# Evidence for a Carrier Conformational Change Associated with Sugar Transport in Erythrocytes*

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ABSTRACT: Sugar transfer through erythrocyte membranes apparently involves a conformational change in the carrier. The main evidence is that inactivation of the transport system by 1-fluoro-2,4-dinitrobenzene (FDNB) is faster when certain sugars are specifically bound at the carrier site, implying that a chemical group attacked by FDNB becomes more exposed or more reactive than in free carrier. The most readily transported sugars accelerate inactivation most, roughly 5-fold in the case of deoxyglucose. Maltose, firmly bound to the sugar site but not transported, protects the system, while cellobiose, which is similarly bound but very slowly transported, gives less protection. Phloretin, bound to the carrier site at low concentrations, protects, while its glucoside phlorizin exposes the system. Urethane accelerates inactivation in a relatively nonspecific manner, which probably does not depend on binding at the carrier site. Two conformational states of the carrier are proposed, one an intermediate in transport that rapidly reacts with FDNB. Transported sugars induce this form, while maltose and phloretin, which are not transported, stabilize the less reactive conformation.

Biological transport remains an obscure phenomenon, with little known about the machinery responsible for translocating molecules within the membrane. Conformational changes in the carrier could be involved however, and this possibility will be explored here.

Of the several techniques capable of demonstrating conformational transitions in purified proteins only a few are readily applied with intact biological systems such as membranes. One of these depends on increased chemical reactivity of a protein in the presence of a ligand specifically bound to it. While lower reactivity can always be explained by shielding of protein groups from an attacking reagent, heightened reactivity is evidence for a greater exposure of reactive groups following ligand binding, indicating an altered protein conformation (Koshland *et al.*, 1962). Two related effects, substrate-induced changes in heat stability of proteins and in susceptibility to attack by proteolytic enzymes (Citri and Zyk, 1965; Markus, 1965) may also be useful with intact systems.

Increased reactivity of the glucose transport system of erythrocytes toward 1-fluoro-2,4-dinitrobenzene (FDNB)[1] in the presence of substrate was in fact reported some years ago (Bowyer and Widdas, 1958). However, the effect was not shown to be restricted to transported sugars, and the unrelated chemicals, urethane and guanidine, had a similar action. The present study was undertaken to decide if the glucose effect is directly related to transport.

## Materials and Methods

Human blood was obtained from an outdated blood bank supply. All chemicals were of commercial reagent grade.

Rates of sugar transport were measured by the light-scattering method described by Sen and Widdas (1962a). Red blood cells were sedimented in a clinical centrifuge and washed twice in isotonic buffer (0.9% NaCl with 5 mM sodium phosphate buffer, pH 7.5). Packed cells (0.2 ml) were added to 3.8 ml of 130 mM sugar in isotonic buffer and the suspension was incubated in a shaking bath at 37° for at least 1 hr, after which it was transferred to a bath at 25°. The final pH of the suspension was in all cases 7.0, whether the cells were suspended in salt–buffer in the absence of sugar, or with 125 mM glucose, mannose, deoxyglucose, xylose, galactose, fructose, maltose, or cellobiose. An 0.5-ml aliquot was injected into 65 ml of saline solution in the photoelectric apparatus, maintained at 25°.

Sugar exit times were computed according to the method of Sen and Widdas (1962a,b), and were divided by the quantity of sugar that escaped. The resulting figure, $\tau$, is the time in minutes required to transfer 1 mmole of sugar/l. of cell water at the initial transport rate. Rates were normally reproducible to within 5%.

