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VARIATIONS WITH TEMPERATURE AND pH OF THE PARAMETERS OF GLUCOSE TRANSFER ACROSS THE ERYTHROCYTE MEMBRANE IN THE FOETAL GUINEA-PIG

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Although the rapid penetration of glucose into erythrocytes from adult blood appears to be peculiar to primates (Kozawa, 1914) it was shown by Widdas (1955) that red cells from the foetal blood of several domestic and laboratory animals had a facilitated transfer for hexoses similar to that in the human erythrocyte. The investigation used the technique of following sugar entry into red cells at increasing concentrations and was carried out at 37° C and pH 7·4.

The measurement of glucose loss from cells previously incubated with sugar, as developed by Sen & Widdas (1962*a*), can more conveniently be used to study the variation of the parameters of the facilitated transfer over a wide range of temperature and pH, and it was decided to use this technique to extend the comparison between the mechanisms for glucose permeability in adult human and foetal guinea-pig erythrocytes.

The facilitated transfer of hexoses is presumed to involve a complexing reaction with some membrane component or 'carrier' and can be considered as having two parameters: (i) the capacity for transfer if the membrane component is fully saturated with glucose (K) and (ii) a parameter depending on the affinity of the membrane component for glucose. This can be characterized by a half-saturation constant (ϕ) following the procedure of Sen & Widdas (1962*a*).

It has been found that, whereas the pH dependence of glucose transfer in foetal guinea-pig cells is similar to that of human cells, the temperature dependence shows a significant difference. This difference concerns the half-saturation constant (ϕ) which, although similar to human cells at 37° C, does not fall in the same regular manner as the temperature is reduced. The parameter K is greater in foetal guinea-pig cells than in human cells throughout the temperature range studied (7–37° C), but its variation with temperature is essentially similar to that in human erythrocytes.

The difference in temperature dependence of the half-saturation constant

ϕ between human and foetal guinea-pig cells is not necessarily evidence for chemical differences in the membrane component since the rate-limiting steps at the various temperatures are not necessarily the same in the two types of cells. This latter possibility has been investigated by a series of calculations with hypothetical rate constants.

METHODS

Foetal guinea-pig blood was used throughout the series of experiments. Foetuses (with ages of gestation between 40 and 69 days) were exposed by Caesarean section under urethane anaesthesia. Blood was then taken from the umbilical vein, dry heparin being used as anti-coagulant. Samples from various foetuses in a litter were stored separately at 2–4° C. Experiments were carried out either on the day of the operation or up to three days subsequently. The washing of the blood and techniques for determining the parameters were as described by Sen & Widdas (1962*a*) except that the line through the points of exit times plotted against outside concentration (Fig. 2, Sen & Widdas, 1962*a*) was not drawn by eye but was the regression line calculated in the usual way. The suspension of cells was made up to contain 0·015 ml. cells/ml. glucose containing saline buffer, as described by Dawson & Widdas (1963), and 0·2 ml. of this suspension was injected into 21 ml. saline buffer in the cuvette of the photo-electric apparatus.

RESULTS

Effect of age of gestation

During an exit experiment foetal guinea-pig erythrocytes, previously equilibrated with 76 mM glucose, lose the contained sugar about twice as fast as human cells. It is correspondingly more difficult to record exits at 37° C or above and the most reliable results are obtained at lower temperatures.

The age of gestation of foetuses from which blood was obtained was estimated from the weight, using the relation $W^{\frac{1}{3}} = 0{\cdot}09\ (t - 16)$, where W is the weight in grams and t the gestation age in days (Huggett & Widdas, 1951).

An examination of results at 22° and 27° C showed that there was no correlation between the parameters of glucose transfer and age of gestation at either temperature and this factor is not further discussed. The majority of samples were taken from foetuses in the last two weeks of pregnancy.

Effect of pH

Foetal guinea-pig erythrocytes were less stable than human red cells at extremes of pH and satisfactory results were obtained only in the range pH 6·5–8·5. The results obtained at 27° C are shown in Table 1 and are similar to those for human cells at 37° C in so far as they do not show any marked pH effect.

