J. Physiol. (1980), **309**, *pp.* 317–327
With 6 text-figures
Printed in Great Britain

ASYMMETRY OF HEXOSE TRANSFER SYSTEM IN ERYTHROCYTES OF FETAL AND NEW-BORN GUINEA-PIGS

BY D. S. AUBBY AND W. F. WIDDAS

From the Department of Physiology, Bedford College, Regent's Park, London NW1 4NS

(*Received 25 April 1980*)

SUMMARY

1. The asymmetries of affinities of two non-transportable competitive inhibitors of hexose transfer across fetal and new-born guinea-pig erythroyctes have been studied.

2. At 16 °C 4,6-*O*-ethylidene-α-D-glucopyranose (ethylidene glucose) inhibited 3-*O*-methyl glucose exchange at 20 mM with a K_i of *ca.* 52 mM when present inside the cells and with a K_i of *ca.* 10 mM when outside. This fivefold asymmetry is qualitatively similar to but smaller than the tenfold asymmetry of human erythrocytes (Baker, Basketter & Widdas, 1978).

3. Methyl-2,3-di-*O*-methyl-α-D-glucopyranoside (trimethyl glucoside) had K_i values of *ca.* 120 mM and *ca.* 160 mM for inside and outside inhibition respectively. This is also qualitatively similar to the inhibition in human erythrocytes.

4. The inhibition produced by phlorizin, phloretin and Cytochalasin B was also studied in the erythrocytes of new-born guinea-pigs. The results were qualitatively similar to those for human erythrocytes but the inhibitory affinities were different. Thus while phlorizin and phloretin had higher affinities for the inhibition of exchange in new-born guinea-pig cells than human cells, the affinity of Cytochalasin B was less for new-born guinea-pig cells than for human cells.

5. It is concluded that the hexose transfer system in fetal and new-born guinea-pig red cells has asymmetric affinities similar to the system in human red cells but with different values of the inhibitory constants. The differences may represent species variations in a structural protein serving identical functions in the two species.

6. The possibility that fetal red cells with their facilitated transfer system play a role in sugar transport is discussed.

INTRODUCTION

Erythrocytes of fetal and new-born guinea-pigs were among those of several domestic and laboratory animals for which the fetal red cells were shown by Widdas (1955) to have a hexose transfer system similar in rate to that of erythrocytes from the human adult. The original observation by Widdas has been confirmed and extended to the rabbit by Augustin, Rohden & Hacker (1967), to the pig by Zeidler, Lee & Kim (1976) and to the dog by Lee, Auvil, Grey and Smith (1976).

Using the Sen & Widdas (1962*a*) exit technique, Dawson & Widdas (1964) studied

0022-3751/80/3120-0939 $07.50

the parameters of the facilitated transfer system in fetal guinea-pig red cells as a function of temperature and pH. Whereas the pH dependence of glucose transfer was similar to that in human cells, the temperature dependence was different. The maximal transfer rate was found to be larger than for human cells throughout the temperature range studied (7–37 °C) and the half-saturation constant (ϕ), although similar to that for human cells at 37 °C, did not fall in the same regular manner as the temperature was reduced. Although this different temperature dependence could indicate a difference in the membrane components involved in the hexose transfer it could have been due to a difference in the rate limiting steps in the membrane environment.

To investigate whether new-born guinea-pig cells have a similar asymmetry of affinities between inward facing sites and outward facing sites in the membrane to that of human red cells, we have studied the inhibition produced by the non-transportable inhibitors ethylidene glucose and trimethyl glucoside and also the inhibitory parameters (for equilibrium exchanges and Sen-Widdas (1962*b*) exits) of the competitive inhibitors phlorizin, phloretin and Cytochalasin B.

A preliminary report of some of these experiments was made to the Physiological Society (Aubby & Widdas, 1979).

METHODS

The majority of the experiments were done at 16 °C.

