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THE ACTION OF INHIBITORS ON THE FACILITATED HEXOSE TRANSFER SYSTEM IN ERYTHROCYTES

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There is now a great deal of evidence indicating that hexoses do not cross the red cell membrane by simple diffusion but by a facilitated mechanism. The results of LeFevre (1948, 1954), LeFevre & LeFevre (1952), Widdas (1951, 1952, 1954), Wilbrandt & Rosenberg (1951) and Rosenberg & Wilbrandt (1955) show that the process behaves as a saturation mechanism, depends on steric configuration and shows competitive and inhibitory effects. It is suggested by most workers that the first stage in the transfer involves the formation of a complex between the hexose and a component of the membrane. Such a reaction is implicit in the kinetic treatments of the above authors (see Bowyer, 1957).

Kinetic treatments show that there are at least three possible mechanisms which would fit the available experimental data:

(1) The carrier system of Widdas (1952) which is equivalent to the non-enzymic carrier of Rosenberg & Wilbrandt (1955). This model postulates the presence of carriers in the membrane which pass backwards and forwards across the membrane owing to thermal agitation, irrespective of whether they are saturated with hexose or not. The carrier could be lipid, lipoprotein, or protein in nature.

(2) The enzymic carrier system of Rosenberg & Wilbrandt (1955). This is similar to the carrier system above except that the formation and dissociation of the carrier complex is catalysed by an enzyme (or enzymes) present in the membrane, which is itself immobile.

(3) The polar 'creep' hypothesis (Bowyer & Widdas, 1956*a*) which is equivalent to the polar pore system suggested for glycerol transfer by Stein & Danielli (1956). In this case no mobile membrane component is postulated, but it is suggested that a series of hexose adsorbing sites exists through the membrane along a polar region from outside to inside. Only one hexose adsorbing site is exposed to each interface. The hexose would then cross the

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membrane, after initial complex formation at the interface, by a chain or 'creep' process along the hexose adsorbing sites. Such a polar region could rather loosely be termed a pore. The possibility that the pore is occupied by a protein helix has been suggested by Burgen (1957).

The above possible mechanisms will be referred to as carrier, enzyme and pore theories respectively.

Until recently the kinetic approach has failed to distinguish between the three possible mechanisms and, in fact, it is difficult to visualize evidence which could not be interpreted in more than one way. Therefore other approaches have been combined with kinetics in the hope that further light may be thrown on the nature of the transfer process.

It has been known for some time that glucose transfer in the human red cell is inhibited by mercuric chloride, *p*-chloromercuribenzoate, iodine and phlorrhizin (LeFevre, 1948), phloretin (Wilbrandt, 1950), polyphloretin phosphate (Wilbrandt & Rosenberg, 1950), the lachrymators allyl*iso*thiocyanate, bromacetophenone and chloropicrine (Wilbrandt, 1954), 2:4-dinitrofluorobenzene (DNFB), dinitrochlorobenzene and dinitrobromobenzene and urethane (Bowyer, 1954; Bowyer & Widdas, 1956*a*).

The mercurial compounds react with sulphydryl (—SH) groups and also with amino (—NH_2) groups, though less readily with the latter (Edsall, 1954). However, the phenyl mercurials are unspecific in their action and have a general depressing action on enzyme activity (Smalt, Kreke & Cook, 1957). It is suggested that they may act by causing denaturation.

Dinitrofluorobenzene reacts irreversibly with —SH, —NH_2 groups and with phenolic hydroxyl. When red cells are inhibited with DNFB, glucose permeability cannot be restored by repeated washing with alcoholic saline, indicating that the inhibition is due to an irreversible action of the inhibitor.

The inhibiting action of phlorrhizin is not understood. The inhibitor is known to have an effect on the energy-supplying reactions of the cell, but these appear to play no part in the glucose transfer. Wilbrandt (1954) suggests a possible inhibition of phosphatase. He points out that lachrymators are known to inhibit hexokinase, but ATP and Mg^{2+}, which accelerate hexokinase activity, did not accelerate glucose transfer in the red cell (Wilbrandt, 1954). Moreover, the action of these enzyme inhibitors is non-specific and possibly indicates the participation of a protein in the transfer mechanism rather than a specific enzyme.

