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A SIMPLE RESOLUTION OF THE KINETIC ANOMALY IN THE EXCHANGE OF DIFFERENT SUGARS ACROSS THE MEMBRANE OF THE HUMAN RED BLOOD CELL

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SUMMARY

1. Using human red blood cells, we measured the time courses of efflux of labelled mannose, glucose or galactose (all at 130 mM) into equimolar concentrations of different sugars.

2. We confirmed that the rates of exchange of mannose with mannose and galactose with galactose are somewhat slower than that of glucose with glucose at the particular concentration studied. The rates of exchange for glucose into mannose and for glucose into galactose are (we confirm) faster, but those for mannose into glucose and galactose into glucose are (we show) slower than for the exchange of either glucose into glucose, mannose into mannose or galactose into galactose.

3. We determined directly, using the single sugars alone, the kinetic parameters K_m and V for exchange transport. The different sugars demonstrated different values for V as well as for K_m.

4. Based on the values obtained, the theoretical time course of the exchange between pairs of sugars at 130 mM was computed according to the conventional carrier model and the recently proposed tetramer model. The experimental results fitted well with the predictions of the tetramer model and to a lesser extent with the carrier model. Thus the previously reported anomaly in the exchange experiments was merely an apparent one due, first, to the fact that different sugars possess different maximum velocities and, second, to the accumulation of the unlabelled sugars within the cell.

INTRODUCTION

Sugars are transferred across the membrane of human red blood cells by one of the facilitated diffusion systems[1-3]. There are, however, considerations suggesting that the conventional carrier model for facilitated diffusion does not fit certain of the experimental results for this system[4-7]. Following a comprehensive study, Miller[4,5] concluded that the carrier model, whether the simple[3] or the "substrate facilitated" one[8,9], does not explain the following results:

(1) Different values of the half-saturation concentration for sugar transport are found using different experimental methods.

(2) There is an increased rate of exchange between different sugars as compared with that between similar ones, when these rates are compared at a sugar concentration of 130 mM.

(3) An experiment on the time course of counter-transport of glucose.

It has been shown recently that the first paradox can be resolved using a new non-carrier model introduced by Lieb and Stein[6]. This model assumes that glucose transport across the red cell membrane is carried out by a protein tetramer embedded in the membrane. Each of the four subunits of the protein includes either a high-affinity or a low-affinity binding site according to the scheme of Fig. 1. The affinity of the L site largely determines the half-saturation concentration for an exchange experiment[6].

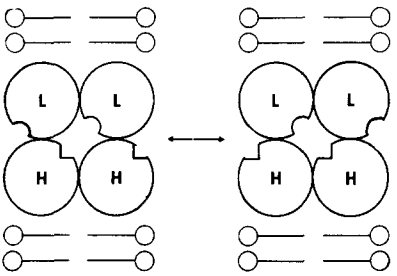

Fig. 1. Internal transfer model for sugar transport. The circles labelled H are the high-affinity binding sites. Those labelled L are the low-affinity sites. The protein tetramer alternates between the two conformations shown[20].

The present work has been undertaken to study the second of the problems listed above, the increased rate of exchange between different sugars as compared with that between similar ones. Naftalin[7] proposed a fixed-site model for sugar transport in order to account for these observations. We show, however, that it is unnecessary to introduce a new model since the phenomenon can be readily understood using the tetramer model or less satisfactorily, a carrier model. We discuss elsewhere[10] how the recent determination of the half-saturation concentration for the efflux of glucose under zero *trans* conditions bears on the validity of the conventional carrier model, on Naftalin's model and on the tetramer model, respectively.

METHODS

Solutions

NaCl–sodium phosphate buffer. This contained 135 mM NaCl, 18.8 mM Na_2HPO_4 and 1.2 mM NaH_2PO_4. The pH was 7.4 and the total osmolarity was 310 mosM.

Stopper solution. This consisted of NaCl, 171 mM; $HgCl_2$, 1 μM; KI, 1.25 mM; phloretin, dissolved in ethanol, was added to give a final concentration of 0.1 mM phloretin and 1 % ethanol[10].

Monosaccharide solutions. The commercial mannose was found to contain some impurities which caused hemolysis of the red blood cells. Therefore, the following purification procedure was adopted: A solution of 1 M mannose was mixed with active charcoal, stirred for about 15 min and filtered several times to remove the charcoal. The resulting clear solution did not now cause hemolysis. The concentration

of the purified solution was determined by a Somogyi–Nelson assay[11]. The solution was kept in the deep freeze until used. Although the commercial glucose and galactose did not cause hemolysis, we adopted a similar purification procedure for the stock glucose and galactose solutions.

