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Permeability of the Human Red Blood Cell to *meso*-Erythritol

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Summary. Using ^{14}C-erythritol, we measured net as well as unidirectional erythritol fluxes. Up to near saturation, net and unidirectional fluxes were virtually identical and linearly related to the erythritol concentration in the medium (isotonic saline). No saturation of the transfer system was observed. At 20 °C, a maximum of 60 to 70% of the erythritol flux could be inhibited by glucose, phlorizin, or a combination of both substances. Dinitrofluorobenzene and $HgCl_2$ also reduce erythritol permeability. These findings confirm the earlier conclusion of F. Bowyer and W. F. Widdas that the glucose transport system is involved in erythritol permeation. Glycerol partially inhibits the glucose-phlorizin-sensitive component of erythritol flux, but not the glucose-phlorizin-insensitive component. Apparently glycerol has a slight affinity to that portion of the glucose transport system which is involved in erythritol transfer, whereas the glucose-phlorizin-insensitive fraction of erythritol movements is not identical with the glycerol system. This latter inference is supported by the observation that, in contrast to glycerol permeability, erythritol permeability is insensitive to variations of pH or to the addition of copper. The apparent activation energy of the glucose-phlorizin-sensitive and -insensitive fractions of erythritol permeation are 22.2 and 20.7 kcal/mole, respectively. These values are not significantly different from one another.

The glucose transport system of the red blood cell membrane exhibits a rather refined substrate specificity. For aldoses, LeFevre (1961) showed that the affinity for the transport system demands the C1 conformation of the pyranose ring and is augmented by increasing numbers of substituents (such as OH groups) in the equatorial position. In view of these rather stringent requirements, it is not surprising that a polyhydric alcohol –mannitol– which has the same molecular weight as glucose, cannot be transferred across the red blood cell membrane by the glucose transport

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system. It is surprising, however, that smaller polyhydric alcohols such as *meso*-erythritol or *penta*-erythritol are accepted as substrates by that transport system. In contrast, an even smaller polyol – glycerol – is thought to penetrate exclusively by an independent pathway (Bowyer & Widdas, 1955, 1956).

The experiments presented below essentially confirm Bowyer and Widdas' conclusions. In addition, it is shown that only about 60% of the total flux of *meso*-erythritol can be accounted for by transport through the glucose pathway. The activation energy for erythritol permeation via the glucose system is nearly identical with that for the permeation by the alternative pathway and amounts to about 21 kcal/mole. A study of the competition between glycerol and erythritol and of the action of a number of specific inhibitors of glucose and glycerol permeability suggest that even the trihydric alcohol has a slight affinity to the glucose system, whereas the tetrahydric alcohol does not seem to interact with the glycerol transport system.

Materials and Methods

Erythrocytes were obtained from freshly drawn citrated blood of healthy donors. The cells were washed three times with isotonic saline and finally resuspended in saline containing, in addition to 166 mmoles/liter NaCl, the desired concentrations of erythritol and other additives (e.g., phlorizin, phloretin, glycerol, glucose, Cu). The hematocrit was about 40%. The cells were incubated at 37 °C for 2 hr. At this temperature, erythritol and other penetrating solutes (e.g., glucose) attained diffusion equilibrium. At the end of the equilibration period, the cell suspension was transferred to the temperature at which the flux measurements were to be performed (usually 20 or 25 °C) and incubated for another 30 min. Subsequently, samples were taken for the determination of hematocrit and water content of the cell suspension. Then the flux measurements were initiated by adding a tracer amount of ^{14}C-erythritol. The disappearance of the radioactivity from the medium was measured by centrifuging samples of the suspension at suitable time intervals, dissolving 0.1 ml of the trichloroacetic acid-treated supernatant (to remove traces of protein) in 10.0 ml of a toluene-methanol liquid scintillation mixture (400 ml methanol, 600 ml toluene, 2.0 g PPO, 0.025 g POPOP), and counting in a Packard Tricarb spectrometer. The determination of the time constant for the disappearance of the radioactivity from the supernatant and the calculation of flux and permeability constant were done as described by Gardos, Hoffman and Passow (1969). Since the surface area of the cells was not known in the present experiments, the fluxes are expressed in moles/cm^3/min (instead of moles/cm^2/min) and the permeability constants (=flux/concentration) in min^{-1} (instead of cm/min).

