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TWO-CARRIER MODELS FOR MEDIATED TRANSPORT

II. GLUCOSE AND GALACTOSE EQUILIBRIUM EXCHANGE EXPERIMENTS IN HUMAN ERYTHROCYTES AS A TEST FOR SEVERAL TWO-CARRIER MODELS

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SUMMARY

1. Equilibrium exchange of glucose and of galactose is measured in human erythrocytes over wide concentration ranges. One-site Michaelis-Menten-type kinetics are shown for the two sugars.

2. In view of the results, two of the two-carrier models discussed in paper I (Eilam, Y. (1975) Biochim. Biophys. Acta 401, 349–363), the different sequential carriers and the antiparallel simultaneous carriers, are rejected for the sugar transfer system. The antiparallel sequential carriers model is consistent with these results.

INTRODUCTION

It was shown in the previous paper [1] that the sugar transport system in human erythrocytes displays two sites on each face of the membrane. This finding led us to examine the applicability of the various two-carrier models discussed earlier [1] to the sugar transfer system.

The kinetics of equilibrium exchange can be considered as a test for the following two carrier models:

(1) Two sequential carriers: These carriers may be "antiparallel" or "different"; "antiparallel" carriers are defined as being 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. Two 'different' carriers are defined as having four different affinity sites. It was shown that the antiparallel sequential carrier model predicts for equilibrium exchange one-site Michaelis-Menten-type kinetics, while the 'different' sequential carriers model predicts two sites.

(2) Simultaneous carriers: Any simultaneous carrier model, in which the maximal velocity of the exchange is higher than the maximal velocity of the zero

TABLE I

VALUES OF PARAMETERS OF EQUILIBRIUM EXCHANGE AT 20° C. FROM DIFFERENT SOURCES

Range of Concentration (mM)	K_m (±S.E.) (mM)	V (±S.E.) (mmol/min/l cell water)	References
Glucose			
20–130	38 ± 3	260 ± 30	Miller [2]
22–130	32 ± 1.1	357 ± 10	Eilam and Stein [3]
5–37	20 ± 1.0	264 ± 42	Lacko et al. [4]
0.8–8	14	300	Edwards [5]
0.5–130	34 ± 6	360 ± 31	Present study
Galactose			
50–400	138 ± 57	432 ± 44	Ginsburg and Ram [6]
13–130	73 ± 13	180 ± 20	Miller [7]
2–300	191 ± 17	453 ± 9	Present study

trans*, predicts that the kinetics of equilibrium exchange are not of the Michaelis Menten type, due to the S^2 term in the equation (S/v against S plot, when S is the concentration and v is the initial rate, would deviate upward at low concentrations from the straight line). A similar deviation is obtained if instead of a single carrier we consider a model of two antiparallel carriers [1].

The parameters for glucose and galactose equilibrium exchange in human red blood cells are reviewed in Table I. It can be seen that different K_m values were obtained in experiments done at different concentration ranges. Such a dependence on concentration is expected for a non Michaelis Menten type kinetics, e.g. in the case of two-site kinetics, the site with the low K_m value would be detected at a low concentration range while at the higher range the site with the high K_m value would be observed.

In the present paper, the equilibrium exchange of glucose and galactose is measured throughout a wide concentration range. One site Michaelis-Menten-type kinetics are shown for both sugars. This result is used to evaluate the two-carrier models discussed in paper I [1].

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 310 mosm.

Stopper. The standard stopper consisted of 170 mM NaCl, 10 μM $HgCl_2$, 1.25 mM KI, 0.1 mM phloretin dissolved in ethanol to a final concentration of 1 %. NaCl was added to the standard stopper according to the osmolarities of the experimental sugar-solutions, when these exceed the osmolarity of the standard stopper.

* For terminology see paper I in this series [1].

Hemolysing solution. This consisted of 0.67 g CsCl, 2.5 ml NH_4OH and 50 μl Triton in 1 l H_2O.

Toluene/Triton scintillation fluid. 5.5 g 2,5-diphenyloxazole; 0.1 g dimethyl POPOP (1,4-bis[2-(4-methyl-5-phenyloxazolyl)]-benzene) in 666 ml toluene and 333 ml Triton.