Effect of temperature

The effect of temperature on the maximal transfer rate (K) and the half-saturation constant (ϕ) is shown in Table 2. Although the former parameter (K) varies by a factor of 36 in the temperature range 7–37° C, the half-saturation constant ϕ varies by a factor of less than 2. With human cells,

TABLE. 1. The effect of pH on the half-saturation constant for glucose (ϕ_g) and maximal transfer rate (K) across the membrane of guinea-pig foetal erythrocytes at 27° C. The number of experiments is given in parentheses.

pH	ϕ_g (mM)	K (isotonic units)
6·4 (1)	3·9	1·03
6·8 (2)	3·15	1·17
7·2 (1)	3·3	1·13
7·4 (8)	3·12	1·12
8·0 (1)	3·1	1·03
8·2 (2)	3·65	1·13
8·5 (2)	3·54	1·05

Sen & Widdas (1962*a*) found that over the same temperature range the factors were 22 for K and 7 for ϕ_g; thus in foetal guinea-pig cells there is a greater temperature effect on K and a smaller one on ϕ_g. The difference in the temperature effect on ϕ_g between 7 and 22° C is the most striking. In this temperature range ϕ_g for human cells increases by a factor of 3, whereas for guinea-pig cells ϕ_g at 7° C is approximately the same as at 22° C.

TABLE 2. Effect of temperature on the half-saturation constant for glucose (ϕ_g) and the maximal transfer rate (K) across the membrane of guinea-pig foetal erythrocytes at pH 7·4. The number of experiments is given in parentheses.

Temperature (°C)	Mean ϕ_g (mM ± S.D.)	Mean K (isotonic units ± S.D.)
7 (6)	2·77 ± 0·68	0·046 ± 0·006
12 (6)	2·56 ± 0·11	0·127 ± 0·036
17 (6)	3·03 ± 0·29	0·292 ± 0·03
22 (8)	2·68 ± 0·44	0·62 ± 0·06
27 (8)	3·12 ± 0·47	1·12 ± 0·21
32 (4)	4·35 ± 0·82	1·28 ± 0·13
37 (2)	4·5	1·68

At 37° C the results were less satisfactory than at the lower temperatures and to make an independent estimate of the half-saturation constant foetal guinea-pig cells were partly inhibited by incubation with either fluoro-2,4-dinitrobenzene (DNFB) after Sen & Widdas (1962*b*) or *N*-ethyl maleimide NEM as described for human cells (Dawson & Widdas, 1963). It was found that cells inhibited by these compounds gave similar half-saturations to un-inhibited cells although the time for an exit experiment

may be increased several fold. In two experiments with DNFB values of ϕ_g at 37° C of 6·0 and 6·1 mM were obtained, and with NEM incubation in one experiment a value of 6·4 mM was obtained.

There is thus no doubt that the parameter ϕ_g increases with temperature above 22° C, but the actual value at 37° C taken as 4·5 mM, can only be regarded as approximate and may be too low.

Kinetic nature of ϕ and K

The half-saturation (ϕ) has been regarded by many investigators as equivalent to the Michaelis constant of an enzyme reaction, but in a carrier system such as that depicted in Fig. 1 the rate constant for transfer through the membrane produces a perturbation and in consequence the half-saturation constant is not a true Michaelis constant.

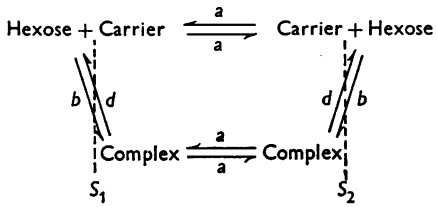

Fig. 1. Simple carrier model for the facilitated transfer of glucose across the erythrocyte membrane. a is the rate constant for transfer between interfaces S_1, S_2, presumed identical for complexes and unsaturated carriers. b and d are rate constants for the formation and dissociation of complexes respectively.

In the scheme given in Fig. 1 the rate for transfer of hexoses across a membrane is given by

$$\underset{S_1 \longrightarrow S_2}{\text{transfer}} = \frac{K'}{2}\frac{ad}{b}\frac{C_1 - C_2}{\left(C_1 + \frac{d}{b}\right)\left(C_2 + \frac{d}{b}\right) + \frac{a}{b}\left(C_1 + C_2 + \frac{2d}{b}\right)}, \qquad (1)$$

where C_1 and C_2 are the concentrations of glucose in the bulk solution at sides S_1 and S_2, a is the rate constant for movement through the membrane (assumed to be the same for saturated and unsaturated carriers), b is the rate constant for the association of glucose and carrier to form a complex, d is the rate constant for dissociation of complexes and K' is a constant proportional to the total amount of carrier in the membrane (Widdas, 1953; Bowyer, 1957).

