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PERMEABILITY OF THE HUMAN RED CELL TO LABELLED GLUCOSE

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The permeability of the human red cell has been much studied particularly by the indirect photo-electric method of Orskov (1935). The results of such experiments have shown that the permeability of the cell membrane is passive in that movement always occurs down the concentration gradient, but that the movement does not obey simple diffusion laws (for reviews see LeFevre, 1954; Wilbrandt, 1954; Bowyer, 1957. Thus, for example, the entry of glucose into the red cell is retarded as the internal concentration rises to a far greater extent than would be expected for diffusion. To explain these phenomena various hypotheses depending on the presence of specific carriers have been put forward (Widdas, 1951, 1952, 1953, 1954; Wilbrandt & Rosenberg, 1951; LeFevre & LeFevre, 1952; Rosenberg & Wilbrandt, 1955). To gain some further insight into the mechanism of glucose transport it was decided to make some attempt to measure the flux of isotopically labelled glucose molecules.

A preliminary account of some of the results has already been given (Britton, 1956). More recently LeFevre & McGiniss (1960) have reported some measurements obtained by using an essentially similar technique.

METHODS

Blood was obtained by venepuncture with 3·8 % sodium citrate as anticoagulant. In most cases the blood of one donor was used (H.G.B.) but occasionally other donors were employed without any significant difference in the results. Out-of-date citrated blood from the blood bank was also tried but showed excessive haemolysis. The cells were washed five times with normal saline at room temperature to remove fibrin and plasma proteins and were then incubated with the suspending medium at 37° C, the suspending medium being replaced several times. Finally, the majority of the medium was removed and the radioactive glucose added (dissolved in a small quantity of suspending medium), and the mixture incubated for about 20 min. The final haematocrit was usually between 80 and 90 %. The suspending medium was based on that of Widdas (1953) and consisted of 90 vol. of NaCl 0·85 g/100 ml. water and 10 vol. of 0·1 M phosphate buffer pH 7·4. The appropriate quantity of glucose was dissolved in this solution.

^{14}C glucose generally labelled was obtained from Radiochemicals, Amersham, and was used both without further purification and with purification by chromatography on No. 3

Whatman paper, *n*-butanol, ethyl alcohol and water, 10:1:2 (v/v) being used as a solvent. The latter step was found to be desirable if the radioglucose was not fresh, since radiochemical degradation products appear to be produced which cross the red cell membrane readily.

Radioactive counting was carried out with an end-window counter. In early experiments samples of the fluid to be counted were dried on paper squares (Long, 1957) or disks (Britton & Long, 1959), estimates being carried out in quadruplicate. In later experiments samples of the solutions were deproteinized with zinc sulphate and barium hydroxide (Somogyi, 1945) and then desalted by running through columns of 'Bio-deminrolit' (Permutit Ltd.) saturated with CO_2. Duplicate samples of the desalted solutions were dried on well polished 1 cm nickel planchettes, a small quantity of 'Teepol' (Shell Chemicals Ltd.) being used as a spreading agent on the planchette. Not more than 100 μg of the solid material was dried on each planchette and counting was therefore at infinite thinness.

Glucose concentrations were determined by the Somogyi (1945) iodimetric method after deproteinizing with zinc sulphate and barium hydroxide.

Sucrose was determined by the Cole–Roe method as described in Bacon & Bell (1948). The appropriate quantities of glucose were incorporated in the blanks and standards.

Haematocrits were determined in duplicate in Wintrobe haematocrit tubes, centrifuging being carried out for 2 hr at 2000 *g*. Trapped fluid was determined with sucrose. The cells were prepared and incubated with glucose 55·6 mM under the conditions of an actual experiment and then sucrose dissolved in a small quantity of the suspending medium was added. After mixing and centrifugation the sucrose concentration in the supernatant was determined. In four separate samples of cells the mean volume of trapped fluid varied between 3 and 5 % of the red cell column. A figure of 4 % of the red cell column has therefore been assumed for all calculations.

In order to study the equilibration of labelled glucose it is necessary to separate the cells from the suspending fluid. However, conventional centrifugation proved to be much too slow for the high rates of equilibration found. Pilot experiments were therefore carried out in which small volumes of packed cells, which had been equilibrated with glucose, were rapidly pipetted into large volumes of ice-cold saline before centrifuging. Despite the cooling, large amounts of glucose were found to have leaked from the cells during the separation procedure. 10^{-3} M-$HgCl_2$ was therefore added to the ice-cold saline, since this was known to inhibit glucose transport (LeFevre, 1948). Under these conditions the exit of glucose from the cells was found to be almost completely arrested. Mercurial solutions of pH 7·4, 6·5 and 4·8 were tried, on the suggestion of W. F. Widdas (personal communication), that the glucose flux may be further reduced at low pH. The impression was gained that pH 6·5 represented an optimum. The final medium usually used was made up of 10^{-2} M-$HgCl_2$ in 1 % NaCl 90 vol. and 0·1 M phosphate buffer pH 6·5 1 vol.: the concentration of $HgCl_2$ was increased to allow for possible uptake by extracellular Hb and the solution was made slightly hypertonic to allow for the tonicity of the glucose contained in the cells. In experiments in which a glucose concentration of 267 mM was used the NaCl concentration was increased to 1·8 % to prevent osmotic haemolysis.

To test the arrest of the exit of glucose, 1 vol. of cells loaded with glucose was transferred rapidly to 5 or 10 vol. of the mercurial solution which had been chilled to 0° C, and the mixture was quickly mixed by inversion and immediately centrifuged at 0° C. A portion of the supernatant was removed for analysis 1½–2½ min after the commencement of the procedure. To test the effectiveness with which radioactive exchange was prevented the cells were initially equilibrated with labelled glucose and unlabelled glucose was added to the mercury solution. Very high concentrations of glucose in the mercury solution were avoided, since the effectiveness of the mercury seemed to be reduced and at the same time the conditions became unrepresentative of the actual experimental conditions. The results are reported below.