Measurements were of two main types, inactivation by FDNB under various conditions, and sugar exit rates in the presence of reversible transport inhibitors. Rates of FDNB inhibition were determined at 25° in cell suspensions containing 0.2 ml of packed cells, 3.6 ml of salt–buffer solution, and 0.2 ml of a freshly prepared solution of 50 $\mu$l of FDNB in 10 ml of ethanol. The final FDNB concentration was 2.0 mM. Reaction was stopped after a recorded time by suddenly cooling the suspension and diluting it with 8 ml of cold salt–buffer solution. The cells were quickly sedimented in a clinical centrifuge and were resuspended in 3.8 ml of isotonic buffer containing 130 mM glucose, after which they were equilibrated as described above. Similar inactivation experiments were carried out in the presence of a transported sugar such as glucose or deoxyglucose. The reaction mixture was the same as above except that the 3.6 ml of salt–buffer solution contained 130 mM sugar. Aliquots (0.5 ml) of the cell suspension were withdrawn at intervals and sugar exit rates determined directly.

In treatment with 2 mM FDNB in the absence of sugar, exit rates conformed rather well to eq 1 during the first hour

$$\log (\tau/\tau_0) = kt/2.3 \qquad (1)$$

(Figure 1). In eq 1 $\tau_0$ and $\tau$ are exit times for normal cells

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[1] Abbreviation used is: FDNB, 1-fluoro-2,4-dinitrobenzene.

TABLE I: Transport Constants for Various Sugars ($K$, the Half-Saturation Constant, and $V$, the Maximum Exit Rate), and the Inactivation Rate Constant for 2 mM FDNB, $k$.[a]

| Sugar | $K$ (mM) | $V$ (mM min$^{-1}$) | $k \times 10^2$ (min$^{-1}$) |
|---|---|---|---|
| 2-Deoxy-D-glucose | 1.59, 1.77, 2.15 | 158, 179, 181, 185, 218 | 9.2, 10.6, 12.9 |
| D-Glucose | 2.08, 2.10, 2.16, 2.44, 2.79 | 138, 159, 168, 191, 215 | 5.1, 5.3, 5.3, 5.5, 5.5, 5.8, 5.8, 6.0, 6.2, 6.7 |
| D-Mannose | 4.76 | 115, 130 | 5.3, 5.8, 6.4, 7.6 |
| D-Galactose | 14.0 | 128 | 2.5, 3.2, 4.1 |
| D-Xylose | 11.1, 11.7, 12.7 | 117, 142, 144 | 2.3, 2.8, 2.8, 3.0, 3.2, 4.1 |
| None | | | 2.2, 2.2, 2.3, 2.3, 2.4, 2.4 |

[a] The latter was determined in the presence of the same sugars at 119 mM, or in the absence of sugar. Transport was observed in pure aqueous media, inactivation in 5% ethanolic media (see Methods). Recorded values are for various blood samples. All rates measured at 25°, pH 7.0.

and for cells treated $t$ minutes, and $k$ is the rate constant for reaction between carrier and FDNB

$$\text{carrier} \xrightarrow{k} \text{inactive carrier}$$

The reaction was therefore routinely stopped after 30 min and exit rates were determined in duplicate or triplicate. The inactivation rate constant, $k$, was calculated from eq 1.

The progress of inhibition in the presence of glucose (119 mM final concentration) is also shown in Figure 1. The calculated rate constant was the same by either method, *i.e.*, where exit rates were determined directly at short intervals, and where reaction was stopped after 30 min and the cells reequilibrated with glucose. The latter procedure for terminating FDNB inactivation is therefore satisfactory.

Parameters of the sugar transfer system were determined by the method of Sen and Widdas (1962a,b), which depends on following the rate of sugar loss from equilibrated cells into saline media containing low sugar concentrations. Exit times, $\tau$, are linearly related to the latter, and the binding constant, $K$, is taken as the sugar concentration required to double $\tau$. Since the experimental binding constants are far lower than the internal sugar concentration (125 mM), the rate of exit into media containing no sugar is taken as the maximum.

Parameters for reversible inhibition of transport were also determined by the method of Sen and Widdas (1962b). An inhibitor was included in the external medium, either at increasing concentrations in the absence of transported sugar, or at a fixed concentration in the presence of increasing concentrations of sugar. The kinetics of these systems have been summarized by Miller (1969).