Loading the cells

The red blood cells were obtained from outdated transfusion blood. The cells were washed 4 times with NaCl–sodium phosphate buffer followed by centrifugation for 5 min at 12000 × *g*. To load the cells with sugar, packed cells were suspended in 20 vol. of a solution containing 21/20 the required concentration of the sugar (glucose, mannose or galactose) in NaCl–sodium phosphate buffer, and incubated for 1 h at 37°. The cells were then centrifuged and most of the supernatant removed, leaving enough to give a hematocrit of approx. 50 %. The radioactive sugar dissolved in a negligible volume of NaCl–sodium phosphate buffer was added to the suspension and exchange of sugar allowed to proceed (to completion) for 15 min at 20°. The loaded cells were then kept at 0° until used (not more than 2 h).

EXPERIMENTAL PROCEDURE

Each sample was treated separately. 0.02 ml of the loaded cells were placed in a large test tube. At zero time 10 ml of the "external" solution, which consisted of the required concentration of the suitable unlabelled sugar in NaCl–sodium phosphate buffer, maintained at 20°, was forced into the tube by an automatic syringe and the mixture pushed back and forth 1–3 times. At the appropriate time (10, 20 or 30 sec) the mixture was forced into a tube containing 30 ml of the stopper solution at 0°. Eight such samples were treated during 15 min and then centrifuged for 15 min at 12000 × *g*.

After centrifugation the supernatant was removed and the next eight samples treated as above. Each series of eight tubes included also a zero time and an "infinite time" control, as follows: The number of counts present in the cell at zero time was determined by adding 30 ml stopper solution mixed with 10 ml of the "external" solution, at 0°, directly to the 0.02 ml of the loaded cells. For the "infinite time" control, which provided the correction for the counts present in the supernatant fluid and trapped in the cell pellet, we allowed the efflux into the external solution to proceed to completion for 8 min (at least 8 half times) and then added the stopper solution. The number of counts obtained (usually no more than 3 % of the zero-time number of counts) were substracted from all the results.

The red cell pellets were suspended in 0.5 ml of NaCl–sodium phosphate buffer, with vigorous mixing. From each suspension two samples were removed, one to determine the radioactivity and one to determine the relative number of cells present. To determine the radioactivity remaining in the cells, 0.1 ml of each suspension was added to an equal volume of 20 % trichloroacetic acid solution and vigorously mixed. The cell debris was removed by centrifugation and 0.1 ml of the clear supernatant added to 10 ml of scintillation fluid (68 % (v/v) of a solution of 0.4 % (w/v) 2,5-diphenyloxazole, 0.04 % (w/v) 1,4-bis-[2-(4-methyl-5-phenyloxazolyl)]benzene in toluene; 32 % (v/v) ethanol). Radioactive counting was done using the Packard liquid scintillation spectrometer. The relative number of cells present in each pellet

was determined by measurement of the hemoglobin content using the standard Drabkin procedure[12]. 0.25-ml aliquot of each of the cell pellet suspension was added with rapid mixing to 3 ml of the Drabkin solution and the resulting absorbance read at 540 nm in a Gilford spectrophotometer. This measure of the hemoglobin provides an internal control for possible variation in the recovery of cells.

To check the efficiency of the stopper solution in blocking the escape of the monosaccharide from the red cells a separate experiment was performed in which cells loaded with sugar were kept in the presence of stopper for various periods of time. 30 ml stopper solution mixed with 10 ml of an "external" solution (130 mM glucose in NaCl–sodium phosphate buffer) at 0°, was added to 0.02 ml cells previously loaded with radioactive glucose to 130 mM. The mixture was left at 0° up to 20 min, followed by centrifugation for 15 min at 12000 × *g* in the cold. The pellets were treated according to the usual experimental procedure. Table I shows that there was no detectable decrease in the number of counts present inside the cell, up to the time of the last measurement, at 20 min. This exceeded the maximum time that, under experimental conditions, the cells were in the presence of stopper before centrifugation.

TABLE I

A TEST OF THE EFFICIENCY OF THE STOPPER SOLUTION IN BLOCKING GLUCOSE ESCAPE FROM LOADED RED CELLS

See Method for details; each point is the mean of three observations.

