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HEXOSE PERMEABILITY OF FOETAL ERYTHROCYTES

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Although the permeability properties of erythrocytes from various species have been the subject of extensive investigations during the present century, few studies have been made on the erythrocytes from foetal blood. Widdas (1951) reported that the haemolysis of foetal sheep erythrocytes in hypotonic saline was markedly slower than that of adult sheep erythrocytes. A similar result for human foetal erythrocytes has been reported by Sjölin (1954) who also studied the mechanical and osmotic resistance (fragility) changes in foetal and neonatal blood.

That there might be a difference in permeability to sugars seemed possible from the observation by Hitchcock (1949), confirmed in this Department by Dobbing & Long (unpublished observations), that the sugars in the blood of the foetal sheep were approximately evenly distributed between cells and plasma, whereas the maternal blood sugar was all in the plasma. A study of the distribution of sugars in foetal bloods of various species has recently been reported by Goodwin (1954).

The distribution of sugar between cells and plasma gives no evidence as to the nature of the permeability, and the present investigations were undertaken to see if the process of penetration was a simple diffusion or a facilitated transfer such as is postulated to account for glucose penetration into the adult human erythrocyte (LeFevre, 1948; Widdas, 1954*a*).

A preliminary account of the results was given to the XIX International Physiological Congress (Widdas, 1953*a*).

METHOD

The apparatus used (Ørskov type) was as described (Widdas, 1953*b*) and the experimental technique and method of analysing the results followed that used for studies on the hexose permeability of human erythrocytes (Widdas, 1954*a*).

The method of collecting foetal blood varied in accordance with the facilities available. In the laboratory, foetuses were delivered by caesarean section under anaesthesia (spinal anaesthesia in the case of the sheep, Nembutal in the case of guinea-pig, rabbit and cat), and blood obtained from

the umbilical vein. The blood was either heparinized or defibrinated, there being no observable difference on the experimental results.

In the case of the pig and deer the uterus was removed from the animal after it had been shot. The foetuses were dead, but the foetal blood was still fluid and sufficient was obtained either by umbilical venepuncture or cardiac puncture.

Human foetal blood was similarly obtained after foetal death, the whole conceptus being removed at a therapeutic caesarean section.

RESULTS

Foetal cells of sheep, deer, pig, rabbit, guinea-pig and human showed reswelling in hypertonic mixtures of glucose and saline (1·0% NaCl solution); that is, they are qualitatively permeable to glucose. In the case of the rabbit, however, the cell volumes were relatively unstable and the results were not suitable for quantitative measurements. Foetal cat red cells did not reswell in glucose solutions and thus are judged to be impermeable.

To decide the type of process involved in hexose penetration of foetal cells three types of experiment were made:

(1) The effect of increasing glucose concentration was studied and the swelling curves analysed on a basis of (*a*) diffusion, and (*b*) near saturation carrier kinetics (Widdas, 1952, 1954*a*).

(2) The swelling curves in solutions of the ketose sugar sorbose were compared with those in glucose solutions in a number of experiments.

(3) Where possible the method of glucose sorbose competition was employed to estimate the value of ϕ_g (the equilibrium constant of the carriers reacting with glucose).

Glucose

Reswelling curves in four different concentrations of glucose (obtained by repeated injections of 0·5 ml. glucose solution in 21 ml. dilute cell suspension) were analysed on a basis of the diffusion equation

$$kt = C' + 1 - (1+C)V + (1+C)\ln\frac{C-C'}{(1-V)(1+C)} \qquad (1)$$

$$= F(C, V), \qquad (1a)$$

where C' refers to the initial hexose concentration and C is the total hexose concentration of the medium. V is the volume of cell water. In equation (1) k is the penetration constant on a basis of diffusion, t is the time in minutes taken to reach volume V and $F(C, V)$ is a function of the glucose concentrations (C and C') and the volume of cell water (V). Plotting the times taken to reach intermediate volumes on the reswelling curve against calculated values of $F(C, V)$ should give a straight line with slope equal to k, the diffusion penetration constant. This is illustrated in Fig. 1, the data being from a typical experiment with foetal guinea-pig erythrocytes.