It was shown by Widdas (1953) that if a^2/b^2 is presumed to be very small, the equation approximates to

$$\underset{S_1 \longrightarrow S_2}{\text{transfer}} = \frac{K'}{2}\frac{ad}{a+d}\left\{\frac{C_1}{C_1 + \frac{a+d}{b}} - \frac{C_2}{C_2 + \frac{a+d}{b}}\right\} \qquad (2)$$

and if this is compared with the simpler equation normally used (e.g. Widdas, 1954*a*, *b*), namely

$$\underset{S_1 \longrightarrow S_2}{\text{transfer}} = K\left(\frac{C_1}{C_1+\phi} - \frac{C_2}{C_2+\phi}\right) \tag{3}$$

it will be seen that the half-saturation constant is given by $(a+d)/b$ and not by d/b which would be the case for a true Michaelis constant. The position is more equivalent to that of the Briggs–Haldane treatment of enzyme kinetics (Dixon & Webb, 1958).

The use of Michaelis terms would only be justified if a were so small as to make the second term of the denominator of equation (1) insignificant. On the other hand, if d were smaller than a, the half-saturation constant might approximate to a/b and depend only on the association rate constant and the constant for movement through the membrane.

This kinetic treatment also shows that the maximal transfer rate contains the rate constant for dissociation of complexes as well as the kinetic term for movement through the membrane. Thus K of the simpler equation corresponds to $(\frac{1}{2}K')ad/(a+d)$ in equation 2. If $a \gg d$ K would approximate to $\frac{1}{2}K'd$, whereas if $a \ll d$ the constant would approximate to $\frac{1}{2}K'a$.

Although these fuller equations have been partly used by Fisher (1962) and by Fisher & Zachariah (1961) in the interpretation of their results on the effect of insulin on the hexose permeability of perfused rat hearts, there has as yet been no need for their use in analyses of results obtained with red cells. However, they may be important when comparing the parameters of glucose permeability in two different species of red cells and also when considering the temperature dependence of these parameters. This is because the rate constants a, b and d may have different temperature coefficients so that their relative magnitude may change with temperature. This aspect is considered in a subsequent section.

Validity of approximations

Since equation (2) appeared to be symmetrical in respect of the rate constants a and d there seemed no reason why equation (3) should not be a satisfactory approximation for any of the following three cases:

$$d \gg a, \quad \phi \simeq d/b, \quad K \simeq \tfrac{1}{2}K'a; \tag{4}$$

$$d \ll a, \quad \phi \simeq a/b, \quad K \simeq \tfrac{1}{2}K'd; \tag{5}$$

$$d \lessgtr a, \quad \phi \simeq \frac{a+d}{b}, \quad K \simeq \frac{K'}{2}\frac{ad}{(a+d)}. \tag{6}$$

The relation in equation (4) is that usually assumed to occur, but there has been no very good evidence for or against this.

In order to investigate the validity of the approximations used in deducing equation (2), equation (1) was used to derive an expression for the entry and exit of glucose. The following is the integrated form of this equation

$$C'-S+\left\{\frac{\frac{d}{b}C\left(1+\frac{a}{d}\right)+\frac{d^2}{b^2}\left(1+\frac{2a}{d}\right)}{\left(C+\frac{d}{b}\right)\left(1+C+\frac{d}{b}\right)+\frac{a}{b}\left(1+2C+2\frac{d}{b}\right)}+C\right\}\ln\frac{C-C'}{C-S}$$

$$=\frac{K(ad/b)\,t}{2\left\{\left(C+\frac{d}{b}\right)\left(1+C+\frac{d}{b}\right)+\frac{a}{b}\left(1+2C+2\frac{d}{b}\right)\right\}}, \qquad (7)$$

where C' is the concentration of glucose inside the cell at the start of the experiment ($t=0$), S the quantity of sugar in the cell at time t and C the concentration of sugar in the medium, all expressed in isotonic units.

The volume of the cell water was presumed to vary to maintain osmotic equilibrium so that

$$\frac{V}{V_0}=\frac{1+S}{1+C}$$

where V is the volume at any time and V_0 the initial volume in isotonic saline.

A programme was prepared in Mercury autocode and the University of London computer was used for the numerical evaluation of equation (7) for a large number of different volumes and sugar concentrations. The parameters were varied in the following two ways:

(i) A value of 0·01 isotonic units was assigned to d/b and six values in the range from 0 to 0·03 were given to a/b. Tables of values of $\frac{1}{2}Kat$ were obtained.

(ii) A value of 0·01 isotonic units was assigned to a/b and six values in the range from 0 to 0·03 were given to d/b. In this case $\frac{1}{2}Kdt$ was evaluated.

From the results under (i) a family of theoretical exit curves was reconstructed such as would be expected from cells equilibrated with 76 mM glucose when placed in saline media of low glucose concentrations.