Using polyphloretin phosphate labelled with ^{32}P, Wilbrandt (1954) showed that the highly polymerized molecule did not penetrate the red cell and suggested that the action of the inhibitor was on the outer surface of the cell membrane. He also showed that polyphloretin phosphate inhibited the exit of glucose more than the entry. From this he concluded that there were two

enzymes on the outer surface, one of which was inhibited by polyphloretin phosphate and preferentially involved in outward transport. More recent experiments by Wilbrandt, Frei & Rosenberg (1956) showed that the rate constant for the entry of glucose into human red cells was higher than that for exit (K_E entry $= 7 \times 10^{-2}$; K_E exit $= 4 \times 10^{-2}$).

The question has therefore arisen as to whether the transfer process is more complex than can be represented by present treatments, which presume a symmetrical transfer system, i.e. one in which rates of entry and exit should bear similar relationships to the concentrations of hexoses when only the direction of the concentration gradient is reversed. A real discrepancy between entry and exit rates as described by Wilbrandt *et al.* (1956) would be of importance in attempting to understand the kinetics of the transfer process and would affect the interpretation of inhibitor results.

The present paper therefore describes (*a*) further experiments to investigate differences in rate constants for entry and exit and (*b*) experiments with inhibitors of three different types:

(1) a competitive inhibitor, urethane, which can penetrate the red cell;
(2) a competitive inhibitor, polyphloretin phosphate, which cannot enter the cell;
(3) the irreversible inhibitor DNFB.

A preliminary account of part of this work has been given to the Physiological Society (Bowyer & Widdas, 1957).

METHODS

The apparatus used (Ørskov type described by Widdas (1953*a*)); the experimental technique and the method of analysing the results of entry experiments have been described previously (Widdas, 1954). Human erythrocytes were used throughout. For exit experiments cells previously equilibrated in a glucose medium were centrifuged and a fixed quantity (about 3 mm^3) of packed cells was resuspended in 0·2 ml. of the supernatant and added rapidly to 21 ml. buffered saline in the cuvette. The resuspension was found necessary in order to obtain a rapid dispersion of the cells so that their volume change could be followed more accurately. The diluted suspension only contributed a concentration of glucose of 0·05 mM to the outside medium, which was negligible compared with concentration inside the cells. This can be allowed for when necessary.

Solutions of inhibitors were made up in buffered isotonic saline. A stock solution of DNFB was prepared by dissolving 44·4 mg in 11·4 ml. ethanol before making up to 100 ml. with saline. Since DNFB is readily hydrolysed a fresh stock solution was made up for each experiment. This was diluted as required, the proportion of alcohol to buffered saline not being allowed to exceed 0·4 ml. in 21 ml., in order to eliminate any effect on the stability of the red cell suspensions in the cuvette or upon hexose transfer.

The polyphloretin phosphate used was obtained from Leo, Hälsingborg, and had an estimated molecular weight of 15,000.

RESULTS

The entry and exit rates for the transport of glucose across the human red cell membrane were measured over the range 38–76 mM. The results were analysed as in the previous paper (Widdas, 1954) using the approximation that for glucose,

$$\text{transfer rate} = K\phi\left(\frac{1}{C_i} - \frac{1}{C_o}\right), \tag{1}$$

where C_o and C_i refer to the outside and inside concentration of hexose, ϕ is the equilibrium constant, and K is a constant. This approximation is derived from the more precise equation

$$\text{transfer rate} = K\left(\frac{C_o}{C_o+\phi} - \frac{C_i}{C_i+\phi}\right), \tag{2}$$

on the assumption that both C_o and C_i are very large relative to ϕ (see Widdas, 1951; Wilbrandt & Rosenberg, 1951).

In order to calculate the transfer rate from equation (2) it is necessary to insert numerical values for ϕ, and these have been taken from Widdas (1953*b*, 1954) and LeFevre (1954).

The rate constants for exit and entry, calculated from equations (1) and (2), are compared in Fig. 1.