Exit experiments were carried out as described by Sen & Widdas (1962*a*, *b*). Cells were pre-incubated to contain 76 mM-glucose and exits made into 21 ml. of suspending medium by the rapid addition of *ca.* 0·15 ml. of cell suspension containing less than 3 μl. cells.

Exchange experiments were performed as described by Baker, Basketter & Widdas (1978).

Blood was obtained either from fetal guinea-pigs, near full term, as described by Dawson & Widdas (1964) or from recently new-born guinea-pigs which were stunned and bled. The blood was collected over dry heparin and rapidly mixed with phosphate buffered saline. The cells were washed three times with buffered saline before using for exchange or exit measurements.

RESULTS

3-O-methyl glucose exchange

Equilibrium exchanges can be treated as arising from a transfer system with simple kinetics in the form:

$$\text{Flux} = V_{EX} \frac{C}{C+\phi_{EX}}, \tag{1}$$

where V_{EX} is the maximal exchange flux, C is the concentration of sugar inside and outside the cells and ϕ_{EX} is the half-saturation concentration for exchange. Eq. (1) can be written in the Hanes plot form (Hanes, 1932) as:

$$\frac{C}{\text{Flux}} = \frac{1}{V_{EX}} (C+\phi_{EX}). \tag{2}$$

Thus the concentration multiplied by the reciprocal of the flux is linearly related to the concentration. In this type of plot, the intercept on the abscissa gives the half-saturation constant for exchange (ϕ_{EX}) and the intercept on the ordinate corresponds to ϕ_{EX}/V_{EX}.

The exchange of 3-*O*-methyl glucose was measured in the range 2–160 mM using ^{14}C-labelled 3-*O*-methyl glucose and the results are shown in the Hanes plot in Fig. 1. The experiments with new-born fetal guinea-pig cells gave a half-saturation constant for exchange at 16 °C of *ca.* 25 mM which was higher than the corresponding value for human cells (*ca.* 15 mM). The value for the V_{EX} (*ca.* 167 m-mole l.$^{-1}$ min^{-1}) was also higher than for human blood (*ca.* 119 m-mole l.$^{-1}$ min^{-1}). The results for human blood are taken from Basketter & Widdas (1978).

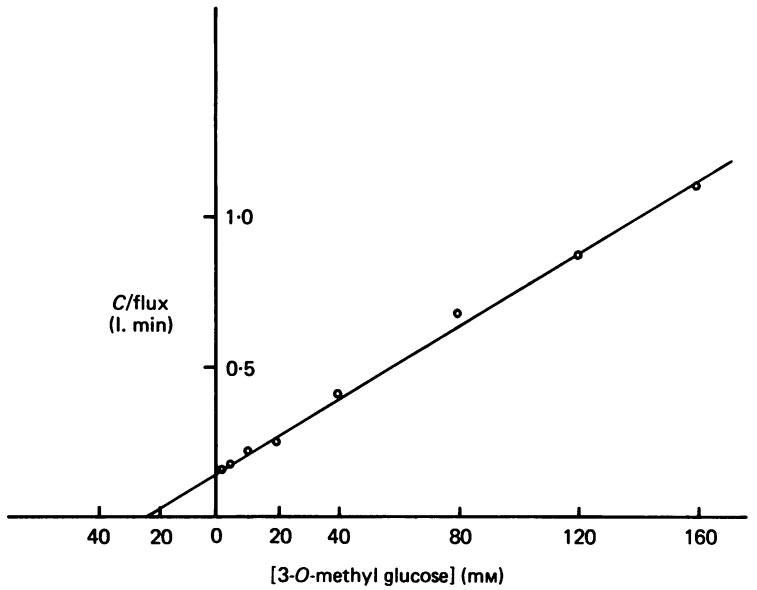

Fig. 1. Hanes plot of 3-*O*-methyl glucose exchange in the range 2–160 mM in fetal and new-born guinea-pig red cells at 16 °C. Points are the means of three experiments with similar results.