Such theoretical curves (Fig. 2) were treated in the way described by Sen & Widdas (1962*a*) in order to determine the half-saturation constant (ϕ_g) and the maximal transfer rate (K) to see how those authors' results might have been affected by the approximations used. Their treatment consisted of producing the straight part of the exit records to cut the abscissa (normally the time axis, but in this case the axis of $\frac{1}{2}K'at$).

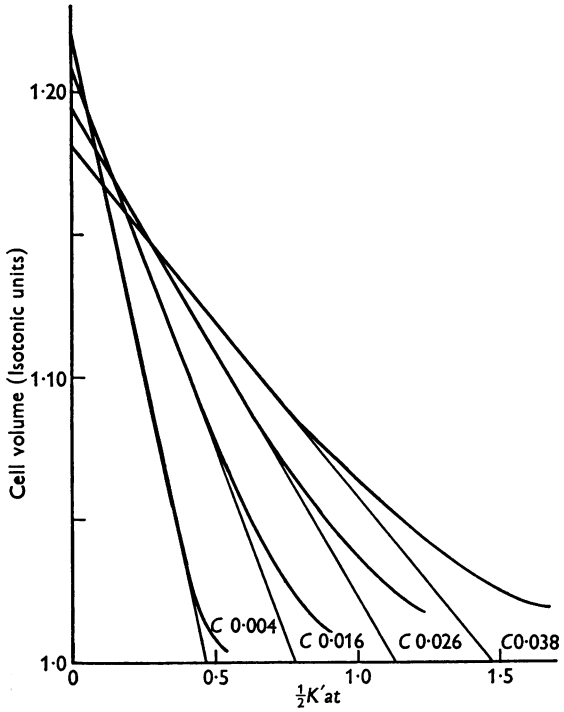

Fig. 2. Theoretical exit curves into low glucose concentrations calculated from equation (7) on the assumption that $a = 0{\cdot}6d$ and $d/b = 0{\cdot}01$ isotonic units. The straight parts of the curves have been produced to cut the abscissa and the values of $\frac{1}{2}K'at$ at these intercepts measured as described in the text.

The equation used to relate the times measured in this way to the outside glucose concentration (C) was

$$t = \frac{S' + \phi_g}{K\phi_g}(C + \phi_g),$$

where S' was the initial concentration of glucose in the cells.

Based on the fuller treatment of equation (2), the corresponding equation is

$$t = \frac{S' + (a+d)/b}{\left(\frac{K'}{2}\frac{ad}{a+d}\right)\left(\frac{a+d}{b}\right)}\left(C + \frac{a+d}{b}\right)$$

but, since the computed values under (i) above were for $\frac{1}{2}K'at$, it is convenient to re-arrange this equation in the form

$$\tfrac{1}{2}K'at = \frac{S' + (a+d)/b}{\left(\frac{d}{a+d}\right)\left(\frac{a+d}{b}\right)}\left(C + \frac{a+d}{b}\right).$$

Thus, by plotting the values of $\frac{1}{2}K'at$ measured in the way illustrated in Fig. 2, against C, there should be a linear relationship. The intercepts of this line with the abscissa ($\frac{1}{2}K'at = 0$) should give $-(a+d)/b$ whilst the intercept with the ordinate ($C = 0$) should occur when

$$\tfrac{1}{2}K'at = \frac{S' + (a+d)/b}{d/a+d)}$$

from which $d/(a+d)$ can be derived by the relation

$$\frac{a+d}{d} = \frac{S' + \left(\frac{a+d}{b}\right)}{\text{intercept}_{(C=0)}}.$$

In Fig. 3 this analysis has been applied to the results from six families of curves of the type shown in Fig. 2 but in which the relative magnitude of a and d have been varied. The value of the half-saturation constant and the derived value of $d/(a+d)$ are compared with the theoretical values in Table 3.

This confirms that, if glucose transfer follows the kinetics of equation (2), the Sen & Widdas method of analysing the results gives $(a+d)/b$ for ϕ_g and not d/b as would be the case if ϕ_g was a true Michaelis constant. The approximation, however, would tend to under-estimate the true value by