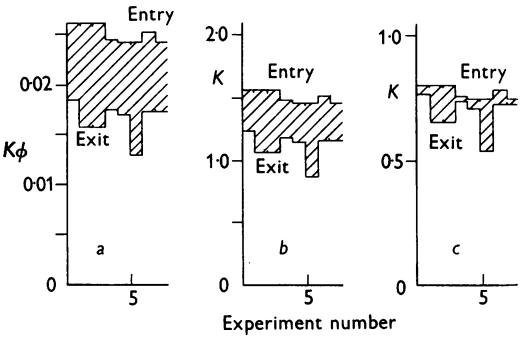

Fig. 1. Rate constants for entry and exit of glucose. Human erythrocytes; temp. 37° C. Exit experiments carried out from 76 to 38 mM glucose; entry from 38 to 76 mM. Results calculated (*a*) from eqn. 1, (*b*) from eqn. 2 ($\phi_g = 7$ mM), (*c*) from eqn. 2 ($\phi_g = 17$ mM).

The results from equation (1) give rate constants for glucose entry, which exceed those for exit by 40%. Wilbrandt *et al.* (1956) reported a difference of the order of 75%. The use of equation (2) reduces the difference to about 12%, showing that the errors due to the approximation fall unevenly on the equations for entry and exit.

In the case of sorbose, the rate constants for exit and entry are not calcu-

lated from the approximate equation (1), as the equilibrium constant (ϕ_S) is high compared with the concentrations. Errors arising from the use of equation (1) are thus not introduced in the sorbose results. Using a diffusion type equation (Widdas, 1954), rate constants for exit and entry differ by 15%, which is the same order of magnitude as experimental error.

It therefore appears justifiable to look at the results of inhibitor experiments in terms of a symmetrical transfer system.

Theoretical considerations

Since the experimental conditions were varied and the processes of inhibition might operate in different ways, it was desirable to express all the results initially in a way which made no assumptions as to the underlying processes.

If P_o represents any rate constant of the hexose transfer system in the absence of inhibitor, and P_i the same constant when inhibitor is present, then the fraction $\frac{P_o - P_i}{P_o}$ represents the fractional inhibition of the transfer system independent of the units used. This treatment could be applied to experiments in which the inhibition of sorbose entry, glucose entry, or glucose exit into a glucose-free medium was measured, although the rate constants found in the three types of experiment have different units. The fractional inhibition should be some function of inhibitor and hexose concentrations, and can conveniently be compared with theoretical predictions.

Competitive inhibitors

If two species of substrate are capable of reaction with the membrane component, the state of adsorption equilibrium of each species separately may be defined by

$$\theta_A = \frac{x}{1+x} \quad \text{and} \quad \theta_B = \frac{y}{1+y}$$

where θ is the fraction of the complex

$$x = \frac{C_A}{\phi_A}, \quad y = \frac{C_B}{\phi_B},$$

and C and ϕ refer to the appropriate concentrations and equilibrium constants respectively.

In a competitive system

$$\theta_A = \frac{x}{1+x+y} \quad \text{and} \quad \theta_B = \frac{y}{1+x+y}.$$

In the treatments which follow, species A is the hexose of which the rate of penetration is measured, and species B is the inhibitor. Subscripts 1 and 2 are used to indicate outside and inside the cell respectively.

The basic assumption in the pore hypothesis is that specific sites in the two interfaces correspond and that transfer can only occur if one site is occupied by hexose and the other is not. The inward flux is thus proportional to the probability that a site in interface S_1 is saturated (θ_1) multiplied by the probability that the corresponding site in S_2 is unsaturated ($1-\theta_2$). Similarly, outward flux will be proportional to the product $\theta_2(1-\theta_1)$.

The net transfer may be represented as

$$\underset{S_1 \to S_2}{\text{Transfer}} = K\{\theta_1(1-\theta_2) - \theta_2(1-\theta_1)\}, \tag{3}$$

which in the simplest experimental conditions reduces to

$$\underset{S_1 \to S_2}{\text{Transfer}} = K(\theta_1 - \theta_2), \tag{4}$$

which is identical to the simplest form of the carrier kinetics. Inserting the conditions appropriate to competitive inhibition, however, we have

$$\underset{S_1 \rightarrow S_2}{\text{Transfer}} = K\left(\frac{x_1}{1+x_1+y_1} \times \frac{1}{1+x_2+y_2} - \frac{x_2}{1+x_2+y_2} \times \frac{1}{1+x_1+y_1}\right), \tag{5}$$

and theoretical dependence of the fractional inhibition on values of x and y for different experimental conditions can be derived from equation (5).