Time in stopper (min)	*Mean number of counts per absorbance unit hemoglobin inside the cell ± S.E.*
0	4895 ± 45
5	5026 ± 164
10	4930 ± 42
15	5064 ± 52
20	4970 ± 112

Materials used were Analar except mannose which was "pure" from the National Biochemical Corp. and galactose which was "extra pure" from Merck. Phloretin was from Fluka A.G. Scintillation reagents were from Packard Instrument Co. D-[^{14}C]glucose, D-[^{14}C]mannose and D-[^{14}C]galactose were from Radiochemical Centre.

RESULTS AND DISCUSSION

Time course of exchange at 130 mM

Miller[4] loaded the red cells with 130 mM [^{14}C]glucose and measured the initial rate of escape of radioactivity from the cells. He found that this rate was significantly higher when the external solution contained 130 mM mannose or galactose (we will term this "hetero-exchange") than when it contained 130 mM non-radioactive glucose ("homo-exchange"). Yet the homo-exchange rates for mannose or galactose (all at 130 mM) were somewhat lower than that for glucose. The rates of hetero-exchange for mannose or galactose when the glucose was outside the cell were not reported.

In order to obtain further information about the problem of exchange, the

following experiment was performed: Some cells were loaded with 130 mM [^{14}C]-glucose and others with 130 mM [^{14}C]mannose. The time course of the escape of radioactivity was measured when the external solution contained 130 mM of non-radioactive glucose or mannose in the following combinations: (1) [^{14}C]glucose (in) – glucose (out); (2) [^{14}C]mannose (in) – mannose (out); (3) [^{14}C]glucose (in) – mannose (out), and (4) [^{14}C]mannose (in) – glucose (out).

The results are shown in Fig. 2. The results of a similar experiment, in which galactose replaced mannose, are shown in Fig. 3. It can be seen that, at the particular concentration used, the rate of the homo-exchange for mannose was marginally, while that for galactose was somewhat, slower than that for glucose. However, the rates of hetero-exchange when glucose was present inside the cell were faster, while the rates when glucose was outside the cell were slower, than those for homo-exchange. We note that the curves for the hetero-exchanges are not exponential.

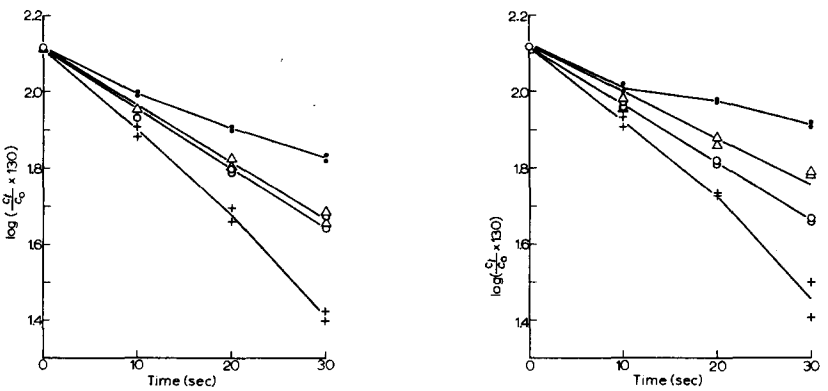

Fig. 2. Experimental time course for exchange of glucose and mannose at 130 mM at 20°. Cells were loaded with radioactive glucose or mannose and the time courses of escape of radioactivity into the same concentrations of non-radioactive sugars were measured. Abscissa: time of efflux in seconds; ordinate: logarithm of the radioactivity per unit hemoglobin remaining inside the cell at the indicated time (c_t) divided by the radioactivity per unit hemoglobin at zero time (c_0), multiplied by the initial concentration (130 mM). Each point is the mean of a duplicate determination. ○, [^{14}C]glucose (in) – glucose (out); △, [^{14}C]mannose (in) – mannose (out); +, [^{14}C]glucose (in) – mannose (out); ●, [^{14}C]mannose (in) – glucose (out).

Fig. 3. Experimental time course for exchange of glucose and galactose at 130 mM at 20°. As for Fig. 2, with galactose instead of mannose. ○, [^{14}C]glucose (in) – glucose (out); △, [^{14}C]galactose (in) – galactose (out); +, [^{14}C]glucose (in) – galactose (out); ●, [^{14}C]galactose (in) – glucose (out).