## Experimental Results

*Inactivation by FDNB in the Presence of Sugars.* Transport rates for cells treated with 2 mM FDNB along with deoxyglucose, glucose, mannose, galactose, or xylose were determined directly by observing transfer of the particular sugar used in the experiment (see Methods). Inactivation rate constants from a number of experiments are listed in Table I. The observed variability in $k$ may be partly due to slightly different properties of various blood samples. The largest enhancement is produced by deoxyglucose, followed by glucose and mannose.

The relation between glucose concentration and the inactivation rate was also investigated. In these experiments reaction with 2 mM FDNB in the presence of various glucose concentrations was stopped after 30 min by cooling and dilution. Calculated inactivation rate constants are plotted in

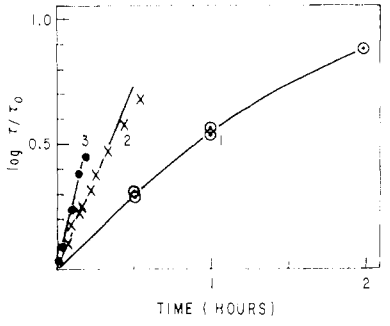


FIGURE 1: Relative glucose exit times, $\tau/\tau_0$, following treatment of cells with 2 mM FDNB for various periods in the presence of 119 mM glucose (curve 2), 119 mM glucose plus 203 mM urethane (curve 3), or with no additives (curve 1).

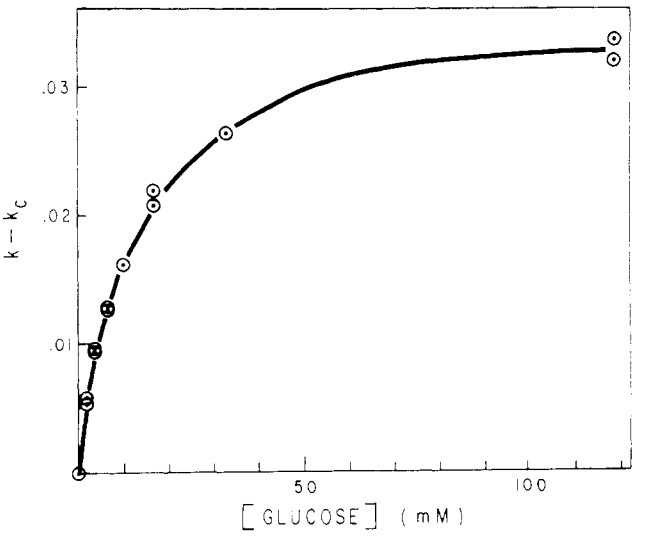


FIGURE 2: Effect of glucose concentration on the rate of inactivation by 2 mM FDNB. Rate constants in the presence and absence of glucose are designated as $k$ and $k_c$, respectively. Half-maximal stimulation is produced by 10.5 mM glucose.

Figure 2 (where the constant in the absence of sugar is $k_c$ and those in its presence $k$). The inactivation rate reaches a maximum at high glucose concentrations, as observed by Bowyer and Widdas (1958). Figure 2 shows pooled results for experiments run on two different occasions 1 month apart; the measured half-saturation constants agreed to within 3%, with an average of 10.5 mM.

To decide if the results conform to 1:1 complex formation between glucose and carrier a kinetic equation was derived for this case and the data replotted. The total rate of inactivation is the sum of rates for free and complexed carrier (eq 2 and 3), where C is the carrier, CS the complex, and C′ and

$$C \xrightarrow{k_c} C' \quad (2)$$

$$CS \xrightarrow{k_x} C'S \quad (3)$$

C′S their inactive forms. The equilibrium between carrier and glucose (S) may be written as eq 4. If the total amount of active

$$C + S \overset{K}{\rightleftharpoons} CS \quad (4)$$

carrier is $C_t = [C] + [CS] = [C](1 + [S]/K)$, then the rate of inactivation may be written as eq 5. After integration this