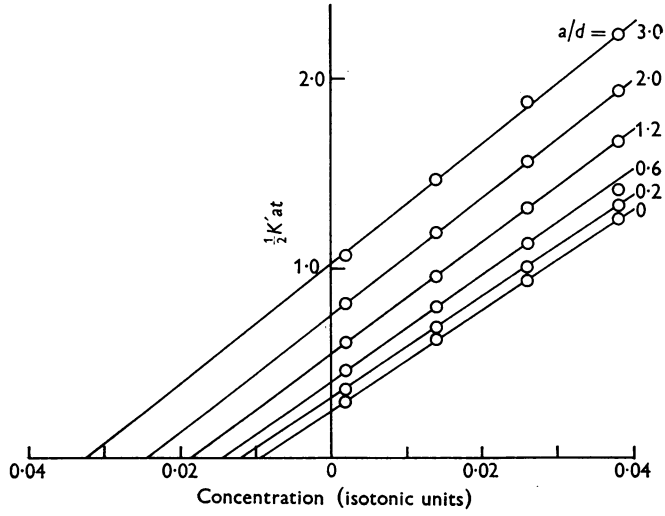

Fig. 3. The measured values of $\frac{1}{2}K'at$ of six families of curves (in which a/d varied between 0 and 3) plotted against the outside glucose concentration. The parameters derived from the intercepts, as described in the text, are compared with the theoretical values in Table 3.

about 10% in the normal range (4 mM = 0·012 isotonic units) and by a larger amount when $(a+d)/b$ is larger.

Table 3 also shows that $d(a+d)$ derived in the way described above is very close to the theoretical value; thus, in the Sen & Widdas method when time (and not $\frac{1}{2}Kat$) is plotted along the ordinate, the maximal transfer constant (K) obtained from the corresponding intercept would be $\frac{1}{2}K'ad/(a+d)$ (see equation (2)).

TABLE 3. A comparison of the theoretical values of the parameters of hexose transfer with those obtained from model exit curves by the experimental technique used by Sen & Widdas (1962*a*)

Theoretical			Measured	Theoretical	Measured
$\frac{a}{d}$	$\frac{d}{b}$	$\frac{a+d}{b}$	ϕ	$\frac{d}{a+d}$	$\frac{S'+\phi}{\text{intercept}_{(G=0)}}$
0	0·01	0·010	0·009	1·0	0·95
0·2	0·01	0·012	0·0115	0·83	0·80
0·6	0·01	0·016	0·014	0·625	0·61
1·2	0·01	0·022	0·018	0·455	0·46
2·0	0·01	0·030	0·024	0·33	0·34
3·0	0·01	0·040	0·032	0·25	0·26

The calculations under (ii) in which d/b was varied relative to a fixed value of a/b yielded essentially similar curves; thus the fit of the simpler equation to experimental results is equally consistent with any of the three cases considered in equations (4), (5) and (6).

Effect of temperature on the rate constants

Rate constants have a temperature dependence given by the Arrhenius equation

$$k = Ae^{-E/RT}$$

or

$$\log_e k = \log A - (E/RT),$$

where k is the rate constant, R the gas constant, T the absolute temperature and E the activation energy.

Similar equations would apply to the three rate constants (a, b and d) discussed above, but their individual values at various temperatures would be needed to determine their respective activation energies E_a, E_b and E_d.

(a) *The half-saturation constant* (ϕ). In enzyme kinetics (see, for instance, Laidler, 1958) the Arrhenius plot of the Michaelis constant can be used to obtain the difference in the activation energies of the two rate constants concerned. Applying this principle to the half-saturation constant one would obtain the relation

$$\log_e \phi = \log_e d/b = (\log_e A_d - \log_e A_b) - \frac{E_d - E_b}{RT} \qquad (8)$$

provided $\phi \simeq d/b$. If $\phi \simeq a/b$ a comparable expression can be derived, but

if $\phi \simeq (a+d)/b$ the temperature dependence of the half-saturation constant would be a more complex function of all three activation energies.

It follows that the Arrhenius plot of $\log_e \phi$ against $1/T$ could only be linear if, over the temperature range concerned, the approximations $\phi \simeq d/b$ or $\phi \simeq a/b$ obtained. Although the results of Sen & Widdas (1962*a*) using human red cells were consistent with this hypothesis, those reported in this paper using foetal guinea-pig cells are not. A possible interpretation is that for foetal guinea-pig cells the rate constants a and d are either of comparable magnitude or become so as the temperature is lowered. This will be discussed further in the next paragraph.

(b) *The maximal transfer rate* (K). As reported by Sen & Widdas (1962*a*) and as found in the present work, the Arrhenius plot of $\log_e K$ against $1/T$ is not linear but convex upwards. This could suggest that the rate limiting step is one with a lower temperature coefficient (Q_{10}) at high temperatures and a different reaction with a higher temperature coefficient at lower temperatures.

Since in the analysis given above the maximal transfer rate is $(\frac{1}{2}K')\,ad/(a+d)$, its temperature coefficient in a given range of temperature will be most closely related to that rate constant which is smaller in actual magnitude.