An interpretation based on the change in the intensity of internal competition

In order to interpret the data of Figs. 2 and 3 it is helpful to take into account the following considerations: the internal volume of the cell cannot be considered as an infinite sink, unlike the external space. Hence, one will find effect of competition within the cell interior, after a short time interval. Considering the exchange of [^{14}C]glucose for glucose, then after such a short time interval the cell contains both radioactive and non-radioactive sugar. The non-radioactive sugar will compete at the transport site with the radioactive sugar (*i.e.* the specific activity of the sugar is lowered) and reduce efflux of label. On the other hand, when [^{14}C]glucose is exchanged for the lower affinity sugars mannose or galactose, the internal competition is now

lower, resulting in an apparently higher exchange rate. Similarly, the rates of hetero-exchange with glucose outside the cell will be expected to be lower than for homo-exchange, due to a higher degree of competition. These effects are reflected in the non-exponential form of the efflux curves in hetero-exchange experiments.

This interpretation applies only to the time-course curves but not to the "initial rates" since initially the cells contain only the radioactive sugars. However, since the exchange is very fast, it can be argued that it is very difficult to measure the true initial rates, and indeed in the present paper we have not attempted to do so.

Experiments to determine the values of V and K_m for glucose and mannose

It was reported by LeFevre[13] that many sugars using the glucose transport system demonstrate rather similar values for the limiting velocity, V. However, we had some preliminary evidence suggesting, rather, that different sugars did demonstrate somewhat different values of V. In order to determine the correct values of the kinetic parameters for the theoretical analysis of Figs. 2 and 3, we found these parameters directly from exchange experiments.

Cells were loaded with different concentrations of the radioactive sugars (ranging from 26 to 140 mM) and suspended in a solution containing that same concentration of the non-radioactive sugar. The escape of radioactivity was measured as a function of time. The values K_m and V are obtained as follows: Let S be the concentration of sugar used, f_t the specific activity, and c_t the radioactive counts present, inside the cell at time t, while f_0 and c_0 are the initial specific activity and counts, respectively. The concentration of label is given by the total concentration of sugar multiplied by the specific activity. Hence, the rate of efflux of label is the total efflux of sugar multiplied by the specific activity. Thus:

$$\frac{d(f_t S)}{dt} = \frac{-f_t \cdot S}{K_m + S} V.$$

Since S is independent of t, upon integration we obtain:

$$\ln\left(\frac{f_t}{f_0}\right) = \frac{-V}{K_m + S} t,$$

which is equivalent to

$$\ln\left(\frac{c_t}{c_0}\right) = \frac{-V}{K_m + S} t.$$

The reciprocal of the slope of the plot of $\ln(c_t)$ *versus* t we write as $-R$, where $R = (K_m + S)/V$ for each substrate concentration S used. Then the plot of R *versus* the concentration S has a slope of $1/V$ and a Y-axis intercept of K_m/V. Such plots for glucose and mannose are shown in Figs. 4a and 4b. All lines were fitted to the experimental points by the least square method. The following values were obtained: K_m for glucose = 32 ($\pm$1.1) mM, V for glucose = 357 ($\pm$10) mmoles/min per l cell water. K_m for mannose = 72 ($\pm$2) mM, V for mannose = 416 ($\pm$14) mmoles/min per l cell water. (The numbers in parentheses are standard errors from the least squares analysis, on 12 points.) It is apparent that glucose and mannose demonstrate different values of V. (It is to be noted that our value for K_m is in fair agreement with

the value of 38 (±3) mM found by Miller[5] but our value for V is higher than his value of 260 (±30) mmoles/min per l cell water. Values of V in general vary more between authors than do those for K_m (ref. 14)).

The kinetic parameters for exchange of galactose were obtained by the same procedure by Miss D. Ram, working in our laboratory[15]. K_m was found to be 147 (±13) mM, while V was found to be 460 (±24) mmoles/min per l cell water.

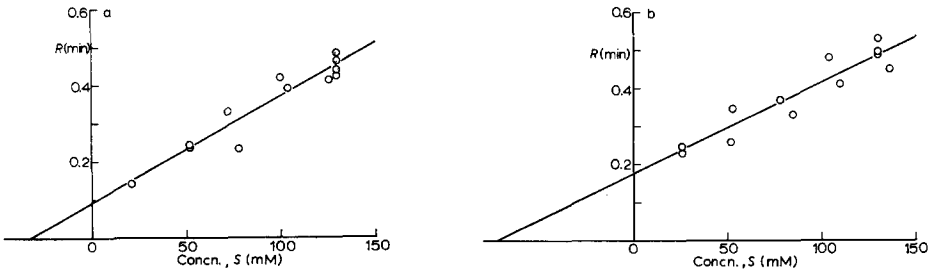

Fig. 4. Exchange of glucose with glucose (a) and mannose with mannose (b) at different concentrations of sugar. Cells were loaded with different concentrations of radioactive sugar and the time course of escape of radioactivity into the same concentration of the non-radioactive sugar was measured. Abscissa: Concentration of sugar in mM; ordinate: R (in min) being the reciprocal of the slope of the natural logarithm of the counts remaining in the cells plotted against time (see text). Each point is the result of an experiment performed in duplicate.

