Effects of erythrocyte lipid and of glucose and galactose concentration on transport of the sugars across a water–butanol interface

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ABSTRACT A property of sugar transport into the human erythrocyte is that a sugar with a high affinity for the hypothetical "carrier" will enter the cell at low concentration more rapidly than a sugar with lower affinity for carrier. At high concentration the sequence will be reversed. This behavior is exemplified by glucose, which enters erythrocytes faster than galactose at 0.015 M and slower than galactose at 1.3 M.

A physicochemical model with the same properties has been found: layers of butanol and water with erythrocyte lipid at the interface. With total lipid from the human erythrocyte incorporated into the model, glucose at low concentration enters the oil phase faster than galactose and at high concentration galactose enters more rapidly. In the absence of lipid, glucose flux exceeds galactose flux at all concentrations.

The hypothetical carrier molecule has not been identified.

SUPPLEMENTARY KEY WORDS   diffusion · carrier-mediated transport · erythrocyte membrane

THE ENTRANCE of metabolically important hexoses into the human erythrocyte may represent the simplest form of biological transport. Elucidation of the mechanism may provide meaningful insights into more complex transport systems.

Investigations over the last 30 years have characterized many of the properties of this process. It is not influenced by the presence of insulin; it is temperature-dependent, and seems therefore to be driven by thermal agitation; entry rates for particular sugars are species-dependent; counter transport occurs; the process can be inhibited; the kinetics of transport for most sugars best fit a "carrier" model in which an unidentified carrier transports the sugar across the cell membrane (1).

Different approaches to the kinetics of penetration of nonelectrolyte into the mammalian erythrocyte have been extensively reviewed by Bowyer (1). These approaches have failed to distinguish between a nonenzymatic carrier system, an enzymatic carrier system, and a "polar creep" mechanism. An alternative approach is to incorporate membrane components into a physical model, repeat the translocation studies in the model, and compare the kinetic analysis with the in vivo data.

The general equation for sugar transport into the erythrocyte takes the form (2):

$$v = \frac{V_{\max}K_m([S]_{\text{out}} - [S]_{\text{in}})}{([S]_{\text{in}} + K_m)([S]_{\text{out}} + K_m)} \qquad \text{Eq. 1}$$

where v = transport rate, $V_{\max}$ = maximal transport rate, K_m = dissociation constant of carrier–substrate complex, $[S]_{\text{in}}$ = substrate concentration inside cell, and $[S]_{\text{out}}$ = substrate concentration outside cell.

When the degree of saturation of the carrier is very low, the equation is simplified to:

$$v = V([S]_{\text{out}} - [S]_{\text{in}})/K_m. \qquad \text{Eq. 2}$$

When the degree of saturation of the carrier is high, the equation is simplified to:

$$v = VK_m \left(\frac{1}{[S]_{\text{in}}} - \frac{1}{[S]_{\text{out}}}\right). \qquad \text{Eq. 3}$$

The values of K_m for D-glucose and D-galactose have been measured by LeFevre and Marshall (3) as 0.007 M and 0.03 M respectively. Equation 2 predicts that at low concentrations of sugar the rate of entry, being inversely proportional to K_m, will be higher for glucose than for galactose. At high saturation of carrier (when $c \gg K_m$), equation 3 predicts that the opposite will be true.

These predictions were confirmed by Wilbrandt (4). At 0.03 M glucose was found to enter the human erythrocyte more rapidly than galactose, and at 1.5 M galactose entered the cell more rapidly than glucose.

If a physical membrane is to provide a qualitatively

satisfactory model for sugar translocation in the intact cell, two requirements must be met. First, the model should display kinetics analogous to equation 2 at low concentration, and to equation 3 at high concentration. Second, if these "carrier" kinetics are caused by some component of the erythrocyte membrane, incorporation of this component in the model should generate the appropriate kinetics, while its exclusion should generate different kinetics.