The curvature of the Arrhenius plot of $\log_e K$ against $1/T$ could arise from a change in relative magnitude of a and d as the temperature is lowered. This is illustrated in Fig. 4 in which a theoretical curve is compared with the experimental results of Table 2. In this theoretical curve it was assumed that the activation energies were $E_a = 12{,}900$ cal/mole, $E_d = 33{,}000$ cal/mole and that $a/d = 0{\cdot}25$ at 37° C. With these activation energies corresponding to Q_{10}'s of 2·0 and 6·0, respectively, a/d would increase as the temperature was lowered and would equal 7·1 at 7° C.

If the components responsible for transfer in human cells are the same as those in guinea-pig foetal red cells, one should be able to fit the results for human cells using activation energies similar to those above. The parameters d and b, representing rate constants for dissociation and association of complexes, may be expected to be identical in the two species of cells, but the rate constant a, depending on the activation energy necessary for movement through the membrane, could well be different. If for human cells one assumes that the activation energy for transfers through the membrane is higher by only 570 cal ($E_a = 13{,}470$), this would reduce a by a factor of 2·5 at 37° C. If d remained unchanged one would have $a/d = 0{\cdot}1$ and $ad/(a+d)$ would be reduced by a factor of 2·2 relative to guinea-pig cells. Using the same activation energy for d, a curve for $\log_e K$ against $1/T$ for human cells can be derived which is in fair agreement with the results of Sen & Widdas (1962*a*) (see Fig. 4).

The half-saturation constant $(a+d)/b$ corresponding to the various values of a and d can also be calculated by assuming an activation energy for b and arbitrarily assigning a value to $(a+d)/b$ at one temperature. This can be illustrated by a calculation assuming $E_b = 20{,}000$ cal/mole ($Q_{10} = 2{\cdot}94$) and by making $(a+d)/b = 4$ mM for human cells at 37° C. Other values for human cells and all the values for guinea-pig cells can then be calculated.

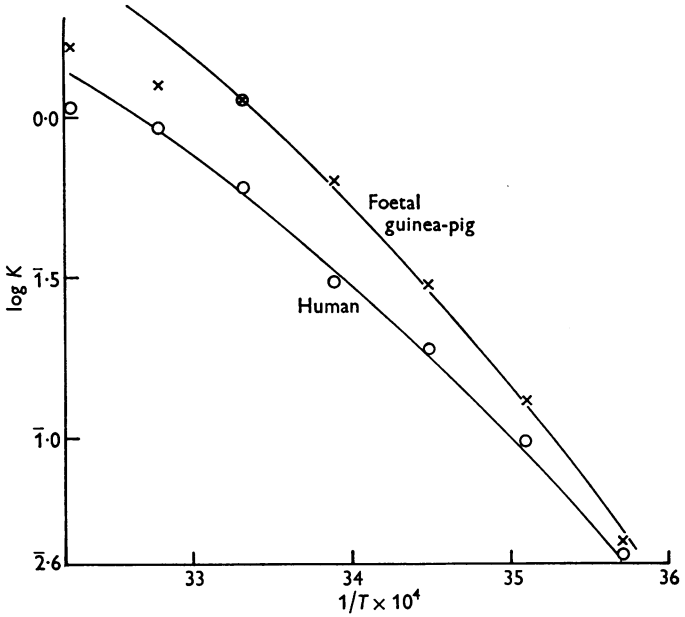

Fig. 4. Experimentally determined variations of log K for foetal guinea-pig red cells (×) and for human red cells (○) plotted against $1/T$. On the assumptions described in the text and taking ⊗ the value for foetal guinea-pig cells at 27° C as reference point, the lines indicate the theoretically predicted variations. The points for human red cells are from Sen & Widdas (1962*a*).

The calculated line for the variation with temperature of the half-saturation constant for guinea-pig cells is compared with the experimental data of Table 2 in Fig. 5. In the same figure the results for human cells obtained by Sen & Widdas (1962*a*) are compared with the predicted values for these cells. The lines are in fair agreement down to 17° C but tend to be unsatisfactory below this temperature. They illustrate how the half-saturation constant may flatten off at low temperatures and in fact predict this as a normal consequence of the changes in the relative magnitude of a and d which are presumed to occur in order to account for the curvature of the plot of log K against $1/T$.