$$\frac{d([C'] + [C'S])}{dt} = \frac{-dC_t}{dt} = k_c[C] + k_x[C][S]/K = \frac{C_t(k_c + k_x[S]/K)}{(1 + [S]/K)} \quad (5)$$

becomes eq 6, where $C_t^0$ is the total carrier at zero time, and $\tau$

$$2.3 \log (C_t^0/C_t) = 2.3 \log (\tau/\tau_0) = \left(\frac{k_c + k_x[S]/K}{1 + [S]/K}\right)t = kt \quad (6)$$

and $\tau_0$ are exit times for treated and untreated cells, as before. The inactivation constant is given by

$$k = \frac{k_c + k_x[S]/K}{1 + [S]/K} \quad (7)$$

which may be rearranged to

$$1/(k - k_c) = (1 + K/[S])/(k_x - k_c) \quad (8)$$

A plot of $1/(k - k_c)$ *vs.* $1/[S]$ should therefore be linear if a single glucose molecule is involved, as is found (Figure 3).

Besides these sugars, which are readily transported, a number of others were tested, as well as the sugar alcohols sorbitol and mesoerythritol, and the glucose derivative, α-methyl D-glucoside (Table II). None of these compounds readily penetrates red blood cells, though some, like fructose and sorbose, are very slowly transferred by the glucose system (LeFevre, 1961). The sugar alcohols did not alter the inactivation rate at all, and ribose, arabinose, and lactose increased the rate slightly (under 10%). Methyl glucoside, sucrose, sorbose, and fructose accelerated inactivation 20–50%.

*Inactivation by FDNB in the Presence of Transport Inhibitors.* The disaccharides maltose and cellobiose reversibly block glucose transport in red cells (Chen and LeFevre, 1965), as do

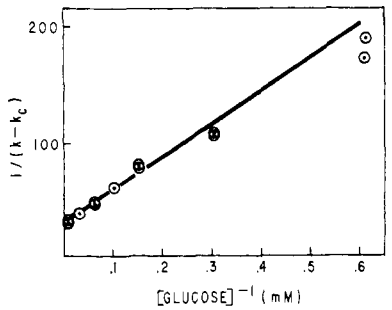


FIGURE 3: Reciprocal plot for glucose stimulation of inactivation by 2 mM FDNB, according to eq 8.

certain chemicals unrelated to sugars such as phloretin and phlorizin. Inactivation rates in their presence are listed in Table III. Maltose, cellobiose, and phloretin retard inactivation, maltose most strongly and cellobiose least. Phlorizin, on the other hand, produces a small acceleration (30%).

*FDNB Inactivation in the Presence of Urethane and Guanidine Hydrochloride.* These substances were reported by Bowyer and Widdas (1958) to accelerate reaction of the carrier with FDNB, confirmed in the case of urethane (Figure 1, curve 3; Table III). A plot of $k$ *vs.* the urethane concentration curves upward (Figure 4), indicating that more than one molecule is involved in accelerating the rate. Guanidine hydrochloride however, at 0.1–0.5 M, did not increase the rate in the presence of glucose, though in its absence there was a small rise (15%).

*Parameters for Sugar Transport and Its Reversible Inhibition.* Binding constants and maximum transport rates for deoxyglucose, glucose, mannose, galactose, and xylose were determined by the method of Sen and Widdas (1962a,b) (see Table I). Pure aqueous solutions were used in these experiments rather than 5% ethanol as in work with FDNB. Therefore to

TABLE II: Inactivation of the Glucose-Transfer System by 2 mM FDNB in the Presence of Various Sugars and Related Compounds, All at 119 mM.[a]

| Compound | Rel Glucose Exit Rate[b] | $k \times 10^2$ (min$^{-1}$) |
|---|---|---|
| None | 50 | 2.30 |
| D-Sorbitol | 50 | 2.30 |
| Mesoerythritol | 50 | 2.30 |
| Maltose | 66 | 1.38 |
| Cellobiose | 53 | 2.12 |
| D-Ribose | 49 | 2.37 |
| L-Arabinose | 48 | 2.44 |
| Lactose | 47 | 2.51 |
| α-Methyl D-glucoside | 43 | 2.81 |
| Sucrose | 43 | 2.81 |
| L-Sorbose | 39 | 3.13 |
| D-Galactose | 37 | 3.32 |
| D-Xylose | 37 | 3.32 |
| D-Fructose | 34 | 3.59 |
| D-Glucose | 17 | 5.90 |