Inhibition of exchange by ethylidene glucose

When inhibitors of hexose exchange are present, a relatively simple treatment (Baker *et al.* 1978) predicts that the reciprocal of the exchange flux should be a linear function of the inhibitor concentration and that such a line should intercept a line through $1/V_{EX}$ parallel to the abscissa at a point corresponding to the half-saturation concentration for the inhibitor.

The inhibition of exchange of 20 mM-3-*O*-methyl glucose was investigated with cells equilibrated with up to 200 mM-ethylidene glucose. With the inhibitor inside the cells, osmotic compensation was provided by having inositol and malonamide in the outside medium as described by Baker & Widdas (1973). This minimizes any volume changes during the time the radioactive samples are taken to measure the exchange flux.

In Fig. 2 the reciprocal of the flux has been plotted against the inhibitor concentration for the case of internal inhibition and also for external inhibition. It can be

seen that there was marked asymmetry of inhibition. The effect of 200 mM-ethylidene glucose inside the cells was less than that of 50 mM outside. Although marked, this asymmetry was less than that obtained by Baker *et al.* (1978) in human cells. The interrupted lines in Fig. 2 represent the corresponding inhibition in human cells and it will be noted that they have similar intercepts on the ordinate. This is because at

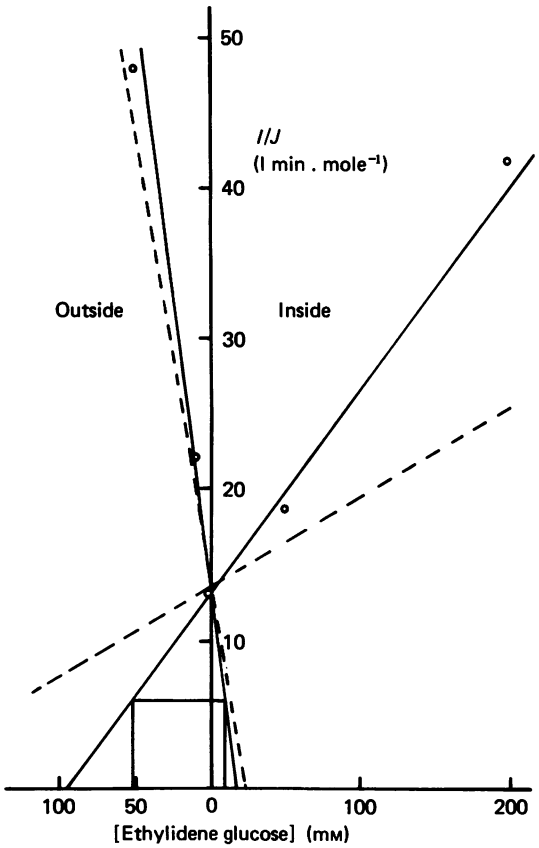

Fig. 2. Asymmetric inhibition of 3-*O*-methyl glucose exchange by purified ethylidene glucose. Points are the means of two similar results for 20 mM-3-*O*-methyl glucose exchange in new-born guinea-pig cells at 16 °C. Interrupted lines represent the corresponding results for human cells obtained by Baker *et al.* (1978).

20 mM the saturation fraction of 3-*O*-methyl glucose for human cells is greater than for new-born guinea-pig cells and this partly compensates for the lower V_{EX} in human cells.

The intersection of the lines with $1/V_{EX}$ derived from the results of Fig. 1 occurs at the K_i values for ethylidene glucose and these were *ca.* 52 mM for inhibition inside and *ca.* 10 mM for inhibition outside. The corresponding values for human red cells (Baker *et al.* 1978) were 110 mM and 11 mM. The asymmetry of the apparent affinities of ethylidene glucose was therefore fivefold in new-born guinea-pig cells in contrast to tenfold in human cells.