Carrier equation with different values of V

In order to test the prediction of a carrier model with different values of V, we prepared a computer program based on the following considerations.

The carrier mediated transport of two sugars can be represented in the scheme of Fig. 5. G and S are the concentrations of two different sugars and GE and SE are the concentrations of their corresponding complexes with the carrier. E is the concentration of the free carrier. The symbol 1 stands for the inner solution and surface,

Inner Solution	Inner Surface	Membrane Phase	Outer Surface	Outer Solution
G1	GE1	L	GE2	G2
S1	SE1	R	SE2	S2
	E1	K	E2	

Fig. 5. Schematic representation of the carrier-mediated transport of two sugars. See text.

and 2 is for the outer solution and surface. R, L and K are the rate constants for the diffusion of SE, GE and E. KS and KG are the affinity constants for sugars S and G.

$$KS = \frac{S1 \cdot E1}{ES1} = \frac{S2 \cdot E2}{ES2} \tag{1}$$

$$KG = \frac{G1 \cdot E1}{EG1} = \frac{G2 \cdot E2}{EG2} \tag{2}$$

Since the amount of carrier ($2T$) remained constant throughout the membrane

$$2T = E1 + ES1 + EG1 + E2 + ES2 + EG2 \tag{3}$$

At the steady state

$$K \cdot E1 + R \cdot ES1 + L \cdot EG1 = K \cdot E2 + R \cdot ES2 + L \cdot EG2 \quad (4)$$

From these four equations we evaluate $E1$ and $E2$ and obtain an equation for the amounts ΔS and ΔG of sugars S or G, respectively, escaping from the cell during a short time interval Δt.

Let ΔES be $ES1$—$ES2$ and ΔEG be $EG1$—$EG2$. Then from Eqns. 1–3:

$$2T = E1\left(1 + \frac{S1}{KS} + \frac{G1}{KG}\right) + E2\left(1 + \frac{S2}{KS} + \frac{G2}{KG}\right) \quad (5)$$

and from Eqns. 1, 2 and 4:

$$E1\left(1 + r\frac{S1}{KS} + l\frac{G1}{KG}\right) = E2\left(1 + r\frac{S2}{KS} + l\frac{G2}{KG}\right) \quad (6)$$

where $r = R/K$ and $l = L/K$.

We write

$$A = 1 + \frac{S1}{KS} + \frac{G1}{KG} \qquad B = 1 + \frac{S2}{KS} + \frac{G2}{KG}$$

$$C = 1 + r\frac{S1}{KS} + l\frac{G1}{KG} \qquad D = 1 + r\frac{S2}{KS} + l\frac{G2}{KG}$$

$$F = AD + CB$$

Then (from Eqn. 6)

$$E2 = E1 \cdot \frac{C}{D} \quad (7)$$

and from Eqns. 5 and 7:

$$E1 = \frac{2TD}{F} \text{ and } E2 = \frac{2TC}{F} \quad (8)$$

Since

$$\Delta S = R \cdot \Delta ES \cdot \Delta t = R\left(\frac{E1 \cdot S1}{KS} - \frac{E2 \cdot S2}{KS}\right)\Delta t \quad (9)$$

and

$$\Delta G = L \cdot \Delta EG \cdot \Delta t = L\left(\frac{E1 \cdot G1}{KG} - \frac{E2 \cdot G2}{KG}\right)\Delta t$$

and from Eqn. 8:

$$\Delta S = \frac{2TR}{F \cdot KS}(D \cdot S1 - C \cdot S2)\Delta t \quad (10)$$

and

$$\Delta G = \frac{2TL}{F \cdot KG}(D \cdot G1 - C \cdot G2)\Delta t$$

TR is the V for sugar S ($V(S)$) and TL is the V for sugar G ($V(G)$). Then

$$\Delta S = \frac{2 \cdot V(S) \cdot \Delta t}{KS \cdot F}\left[\left(1 + r\frac{S2}{KS} + l\frac{G2}{KG}\right)S1 - \left(1 + r\frac{S1}{KS} + l\frac{G1}{KG}\right)S2\right] \quad (11)$$

and

$$\Delta G = \frac{2 \cdot V(G) \cdot \Delta t}{KG \cdot F}\left[\left(1 + r\frac{S2}{KS} + l\frac{G2}{KG}\right)G1 - \left(1 + r\frac{S1}{KS} + l\frac{G1}{KG}\right)G2\right] \quad (12)$$

