tracer equilibration in relation to its net movement, which was seen with the high-affinity glucose. Such rapid tracer movement appears at first sight to be analogous to the findings of Heinz and his associates in connection with amino acid transfer in Ehrlich ascites tumor cells (7–9) and chloride ion transfer through the frog's gastric mucosa (10). In these systems, an increased speed of tracer migration was found in response to the elevation of the *trans*-concentration of the (unlabeled) transported species (or of some allied molecular species sharing the same carrier). This phenomenon was attributed to the enhancement of flux in the direction, *cis* → *trans*, resulting from the elevation of the back-flux in response to increase in the *trans*-concentration; the mechanism of this coupling was presumed to be that the delivery of one carrier-passenger complex at one side of the membrane, with release of the free passenger at that point, leads to a rearrangement in the reactive site such as to favor the return passage of the carrier bearing another passenger molecule (of the same or a competing species). Alternatively, the phenomenon might simply have been attributed to the saturation of the carrier on the *trans*-side (cor-

responding to the original interpretation of the glucose behavior), since the back-flux of tracer would accordingly be depressed by competition with the unlabeled molecules when the *trans*-concentration was elevated, and this would lead to the observed increase in the net tracer movement under these circumstances. However, the latter explanation seemed untenable in the case of the amino acid transport, since Heinz observed (7) that the net exit process was not yet saturated at intracellular glycine levels which sufficed to produce a definite acceleration of tracer glycine uptake; this appeared to make the more complex interpretation inescapable.

It should be emphasized that with this coupled exchange hypothesis, the high *trans*-concentration of unlabeled material is held not simply to diminish the tracer back-flux by competition, but actually to increase the unidirectional tracer flux, *cis* → *trans*. Heinz and Durbin (10) developed general equations characterizing such a linked exchange-diffusion carrier model, which provided a satisfactory formal description of the observed behavior of the amino acid- and chloride-transporting systems. This involved the assumption that the mobility in the membrane of the loaded carrier-complex is distinctly greater than the mobility of the free carrier.

In view of the apparent suitability of this sort of analysis in application to these comparable phenomena in other carrier systems, it would appear reasonable *a priori* to invoke an exchange-diffusion component in the carrier-mediated sugar fluxes in the red cell system as the basis for interpretation of the behavior of tracer glucose, in lieu of the analysis originally proposed in terms of the simple symmetrical model. However, the present observations with ribose would then require that this sugar, even though making use of the same carrier mechanism as glucose for its net transport (5, 6), must be denied participation in the associated exchange-diffusion process. Moreover, it would appear necessary to postulate that the mobility of the glucose-carrier complex in the membrane greatly exceeds the mobility of the unattached carrier, although the general properties of this system (in contrast with the actively accumulating transport systems) have never given any indication that a cyclic inactivation and reactivation need be involved. The relative complexity of such a picture, while not conclusively ruling out this combination of hypotheses, favors the retention of the unitary interpretation provided by the simple model system (1), according to which the pronounced contrast in the tracer behaviors of glucose and ribose follows directly from the known quantitative difference between the two sugars in respect to their affinities for the transport sites. The apparent inadequacy of this uncomplicated interpretation in the amino acid- and chloride-transporting systems described by Heinz and his coworkers is presumably associated with the well recognized distinction that the latter systems are capable of *active* transport (powered by a metabolic energy supply), whereas the sugar transport system con-

cerned in the present study appears to operate only as a carrier-mediated "facilitated diffusion."

This study was supported by a grant from the National Science Foundation and by United States Public Health Service Research Career Development Award No. GM-K3-15, 469.
Received for publication, June 27, 1962.

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