Inhibition of exchange by trimethyl glucoside

Methyl-2,3-di-*O*-methyl-α-D-glucopyranoside (trimethyl glucoside) was found to produce haemolysis of new-born guinea-pig cells when incubated at 200 mM to equilibrate the cells so that the inhibitor was inside. Concentrations of 100 mM inside could however be obtained with only moderate haemolysis and it was possible

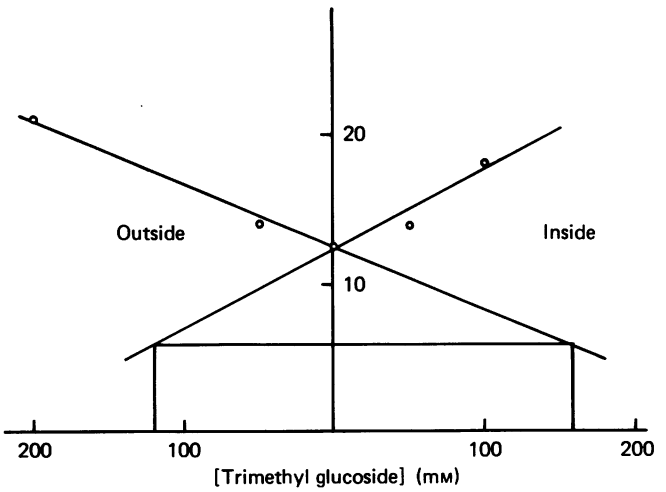

Fig. 3. Asymmetric inhibition of 3-*O*-methyl glucose exchange by trimethyl glucoside. Points are from two experiments with similar results and are for 20 mM exchange at 16 °C.

to make exchange measurements. The results are shown in Fig. 3. Although the results were rather variable, it was possible to confirm that with this reagent, inhibition was greater when the compound was inside the cells than when it was outside. That is, the asymmetry was in the opposite direction from that of ethylidene glucose. Qualitatively this was similar to the results obtained with human cells (Baker *et al.* 1978).

Inhibition by phlorizin

The inhibition of 3-*O*-methyl glucose exchange was studied at 2 and 20 mM with varying concentrations of phlorizin from 0·05 to 0·5 mM and also at varying concentrations of 3-*O*-methyl glucose from 2 to 40 mM with a constant concentration of phlorizin (0·5 mM).

A Dixon plot of the results at 2 mM and 20 mM-3-*O*-methyl glucose exchange is given in Fig. 4. The estimations of the K_i values tended to be higher at the larger concentrations of phlorizin and this suggested that the inhibitor may not have completely equilibrated with the cells at these concentrations. Experiments with phlorizin on human red cells gave similar variations of the K_i values. Calculations of the latter were made at each of the concentrations used between 0·05 and 0·5 mM and an estimate of the true K_i was obtained by extrapolation to zero phlorizin concentration.