A carrier system may be regarded as made up of many individual units. A cycle of activity for one unit would result in transfer if the carrier crossed from S_1 to S_2 saturated with hexose and returned from S_2 to S_1 unsaturated. To a first approximation the probability of this event would be proportional to $\theta_1(1-\theta_2)$. Similarly, the probability of hexose transfer from S_2 to S_1 would be $\theta_2(1-\theta_1)$, and net transfer would be given by equation (3).

In a simple carrier model it is also necessary to allow for the possibility of exchange. Thus it would be sufficient for net transfer of one sugar if the 'carriers' returned unsaturated or saturated with the other sugar.

This can be represented by

$$\underset{S_1 \rightarrow S_2}{\text{Transfer}} = K\left(\frac{x_1}{1+x_1+y_1} \times \frac{(1+y_2)}{1+x_2+y_2} - \frac{x_2}{1+x_2+y_2} \times \frac{(1+y_1)}{1+x_1+y_1}\right) \tag{6}$$

Evaluation of x and y

In order to use the above equations, it is necessary to determine values of ϕ for glucose and the inhibitors so that concentrations can be expressed as multiples of the equilibrium concentration.

To do this the inhibition of sorbose entry by inhibitor or by glucose can be used (in this case glucose is treated as an inhibitor). There is considerable evidence (LeFevre, 1953; Widdas, 1953*b*, 1954) that the equilibrium constant for sorbose is high and that, for the concentration at which the experiments are carried out, $x < 0{\cdot}05$ and may be neglected without serious error.

With this approximation, equation (6) reduces to

$$\frac{P_o - P_1}{P_o} = \frac{y}{1+y};$$

that is, 50% inhibition will occur when $y=1$ and ϕ will equal the concentration of inhibitor at which sorbose entry is reduced to 50%.

Equation (5) reduces to

$$\frac{P_o - P_1}{P_o} = \frac{y(2+y)}{(1+y)^2},$$

and 50% inhibition occurs when $y=0{\cdot}414$; thus $\phi = 2{\cdot}42\ C$, where C is the concentration at which sorbose entry is 50% inhibited. This would represent the case of an inhibitor which reacted with the glucose adsorbing site or carrier, but did not move through the membrane on the hexose system.

The value of ϕ obtained thus depends on the different approaches and assumptions made, but as equations (5) and (6) apply equally to a pore or carrier model, they have been used to predict fractional inhibitions which are compared with experimental results.

For this purpose equation (5) can be written in the form

$$\frac{P_o - P_1}{P_o} = \frac{y_1 y_2 + y_1(1+x_2) + y_2(1+x_1)}{(1+x_1+y_1)(1+x_2+y_2)}, \tag{7}$$

which can be further simplified when $y_1 = y_2$ or when $x_1 = 0$, as in exit experiments.

If $y_2 = 0$, i.e. if the inhibitor cannot penetrate the cell, equation (7) becomes

$$\frac{P_o - P_1}{P_o} = \frac{y_1}{1+x_1+y_1} \tag{8}$$

and for exit experiments when $x_1 = 0$

$$\frac{P_o - P_1}{P_o} = \frac{y_1}{1+y_1}. \tag{9}$$

Inhibitor results

Competitive inhibition by urethane. The penetration of urethane into human red cells is very rapid. The rate of penetration is greater than that for glycerol and is not suitable for quantitative measurement by the methods used in these experiments. Tests with cells in which the hexose transfer had been inhibited more than 95% by DNFB still showed urethane entry too rapid to measure quantitatively and it may be presumed that the hexose system is not essential for urethane penetration of red cells. The evidence for the reaction of urethane with the hexose transfer system is based solely on its behaviour as an inhibitor.