[a] Incubation 30 min at 25°, pH 7.0. [b] Exit rate of untreated cells taken as 100. Rates cited are averages of several determinations.

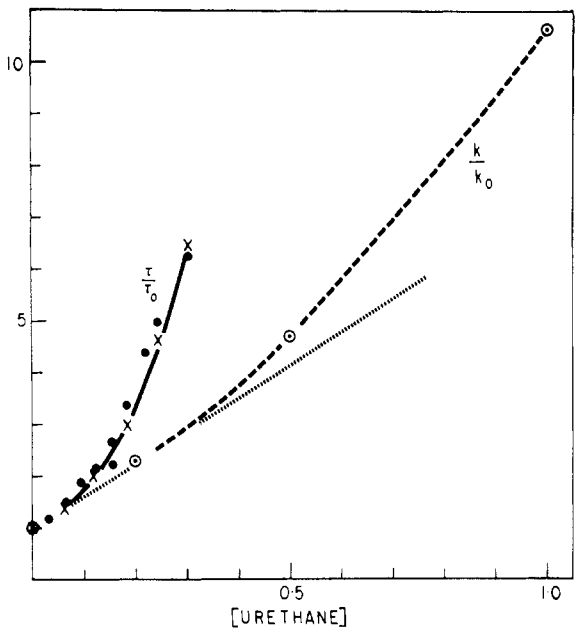


FIGURE 4: Relative exit times for glucose transport ($\tau/\tau_0$) and relative rates of inactivation by 2 mM FDNB ($k/k_0$) in the presence of increasing urethane concentrations, up to 1 M. Inactivation was determined in solutions containing 5% ethanol, and transport rates in pure aqueous solutions (crosses) or 5% ethanol (filled circles).

compare the glucose half-saturation constant for transport and for promotion of FDNB reaction, the transport constant was also measured in 5% ethanolic solution. It was found to be 6.2 mM, about three times higher than without ethanol.

TABLE III: Half-Saturation Constants, $K_i$, for Transport Inhibitors, and Rate Constants, $k$, for Inactivation of the Sugar-Transfer System by 2 mM FDNB in the Presence of Inhibitors and Glucose.[a]

| Inhibitor | Concn (mM) | Glucose Concn (mM) | $k \times 10^2$ ($min^{-1}$)[b] | $K_i$ (mM)[c] |
|---|---|---|---|---|
| Maltose | 119 | 0 | 1.33, 1.38 | 14 |
| Cellobiose | 119 | 0 | 1.93, 2.05, 2.12 | 28 |
| Cellobiose | 238 | 0 | 1.64, 1.77, 2.00 | |
| Phloretin | $4.4 \times 10^{-3}$ | 0 | 1.82, 1.84, 1.86 | $6.6 \times 10^{-4}$ |
| Phloretin | $8.8 \times 10^{-3}$ | 119 | 5.3, 5.5 | |
| Phlorizin | 0.28 | 0 | 2.81, 2.92, 3.13, 3.18 | 0.29 |
| Phlorizin | 0.26 | 119 | 4.8, 5.3 | |
| Urethane | 205 | 0 | 4.8, 5.1, 5.1 | 65[d] |
| Urethane | 205 | 119 | 9.2 | |

[a] All measurements at 25°, pH 7.0. [b] Reaction of 2 mM FDNB in 5% ethanolic media. [c] Determined in the absence of ethanol. [d] Determined with 0.12 M urethane (ethyl carbamate); $K_i$ is concentration dependent (Figure 4).