It is seen that as the concentration of glucose in increased the value of k is markedly reduced. If log k is plotted against log C as in Fig. 2 it is found that

the points fit a line with a slope of approximately −2. This result is similar to that obtained by Wilbrandt, Guensberg & Lauener (1947) for human erythrocytes and may be expected from the kinetics of a hexose carrier (Widdas, 1952).

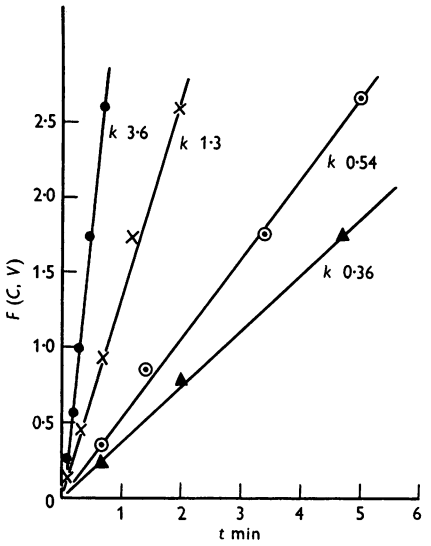

Fig. 1

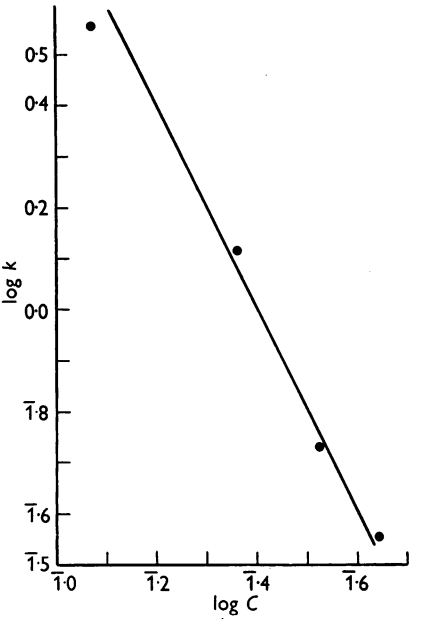

Fig. 2

Fig. 1. Guinea-pig erythrocytes from foetus of 63 days gestation age. Reswelling curves in glucose solutions analysed by plotting times to reach intermediate volumes against $F(C, V)$. The concentration was increased in stages. Points ● represent a glucose concentration of 0·1165; points ×, 0·227; points ⊙, 0·333; points ▲, 0·435 isotonic units. The slopes indicate the values of k the penetration constant on a basis of diffusion.

Fig. 2. Plot of log k against log C. The values of k and C were obtained from the result shown in Fig. 1. The continuous line has been drawn with a slope of −2.

Analysing the same data by the near saturated carrier equation of the earlier paper (Widdas, 1954*a*)

$$k't = C(1+C)\left\{C' + 1 - (1+C)\,V + C\ln\frac{C-C'}{(1-V)\,(1+C)}\right\} \qquad (2)$$

$$= F'(C, V), \qquad (2a)$$

the result illustrated in Fig. 3 is obtained. The points lie about a common line whose slope ($k' = 0{\cdot}058$) gives the near saturated carrier constant.

This equation is an approximation of the full carrier equation such that $k' = K\phi$, where K represents the maximum transfer of sugar into the cell if the externally placed carriers were fully saturated with glucose and the internally situated carriers unsaturated. ϕ represents the equilibrium constant of the carriers reacting with glucose.

Erythrocytes of foetal sheep, deer, pig and human show the same features and the method of analysis illustrated in Fig. 3 has been used to estimate the value of k' in each case.

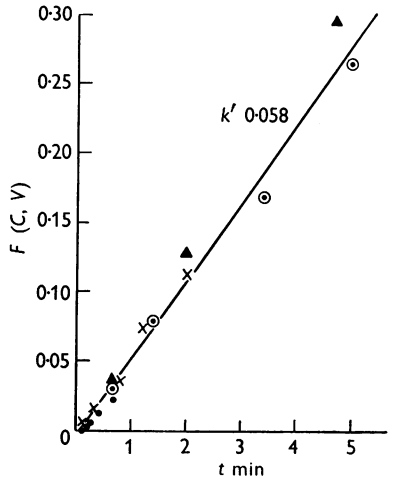

Fig. 3. Result of Fig. 1 analysed by the near saturated carrier equation. Measured times to reach intermediate cell volumes have been plotted against calculated values of $F'(C, V)$ in the four different glucose concentrations: ●, 0·1165; ×, 0·227; ⊙, 0·333; and ▲, 0·435 isotonic units.