The concentration of sugar S inside the cell at the time $t+\Delta t$ is equal to the concentration St at the time t *minus* ΔS. Similarly $G(t+\Delta t)$ is equal to Gt—ΔG. Δt is the iteration step chosen for the computer program and is equal in our program to 0.01 min.

TABLE II

THE KINETIC PARAMETERS FOR TRANSPORT OF GLUCOSE, MANNOSE AND GALACTOSE AT 20°

The parameters K_m and V are determined directly from Figs. 4a and 4b and from ref. 15. K_L and K_H are the affinities of the L and H sites, respectively, in the model of Fig. 1 (see ref. 6). K_L was computed from K_m using Eqn. 12 in ref. 18. K_H was computed from values of K_m for the "infinite *cis*" procedure reported in the literature, using Eqn. 11 in ref. 18.

Sugar	*V for exchange*		*K_m for exchange*		*K_L (mM)*	*K_H*	
	mmoles/min per l cell water	*Ref.*	*(mM)*	*Ref.*		*(mM)*	*Calculated from ref.*
Glucose	357 ± 10	The present	32 ± 1.1	The present	25.1	1.35	6
Mannose	416 ± 14	paper	72 ± 2	paper	56.4	5.0	17
Galactose	460 ± 24	15	147 ± 13	15	114.8	12.0	19

The values of V and K_m for glucose, mannose and galactose used for the computer program were those determined in Fig. 4, or by Miss Ram, and quoted in Table II. The value of r for glucose ranged from 1 to 4 (ref. 16) and 1 for mannose or galactose was the ratio of their V values to the value of V for glucose multiplied by 1 or 4. The curves obtained are shown in Figs. 6 and 7. It can be seen that the theoretical curves fit reasonably well with the experimental results.

Exchange between different sugars according to the tetramer model

The tetramer model was first developed for glucose transport across the membrane of red blood cells[6]. It was assumed that a transition between the two conformations of the protein tetramer (Fig. 1) mandatorily demanded prior binding of substrate. For the present data where we use different substrates, the rate of transition might well depend on the particular substrate bound and will be proportional to the relevant V. When different substrates are simultaneously bound to the tetramer we now assume that each contributes to the overall V in proportion to the amount of that substrate bound, multiplied by its relevant V.

This assumption was incorporated into the computer program prepared by Lieb and Stein[6] for [^{14}C]glucose–glucose exchange, and we obtained the predicted time course curves shown in Figs. 6 and 7. The parameters chosen for the program

are those derived in Table II. It is obvious from Figs. 6 and 7 and Table III that the prediction of the tetramer model fits well with the experimental results.

It is clear, therefore, that from exchange experiments using single sugars, it is possible to predict accurately the experimental data obtained with pairs of sugars.