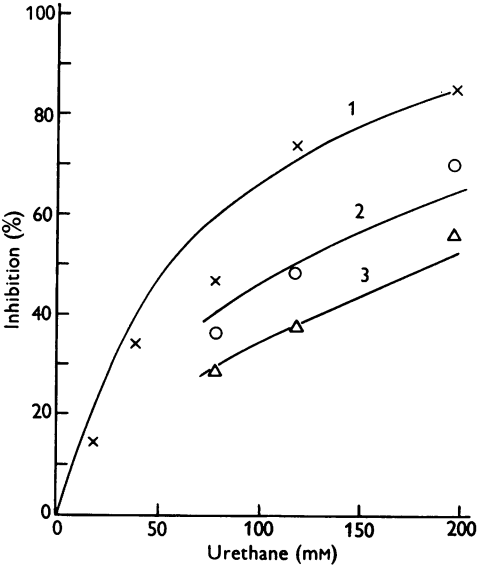

Fig. 2

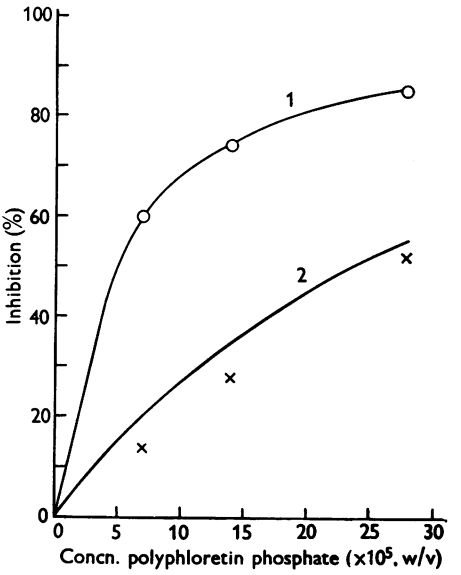

Fig. 3

Fig. 2. Inhibition of hexose transfer by increasing concentrations of urethane. ×, sorbose entry; ○, glucose exit; Δ, glucose entry. Curve 1 drawn according to eqn. 5 to fit the experimental results (y = urethane, mM/137). Curves 2 and 3 are lines predicted from eqn. 7 using same values of y. Values of x obtained from $x = C_g/\phi_g$ where $\phi_g = 7$ mM.

Fig. 3. Inhibition by polyphloretin phosphate. ○, glucose exit; ×, glucose entry. Curve 1 drawn according to eqn. 9 to fit experimental results (y = concn. polyphloretin phosphate (w/v) (5×10^{-5})). Curve 2: predicted line drawn from eqn. 8 using same values of y. Values of x obtained from $x = C_g/\phi_g$, where $\phi_g = 7$ mM.

Its rapid penetration, however, makes it justifiable to assume that it will maintain an equilibrium in which its concentration is equal inside and outside the cell and its inhibitory activity agrees with predictions made on this basis.

In Fig. 2 are shown the percentage inhibitions of sorbose entry, glucose exit and glucose entry as the urethane concentration is varied. For a given urethane concentration it is found that inhibition of sorbose entry > inhibition

of glucose exit > inhibition of glucose entry. This is the order expected on theoretical grounds for a competitive inhibitor and is quantitatively of the right order when the results are treated as described in the Theoretical section. The lines 2 and 3 of Fig. 2 are the predicted values using values for ϕ_g and ϕ_u derived from sorbose inhibition. It is assumed that the inhibitor does not move through the membrane on the hexose transfer system but competitively inhibits at each interface.

Competitive inhibition by polyphloretin phosphate. Two types of experiment were performed on each cell suspension, which gave respectively the inhibition of rates of exit and entry. Repeated experiments at different concentrations of polyphloretin phosphate were carried out. Since this inhibitor does not penetrate the cells, the inhibition of exit into a glucose-free medium should depend on inhibitor concentration in a relatively simple manner, indicated by equation (9). This is a saturation type of equation and fits the experimental results when the half-saturation value ($y=1$) is fixed at 5×10^{-5} (w/v). This has been done in Fig. 3. Using this value, the percentage inhibition of entry can be predicted from equation (8) by inserting the appropriate values for x_1. Line 2 in Fig. 3 has been drawn in this way. The observed inhibitions of glucose entry agree reasonably well with prediction.