Loading the cells

The red cells were obtained from outdated transfusion blood after 21–28 days of storage. The cells were washed 4 times with NaCl sodium phosphate buffer at 20–25 C. To load the cells with galactose, cells were suspended in 20 vol. of solution containing 21/20 the required concentration of galactose in NaCl sodium phosphate buffer and incubated for 1 h at 37 C. The cells were then centrifuged and the [^{3}H]-galactose was added to the cell suspension at hematocrit of approx. 50 %. Exchange of sugar was allowed to proceed for 20 min at 37 . To load the cells with low concentrations of glucose the procedure was shortened to avoid a possible metabolic breakdown of the labeled glucose. The loading was done all in one stage; each sample was loaded separately by incubating the blood with the radioactive sugar at the required concentration for 5 min at 33 C and the cells were used immediately for the experiment.

Experimental procedure

Portions of 0.05 ml of the loaded blood suspension were placed in large test tubes. At zero time 10 ml of a solution of the non-radioactive sugar at identical concentration were forced into the tube by a syringe and pushed back and forth 1–3 times. The exchange was terminated after either 20 or 30 s for sugar at high concentration and either 5 or 10 s for sugar at low concentration, by forcing the mixture into a tube containing 30 ml of stopper solution at 0 C. For each concentration zero time and infinite time control was determined, as described previously [3]. Groups of six tubes were immediately centrifuged for 5 min at 10 000 rev./min, the supernatant discarded and the walls of the tubes wiped. The cell pellets were dissolved in 5 ml of hemolysing solution. Portions of 2 ml were transferred to conical centrifuge tubes and 0.2 ml of 100 % trichloroacetic acid were added. After centrifugation, samples of 1 ml were transferred to toluene/Triton scintillation fluid, and radioactivity was counted with a Packard liquid scintillation spectrometer. The remainder of the hemolysing solutions was used to determine the absorption at 540 nm in a Gilford spectrophotometer. It was previously determined that absorption at 540 nm is proportional to the concentration of hemoglobin up to 1.5 .4 units. The counts in each sample were then calculated per unit of optical density.

Calculation

It was previously shown [2] that in the equilibrium exchange experiment $\ln (C_t/C_0)$ $V_t/(K_m \cdot S)$. C_t and C_0 are the number of counts at time t and 0, respectively; S is the concentration and t is the time in min. For each concentration the slope of $\ln (C_t/C_0)$ against t was determined from six experimental points by linear regression. The standard deviations of the slopes and the coefficient of correlation were also calculated. The reciprocals of the slopes, B, obtained in the different experiments were plotted against their respective concentrations, to yield the required parameters.

RESULTS AND DISCUSSION

Galactose equilibrium exchange. The results are shown in Fig. 1. The concentration range was 2–300 mM. The values obtained for the parameters are K_m 191 ± 17 mM (± S.E., n = 13) and V = 453 ± 9 mmol/min/l cell water (± S.E., n = 13). The data were statistically examined for their fit with one site or two site kinetics. The following results were obtained: (1) The correlation coefficient with a straight line equals 0.97 probability for non-correlated population being less than 0.001. (2) The Run Test [8], to determine whether the scatter of data about the line is random, showed that the hypothesis of a non-random scatter at any particular region should be rejected ($P < 0.05$). (3) Using a computer program based on the non-linear least-square method, the experimental results were fitted into two models, a two-parameter Michaelis-Menten model with $v = SX(1)/(S + X(2))$ and a four-parameter model with $v = SX(1)/(S + X(2)) + SX(3)/(S + X(4))$, v being the initial rates and S the concentrations. X (1–4) are the parameters determined by the program. χ^2 values for the best fits were subjected to the F test for additional term [9]. No significant improvement of the fit was obtained by introducing four parameters rather than two (P = 0.33).

Glucose equilibrium exchange. The experiments were carried out mainly at low concentrations (0.5–10 mM), the total concentration range being 0.5–115 mM. When cells are loaded with low concentrations of glucose a significant fraction might be metabolically consumed, thus reducing the actual concentration available for transport. If this were the case, slopes of $\ln C_t/C_0$ against time would not be linear. In order to test this the correlation coefficients of the linear regression were calculated. The mean correlation coefficient at low concentrations of glucose (0.5–5 mM) was found to be 0.99 (the smallest value being 0.98). This indicates that in the present work no significant metabolic breakdown occurred during the experiments.