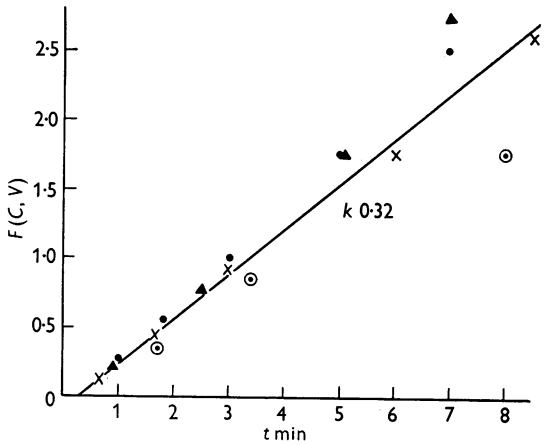

Fig. 4. Analysis of reswelling curves in four increasing concentrations of sorbose according to the diffusion type equation. Measured times to reach intermediate volumes have been plotted against calculated values of $F(C,V)$. The concentrations were: ●, 0·1165; ×, 0·227; ⊙, 0·333; and ▲, 0·435 isotonic units.

Sorbose

Swelling curves of foetal guinea-pig cells in sorbose solutions analysed by the diffusion equation (1) are shown in Fig. 4 and satisfactorily fit a line of

slope $k = 0{\cdot}32$, whereas plotted according to the near saturated carrier equation four lines of widely differing slopes are obtained.

This means that the kinetics of sorbose penetrations are somewhat different from glucose as they are in the case of the human erythrocyte. In the previous paper (Widdas, 1954*a*) it was explained that the kinetics of the carrier transfer approximated to the diffusion equation when the carrier equilibrium constant was high relative to the hexose concentrations used. In this approximation the diffusion penetration constant k can be replaced by K/ϕ, where K and ϕ have the same significance as referred to above.

Since the values obtained from foetal bloods for k'_g and k_s differ from those found in adult human erythrocytes the differences may lie in the respective values of K, of ϕ, or of both. The ratio

$$\frac{k'_{\text{glucose}}}{k_{\text{sorbose}}} = \frac{K\phi_g}{K/\phi_s} = \phi_g \cdot \phi_s, \tag{3}$$

where the subscripts g and s refer to glucose and sorbose respectively, is independent of K, and it seemed of interest to calculate this value for the results of the different species.

TABLE 1. Species variation in hexose permeability of erythrocytes

Showing the range of values of k' glucose, k sorbose and the ratio k'_g/k_s found in the different species examined. The constants are derived from isotonic units and equations previously described (Widdas, 1954*a*). The numbers in brackets indicate the number of samples on which the measurements have been made.

Species	k'_{glucose}	k_{sorbose}	k'_g/k_s
Foetal pig	0·007 (2)	0·073 (1)	0·15 (1)
Foetal deer	0·013 (1)	0·128 (1)	0·10 (1)
Foetal sheep	0·027 (24)	0·275 (18)	0·10 (18)
Human adult	0·027 (7)	0·22 (7)	0·12 (7)
Human foetus	0·051 (3)	—	—
Foetal guinea-pig	0·073 (8)	0·45 (3)	0·15 (3)

Table 1 gives a summary of the values of k'_{glucose}, k_{sorbose} and the above ratio obtained from the experiments in which sorbose penetration was measured. In the case of the deer and pig only a limited number of samples were available, and quantitative measurements of sorbose penetration were only made on one sample in each case. Average values for erythrocytes from one human adult are given for comparison with the foetal results.

Although there were few samples of pig and deer foetal bloods examined and there exists a considerable range of values of k'_{glucose} between species it is felt that the ratio k'_g/k_s is in reasonable agreement, and that the major differences which exist are most probably in the values of K and not of ϕ. This is what might be expected if the carrier molecules in the various species were similar biochemical entities.