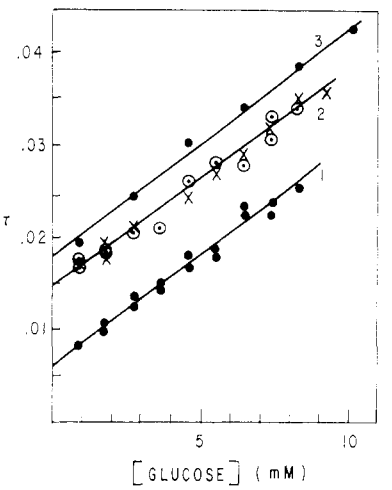


FIGURE 5: Glucose exit times ($\tau$) with varying external glucose concentrations, cells preequilibrated with 125 mM glucose. Curve 1: no added inhibitor. Other points are for transport in the presence of a fixed concentration of a reversible inhibitor. Curve 2: crosses, 18 mM maltose; circles, 40 mM cellobiose. Curve 3: $1.06 \times 10^{-6}$ M phloretin.

Half-saturation constants, $K_i$, for transport inhibitors used with FDNB were also determined (Table III). Phloretin, maltose, and cellobiose acted as competitive, nontransported inhibitors (Miller, 1969). Glucose half-saturation constants were raised in their presence, showing that they interferred with glucose binding (Figure 5). The involvement of a single inhibitor molecule is demonstrated by the linear relationship between $\tau$ and concentration for maltose (Figure 6) and phlorizin (Sen and Widdas, 1962b). Urethane is abnormal in this respect (Figure 4). The upward curvature is similar to that for urethane promotion of FDNB inhibition, and indicates that more than one molecule is involved.

*FDNB Inactivation in the Presence of Fructose Together with Maltose.* To decide if the action of fructose depends on formation of a specific complex the combined effect of fructose and maltose was observed. Maltose does not penetrate the cell, and the entry of fructose, which is normally slow, should be largely blocked by 119 mM maltose, a transport inhibitor. Both sugars should therefore act from outside the cell. In several experiments 119 mM fructose increased $k$ by roughly 30% and 119 mM maltose reduced it to nearly half. Maltose and fructose together, at the same concentrations, did not alter the rate. Maltose, being much more strongly bound than

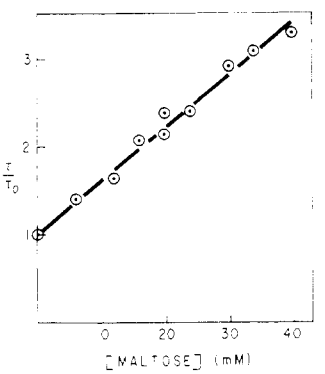


FIGURE 6: Relative glucose exit times ($\tau/\tau_0$) into media containing various maltose concentrations.

fructose (LeFevre, 1961), should have exercised almost its full effect, and the action of fructose may therefore depend only partly on specific complex formation. An altered membrane structure may also be involved, as suggested by the following observations. After incubation of cells with FDNB and 238 mM fructose in the usual manner, the cells became fragile and lysed during rate assays. On the other hand, cells incubated with the same total sugar concentration, but with 119 mM fructose together with 119 mM maltose, remained intact.

## Discussion

Enhancement of FDNB inhibition could result from specific interactions of sugars and reversible inhibitors with the carrier, or from exposure of the latter following general alterations in membrane structure. For various reasons the second alternative can be ruled out. Gross membrane stretching cannot be the cause, since the cell volume did not change during reaction with FDNB and in the case of permeable sugars was identical to that in pure salt solution. Furthermore glucose acts by forming a 1:1 complex, which if not with carrier must involve another specific site in the membrane. The relationship between such a site and the carrier would have to be extremely intimate, and at present the two would be indistinguishable. Other evidence pointing to specific interactions at the carrier site will now be taken up.