To study the equilibration of radioactive glucose the final procedure adopted was as follows. 0·5 ml. portions of packed cells (haematocrit 80–90 %) which had been equilibrated with labelled glucose were placed in small tubes in a water-bath. Each tube was taken separately and at zero time 0·5 ml. of phosphate saline containing the same glucose concentration (but no radioactive glucose) and sucrose 1000 mg/100 ml. (to act as a volume marker) were injected forcibly from a warmed syringe. Agitation was continued manually and when it was desired to interrupt the equilibration the mixture was rapidly sucked into 10 ml. (5 ml. in early experiments) of the ice-cold mercurial solution. The supernatant which was removed after centrifuging was deproteinized with zinc sulphate and barium hydroxide and desalted as described above. A portion was then taken for counting and sucrose estimation. In some cases an independent estimate of the final equilibrium value was made by omitting the $HgCl_2$ from the saline and warming to 37° C for a few minutes before centrifugation. In this way the glucose became evenly distributed, and by assuming a water content of the cells of 65 % it was possible to calculate the equilibrium value. The initial value for the radioactivity in the supernatant was obtained by calculation from the haematocrit. No correction was attempted for any exchange that might occur after treatment with the mercurial solution.

RESULTS

Effectiveness of the mercurial solution in preventing glucose exchange

The results of a series of experiments designed to show the effectiveness of mercurial saline in arresting glucose exchange are shown in Table 1. The amount of exchange was considerably reduced when freshly labelled glucose was used, presumably because of smaller amounts of radiochemical break-down products. Because of this, and of uncertainties in the trapped fluid volume in the haematocrit, no great accuracy can be claimed. However, the exchange is seen to be small with a probable tendency to decrease with higher concentrations of glucose. Where no glucose was added to the mercurial saline the results indicate the net loss of glucose that occurs. The small negative values at high concentrations of glucose probably reflect small differences in the trapped fluid volume in the haematocrit, although the possibility that some adsorption occurs on the cell surface cannot be excluded. If the rate of reaction of the mercury with the carrier was independent of glucose concentration, it might be expected that the exchange and loss of glucose would increase very markedly at low concentrations. Since this does not appear to be the case it seems that at the higher glucose concentrations the rate of reaction of the carrier with the mercury must fall. In this connexion it was also noticed in some preliminary experiments that if the glucose concentration in the mercurial saline was made equal to that in the cell suspension the amount of exchange was increased. In other experiments mercurial saline at room temperature was used, but a considerable loss of glucose then took place. One of the purposes of using a large volume of mercurial saline in relation to the cell suspension would therefore seem to be to minimize the rise in temperature when the suspension is added.

TABLE 1. Effectiveness of mercurial saline in arresting glucose exit and exchange

Blood sample	Concentration of glucose solution with which cells equilibrated (mM)	No. of observations	Vol. of mercurial saline / Vol. of cell suspension	Amount of inactive glucose in mercurial saline / Amount of radioactive glucose in cell suspension	% Loss of counts from intracellular part of red cell suspension (%)
1*	266	4	10	7·2	0·4
		4	10	0	− 1·4
2	133	4	10	7·1	5·6
		6	5	3·6	7·4
3*	133	4	10	5·5	0
		4	10	0	− 2·0
4	55·6	4	10	5·9	5·7
		4	5	2·9	8·2
5*	55·6	5	10	7·1	2·4
		5	10	0	− 0·6
6*	16·7	5	10	6·0	7·0
		5	10	0	4·6

* Fresh labelled glucose.

Exchange of glucose between cells and suspending medium

In five series of experiments the exit of radioglucose from the cells was studied at a concentration of 133 mM glucose. The results are shown in Fig. 1. The haematocrits of the cell suspensions used varied between 80