Irreversible inhibition by DNFB. Quantitative experiments with this inhibitor previously reported (Bowyer & Widdas, 1956*a*, *b*), indicated that the over-all reaction of DNFB with the red cell was complex and had a high Q_{10}. The reaction of DNFB with the membrane is presumed irreversible, as the inhibition cannot be reversed by repeated washing with alcoholic saline buffer.

At concentrations of DNFB of the order of 1 mM the development of inhibition required some hours' incubation at room temperature, but the progress of the reaction could be arrested at any time by washing the cells with DNFB-free buffered saline. By carefully controlling the experimental conditions, reasonably reproducible fractional inhibitions were obtained. However, entry inhibition developed more slowly than inhibition of exit (see Fig. 4).

It has been postulated (Stein & Danielli, 1956) that in the human red cell glycerol is transferred by a facilitated diffusion, but independently of the hexose system. A comparison of the inhibition of the entry of glucose and of glycerol by DNFB in human red cells also showed a difference in the rate of development of inhibition. Glycerol transfer was affected more slowly. This is shown in Fig. 5.

In the course of this study it was observed that when cells were incubated with DNFB in the presence of glucose, the inhibiting reaction proceeded more rapidly. This effect was investigated with varying glucose concentrations, with the result shown in Fig. 6. The shape of this curve suggests a saturation phenomenon, and the glucose concentration giving half the maximum effect

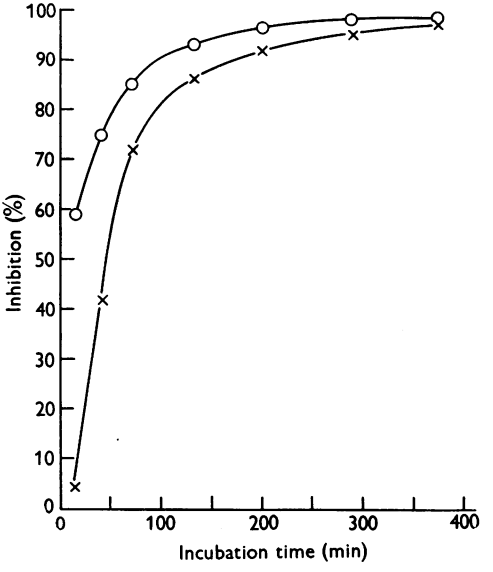

Fig. 4

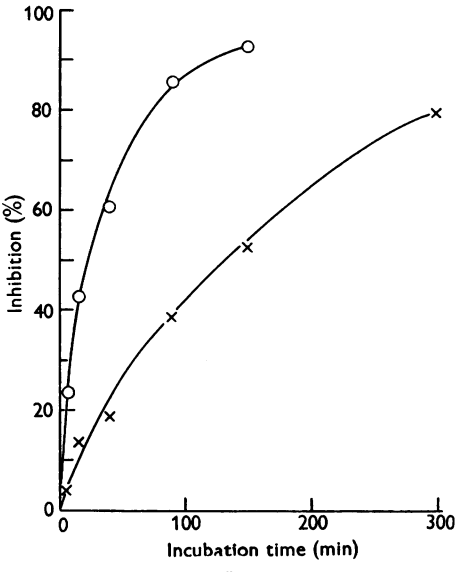

Fig. 5

Fig. 4. Development of inhibition of glucose entry and exit on incubation with 1·4 mM DNFB at 20·5° C in presence of 100 mM glucose. ○, glucose exit; ×, glucose entry. Exit and entry measured at 37° C.

Fig. 5. Development of inhibition of glucose and glycerol entry on incubation with 1·4 mM DNFB at 24° C. ○, glucose entry; ×, glycerol entry. Entries measured at 27° C.