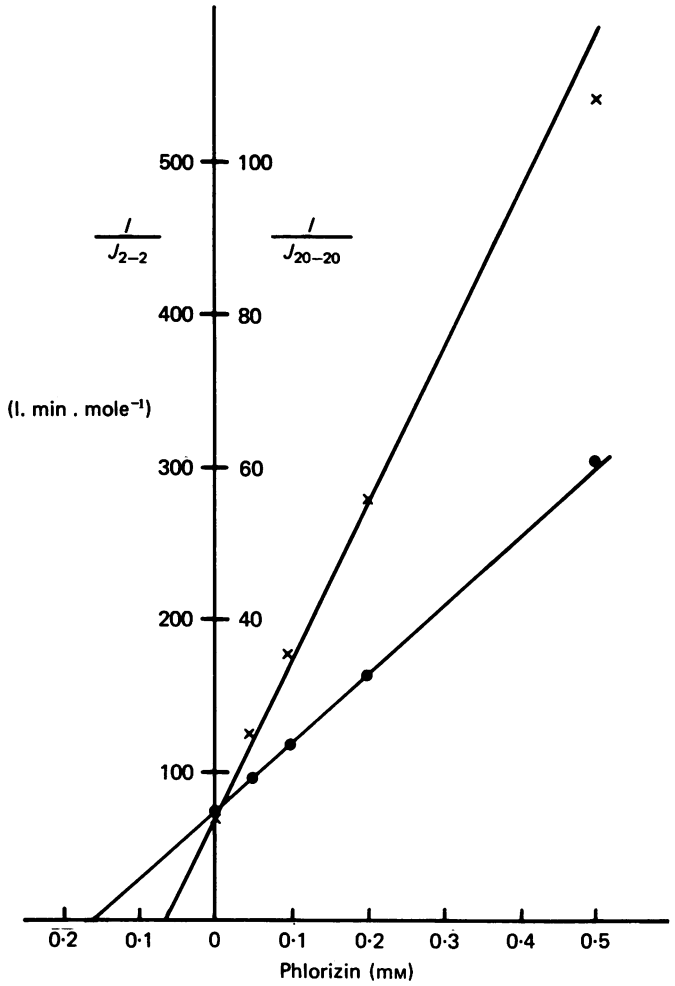

Fig. 4. Effect of phlorizin concentration on 3-*O*-methyl glucose exchange at 16 °C in new-born guinea-pig red cells. Points X, 2 mM exchange; points ●, 20 mM exchange. Points are means of two similar results at each sugar concentration.

Glucose exits were also carried out in the presence of phlorizin and the results from these and the exchange experiments are collected in Table 1.

Inhibition by phloretin

The inhibition of exchange of 3-*O*-methyl glucose by phloretin was studied in the range 0·5–2·0 μM. Sen-Widdas (1962*b*) exits of glucose were also studied in the presence of different concentrations of phloretin. Glucose exit from new-born guinea-pig cells at 16 °C was more inhibited by phloretin than from human cells and the apparent K_i for exit was only a fifth of that for inhibition of exchange.

Fig. 5 shows a typical result in which the inhibition of glucose exit in new-born guinea-pig cells is compared with that in human cells. Not only is the intercept on the

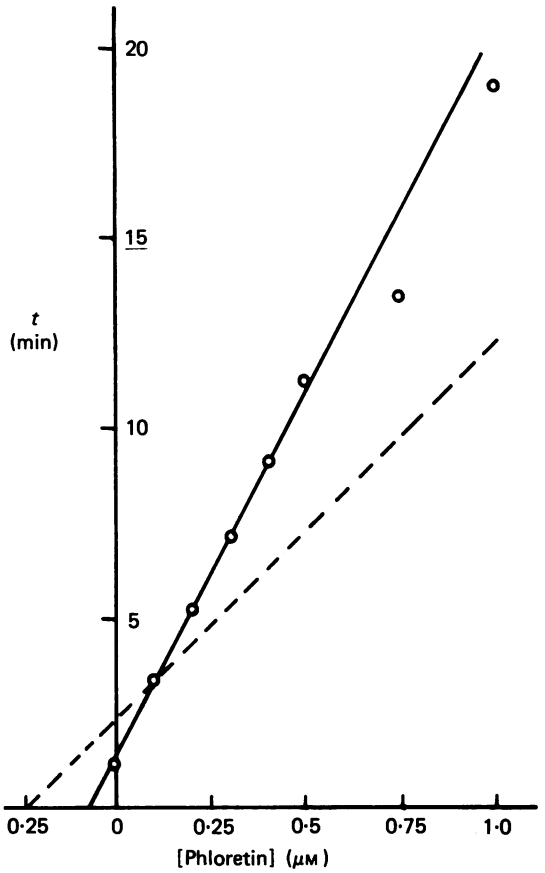

Fig. 5. Effect of phloretin concentration on glucose exit times at 16 °C. Exits were measured in a photoelectric apparatus with cells pre-incubated to 76 mM. Points and continuous line are typical results from new-born guinea-pig red cells. The interrupted line represents the corresponding results for human red cells as obtained by Basketter & Widdas (1978).

ordinate smaller, indicating a faster maximal exit rate in the absence of inhibitor, but the slope of the line is greater.

These and the results from inhibition of exchange are collected in Table I.