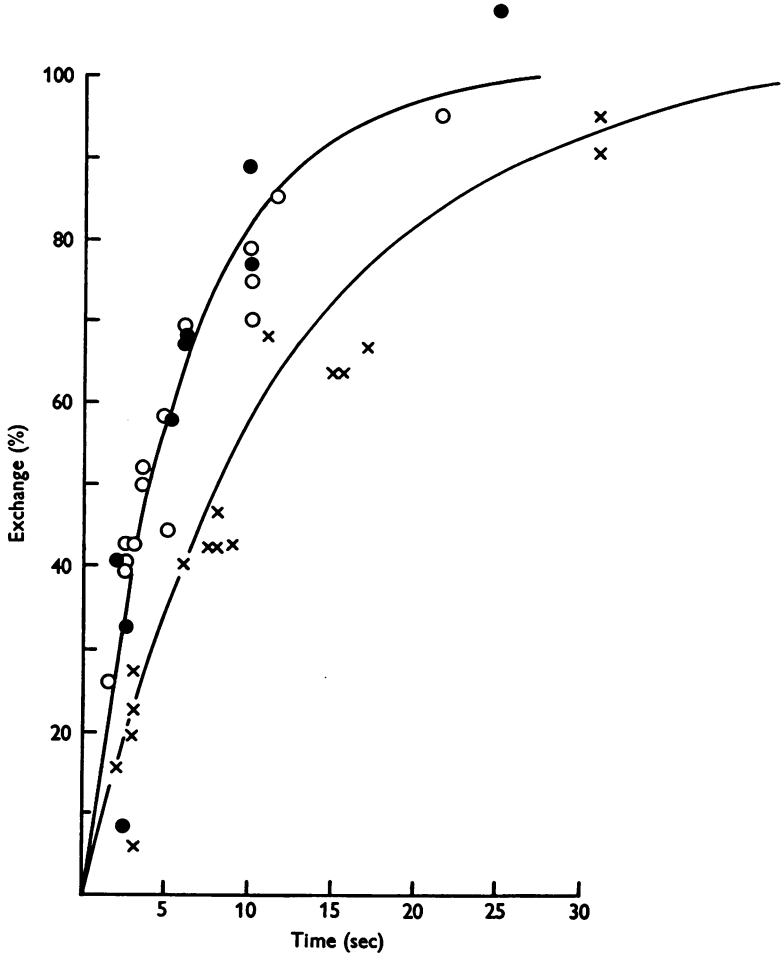

Fig. 1. Exit of radioactive glucose from red cells at 37° C. ○ Results from four series of experiments; concentration of glucose 133 mM; range of haematocrits of packed cells 80–92 %; volume of mercurial saline: volume of cell suspension = 5:1. ● Results from one experimental series; concentration of glucose 133 mM; haematocrit of packed cells 87·5 %; volume of mercurial saline: volume of cell suspension = 10:1. ×, Results from two series of experiments; concentration of glucose 266 mM; haematocrits of packed cells 77 and 78; volume of mercurial saline: volume of cell suspension = 10:1. Solid lines are exponential curves for times of half-exchange of 4 and 8 sec.

and 92 %, and as shown in the Appendix (Part I) the rate of equilibration will vary with the haematocrit. The effect, however, will be small and the results have therefore been superimposed. No correction has been applied for any exchange of labelled glucose that may occur after the cell suspension has been transferred to the mercurial saline. From the results it is clear that

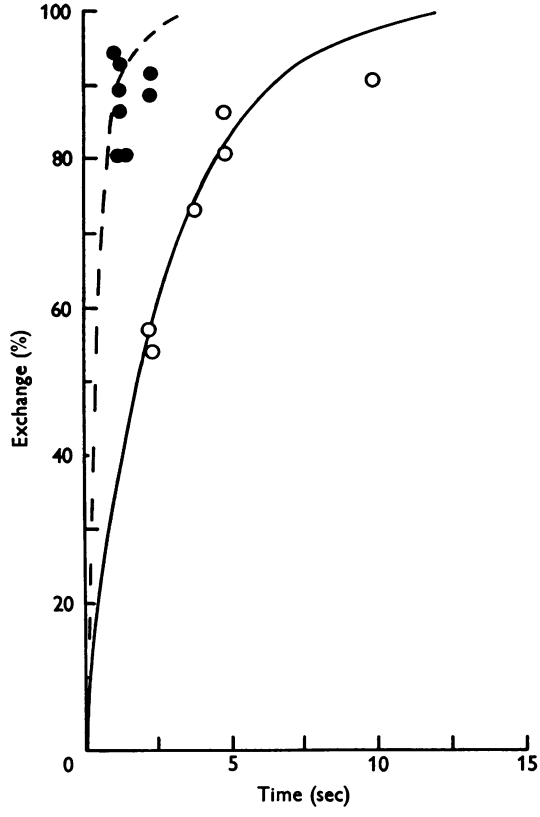

Fig. 2. Exit of radioactive glucose from red cells at 37° C. ○, Results from an experimental series with glucose concentration of 66·7 mM; haematocrit of packed cells 81 %. ●, Results from two series of experiments with glucose concentration of 16·7 mM; haematocrits of packed cells 85 and 82 %. In all experiments volume of mercurial saline: volume of cell suspension = 5:1. Solid line, exponential curve with time of half-exchange 4 sec; interrupted line, exponential curve with time of half-exchange 0·5 sec.

a very rapid exchange of labelled glucose occurs between the cells and suspending medium. An exponential curve with a time of half-exchange of 4 sec is shown in Fig. 1 and this line passes approximately through the experimental points. As calculated in the Appendix (Part I) such an exponential curve would be expected if the glucose flux across the membrane

were 0·052 isotonic units/sec, and the results are thus consistent with a flux of this order of magnitude.

Several series of experiments were carried out at glucose concentration other than 133 mM, and the results are shown in Figs. 1 and 2. At a concentration of 16·7 mM glucose (Fig. 2) it is seen that equilibration was

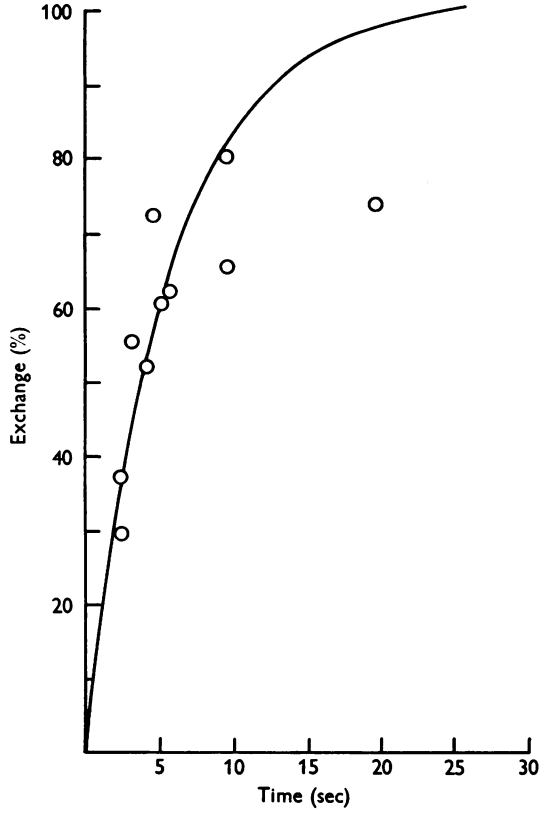

Fig. 3. Entry of radioactive glucose into red cells at 37° C. Results from two series of experiments; concentration of glucose 133 mM; haematocrits of packed cells 79 and 87%; volume of mercurial saline:volume of cell suspension = 5:1. Solid line is an exponential curve with a time of half-exchange of 4 sec.

extremely rapid, and this result is of interest since it indicates that equilibrium is not being greatly delayed by the mixing of cells and suspending medium. At a concentration of 66·7 mM glucose (Fig. 2) the time of half-exchange appeared to be of the order of 2 sec while at a concentration of 267 mM glucose (Fig. 1) the time of half-exchange was of the order of 8 sec. Comparison with the results at 133 mM shows that the time for equilibration

increases with increasing glucose concentration, and that in the concentration range 66·7–267 mM there is an approximately inverse relation between glucose concentration and rate of equilibration. As shown in the Appendix (Part 1) an inverse relation between glucose concentration and rate of equilibration would be expected if the glucose flux remained constant across the membrane and independent of glucose concentration.