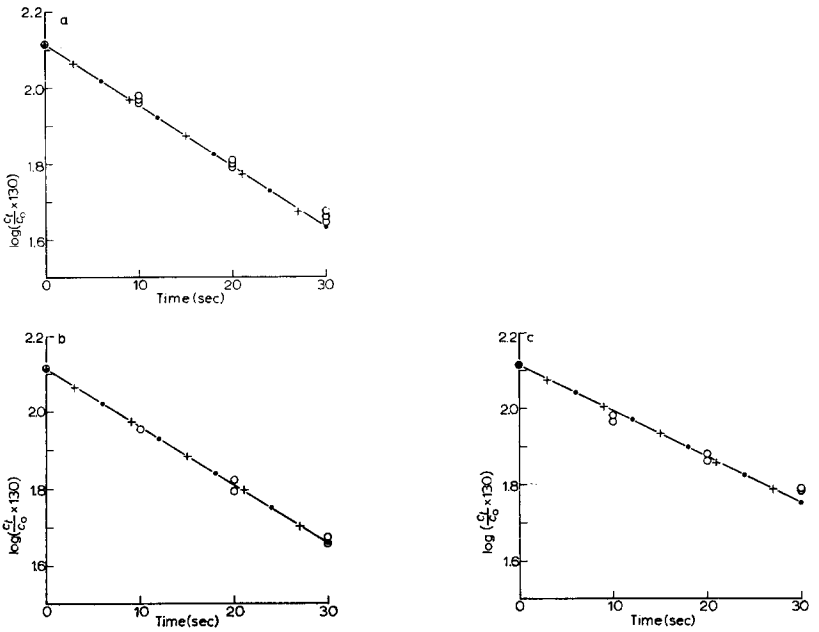

Fig. 6. Theoretical and experimental plots of time courses of homo-exchange of sugars at 130 mM and at 20°. Abscissa and ordinate are as in Fig. 2. ●, theoretical time course according to the tetramer model. +, theoretical time course according to the carrier model (different values of the parameters r give identical predictions; see Table II for the parameter used and text for the computation procedure). ○, experimental results, taken from Figs. 2 and 3. Each point is the mean of a duplicate determination. (a) [^{14}C]glucose (in) – glucose (out); (b) [^{14}C]mannose (in) – mannose (out); (c) [^{14}C]galactose (in) – galactose (out).

Table III shows the experimental and computed concentration of the radioactive sugar inside the cell at 30 sec. This time was chosen because here the differences between the predictions of the various hypotheses are at their maximum. The computation is based on the carrier theory with different parameters for r and V and on the parameters of Table II in the case of the tetramer theory. The fit between the predictions of each of the theories and the experimental value was examined by the χ^2 analysis. It was found (see Table III) that the tetramer theory has a probability of 90 % to fit the experimental results. While the differences between the predictions of the tetramer and the various forms of the carrier theory are not large enough to reject the carrier theory on this ground only, it is clear that their probability to fit the experimental results is substantially lower (Table III).

Naftalin's fixed site model, which was put forward to explain the problem of increased rate of exchange between dissimilar sugars over that between similar ones is now seen to be unnecessary. The problem arises in part because the exchange experiments were performed at a particular sugar concentration, within the range