Inhibition by Cytochalasin B

Cytochalasin B competitively inhibited 3-*O*-methyl glucose exchange with a K_i of *ca.* 0·24 μM. The inhibition of glucose exits was also studied and the Sen-Widdas K_i was *ca.* 0·42 μM. These results are compared with corresponding results for human cells in Table 1. It will be noted that the concentration of Cytochalasin B which half-inhibited exchange was about double that for human cells but the Sen-Widdas inhibitory constant was slightly less than for human cells.

DISCUSSION

Widdas (1955) found fetal guinea-pig cells to have a faster rate of hexose transfer than human cells using glucose entry measurements, and Dawson & Widdas (1964) showed that this was true for glucose exits. The present experiments show that this also applies to hexose exchanges in fetal and new-born guinea-pig cells.

TABLE 1. Exit and exchange inhibitory constants

Inhibitor and cells	Concentration which half-inhibits glucose exit (S–W constant) μM	Inhibitory constant for exchange μM	Ratio exchange constant / S–W constant
Phlorizin			
N–b guinea-pig	50	53	1·1:1
Human	80	95	1·2:1
Phloretin			
N–b guinea-pig	0·068	0·34	5:1
Human*	0·24	0·4	1·67:1
Cytochalasin B			
N–b guinea-pig	0·42	0·24	0·57:1
Human*	0·5	0·11	0·22:1

* Data from Basketter & Widdas (1978).

This inequality does not necessarily reflect on the hexose transfer system itself since the responsible membrane components may be present in greater quantities in the fetal and new-born guinea-pig cells. The different affinities for the various inhibitors of transfer, however, more strongly suggest that there are chemical differences between the hexose transfer system of new-born guinea-pig cells and human cells, but these cannot be large since qualitatively the reactions with inhibitors are similar.

Thus the fivefold asymmetry shown towards ethylidene glucose compares with a tenfold asymmetry in human cells, but the asymmetry is in both cases a lowered affinity for the inward facing sites of the hexose transfer system. The reverse asymmetry shown by trimethyl glucoside is also seen in human cells. New-born guinea-pig cells have a greater affinity for phloretin and phlorizin than have human cells. The affinity for Cytochalasin B is less than in human cells for inhibition of exchange but is slightly greater for the inhibition of glucose exit.

The ratios of the K_i values for inhibition of exchange to those for inhibition of Sen-Widdas exits were used by Basketter & Widdas (1978) as indications of the sites of inhibition: a high value of *ca.* 6 is characteristic of outside inhibition only and a low value is characteristic of internal inhibition; inhibitors acting both inside and outside have intermediate values. It will be noted that these ratios are different for human and new-born guinea-pig cells (Table 1). In particular, the high value for phloretin may be taken to indicate that in new-born guinea-pig cells that inhibitor was acting mostly on the outside of the cell membranes whereas in human cells there was a considerable contribution from internal inhibition.

It is considered that the differences in affinities are not greater than might be

expected of a structural protein evolved in different species but serving an identical physiological function.

The physiological function of the facilitated transfer system for sugars in human red cells has never been clearly established. Since the maximal rate of transfer is at least 250 times the rate of metabolic utilization (Widdas 1954) and since the normal blood sugar would more than half-saturate the outside sites at 37 °C (Sen & Widdas