MATERIALS AND METHODS

Lipid Extraction

Venous blood was drawn into heparinized tubes from 10 healthy adult donors. Total lipids were extracted by procedure III of Ways and Hanahan (5). The extracted lipid, was a clear, amber oil, was stored in benzene or butanol at $-20°C$ before use in the membrane experiments. When the extraction method was standardized, nine determinations on erythrocytes from normal adult volunteers gave gravimetric estimations of lipid content as 4.79 (range, 4.48–5.09) mg of lipid per ml of packed cells (obtained at 1,000 g).

Membrane Model

The principle of the Schulman chamber for investigating model membranes (6–8) is that two immiscible liquids having a stable interface are stirred at the same rate by two blades mounted on a single stirrer shaft. An amphiphilic solute (lipid), introduced into the less polar (butanol) phase, forms a film at the interface. Radioactive, hydrophilic molecules (sugars in this case) are introduced into the more polar phase (water) and their rate of appearance in the butanol is measured. Different parameters of the system can be varied to measure their influence on the translocation process.

The apparatus consists of battery jars with glass covers and double-bladed stirrers on each stirring shaft. The stirring blades were rotated at a constant speed of 15 rpm. A six unit Phipps & Bird electric stirrer allowed four simultaneous experiments to be conducted. For all experiments 10 ml of 1 M sodium and potassium propionate (included to provide a counter-ion for the interfacial lipid), 200 ml of 1-butanol, and sufficient distilled water to bring the final volume of water—after addition of the sugar solution—to 280 ml, the two layers being mutually saturated, were placed in the jars and allowed to equilibrate. In some experiments, 4 mg of total lipid from erythrocytes was introduced into the butanol in each cell. Then a solution of D-glucose-U-^{14}C or D-galactose-U-^{14}C was injected into the aqueous phase, so that the sugar concentration of the aqueous phase at time zero was either 0.015 M or 1.3 M. Eight 1.0 ml samples were taken from the butanol phase at 10-min intervals. After

the samples had been collected, the whole system was vigorously stirred to equilibrate the sugar between both phases, and allowed to clear for several hours before samples were drawn from each phase for the determination of the butanol–water distribution coefficient (P_{ow}). Samples were transferred to scintillation vials containing 15 ml of Bray's solution (9) and counted in a Packard model 4000 liquid scintillation spectrometer. Counts per minute were converted to disintegrations per minute by means of an external standard correlation curve. The temperature in the Schulman chamber was maintained at 34°C in a 120 liter circulating water bath.

Theoretical Considerations (8)

The kinetic equation for measuring the interfacial transfer coefficient in the Schulman chamber has already been derived. It states that

$$dn_o/dt = AK_{wo}C_w \qquad \text{Eq. 4}$$

$$K_{wo} = (P_{ow})[-\ln\{1 - (C_o/C_o^e)\}]$$

$$[At(aP_{ow} + 1)/V_o]^{-1} \qquad \text{Eq. 5}$$

where C_o = concentration of the permeator in the butanol phase at time t; C_o^e = concentration of the permeator in the alcohol phase at equilibrium, $t = \infty$; C_w = concentration of the permeator in the aqueous phase at time t; n_o = number of moles of permeator in the butanol phase; P_{ow} = distribution coefficient for the volumes of butanol and water used = C_o^e/C_w^e; A = area of the interface; K_{wo} = interfacial transfer coefficient from water to butanol (cm-hr^{-1}); V_o = volume of the alcohol phase; and $a = V_o/$(Volume of the aqueous phase).

RESULTS

The translocation of the sugars D-glucose and D-galactose from the aqueous phase into the oil phase was examined at 34°C in every experiment. Figs. 1 and 2, representative experiments, are plots of $K_{wo}t$ (calculated from equation 5) against time in minutes. The slope of the plot represents K_{wo}, the interfacial transfer coefficient from water into oil (butanol). Despite the excellent straight lines obtained in any one experiment, the variability in values from one experiment to another was considerable (see later, in Table 1).