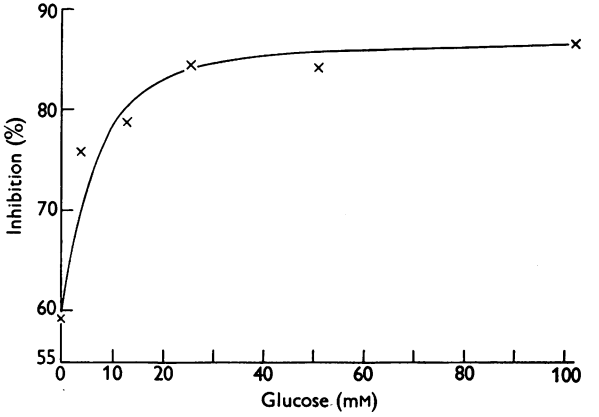

Fig. 6. Acceleration of inhibitory reaction of DNFB on glucose entry into red cells by incubation in the presence of glucose. Incubation at 20° C for 132 min with 1·4 mM DNFB.

corresponds reasonably with the concentration of glucose which half saturates the hexose transfer system at this temperature. This effect is the opposite of what might be expected if glucose and DNFB were reacting with the same component of the membrane, and its full interpretation is not yet clear.

It has been suggested that hydrogen bonds play an important part in the formation of the complex with the membrane component (Danielli, 1954; Bowyer & Widdas, 1955). In view of this possibility it was considered that the reaction of glucose with the membrane component might expose reactive groups by forming external hydrogen bonds, thus facilitating DNFB reaction. To test this hypothesis, known hydrogen bonding reagents, e.g. urea, urethane and guanidine, were substituted for glucose in the DNFB incubating medium. The high concentrations normally used for denaturation of proteins by these substances could not be used, as they led to instability of the cell suspensions and in some cases caused haemolysis. However, positive acceleration of DNFB inhibition was obtained with urethane and guanidine at lower concentrations. The results of such experiments are shown in Table 1. Experiments with urethane previously described indicate that it competes with hexoses for the transfer system, and thus its action is presumably similar to glucose in accelerating DNFB inhibition.

TABLE 1

Inhibition of glucose entry into red cells after incubation at 20° C with 1·4 mM DNFB

Time of incubation (min)	Inhibition (%)			
		Incubated in presence of		
	Control	Glucose 50 mM	Urethane 100 mM	Guanidine 100 mM
132	53	80	—	80
180	64·5	92·5	84·5	—

DISCUSSION

The results described in this paper show that the use of fuller equations in analysing experiments of exit and entry reduces the difference in the rate constants to the order of 12%, which approximates to experimental error. This error is usually of the same sign, i.e. the rate constant for exit < for entry, but this may be due to systematic errors. For example, the contribution of haemoglobin to the internal osmotic pressure is not a linear function of concentration and may introduce a small systematic error between conditions of shrinking and swelling used in entry and exit experiments in the Ørskov technique. In the studies with urethane and polyphloretin phosphate the greater degree of glucose exit inhibition can be accounted for on the basis of a symmetrical system, provided the reactions at both interfaces are regarded as essential to the transfer process and are provided for in the kinetic treatments.

These results do not disprove the enzyme theory, which gives similar kinetics. It is unnecessary to suggest separate enzymes for the formation and hydrolysis of the complexes and that these are differently affected by polyphloretin

phosphate. In fact, such a system would be a case of Maxwell's Demon and would cause accumulation of glucose. This has not been reported. Further, since urethane acts on both the outer and the inner interfaces, a preferential effect on the hydrolysis of complexes would cancel out and entry and exit should be inhibited equally. The increased inhibition by urethane of exit relative to entry would need to be explained in a different way.

The reaction of DNFB with the membrane appears to be irreversible, as the inhibition cannot be removed by washing. Therefore DNFB cannot be acting as a competitive inhibitor in the normal manner. However, if the inhibition is non-competitive there should be no difference in the development of the inhibitions of entry and exit.

An alternative possibility is that DNFB causes 'internal competition'. That is, the DNFB is not bound to the specific glucose-adsorbing site but to a neighbouring site. The DNB–membrane component could then compete for the glucose site. The gradual production of an 'internal competitor' on both sides of the membrane would behave like increasing amounts of urethane, and would give a result in which inhibition of entry developed more slowly than inhibition of exit.

That the inhibition by DNFB of glycerol entry develops more slowly than the inhibition of glucose entry might be due to a difference in chemical reactivity of the two mechanisms, and offers additional evidence of the independence of the respective transfer systems.