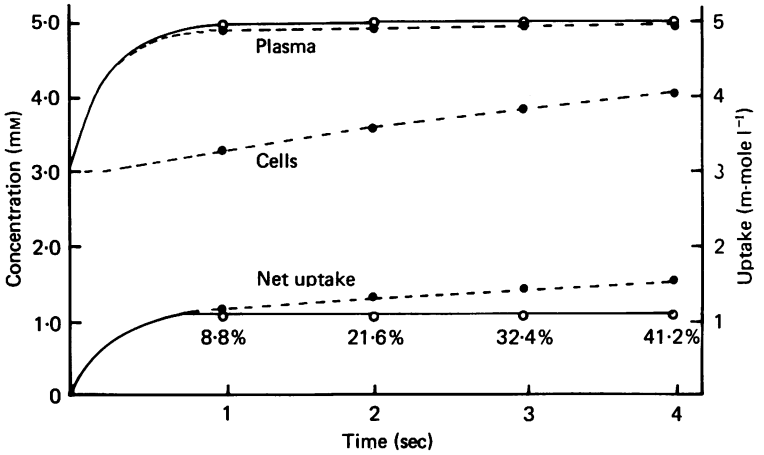

Fig. 6. Results from model calculations to show the concentration changes and uptake of glucose by blood initially equilibrated at 3 mM while in a capillary where the tissue glucose concentration was 5 mM. The uppermost lines represent the rise in plasma concentration with time in the absence (points ○ and continuous line) and presence of glucose permeable red cells (points ● and interrupted line). The middle line represents the rise in concentration in red cells whose permeability was similar to fetal guinea-pig red cells at 37 °C. The lower lines (and right hand scale) represent the net uptake of glucose if carried by plasma alone (continuous line) and if carried in both cells and plasma (interrupted line). The increment due to sugar carried in the red cells is indicated as a percentage at each sec.

1962*a*), the facilitated transfer is two orders of magnitude greater than needed for cellular metabolism. The occurrence of a facilitated transfer system with similar rates in red cells from the fetal blood of animals suggests that it may have a role in sugar transport, particularly from the placenta to the developing fetus. Thus, if transfer across the red cell membrane could occur fast enough, carriage in the red cell would give an additional capacity for transport over and above that of the fetal plasma. The cells would also have a sort of 'buffering' function in maintaining a small concentration gradient across the placenta (Widdas, 1980).

To see if this transport function would offer any practical advantage, calculations were made on a programmable calculator using the simple kinetics for asymmetric transfers described by Baker & Widdas (1973) with parameters appropriate to fetal guinea-pig cells. The calculations covered the cases of (i) uptake of glucose by blood in which the cells contributed to the uptake and (ii) the uptake of glucose by blood in which only the plasma could take up glucose. Simulations of transfer from blood to tissues were also considered.

The calculations were made at low sugar concentrations such as are likely to be met *in vivo* and it was assumed that diffusion of glucose from tissue to blood plasma would be sufficiently rapid to equilibrate in about one second, but that the equilibration between plasma and red cells would follow the facilitated transfer kinetics.

Fig. 6 shows the expected uptake by blood in a capillary bed held at a concentration of 5 mM-glucose when the blood is initially in equilibrium at 3 mM.

The result in Fig. 6 is typical of a number of similar calculations but with other starting concentrations. It shows that only a small contribution would be made by uptake in the cells during the first second although the plasma concentrations would be changing rapidly. If the blood remained in the capillary bed for 2 sec or longer, however, a significantly increased uptake would occur over and above that in a blood in which sugar carriage was in the plasma alone.

Thus blood with cells having a hexose transfer similar to that in fetal and new-born guinea-pigs would take up 22% more glucose in 2 sec, 32% more glucose in 3 sec and 41% more in 4 sec. During the first second the excess over carriage by plasma alone would only be about 9%.

The model calculations for Fig. 6 assumed a haematocrit of 45%. Carriage by the red cells could assume greater significance at higher haematocrits. Hochachka, Murphy, Liggins, Zapol, Crensy, Snider, Schneider & Quist, 1979 found the haematocrit in Weddell seal fetuses to be 70% and although the plasma glucose was always less than that of the adult the whole blood glucose was higher because of the sugar carried in the cells of fetal blood but not in the cells of the adult seal. The authors deduced a relatively slow turnover rate for glucose in that species. Rates of transfer across the red cells were not measured, but if they were as fast as in fetal guinea-pig cells the glucose uptake by blood in the placental capillaries of the seal would be incremented by more than double the percentages given in Fig. 6.

The equilibrium between cells and plasma which would be displaced due to the more rapid change in plasma concentration would be re-established (to within about 1%) in 10 sec. Thus there would be a further reduction in plasma glucose concentration before the blood reached the fetal tissues. In the tissues however, loss of glucose from the cells would help to keep up the plasma concentration.