Glucose-sorbose competition

Experiments on glucose-sorbose competition such as those described for the human erythrocyte (Widdas, 1954*a*) have been carried out on selected samples of red cells from foetal sheep and foetal guinea-pigs. The cases were necessarily selected since it was found that cell suspensions prepared from some foetal bloods were not stable over long periods in the cuvette. Two samples of foetal sheep's blood and four samples of foetal and neonatal guinea-pig blood have been tested in this way and the mean values of ϕ_g obtained were 11 and 14 mM for foetal sheep and guinea-pig cells respectively. As pointed out in the previous paper, the estimation of ϕ_g by this method may be in error by a factor of two, but these values agree well with that found in the human erythrocyte by the same technique. The results give further support to the suggestion that the equilibrium constants of the hexose carriers are similar in the different species.

Post-natal change

The post-natal change in permeability to glucose was followed in a guinea-pig over a period of 65 days. It was found that the value of k' fell from 0·058 at the 2nd day to 0·039 by the 9th day, and thereafter was difficult to estimate accurately but was in the range 0·027 to 0·012 on the 23rd day. Although penetration of glucose continued to be detectable up to the 51st day, the amplitude of the reswelling part of the record obtained with the apparatus

TABLE 2. Post-natal change in glucose permeability of young guinea-pig erythrocytes

Results from erythrocytes of a young guinea-pig at different ages. The reduction in light change due to reswelling of the cells makes assessment of $k'_{glucose}$ unsatisfactory but the estimated range in which $k'_{glucose}$ lay is shown.

Days post-natum	Weight (g)	$k'_{glucose}$	Reswelling change as percentage of total light change (%)
2	119	0·058	49
9	185	0·039	44
16	238	0·024–0·040	20
23	275	0·012–0·027	17
37	342	0·017–0·030	19
51	409	0·012–0·029	9
65	470	Negligible	3

decreased with age, and this made quantitative assessment unreliable. By the 65th day the change in the trace due to reswelling was less than 3% of the total change in light transmission (i.e. that due to the dilution effect and cell shrinkage in the hypertonic mixture of glucose and saline). The results of this experiment are summarized in Table 2.

Although the fall in k' may be interpreted as a loss of carriers by the cells in the early post-natal period the diminished amplitude of the light change due to swelling of the cells is suggestive of a mixing of permeable red cells with

cells whose permeability is negligible, and which remain in a shrunken condition. The initial light change due to shrinkage of the cells would be unchanged in such a mixture (since all the cells take part), but only the glucose permeable cells would return to their former volume and the amplitude of light change during reswelling would thus be reduced. Such diminished amplitudes are given by suspensions made up of a mixture of foetal and adult guinea-pig erythrocytes. More experiments of this nature are required, however, before the possible factors responsible for bringing about this change can be elucidated with certainty.

The post-natal changes in the sheep have not been studied.

DISCUSSION

Kozawa (1914) demonstrated that human erythrocytes were permeable to hexoses and pentoses as were the erythrocytes of *Macacus rhesus*. The red cells of ox, pig, rabbit, guinea-pig, goat, horse, ram and cat were all impermeable but dog cells were slowly permeable to glucose.

Since Kozawa's work (1914), glucose permeability has been regarded as a specialization of human and ape erythrocytes (Jacobs, 1939; LeFevre & LeFevre, 1952). Wilbrandt (1938) studied the slow permeability shown by dog erythrocytes, but in that species the time taken to attain equilibrium is over 1 hr in relatively low hexose concentrations compared with 1 or 2 min taken by the human erythrocyte.

The erythrocytes from the foetal blood of the pig, rabbit, guinea-pig, sheep and deer display a glucose permeability of the same order of magnitude as that of the human erythrocyte. In six species so far examined only the erythrocytes of the foetal cat failed to show glucose permeability. The erythrocytes from human foetal blood were found to have a greater permeability than the adult but only by a factor of two. Their permeability compares with that of foetal guinea-pig erythrocytes. The specialization of the primate erythrocyte in regard to glucose permeability is thus not unique and the differences observed in the adult animal do not extend to foetal bloods.