In two series of experiments the entry of labelled glucose into the cell at a glucose concentration of 133 mM was studied; the results are shown in Fig. 3. It is apparent that, as would be expected, the time course of entry of labelled glucose is similar to that of exit.

TABLE 2. Effect of temperature on glucose flux. Concentration of glucose 133 mM

Temperature (° C)	Flux (isotonic units/sec)
37	0·052
27	0·028
22·5	0·017

In several experiments the effects of temperature on the rate of equilibration were examined and the results are given in Table 2. If the logarithm of the glucose flux is plotted against the reciprocal of the absolute temperature the three points lie in an approximately straight line corresponding to a Q_{10} of 2·0. In view of the experimental difficulties this figure for the Q_{10} can only be regarded as very approximate.

DISCUSSION

The data shown in Table 1 indicate that the mercurial saline technique for arresting glucose flux should be an effective method for studying glucose fluxes at high concentrations of glucose. The residual flux after treatment with the mercury will represent a small error in comparison with errors of timing and delays in mixing. At low concentrations of glucose the method may be less effective, but at the same time the rate of equilibration becomes too rapid for study. The technique should also be suitable for examining the net entry of glucose into the cell, but again the method may break down when the concentration of glucose in the cell is low. LeFevre & McGiniss (1960) have used a similar technique to measure the entry of glucose into the cell at 20° C.

The glucose exchange experiments show that there is a very rapid flux of glucose across the red cell membrane at high concentrations of glucose. The majority of measurements were made at a glucose concentration of 133 mM, where a flux of about 0·052 isotonic units (10·2 m-mole/l. cells/

sec) was found, but experiments at 67 and 267 mM glucose indicated fluxes of the same order, so that, to a first approximation, the glucose flux seems to be independent of glucose concentration within this concentration range. These results may be compared with those of LeFevre & McGiniss (1960), who used an essentially similar technique, and who obtained a value of 0·0157 isotonic units/sec for cells equilibrated with 205 mM glucose at

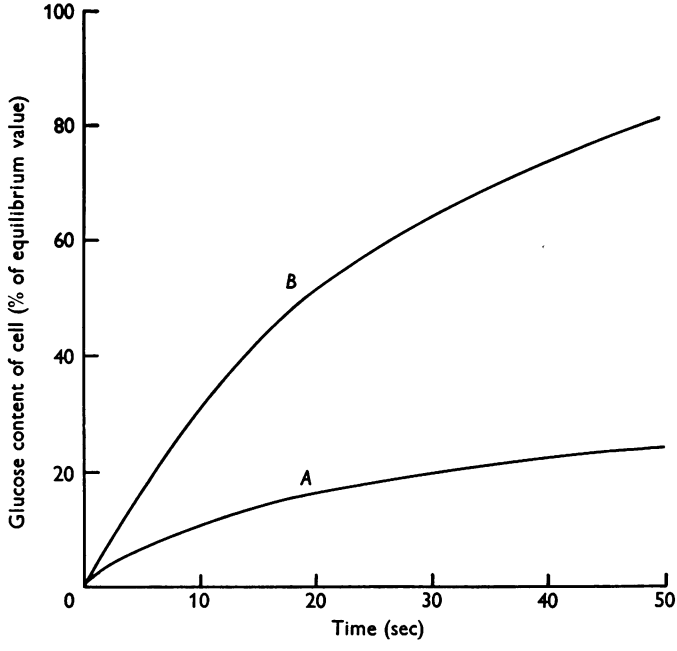

Fig. 4. Graph to show net entry of glucose into red cells placed in a large volume of a solution containing 133 mM glucose. Curve *A* computed from equation (4) taking *K* and $an/2$ as 4 mM and 0·018 isotonic units respectively. Curve *B* calculated from simple diffusion for the same initial rate of entry. Osmotic equilibrium has been assumed. Equilibrium glucose content of cells 0·444 isotonic units.

20° C. If the present results (Table 2) are extrapolated to 20° C a flux of about 0·016 isotonic units/sec is found, so that the results are in good agreement.

If the flux of glucose through the membrane is independent of concentration it suggests that the movement of glucose cannot be by simple diffusion, since if simple diffusion was occurring the flux should be proportional to the concentration. The net transport of glucose also does not follow simple diffusion kinetics but obeys an equation of a type predicted by the mobile-carrier hypothesis (equation 4). In Fig. 4 an entry curve

for cells placed in 133 mM glucose has been plotted from this equation, so that the net entry can be compared with the glucose flux: for this purpose the constants K and $an/2$ in equation (4) have been taken as 4 mM and 0·018 isotonic units respectively (Sen & Widdas, 1962*a*, *b*). Also shown is the curve that would be expected for simple diffusion, the same initial rate of entry being assumed and it is seen that the net rate of entry of glucose decreases rapidly when compared with simple diffusion as the glucose enters the cell. The initial rate of entry (0·0175 isotonic units/sec) and the glucose flux for cells equilibrated with 133 mM glucose (0·052 isotonic units/sec), on the other hand, are of the same order of magnitude, and this suggests that the inward flux of glucose must remain at least approximately constant. If this is the case the pronounced reduction in the net rate of entry as glucose enters the cell must be due to a high outward flux. As a corollary, the ratio (flux in):(flux out) should become very nearly equal to unity, even when substantial concentration gradients still exist across the membrane and the concentration ratio should exceed the flux ratio. The membrane should therefore show exchange diffusion (Ussing, 1949). Confirmation of this property comes from the work of Rosenberg & Wilbrandt (1957) who showed qualitatively that glucose or mannose could cause induced uphill transport of radioglucose at low temperatures. Induced uphill transport and exchange diffusion may be regarded as basically the same phenomenon: this is apparent from the work of Rosenberg & Wilbrandt (1957) and the relation has been examined in some detail recently (Britton, 1963).