The figures show that at 0.015 M, with or without lipid in the chamber, K_{wo} is greater for D-glucose than for D-galactose. The fact that the lines do not pass through the origin must mean that at the slow rate of stirring the sugar does not become effectively dispersed and begin to traverse the butanol boundary until several minutes have elapsed.
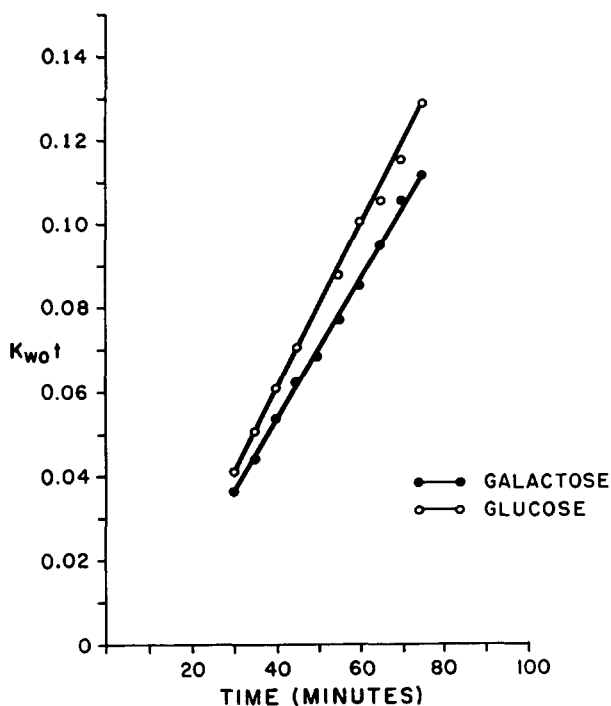
FIG. 1. K_{wo} (a measure of transport rate from water into butanol, defined by equation 5) for D-glucose exceeds K_{wo} for D-galactose when no lipid is in the chamber and the initial aqueous sugar concentration is 0.015 M. Temperature, 34°C. Very similar values were obtained when erythrocyte lipid was at the interface.

TABLE 1 RATIO OF THE INITIAL RATE OF FLUX (dn_o/dt) OF GALACTOSE FROM WATER INTO BUTANOL RELATIVE TO THAT OF GLUCOSE, WITHOUT AND WITH LIPID* PRESENT

Initial Sugar Concentration in Aqueous Phase	Ratio of Initial Fluxes (Glucose 1.0)	
	Without Lipid	With Lipid
0.015 M	0.80†	0.82
	(0.67–0.93)	(0.75–0.94)
1.3 M	0.78	1.65
	(0.62–0.94)	(1.17–2.22)

* 4 mg of erythrocyte lipid added to the butanol.
† Mean for four determinations, range in parentheses.

Table 1 summarizes data of 16 experiments. The determined value of K_{wo} was substituted in equation 4 for zero-time conditions to yield dn_o/dt. The ratios of dn_o/dt for galactose to dn_o/dt for glucose are shown in Table 1. When no lipid was in the chamber, changing the concentration of sugar did not influence the rate of transport of galactose relative to that of glucose; initial galactose transport was consistently less than initial glucose transport from water to butanol. When erythrocyte lipid was present in the butanol and at the interface, glucose transport again exceeded galactose transport at a low concentration of sugar; the ratio of initial fluxes was about the same as in the absence of lipid. At high sugar concentration (1.3 M), however, the situation was
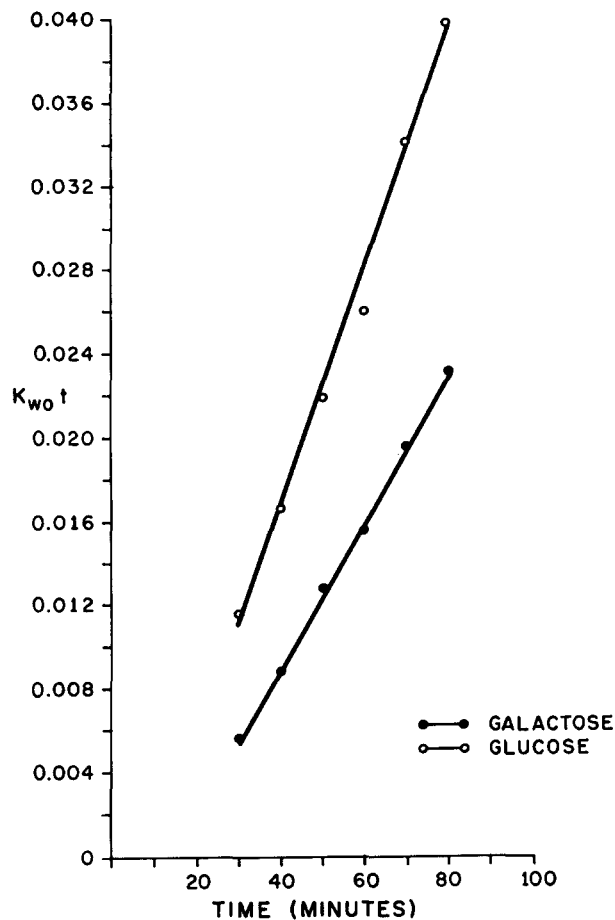