The curve fitting illustrated in Figs. 4 and 5 is consistent with the hypothesis that the chemical nature of the membrane component is similar in foetal guinea-pig cells and human cells but that there is a difference in the rate constant for transfer through the membrane of the order of 2·5. This difference accounts both for the ratio in maximal transfer rate $(\frac{1}{2}K')ad/(a+d)$ between the two species (without assuming any difference

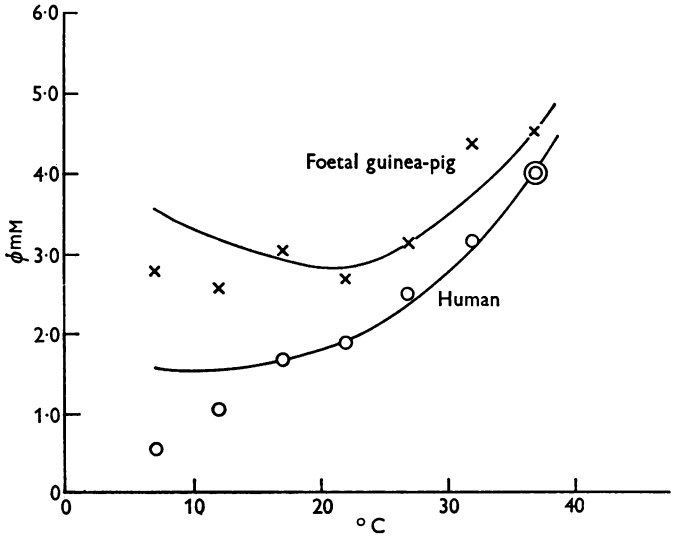

Fig. 5. Variation of the half-saturation constant with temperature. Points (×) foetal guinea-pig red cells, points (○) data for human red cells from Sen & Widdas (1962*a*). On the assumptions described in the text and taking (◎) the value of 4 mM for human cells at 37° C as reference point, the lines indicate the theoretically predicted variations.

in the constant K') as well as for the two ranges of half-saturation constants $(a+d)/b$ between 17 and 37° C (using common values for the dissociation (d) and association (b) rate constants and their activation energies).

DISCUSSION

Although the transfer of glucose across the red cells of foetal guinea-pig blood has several common features with that in adult human blood (Widdas, 1955), the study of the effect of temperature has displayed a different behaviour in respect of the half-saturation constant in the two species.

In the foetal guinea-pig cells this parameter becomes relatively temperature independent below 30° C although clearly it is lower than the value at 37° C. This would not be expected of a true Michaelis constant with which this half-saturation value has usually been equated. It is,

however, consistent with the kinetics of a carrier model for hexose transfer in which the half-saturation constant can be shown to contain a term depending on the mobility or diffusion of the carriers (or carrier complexes) through the membrane.

If the over-all process involves enzyme action at the two interfaces (Wilbrandt, 1954) there would be many rate constants with different activation energies and one might expect the temperature dependence to be complex. The present analysis on the basis of only the three rate constants fundamental to the simplest carrier model does not exclude these possibilities, since over the covered temperature range other processes may not become rate limiting or may be contributing to the rate constants actually used. The inability to make a satisfactory fit for results for the half saturation constants below 17° C may suggest that factors outside the present analysis are involved, but unfortunately the discrepancies occur at low temperatures where difficulties in technique may have introduced uncertainties.

Good (1962), working on the delay in osmotic haemolysis of a number of non-electrolytes, has argued that glucose, by promoting a lattice structure in water, retards the movement of water through the membrane. A non-specific effect of this type at low temperatures could introduce a systematic error which might be expected to apply equally to human and guinea-pig foetal cells.

It is also assumed in the kinetics that the rate constant for transfer through the membrane a is the same for saturated and unsaturated carriers, but this may not be so (Widdas, 1952). If the rate constant for unsaturated carriers is less than that for saturated carriers, the influence of a on the half-saturation constant could be lessened more than its effect on the maximal transfer rate, and if the differential effect increased as the temperature was lowered this might explain the lower half-saturation values at temperatures under 17° C.

Most laboratory animals have red cells which are either impermeable to glucose or into which glucose enters so slowly that the cells would not be suitable for study at low temperatures. A survey of other primate red cells which are penetrated rapidly by glucose would be desirable, as well as further work to extend these studies at low temperatures.

The activation energies used in the analysis, although chosen by trial and error and to that extent arbitrary, are reasonably consistent with the experimental results. Thus the difference in activation energies allotted to d and b, i.e. 13,000 cal/mole (33,000–20,000), might be thought large compared with the 10,000 cal/mole obtained by Sen & Widdas (1962*a*) from the plot of $\log_e \phi$ against $1/T$. In human cells the half-saturation constant at 37° C probably approximates to d/b, but the discrepancy of

3000 cal/mole is accounted for by the influence of the term a in the full expression for the half-saturation constant ($\phi = (a+d)/b$.