If the inward flux (in Fig. 4) remained absolutely constant, the initial rate of entry (0·0175 isotonic units/sec) and the rate of exchange at 133 mM glucose (0·052 isotonic units/sec) should be identical. Since different methods were used in the two measurements and very rapid movements were involved it may be that the agreement is satisfactory. It may also be noted that LeFevre (1961) has reported a rather higher value of 0·03 isotonic units/sec for $an/2$ in equation (4), which corresponds to an initial rate of entry of 0·029 isotonic units/sec. However, it is entirely possible that the flux does increase as the internal concentration of glucose rises, since this type of phenomenon has been observed in other fields of permeability, e.g. sodium (Glynn, 1956; Keynes & Swan, 1959), amino acids (Heinz, 1954; Heinz & Walsh, 1958), chloride (Heinz & Durbin, 1957) and (in extreme form) phosphate (Mitchell, 1954).

The action of specific inhibitors, competition between sugars and the presence of saturation phenomena in the kinetics suggest that there must be a limited number of specific sites in the membrane to which the sugar must become attached if it is to cross the membrane. The minimum number of reaction steps for glucose entering the cell would seem to be

$$G(\text{outside}) + [X]_{\text{I}} \rightleftharpoons [GX]_{\text{I}}, \tag{1}$$

$$[GX]_{\text{I}} \overset{a}{\underset{a}{\rightleftharpoons}} [GX]_{\text{II}}, \tag{2a}$$

$$[X]_{\text{I}} \overset{b}{\underset{b}{\rightleftharpoons}} [X]_{\text{II}}, \tag{2b}$$

$$[GX]_{\text{II}} \rightleftharpoons [X]_{\text{II}} + G(\text{inside}). \tag{3}$$

G represents glucose and $[X]$ denotes a specific site. The transformations shown in (2) represent the interconversions of the site from the form which can combine with the glucose on the outside of the membrane to the form which can combine with glucose on the inside of the membrane: it is necessary to postulate that the site must exist in two such forms if it is obligatory for the sugar to combine with the site in order to cross the membrane. Let step (2) be rate limiting and let the rate constants a and b of reactions (2a) and (2b) be equal. Since reaction (2) is rate limiting, reactions (1) and (3) will be near equilibrium. Suppose that the equilibrium constant $K = ([G][X])/[GX]$ is the same for reaction (1) and (3);

then the net flux = [flux in] − [flux out]

$$= \left[\frac{an}{2}\frac{C_{\text{e}}}{C_{\text{e}}+K}\right] - \left[\frac{an}{2}\frac{C_{\text{i}}}{C_{\text{i}}+K}\right], \tag{4}$$

where n = total number of active sites, C_{e} = concentration of glucose on outside, C_{i} = concentration of glucose on inside, a and K as in text.

This equation is the mobile carrier equation of Widdas (1951, 1952, 1953, 1954) and Wilbrandt & Rosenberg (1951; Wilbrandt, 1954; Rosenberg & Wilbrandt, 1955) and it provides a satisfactory description of the net kinetics of glucose movement. (For reviews see LeFevre & LeFevre, 1954; Wilbrandt & Rosenberg, 1954; Bowyer, 1957.) Since K is of the order of 4 mM (Sen & Widdas, 1962a, b) the equation indicates that the flux should be substantially independent of glucose concentration in the range 67–267 mM, as is found to be the case. The equation also indicates that the initial rate of entry in Fig. 4 should be equal to the glucose flux after equilibration with 133 mM glucose, but as discussed above it may be that the glucose flux increases to some extent as the internal concentration rises. This possibility could be accommodated in the same kind of model if the rate constant a was greater than the rate constant b, as is shown in the Appendix (Part III). Thus if $a = 3b$ the inward flux would rise by a factor of two as glucose entered the cell. The equation for the net entry becomes rather more complicated (Widdas, 1954), but the deviations from the more simple equation would not be serious and

would only be apparent at the beginning of the entry curve. In terms of the mobile-carrier hypothesis, the carrier would be more mobile in the membrane when combined with glucose than in the uncombined state.

It has been suggested by Widdas (1954) that the reaction of the sugar with the specific sites might be partly rate limiting, since the equation for the net entry would not be greatly altered. In the analysis in the Appendix (Part III), however, it is shown that there would be a marked effect on the fluxes. Thus, for example, if the reaction of the sugar with the specific sites were slow enough to reduce the initial rate of entry in Fig. 4 to one half, the glucose flux after equilibration with glucose would be reduced to one third. The fact that the glucose flux is at least as great or greater than the initial rate of entry therefore suggests that the reaction of the sugar with the specific sites must be rapid, although this argument may not be valid if the rate constant a is much greater than the rate constant b.

The kinetics thus indicate that reaction (2) is the rate-limiting step and it is of interest to consider its physical nature. One possibility is that it represents the physical migration of the carrier from one side of the membrane to the other as has been suggested by Widdas (1951, 1952, 1953, 1954) and Wilbrandt & Rosenberg (1951; Wilbrandt, 1954; Rosenberg & Wilbrandt, 1955). On the other hand, it might represent an intramolecular change in an adsorption site mounted in a pore, and actual physical migration of the site might not occur. Another possibility is that of an adsorption site mounted in a pore which is blocked at each end by lipoid groups. Brownian motion might open such a pore at one or other end periodically: and if the duration of opening of the pore was sufficient for adsorption equilibrium to be obtained appropriate kinetics could result. The transformation represented by step (2) would then be the change from a pore open at one end to a pore open at the other end. An adsorption site situated on a part of the membrane where pinocytosis was taking place might function in the same way, although it is difficult to see how the entry of other small molecules could be prevented.