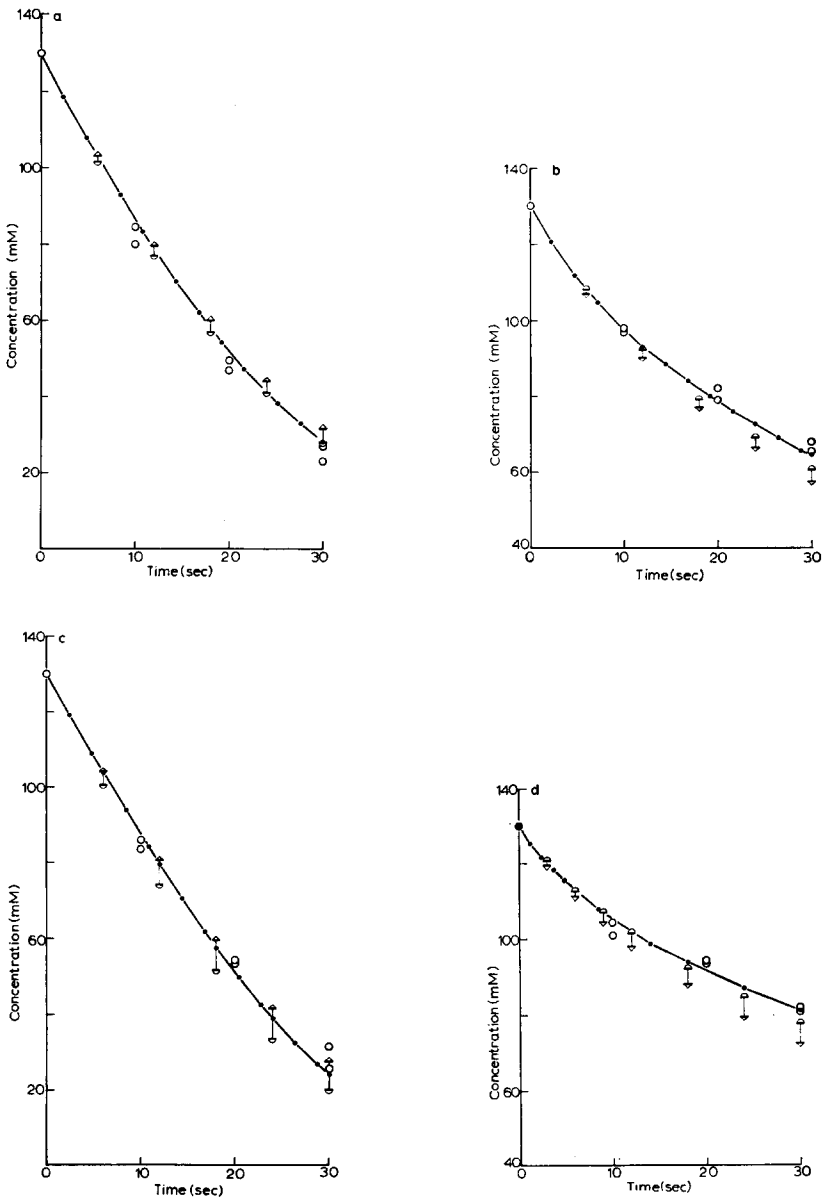

Fig. 7. Theoretical and experimental time course of exchange between different sugars at 130 mM and at 20°. Abscissa: time in sec; ordinate: concentration of that sugar which was initially present inside the cell. ●, theoretical time course according to the tetramer model. ⌓, theoretical time course according to the carrier model (for computation procedure see text). r, the ratio of rates of movement of carrier loaded with glucose to free carrier ranges from $r = 1$(⌓ or ⌔) to $r = 4$(△ or ▽). For the parameters used see Table II. ○, experimental results (taken from Figs. 2 and 3). Each point is the mean of duplicate determinations. (a) [^{14}C]glucose (in) – mannose (out); (b) [^{14}C]mannose (in) – glucose (out); (c) [^{14}C]glucose (in) – galactose (out); (d) [^{14}C]galactose (in) – glucose (out).

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TABLE III

EXCHANGE OF SUGARS

Experimental and theoretical concentrations (computed according to text) of the radioactive sugar inside the cell at 30 sec, at 20°, are given below.

The sugar		*Mean experimental value ± S.E. Number of observations in brackets*	*Computed according to the tetramer theory*	*Computed according to carrier theory, each sugar demonstrating a different V value according to Table II*		*Computed according to the current carrier theory (all sugars demonstrating the same V value in mmole/min. l cell water)*					
						$r = 1$			$r = 4$		
In ([^{14}C]-)	*Out*			$r = 1$	$r = 4$	$V = 357$	$V = 416$	$V = 460$	$V = 357$	$V = 416$	$V = 460$
Glucose	Glucose	46.1 ± 0.52 (8)	42.9	42.7	42.7	42.7	35.4	30.8	42.7	35.4	30.8
Glucose	Mannose	26.0 ± 0.97 (4)	28.0	28.4	31.6	29.9	21.8	17.0	34.2	25.7	20.5
Glucose	Galactose	28.7 ± 1.68 (4)	24.0	20.2	27.5	22.4	14.0	9.6	33.0	23.1	17.1
Mannose	Mannose	47.2 ± 0.95 (4)	46.3	45.9	45.9	53.3	45.9	41.1	53.3	45.9	41.1
Mannose	Glucose	67.1 ± 1.77 (4)	64.9	58.0	57.5	65.7	59.8	55.8	62.4	56.3	52.3
Galactose	Galactose	61.4 ± 0.29 (4)	56.7	56.3	56.3	68.0	61.0	56.3	68.0	61.0	56.3
Galactose	Glucose	81.5 ± 1.16 (4)	81.5	77.7	72.5	85.5	81.1	78.1	79.7	75.0	71.8
χ^2 Value ("Goodness of fit" test)			2.212	6.163	4.534	4.107	19.78	54.168	4.530	7.270	23.60
The probability at 6 degrees of freedom (%)			90	41	60	66	<0.01	<0.01	60	30	<0.01

of values of the half-saturation concentrations for exchange with these sugars. From the parameters that we have derived one might predict that, had the experiments been performed at a much higher sugar concentration, sufficient to saturate the system in the case of galactose, it would be clear that the rates of homo-exchange fall in the order galactose–galactose, mannose–mannose, glucose–glucose.

It seems, therefore, that the faster rate of [^{14}C]glucose–mannose or [^{14}C]glucose–galactose exchange over that of glucose–glucose exchange at 130 mM is due to the combination of two factors: (1) The reduction in the internal competition due to higher values of K_m for mannose and galactose, and (2) the situation that the different sugars used possess different maximum velocities of transport.

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