The penetration of glucose into foetal erythrocytes of various species is not only qualitatively similar to that of the adult human erythrocyte but has the same kinetic characteristics which for the human erythrocyte (LeFevre, 1948; LeFevre & LeFevre, 1952; Widdas, 1954*a*) have been adduced to indicate the existence of a carrier mechanism or facilitated transfer. Their behaviour towards ketose sugars and the estimations of the carrier equilibrium constant in the case of foetal guinea-pig and foetal sheep cells suggest that all species possess a similar hexose carrier mechanism but that they possess it to different degrees.

The anomaly of the erythrocytes of foetal cats brings to mind the fact that adult cat erythrocytes are characterized by a low potassium content and this has been confirmed also for red cells of cat foetuses. On the other hand, Wise,

Caldwell, Parrish, Flipse & Hughes (1947) suggested that high potassium content may be a feature of foetal erythrocytes in the ox, and this has been found to apply also in the sheep (Hallman & Karvonen, 1949; Widdas, 1954*b*). Foetal sheep cells have a glucose transfer mechanism and from results (Goodwin, 1954) on the distribution of sugars between cells and plasma it would appear that a similar mechanism exists in foetal erythrocytes of the ox. Thus in the ruminant the foetal erythrocytes may have a further difference from the adult erythrocytes in regard to their potassium and sodium concentrations.

It may be thought that since the accumulation of potassium and the extrusion of sodium by cells requires metabolic energy, those cells provided with a glucose transfer mechanism of the type described, would be at an advantage and that this might be a factor in the higher potassium content found in foetal sheep erythrocytes relative to the adult cells. However, in the guinea-pig (Widdas, 1954*b*) the rapid penetration of sugar into foetal erythrocytes would appear to confer no advantage since both foetal and adult cells have about equal potassium concentrations. There are several possible explanations for this finding. (i) Glucose may be metabolized at the cell surface without penetration. Various workers, e.g. Conway & Downey (1950), Rothstein, Meier & Hurwitz (1951) suggest that this occurs in yeast, but there is no evidence of its occurrence in erythrocytes. (ii) The potassium accumulation and sodium extrusion mechanism may use an alternative substrate. Thus Clarkson & Maizels (1954) have found that lactate was effective for the sodium extrusion mechanism of chick erythrocytes. (iii) It has been pointed out (Widdas, 1954*a*) that the inward transfer of glucose into the human erythrocyte is about 250 times the rate which would be necessary for the cell's metabolic requirements. By analogy if the adult guinea-pig cells were to possess a carrier transfer to a degree only about 0·5 % of that observed in the foetal cells it might still be adequate to supply the metabolic needs. Such a low glucose permeability may have escaped detection by the methods employed by Kozawa and is not suitably studied by the Ørskov technique since the metabolic removal of glucose may not allow its accumulation within the cell and hence osmotic equilibration with the external medium, on which the method depends, will be incomplete. A few results suggest that such a slow penetration of glucose does exist in adult guinea-pig erythrocytes, and further work on this problem is proposed.

For the present a more cautious view of the erythrocyte impermeability to glucose reported by Kozawa is advisable until adult cells from each of the species concerned can be re-examined by other methods, but even if some erythrocytes prove to be slowly permeable there will still be differences of two orders of magnitude between their rates of glucose penetration and those found in erythrocytes of human adult blood.

SUMMARY

1. In experiments using foetal blood, glucose permeability was displayed by erythrocytes of five out of six species examined (sheep, deer, pig, rabbit and guinea-pig). Erythrocytes of foetal cats did not show glucose penetration.

2. The rates of glucose penetration were of the same order as found in the adult human erythrocyte. In addition, the response to increasing glucose concentration and the penetration of sorbose showed features which are characteristic of a facilitated transfer by a carrier mechanism.

3. In erythrocytes of foetal guinea-pigs and foetal sheep the carrier equilibrium constant for glucose was estimated by sorbose-glucose competition experiments and was similar to that found for human erythrocytes.

4. The results are consistent with the foetal cells of these mammals possessing a facilitated hexose transfer mechanism essentially similar to that of the human erythrocyte but different in degree.

5. The foetal erythrocytes of the sheep (but not of the guinea-pig) also have a higher potassium concentration than the adult cells and a possible relationship to glucose permeability is discussed.

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