If two or more 'systems' giving the kinetics shown in equation (4) are placed in series, the kinetics remain of the same type. This is discussed in the Appendix (Part II). Complete mixing is necessary in the intermediate glucose pools between the 'systems', since if this does not take place the fluxes (but not necessarily the net flux) and the flux ratio will decrease (Hodgkin & Keynes, 1955). Any of the physical models mentioned above may be put in series, but other possibilities exist. Thus two 'systems' in series might correspond to a model incorporating enzymes which are located at each surface of the lipoid membrane. The function of the enzymes would be to convert the sugar into a lipoid-soluble form, which could then cross the membrane by diffusion. In such an enzymic model the transitions of

the glucose enzyme complexes from one form to the other represents reaction (2), and these should be the rate-limiting steps. Three 'systems' in series might correspond to an enzyme at each surface with a mobile carrier within the membrane. Such enzyme models were originally suggested by Rosenberg & Wilbrandt (1955), although an analysis of the individual fluxes was not made.

Models have been proposed where two or more adsorption sites are mounted in series along a pore in which migration from one site to the next can only occur if a site is vacant (Widdas, mentioned by Bowyer, 1957; Rosenberg & Wilbrandt, 1957). Such models may give appropriate net kinetics, but they do not give exchange diffusion (Rosenberg & Wilbrandt, 1957) and the fluxes should fall with increasing concentration, so that they are incompatible with the present data.

A special case of an enzyme model is the dimer hypothesis of Stein (1962), in which it is supposed that there is an enzyme at each surface of the membrane which can convert the sugar into a lipoid-soluble dimer. According to Stein the permeability equation is

$$\begin{aligned}\text{net flux} &= (\text{flux in}) - (\text{flux out}),\\ &= \left\{\frac{\alpha C_e^2}{(C_e+\beta)^2}\right\} - \left\{\frac{\alpha C_i^2}{(C_i+\beta)^2}\right\},\end{aligned}$$

where α and β are constants.

At 24° C β was found to be about 20 mM, but further measurements at 40° C suggested that the figure may have to be revised. It is clear that with a value for β of this order, the theory is compatible with the constancy of the radioactive fluxes, and with the similarity between the flux and the initial rate of entry in Fig. 4. However, the net entry curve would be of a somewhat different shape and the curve would not appear to be compatible with data of Sen & Widdas (1962*a*, *b*). When C_e and C_i are large in relation to β the theory predicts exchange diffusion, but when C_e and C_i are small the flux ratio should be equal to the square of the concentration ratio, and to demonstrate this phenomenon would be a critical test of the hypothesis. It would be difficult to observe with glucose, but measurements might be possible for example with ribose (where β must be large).

In the above discussion the assumption has been made that the mechanisms for the net movement and glucose exchange are the same. It is conceivable, however, that there is a system purely for exchange (Rosenberg & Wilbrandt, 1957; LeFevre & McGiniss, 1960), although the recent work of LeFevre (1963) on ribose suggests that this may not be so. Another possibility is that there may be exchange reactions of the type

$$G^* + [GX] \rightleftharpoons G + [G^* X].$$

It is clearly very difficult to exclude such alternative pathways.

To conclude, therefore, the results in this paper may be of some value in elucidating the kinetics of glucose transfer through the red cell membrane. As with other kinetic data, however, the results can be explained by a number of physical models and the precise mechanism must remain uncertain.

SUMMARY

1. A method is described for studying the flux of radioglucose across the red cell membrane.

2. At a concentration of 133 mM glucose the flux at 37° C was found to be of the order of 0·05 isotonic units/sec. This is of the same order as the maximum rate of net transport. Evidence was obtained that the flux was approximately constant over the concentration range 66·7–267 mM glucose. The effect of temperature was studied.

3. The kinetics of glucose transport across the red cell membrane are discussed. It is concluded that the data are compatible with a number of different physical models.

APPENDIX: PART I

Let V_i = intracellular fluid volume, V_e = extracellular fluid volume, C_i = concentration of glucose in cell, C_e = concentration of extracellular glucose, a_i = specific activity of intracellular glucose, a_e = specific activity of extracellular glucose, R = total radioactivity in system, m_a = flux of glucose into cell, m_b = flux of glucose out of cell.

At equilibrium with respect to inactive glucose

$$C_i = C_e = C, \quad \text{and} \quad m_a = m_b = m.$$

Consider the exit of labelled glucose:

$$\text{net exit} = CV_e\frac{da_e}{dt} = ma_i - ma_e,$$

and since the total radioactivity is constant

$$CV_e a_e + CV_i a_i = R.$$

From (2) and (3)

$$CV_e\frac{da_e}{dt} = \frac{m(R-CV_e a_e)}{CV_i} - ma_e = \frac{mR}{CV_i} - m\left(1+\frac{V_e}{V_i}\right)a_e.$$

Integrating,

$$a_e = a_e' + (a_e'' - a_e')\left[1-\exp\left\{-\frac{mt}{CV_i}\left(1+\frac{V_i}{V_e}\right)\right\}\right],$$

where a'_e = initial specific activity of extracellular fluid, and a''_e = final specific activity of extracellular fluid. If

$$t_{1/2} = \text{time of half exchange,}$$

then

$$\frac{mt_{1/2}}{CV_1}\left(1+\frac{V_1}{V_e}\right) = 0{\cdot}694.$$

If $C = 133$ mM and $t_{1/2} = 4$ sec (a haematocrit of 85% for the cell suspension and an intracellular fluid content of 65% of the red cell volume being assumed)

$$m = \frac{0{\cdot}694 \times 133 \times 0{\cdot}65}{4 \times 1{\cdot}48},$$

$$= 10{\cdot}2 \text{ m-mole/l. cells.}$$

Let m' = flux in isotonic units. Then taking 300 mM glucose as an isotonic solution,

$$m' = \frac{m}{300 \times 0{\cdot}65} = 0{\cdot}052 \text{ isotonic units/sec.}$$