Fig. 2. shows the data from this work, as well as the data obtained previously at higher concentrations [3] and the data of Lacko et al. [4]. The straight line obtained by linear regression yields K_m = 34 ± 6 mM(± S.E., n = 37) and V = 360 ± 31 mmol/min/l cell water (± S.E., n = 37). The correlation coefficient equals 0.97 and the

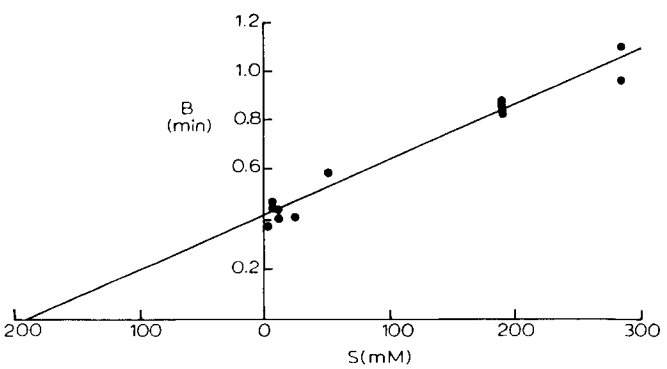

Fig. 1. Equilibrium exchange of galactose. Abscissa: concentration of sugar (mM). Ordinate: B (min) being the reciprocal of the slope of $\ln (C_t/C_0)$ against time (see text). Each slope was obtained from six points. The mean fractional error of the slopes is ± 0.05, largest value being ± 0.09. Temperature was 20 °C.

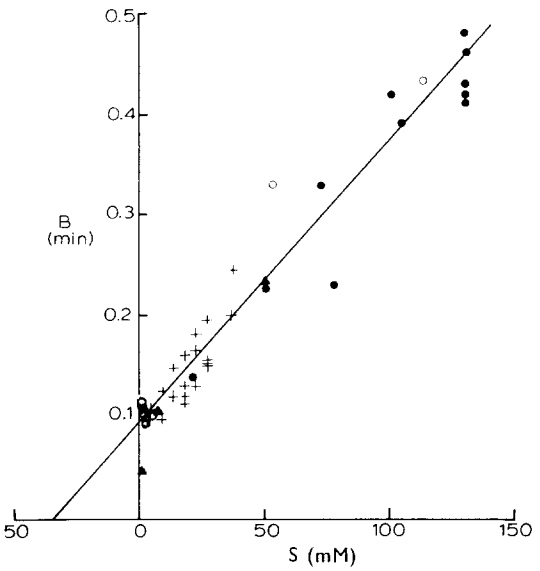

Fig. 2. Equilibrium exchange of glucose. Abscissa and ordinate as in Fig. 1. Present experiments, ○; data from Eilam and Stein [3], ●; data from Lacko et al. [4], +; data from Edwards [5], ▲ (excluded from the linear regression). Mean fractional error of the slopes is 0.065, largest value being 0.11. All experiments done at 20 °C.

probability for random population is less than 0.001. By the Run Test, the hypothesis of a non-random scatter about the line is rejected ($P < 0.05$). F test for additional term shows no significant improvement in the fit of four parameters over two parameters (P 0.37).

It is clear from Fig. 2. that the apparent discrepancy between the parameters obtained by Eilam and Stein [3] and by Lacko et al. [4] is due to the limited range of concentrations used in both studies. On the other hand, the parameters obtained by Miller [7], using a less reliable method of estimating the initial rate (by eye), deviate specifically for galactose. It is not clear why the rates of glucose exchange obtained by Edwards [5] at 0.8 mM are higher than those in the present experiment (Fig. 2).

It can be concluded that the kinetics of equilibrium exchange of glucose and galactose at 20 °C are of a one-site Michaelis-Menten type. In view of this conclusion the model of two antiparallel simultaneous carriers, which predicts non-Michaelis-Menten type kinetics for equilibrium exchange (see ref. 1), is rejected. The model of two different sequential carriers predicts two-site kinetics for equilibrium exchange (see ref. 1) and therefore is also rejected.

On the other hand, the model of two antiparallel sequential carriers predicts one site Michaelis-Menten type kinetics for equilibrium exchange and is not rejected.

CONCLUSIONS

Out of the four possible two-carrier models discussed in paper I [1] two models, the different sequential carriers and the antiparallel simultaneous carriers, are rejected here by the equilibrium exchange experiments. The model of antiparallel sequential

carriers is not rejected but a further analysis of the V/K_m ratios of equilibrium exchange and zero trans is required, as shown in paper I. The different simultaneous carriers model predicts that the equilibrium exchange results, when plotted as S/v against S, may yield one straight line only accidentally [1]. Therefore the probability of finding one straight line for more than one sugar and at more than one temperature is small. Measuring equilibrium exchange kinetics at different temperatures would provide a further test for this model.

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