A striking observation is that the most readily transported sugars (deoxyglucose, glucose, and mannose) produce far larger accelerations than others. This is not simply a reflection of the concentrations of carrier–ligand complex. For example with 119 mM deoxyglucose, glucose, mannose, galactose, or xylose, most of the carrier was in the complexed form, and yet inactivation was accelerated 5-fold in one case (deoxyglucose) and only 30% in another (xylose). The binding constants in Table I were determined in aqueous solution rather than 5% ethanol (as in inactivation experiments) and here, judging by the observations on glucose, they would be roughly 3-fold higher. Even so the carrier would be between 77 and 95% in the form of complex with the various sugars, which is not nearly enough variation to explain the different accelerations.

The situation with transport inhibitors is similar. Thus phloretin and phlorizin have opposite effects, the first reducing the FDNB reaction rate, the second increasing it. The large acceleration due to glucose or deoxyglucose may be contrasted with the protection afforded by maltose. All three inhibitors are competitive and are probably bound at the sugar site. As expected, therefore, the accelerations by glucose and either phloretin or phlorizin are not additive. Phloretin, it may be noted, alters the rate at the same low concentrations required to block transport ($K_i = 7 \times 10^{-7}$ M).

The mechanism by which urethane accelerates FDNB reaction is probably fundamentally different from that of specific sugars, as shown by the following observations. (1) While the combined effects of phlorizin and glucose are not additive, those of urethane and glucose are, implying that urethane acts outside the sugar binding site. (2) The action of urethane on both transport and FDNB reaction depends on more than the first power of its concentration, and no sign of saturation is seen. Two or more urethane molecules must therefore be involved, in contrast to the one molecule of glucose. (3) Similar behavior is exhibited by a variety of substances, including alcohols and detergents. This topic will be pursued elsewhere (Krupka, 1971).

The significance of behavior with very slowly transported and nontransported sugars is uncertain. For example, sorbose and fructose increased inactivation by 36 and 56%, and even sucrose, which neither penetrates the cell nor inhibits transport (Chen and LeFevre, 1965; Lacko and Burger, 1962), accelerated inactivation about 20%. These increments are small compared to those due to deoxyglucose, glucose, and mannose, but are none the less real. It will be recalled that 238 mM fructose made cells more fragile, whereas the combination of 119 mM fructose and 119 mM maltose did not, and this suggests an altered membrane structure in the presence of fructose. Furthermore maltose only partially overcame the fructose enhancement of FDNB reaction, indicating that fructose need not add to the sugar site. On the other hand, fructose is transported, even though very slowly (LeFevre and Marshall, 1958), partially accounting for its action. The effect of sucrose may be entirely indirect, but in view of its smallness is quite distinct from the large specific acceleration resulting from complex formation with glucose.

Since sugars forming a specific complex with carrier accelerate the FDNB reaction, it may be concluded that they induce a conformational change in the carrier. The protection given by maltose and phloretin is in accord with this conclusion, though at first sight it suggests that protection could be due to steric hindrance. For example, if FDNB attacked a point just outside the site to which monosaccharides are bound, reaction could be obstructed by maltose, which is probably bound at the same place, but which would extend further, being a disaccharide. Phloretin is also fairly large and could protect in the same way. If so, phlorizin, the glucoside of phloretin, and cellobiose, another disaccharide, should also protect. Instead phloretin promotes the reaction and cellobiose protects much less than maltose. It is therefore more likely that a conformational state having low reactivity toward FDNB can be stabilized by certain ligands.

Among specific agents there appears to be a functional relationship between transport and enhancement of FDNB reaction. The most readily transferred sugars were seen to be the most efficient in promoting reaction with FDNB, while maltose, a nontransported sugar (Lacko and Burger, 1962) has the opposite effect and reduces the reaction rate. Both maltose and cellobiose are competitive inhibitors of transport, but cellobiose, unlike maltose, is transferred at an extremely low rate (Lacko and Burger, 1962). The smaller protection given by cellobiose could be related to this. Transport may therefore depend on conversion of a conformational form of the carrier with low FDNB reactivity into another with high reactivity. Thus the latter is probably an intermediate in transport.