PART II

Over-all fluxes when diffusion barriers are placed in series

Consider the model depicted in Fig. 5 and let the fluxes through the diffusion barriers be m_1, m_2, etc., as shown. Assume a steady state and complete mixing in pools I, II, III and IV. Then flux from pool I to II = m_1; and probability that a molecule entering pool II will be transferred to pool III

$$= \frac{\text{flux from II to III}}{\text{flux from II to III} + \text{flux from II to I}},$$

$$= \frac{m_3}{(m_3+m_2)}.$$

Hence, $$\text{flux from pool I to III} = m_1\frac{m_3}{(m_3+m_2)}.$$

Conversely, $$\text{flux from pool III to I} = m_4\frac{m_2}{(m_2+m_3)}.$$

Probability that a molecule entering pool III from pool I will be transferred to pool IV

$$= \frac{\text{flux from III to IV}}{\text{flux from III to IV} + \text{flux from III to I}},$$

$$= \frac{m_5}{m_5 + m_4\dfrac{m_2}{(m_2+m_3)}}.$$

Hence,

$$\text{flux from pool I to IV} = m_1 \frac{m_3}{(m_3+m_2)} \frac{m_5}{\left(m_5+m_4\dfrac{m_2}{m_2+m_3}\right)}. \quad (5)$$

Conversely,

$$\text{flux from pool IV to I} = m_6 \frac{m_4}{(m_4+m_5)} \frac{m_2}{\left\{m_2+m_3\dfrac{m_5}{(m_5+m_4)}\right\}}. \quad (6)$$

The argument may be appropriately extended if more than three diffusion barriers are present. Let each diffusion barrier have kinetics given by eqn. 4

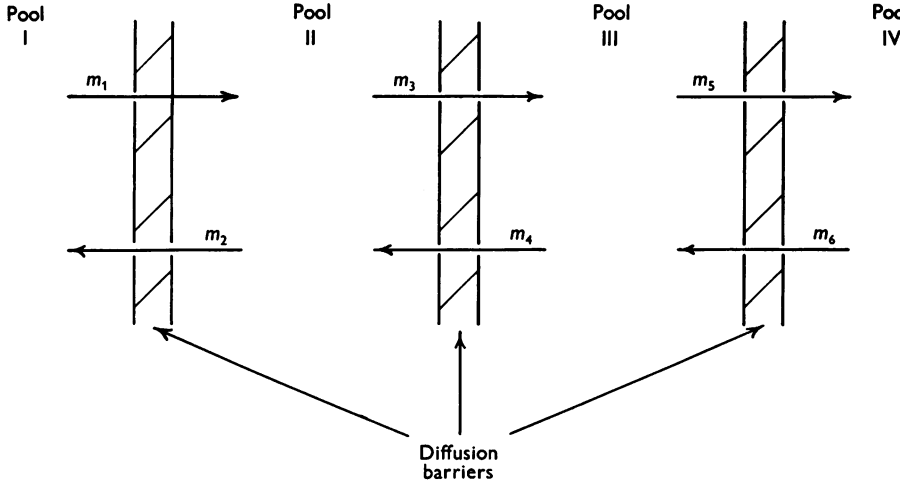

Fig. 5. Model to illustrate effect of diffusion barriers.

and let the constant K be the same in each case. Since the flux through each barrier is then a function of only the concentration on the side from which the flux originates, the probability terms $m_3/(m_3+m_2)$, $m_5\Big/\left\{m_5+m_4\dfrac{m_2}{m_2+m_3}\right\}$, etc., become constants independent of concentration.

Hence

net over-all flux = [over-all inward flux] − [over-all outward flux]

$$= \left[r_1 \cdot \frac{a_1 \cdot n_1}{2} \frac{C_e}{(C_e+K)}\right] - \left[r_0 \cdot \frac{a_n \cdot n_n}{2} \frac{C_i}{(C_i+K)}\right],$$

where r_1 and r_0 = constants independent of concentration, a_1 and n_1 = constants for outermost permeability barrier, a_n and n_n = constants for innermost permeability barrier, and C_e, C_i and K have the values in equation (4).

In a steady state the net over-all flux must be zero when $C_e = C_1$, so that r_1 and r_0 are related thus

$$\frac{r_1}{r_0} = \frac{a_n . n_n}{a_1 . n_1}.$$

From equations (5) and (6) it follows that the over-all flux ratio is the product of the individual flux ratios. Thus if the over-all system shows exchange diffusion, one or more of the permeability barriers must show this phenomenon. This would also be expected from thermodynamic considerations.

PART III

General equations for the fluxes in a carrier system

Consider a symmetrical system

$$S + [X]_I \underset{d}{\overset{c}{\rightleftharpoons}} [SX]_I \underset{a}{\overset{a}{\rightleftharpoons}} [SX]_{II} \underset{c}{\overset{d}{\rightleftharpoons}} [X]_{II} + S$$

(outside) (inside)

and

$$[X]_I \underset{b}{\overset{b}{\rightleftharpoons}} [X]_{II},$$

where a, b, c, d are the appropriate rate constants, $[X]$ = specific site, and S = substrate. Such a model may be regarded as having two intermediate pools of substrate represented by $[SX]_I$ and $[SX]_{II}$, separated by diffusion barriers.