In human and primate blood the facilitated transfer system in the cells of the adult could have a 'buffering' type action on the glucose concentration gradient on the maternal side of the placenta by the cells giving up glucose as the plasma concentration is lowered and this could add to the beneficial action of the facilitated transfer system in the cells of the fetal blood. Thus the facilitated transfer system which gives such fast rates of hexose transfer in fetal red cells of several non-primate mammals and in fetal and adult red cells of primates may be seen as serving a physiological function in the more efficient transport of glucose between mother and fetus. There is the possibility that red cells with a fast transfer of glucose were primarily evolved to serve this function during fetal life and that persistence of this property into adult life in primates is a peculiarity of primate evolution.

REFERENCES

AUBBY, D. S. & WIDDAS, W. F. (1979). Asymmetry in the hexose transfer system of erythrocytes from new-born guinea-pigs. *J. Physiol.* **293**, 73*P*.

AUGUSTIN, H. W., ROHDEN, L. F. & HACKER, M. R. (1967). Uber einige eigenschaften des monosaccharid transport systems in erythrozyten neuge borenen und envashsener kaninchen. *Acta biol. med. germ.* **19**, 723–735.

BAKER, G. F., BASKETTER, D. A. & WIDDAS, W. F. (1978). Asymmetry of the hexose transfer system in human erythrocytes. Experiments with non-transportable inhibitors. *J. Physiol.* **278**, 377–388.

BAKER, G. F. & WIDDAS, W. F. (1973). The asymmetry of the facilitated transfer system for hexoses in human red cells and the simple kinetics of a two component model. *J. Physiol.* **231**, 143–165.

BASKETTER, D. A. & WIDDAS, W. F. (1978). Asymmetry of the hexose transfer system in human erythrocytes. Comparison of the effects of Cytochalasin B, phloretin and maltose as competitive inhibitors. *J. Physiol.* **278**, 389–401.

DAWSON, A. C. & WIDDAS, W. F. (1964). Variations with temperature and pH of the parameters of glucose transfer across the erythrocyte membrane in the foetal guinea-pig. *J. Physiol.* **172**, 107–122.

HANES, C. S. (1932). Studies on plant amylases. I. The effect of starch concentration upon the velocity of hydrolysis by the amylase of germinating barley. *Biochem. J.* **26**, 1406–1421.

HOCHACHKA, P. W., MURPHY, B., LIGGINS, G. C., ZAPOL, W., CRENSY, R., SNIDER, M., SCHNEIDER, R. & QUIST, J. (1979). Unusual maternal-fetal blood glucose concentrations in Weddell seal. *Nature, Lond.* **277**, 388–389.

LEE, P., AUVIL, J., GREY, J. E. & SMITH, M. (1976). 3-*O*-methyl glucose transport in new-born and adult dog red cells. *Fedn Proc.* **35**, 780.

SEN, A. K. & WIDDAS, W. F. (1962*a*). Determination of the temperature and pH dependence of glucose transfer across the human erythrocyte membrane measured by glucose exit. *J. Physiol.* **160**, 392–403.

SEN, A. K. & WIDDAS, W. F. (1962*b*). Variations of the parameters of glucose transfer across the human erythrocyte membrane in the presence of inhibitors of transfer. *J. Physiol.* **160**, 404–416.

WIDDAS, W. F. (1954). Facilitated transfer of hexoses across the human erythrocyte membrane. *J. Physiol.* **125**, 163–180.

WIDDAS, W. F. (1955). Hexose permeability of foetal erythrocytes. *J. Physiol.* **127**, 318–327.

WIDDAS, W. F. (1980). The asymmetry of the hexose transfer system in the human red cell membrane. *Curr. Top. Membranes & Transp.* **14**, pp. 165–223. New York and London: Academic Press.

ZEIDLER, R. B., LEE, P. & KIM, H. D. (1976). Kinetics of 3-*O*-methyl glucose transport in red blood cells of new-born pigs. *J. gen. Physiol.* **67**, 67–80.