Recent results by Park, Post, Kalman, Wright, Johnson & Morgan (1956) confirmed the prediction made by Widdas (1952) of 'counter transport'. This involves the transport of hexose against a concentration gradient under certain competitive conditions. Park *et al.* (1956) incubated red cells in xylose until the external and internal concentrations were approximately equal. They then added glucose to the external medium and the internal xylose concentration fell, i.e. xylose had left the cell against a concentration gradient as glucose entered. Such an 'uphill' transfer is only temporary. The prediction was made on the basis of the carrier hypothesis, and the phenomenon can readily be explained in terms of a mobile membrane component with which xylose and glucose combine competitively at the outer interface, while initially xylose alone combines with it at the inner interface. Similar results have been obtained by Rosenberg & Wilbrandt (1957) for glucose and mannose. The 'counter transport' cannot easily be explained in terms of a non-mobile membrane component if it is postulated that only one site is exposed to the medium at each interface (see below).

The possible role of enzymes in the transfer process might be re-examined in view of the increased knowledge of transferase activity of hydrolytic enzymes (Morton, 1955). Morton has shown that during the hydrolysis of a phosphate ester molecule (the donor) by an enzyme, the phosphate group is

transferred to a second molecule (the acceptor); e.g. when phenyl phosphate is hydrolysed by acid phosphatase, primary alcohols, glycols, glycerol and glucose can act as acceptors. Hydrolysis by alkaline phosphatase transfers phosphate to glucose, fructose and glycerol, while hydrolysis by hexose diphosphatase transfers phosphate to sorbose. It is possible for the same enzyme to hydrolyse the phosphate ester produced, and so the original molecule is reformed.

Phosphatases are known to be present in regions where transfer of glucose takes place, e.g. proximal kidney tubule, intestinal mucosa and red cells, and it has long been postulated that they play some part in the transfer mechanism (Danielli, 1953).

In the light of recent work it is possible to attempt a synthesis of the enzyme, carrier and pore models. The basic postulate would be an enzyme molecule (e.g. we might consider a phosphatase) at each interface. The molecule, following Morton's suggestions, would have a donor site and an acceptor site. The first stage in transfer would be the attachment of glucose to the acceptor site. It is necessary to postulate the existence of a donor molecule attached to the donor site, e.g. it could be a phosphate molecule similar to Morton's phenyl phosphate, or a physiological compound. When this molecule is hydrolysed the glucose is esterified and thereby displaced from the site. At least two models would then be reasonable to explain transfer through the membrane:

(1) The glucose ester could be transported across the lipid membrane by diffusion. At the inner interface the molecule would become attached to the donor site on the enzyme and be hydrolysed. The glucose would be liberated to the inside of the cell and the phosphate, for example, would become attached to another acceptor molecule. An essential step in the transfer is the return of the (phosphate) compound to the outer interface. Thus the complete circulation of one phosphate or similar molecule is part of the transfer and in this respect the system could be called a 'carrier' system. No discontinuity of the non-polar membrane need be presumed, and this model is essentially similar to Rosenberg and Wilbrandt's enzymic carrier transport.

(2) The enzyme sites may be located at discontinuities in the lipid membrane (polar pores) and the glucose phosphate could either diffuse or be transferred directly (or via intermediate sites) to a donor site at the inner interface. The same circulation of phosphate would be necessary either across the membrane or in local circuits between intermediate sites in the pore. This model would be adequate to explain 'counter transport'.

SUMMARY

1. The entry and exit rate constants for the transport of glucose across the human red cell membrane have been determined over a range of concentrations from 38 to 76 mM. Although the constant for entry exceeds that for exit, it has been shown that the major part of this difference is due to approximations in the equations used, and that to postulate an asymmetrical transfer system is unjustifiable.

2. A symmetrical system of transfer can also account for different degrees of entry and exit inhibition observed in experiments with inhibitors such as urethane and polyphloretin phosphate, provided that the reaction at both interfaces is allowed for in the kinetic treatment.

3. The inhibitory reaction of dinitrofluorobenzene (DNFB) with the red cell appears to be irreversible and the development of inhibition to be accelerated by glucose. During development of inhibition on incubation with DNFB exit is affected more than entry. It is difficult to account for these results in terms of a non-competitive inhibition. The possibility of an 'internal competitor' has been discussed.

4. Some proposed models of transfer have been discussed in the light of the present and recent work.

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