FIG. 2. K_{wo} for D-glucose exceeds K_{wo} for D-galactose when no lipid is in the chamber and the initial aqueous sugar concentration is 1.3 M. Temperature, 34°C. Note difference in ordinate scale from that in Fig. 1. Values of the same order of magnitude were obtained when erythrocyte lipid was at the interface, but the relative positions of the two lines were reversed.

changed radically. Initial galactose transport from water into butanol was faster than initial glucose transport (ratio > 1). This reversal of rates exactly parallels what happens in the intact erythrocyte (4).

DISCUSSION

A major difficulty for investigators studying transport across a liquid–liquid interface is the variability of the experimental results (10). Variations in stirring speed, viscosity of the subsolution, and turbulence at the interface contribute to this variability. The use of a multiple stirring device allows several experiments to be performed simultaneously at the same stirring speed. Comparisons between two chambers run simultaneously yield *relative* rates that are reproducible on subsequent days although the absolute values measured show marked variation. For this reason the data in Table 1 are expressed as ratios. Even then the ratios show a wide range,

but this is not sufficient to obscure the difference between behavior at 0.015 M and 1.3 M initial concentrations in the presence of lipid.

Ting, Bertrand, and Sears (8) have studied the diffusion of salts across a water–butanol interface and have found that the free-energy barrier to diffusion at the interface is much greater than for ordinary bulk diffusion. This is attributed to structuring of water as the interface is approached. These properties are thought to distinguish diffusion across an interface from Fick diffusion in the bulk phase. Table 1 indicates that in the absence of lipid in the model membrane, net flux of both sugars at low and high concentration is qualitatively consistent with a diffusion-like process. No attempt has been made to estimate the magnitude of deviation from Fick diffusion. A clean interface does not give rise to the anomalous kinetics observed for the erythrocyte.

Incorporation of lipid into the model profoundly alters the flux kinetics. No diffusion-like process can explain the observation that at high concentration the entry rates are reversed. The data are consistent with the "carrier" model (equation 1) proposed for the transport of sugar into the intact cell.

10 yr ago Lefevre and Marshall (3) discussed the conformational specificity of the transport system for sugar into the erythrocyte. The carrier was shown to react preferentially with those sugars in which the pyranose ring assumes the C_1 conformation. For this conformation it can further be demonstrated that the affinity for carrier decreases as the number of bulky groups in the axial position increases. Thus D-galactose, possessing a single axially oriented hydroxyl group at C–4, is assigned an instability rating one greater than D-glucose, which has no axial OH group. The possibility exists that the 1-butanol, which saturates the aqueous solution, differentially affects the position of equilibrium between α- and β-forms for both groups.

Implicit in the model studies is the assumption that the hypothetical carrier will be experimentally provided at the interface and that the affinity of glucose for the carrier exceeds that of galactose. Remarkable as it may seem, the data indicate that the addition of erythrocyte lipids to the butanol produces a model having the characteristics of a carrier transport system. The carrier molecule has not been identified.

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