The relationship between FDNB reaction rates and the transport parameters for various sugars, such as the series deoxyglucose, glucose, mannose, galactose, and xylose, can be seen to be nonlinear (Table I). This is to be expected however, since the transport parameters are known to be complex. For example, the apparent binding constant varies widely depending on the experiment to measure it; it was less than 2 mM for inhibition of glucose exit by glucose in the external medium, roughly 40 mM in glucose exchange experiments, and 20 mM for inhibition of sorbose entry by equilibrated glucose (Miller, 1968). The constants are therefore not simple indicators of the free energy of binding. Similarly the maximum transport velocity varies depending on whether sugar is present on one or both sides of the membrane. Accordingly the concentration of the altered carrier form could not be directly related to the apparent binding constant, and it would be pro-

portional to the maximum transport rate only if the same intermediate determined the rates of both processes, which is possible but not particularly likely in a reaction involving several intermediates. Furthermore transport is a cyclic process depending on movement across the membrane and back, in contrast to the inhibition reaction.

In view of the complex nature of the measured binding constants it is also not surprising that the half-saturation constant for glucose in promoting FDNB reaction is not identical with that found for transport inhibition. However the comparable magnitude of the constants supports the contention that both are related to binding at the carrier site.

Acknowledgments

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# Inhibition of Sugar Transport in Erythrocytes by Fluorodinitrobenzene*

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ABSTRACT: Inactivation of sugar transport in erythrocytes by 1-fluoro-2,4-dinitrobenzene (FDNB), previously believed to be second order with respect to FDNB and carrier (fourth order overall), is now shown to be first order in both. Apparent second-order behavior for FDNB was due to a secondary, physical action of this compound, which accelerates the reaction with carrier. Apparent second-order behavior for carrier resulted from a gradual decline in FDNB concentration during treatment of the cells. The physical action of FDNB was shared by various agents, including urea, alcohols, and detergents. The evidence shows that urea, urethane, and alcohols add reversibly at two regions in the carrier—one within the sugar site, causing competitive transport inhibition, and another outside bringing about a conformational change similar to that induced by sugars. It is suggested that the second action involves exposure of a hydrophobic region of the carrier, allowing it to pass through a lipid barrier in the membrane.

Inactivation of sugar transport in erythrocytes by 1-fluoro-2,4-dinitrobenzene (FDNB)[1] was reported to be proportional to the square of the concentrations of both FDNB and functional carrier sites, being fourth order overall (Bowyer and Widdas, 1958; Stein, 1969). The relationship for FDNB was taken to mean that inhibition ensues only when pairs of chemical groups in the carriers have reacted with two FDNB molecules. The dependence on carrier concentration could not be explained simply by the interaction of pairs of carriers, however, and suggested that all the units in the system undergo cooperative changes in conformational state, as in the membrane model of Changea ux *et al.* (1967).

If these observations have been correctly interpreted they may provide a unique clue to the mechanism of biological transport. Their importance appears to justify a reexamination of the evidence, which has been undertaken here.

## Methods

Determination of glucose exit rates from human red blood cells, as well as methods of determining rates of inactivation of transport by FDNB, were described previously (Krupka, 1971). The FDNB inactivation rate constant, $k$, was calculated from eq 1, where $\tau_0$ and $\tau$ are sugar exit times for un-

$$2.3 \log (\tau/\tau_0) = kt \quad (1)$$

treated cells and cells suspended in 2.0 mM FDNB for $t$ minutes, respectively. Exit times are expressed in units of minutes per millimole of sugar per liter of cell water (min mM$^{-1}$). The incubation medium consisted of 0.1 ml of red blood cells, 3.6 ml of glucose–salt–buffer solution (130 mM glucose in 0.9%

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[1] Abbreviation used is: FDNB, 1-fluoro-2,4-dinitrobenzene.