The absolute values allocated to the activation energies for a and d are consistent with the temperature variation of the maximal transfer rate $(\frac{1}{2}K')ad/(a+d)$ in both human and guinea-pig cells (on the assumption that K' does not change with temperature). It is possible to fit the guinea-pig results with activation energies for a and d of 10,000 and 33,000 cal/mole respectively and $a/d = 0{\cdot}1$, but these figures make a fit for the results on human cells impossible. Lower values would not fit guinea-pig cells.

The activation energy for the rate constant b is similarly capable of an arbitrary lowering by about 2000 cal/mole with only moderate loss in accuracy, provided that the activation energy for d is lowered correspondingly. Thus, although great accuracy cannot be claimed for any of the alloted activation energies, they must be of the right order of magnitude if the hypothesis of the simple carrier model is to hold.

The high activation energies necessarily attributed to the separate rate constants for formation and dissociation of complexes in this analysis, however, are in the range where reactions would be expected to be very slow unless catalysed by enzymes. It is interesting to note that Rosenberg & Wilbrandt (1963) have recently made a theoretical study of the kinetics of enzymic carrier models in which carriers may be structurally modified as the result of metabolism and so be capable of effecting uphill transfer. In this treatment they also show that the half-saturation constant is not a simple Michaelis term but contains a second term which, in their case, is a more complex function than that which is used here.

As pointed out earlier, the equations which are used in this paper are approximately symmetrical in a and d so that many of the same arguments could be made if these terms were reversed. In this event the rate constant of larger magnitude at 37° C (but with higher activation energy) would be that for transfer of complexes through the membrane. Accepting this possible reversal, however, it is difficult to see how the half-saturation constant as measured by competition between different sugars (Widdas, 1954*a*; Sen & Widdas, 1962*b*) could come out to be similar to that measured in the exit experiments.

One consequence of reversal would be to make the maximal transfer rate proportional to $\frac{1}{2}K'd$ when $a \gg d$, that is, it would depend on the rate of dissociation of complexes as proposed originally by LeFevre (1954). However, the kinetics which LeFevre developed on this hypothesis assumed a to be infinitely large and the equations gave a less satisfactory fit in their integrated form (Widdas, 1954*b*). By reference to equations (1) and (7) it will be seen that a appears in the form a/b, and in isotonic units this can never be a large quantity although it could be large relative to

d/b. If the maximal transfer rate approximates to $\frac{1}{2}K'd$ it would be impossible to explain the difference between the foetal guinea-pig and human red cells at 37° C on the basis of a change in the rate constant for transfer through the membrane (a). Thus either a difference in K' or a difference in d would be necessary as an alternative hypothesis to that developed here.

Although reversal of magnitude of a and d may be possible kinetically at 37° C it would appear to be unlikely. That reversal occurs as the temperature is lowered, owing to the differential temperature effect, is a necessary part of the hypothesis and it is also suggested that a is of a similar order of magnitude to d, even at 37° C. Even in human cells a/d could be about 0·1 at 37° C, rising to 0·86 at 17° C; thus the half-saturation constant $(a+d)/b$ will be very different from a true Michaelis constant d/b and this must be borne in mind in interpreting experimental results.

SUMMARY

1. The parameters of glucose transfer across the foetal guinea-pig erythrocytes have been determined between 7 and 37° C and in the pH range 6·5–8·5 at 27° C.

2. Although the maximal transfer rate of glucose transfer in foetal guinea-pig red cells is greater than that in human red cells, the fall off with temperature is generally similar.

3. The temperature dependence of the half-saturation constant of guinea-pig cells contrasts with that of human cells particularly at temperatures below 27° C. The variation with temperature is not consistent with a true Michaelis constant.

4. In developing the kinetics of a carrier transfer, Widdas (1953) showed that the half-saturation constant would contain a term due to transfer through the membrane as well as the terms for the dissociation and formation of complexes. Owing to differences in the effect of temperature the relative magnitude of these constsnts is presumed to change as the temperature is lowered, and, assuming the term for transfer through the membrane is larger in the case of foetal guinea-pig cells than in human cells, the results are consistent with components of similar chemical affinities.

5. Computations have been made with the fuller equations to show that the half-saturation constant and maximal transfer rate obtained by the technique used by Sen & Widdas (1962*a*) contain the terms predicted by theory. It is concluded that the half-saturation constant and Michaelis constant should not be used synonymously when describing the parameters of the facilitated hexose transfer system of erythrocytes.

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