From PART II, and identifying pools I and II as inside and outside and pool II and III as $[SX]_I$ and $[SX]_{II}$,

$$\text{flux from outside to inside} = m_1 \frac{m_3}{(m_3+m_2)} \frac{m_5}{\left\{m_5 + m_4 \frac{m_2}{(m_2+m_3)}\right\}},$$

and, since there is a steady state,

$$m_1 - m_2 = m_3 - m_4 = m_5 - m_6.$$

Substituting,

$$\text{flux from outside to inside} = \frac{(m_2+m_3-m_4)m_3 m_5}{(m_3+m_2)\{m_5 + m_4 m_2/(m_2+m_3)\}}. \quad (7)$$

The following relations also apply

$$m_2 = d.[SX]_I; \quad m_3 = a.[SX]_I; \quad m_4 = a.[SX]_{II}; \quad m_5 = d.[SX]_{II}.$$

Substituting in equation (7) and simplifying,

$$\text{flux from outside to inside} = a/(2a+d)[(a+d).[SX]_I - a.[SX]_{II}], \quad (8)$$

and conversely,

$$\text{flux from inside to outside} = a/(2a+d)[(a+d)[SX]_{II} - a[SX]_I] \quad (9)$$

Since there is a steady state the rates of formation of $[SX]_I$, $[SX]_{II}$ and $[X]_I$ must be equal to their respective rates of removal. Consequently,

$$\begin{aligned} a[SX]_I + d[SX]_I &= C_e c[X]_I + a[SX]_{II}, \\ a[SX]_{II} + d[SX]_{II} &= C_i c[X]_{II} + a[SX]_I, \\ C_e c[X]_I + a[X]_I &= d[SX]_I + b[X]_{II} \end{aligned}$$

and

$$[X]_I + [SX]_{II} + [X]_{II} + [SX]_{II} = n,$$

where n = total number of specific sites, C_e = concentration outside, C_i = concentration inside. These equations may be solved for $[SX]_I$ and $[SX]_{II}$ and substitution made in equations (8) and (9), but the result is algebraically very cumbersome. Two special cases will therefore be considered.

When $a = b$

$$\begin{aligned} \text{net flux} &= [\text{flux in}] - [\text{flux out}] \\ &= \left[\frac{n}{2}\frac{acd}{(2a+d)}\frac{(2a+d+C_i c)C_e}{\{(a+d+C_i c)(a+d+C_e c)-a^2\}}\right] \\ &\quad - \left[\frac{n}{2}\frac{acd}{(2a+d)}\frac{(2a+d+C_e c)C_i}{\{(a+d+C_i c)(a+d+C_e c)-a^2\}}\right]. \end{aligned} \tag{10}$$

When $d \gg a$, i.e. reactions of sugar with the specific site are not rate-limiting, this reduces to equation (4).

When $a \gg d$, i.e. reaction with specific-site rate-limiting, equation (10) reduces to

$$\begin{aligned} \text{net flux} &= [\text{flux in}] - [\text{flux out}] \\ &= \left[\frac{ncd(2a+C_i c)C_e}{4(2ad+ac(C_e+C_i)+C_e C_i c^2)}\right] - \left[\frac{ncd(2a+C_i c)C_e}{4(2ad+ac(C_e+C_i)+C_e C_i c^2)}\right]. \end{aligned} \tag{11}$$

Further, if $a \gg C_e c$ and $C_i c$,

$$\text{net flux} = \left[\frac{ncdC_e}{2(2d+c(C_e+C_i))}\right] - \left[\frac{ncdC_i}{2(2d+c(C_e+C_i))}\right]. \tag{12}$$

Equation (12) obeys the Ussing (1949) relation and the expression for the net flux is of the same type as that obtained by LeFevre & LeFevre (1952), who, however, considered an asymmetrical membrane.

Equation (11) is of interest since it indicates that in such a case the best conditions to detect a deviation from the Ussing criterion are when C_e and C_i are very large.

When $a = d$,

$$\text{net flux} = \left[\frac{n.a.c}{6}\frac{(3a+C_1c)C_e}{\{(2a+C_1c)(2a+C_ec)-a^2\}}\right] - \left[\frac{n.a.c}{6}\frac{(3a+C_1c)C_e}{\{(2a+C_1c)(2a+C_ec)-a^2\}}\right],$$

and maximum net rate of transport $(C_e \to \infty, C_1 = 0) = na/4$. Flux when $C_e = C_1 \to \infty = na/6$. Thus the flux when $C_e = C_1 \to \infty$ is reduced to one third, and maximum net rate of transport to one half of the figure of $na/2$ when $d \gg a$.

When $a \neq b$ and $d \gg a, b$, i.e. reaction of substrate with specific site not rate limiting. Let $d/c = K$, where K = equilibrium constant for reaction $S + [X] \rightleftharpoons [SX]$.

Then net flux = [flux in] − [flux out]

$$= \left[\frac{na(aC_1+bK)C_e}{K(a+b)(C_e+C_1)+2aC_eC_1+2bK^2}\right] - \left[\frac{na(aC_e+bK)C_1}{K(a+b)(C_e+C_1)+2aC_eC_1+2bK^2}\right].$$

Maximum net transport

$$(C_e \to \infty, C_1 = 0) = \frac{nab}{(a+b)}.$$

Flux when $C_e = C_1 \to \infty = na/2$. Thus if $a = 3b$, maximum net transport would be $na/4$ or one half of the flux, when $C_e = C_1 \to \infty$ of $na/2$.

If $a \neq b$, but the results are fitted to equation (5) the apparent value of K will not necessarily be the true equilibrium constant (Widdas, mentioned in Bowyer, 1957). In the method of Sen & Widdas, (1962 *a*,*b*) the apparent value will be approximately $[(1+q)/2q]K$ where $a = qb$. Hence if $a = 3b$ and the apparent value is 4 mM, the true equilibrium constant will 6 mM. On the other hand, estimation of the equilibrium constant by the inhibition of sorbose transport by glucose (Widdas, 1954) will give approximately the true value. This may explain the rather higher value found by the latter method.

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