In the experiments on the action of dinitrofluorobenzene (DNFB) and $HgCl_2$, the back-exchange of erythritol or sorbose from ^{14}C-erythritol- or ^{14}C-sorbose-loaded cells was measured. Two parts of cells were first mixed with eight parts of isotonic saline containing 20 mmoles/liter sucrose and 11.5 mmoles/liter ^{14}C-erythritol or ^{14}C-sorbose. After 60 to 90 min of incubation at 37 °C, the cell suspension was diluted 1:1 with additional labeled medium. Sufficient inhibitor was added to obtain the concentrations indicated on the abscissa of the figures. The resulting cell suspensions (hematocrit

10 vol%) were incubated at either 4 °C ($HgCl_2$) or 37 °C (DNFB) for 15 or 30 min, respectively. Subsequently, the suspensions were centrifuged at 4 °C, and the supernatant was removed. The sediment was rewarmed to 25 °C and mixed at that temperature with saline containing 20 mmoles/liter sucrose and 10 mmoles/liter of unlabelled erythritol or sorbose (=start of the flux measurements). The appearance of radioactivity in the supernatant was followed. The curves relating cpm's in the supernatant to time followed a single exponential from which the time constant (min^{-1}) was calculated by the method of least squares. The cell density was 2.5 vol%.

DNFB (Berg, Diamond & Marfey, 1965; Passow, 1969) and $HgCl_2$ (Weed *et al.* 1962) cause K loss and Na uptake. The ensuing colloid-osmotic swelling and hemolysis can be retarded but not completely prevented by the addition of a slowly penetrating nonelectrolyte to the medium. In our experiments where, in contrast to Bowyer and Widdas' (1958) experiments on glucose permeability, DNFB was allowed to react with the cells at 37 °C, the supernatant of the cell suspension contained 20 mmoles/liter sucrose. Under these conditions, it was possible to preincubate the cells prior to the flux measurements without measurable hemolysis for 30 to 60 min.

Under the specified conditions, at the highest DNFB concentrations employed, DNFB binding amounted to more than 10^7 molecules/cell membrane. Details of the methods employed for measuring DNFB binding to membranes isolated from intact cells which were exposed to the agent for various lengths of time will be published in another paper (Poensgen & Passow, *in preparation*).

In some of the back-exchange experiments, phlorizin or phloretin was used. In contrast to DNFB or $HgCl_2$, these agents are reversible inhibitors. Therefore, in these experiments, the inhibitor was present in the final resuspension medium in which the flux measurements were performed. The concentration of the inhibitor was high enough to ensure virtually complete inhibition of the glucose-phlorizin-sensitive fraction of erythritol flux.

Results

Net erythritol movements as well as the disappearance of ^{14}C-erythritol from the medium at diffusion equilibrium followed the same kinetic pattern: they could be described by a single exponential (Fig. 1). The fluxes calculated from these exponentials are proportional to the erythritol concentration in the medium nearly up to the maximum of erythritol water solubility. No saturation of the transport system was observed. The results obtained for net and unidirectional fluxes were virtually identical (Fig. 2). These results and the absence of saturation kinetics indicate that if facilitated transport is involved in the transfer of erythritol into human erythrocytes, the transport system must be far from saturation, even when the medium is nearly saturated with erythritol.

The participation of the glucose transfer system in erythritol permeation is demonstrated in Fig. 3. With increasing glucose concentration in the medium, the permeability constant for erythritol decreases. The effect of glucose reaches the half-maximal value at approximately 25 mmoles/liter, and maximum inhibition can be obtained at a glucose concentration of 200 mmoles/liter in the medium. At maximal inhibition, the permeability

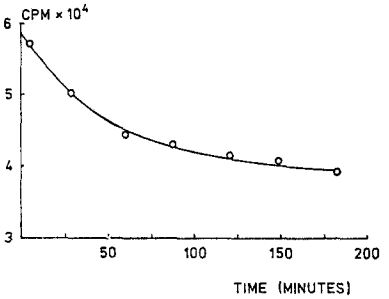

Fig. 1

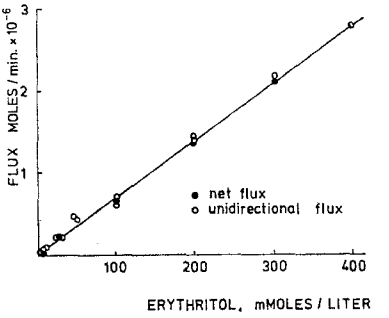

Fig. 2

Fig. 1. Penetration of ^{14}C-erythritol into human red blood cells. The cells were first equilibrated in isotonic saline containing 20 mmoles/liter erythritol at 37 °C for 2 hr. Subsequently, the cell suspension was transferred to 20 °C. Thirty minutes later, ^{14}C-erythritol was added (=zero time), and the disappearance of the radioactivity from the medium was measured. The curve represents an exponential which was fitted to the data by the method of least squares. Hematocrit: 40 vol%. Erythritol concentration in the medium at the end of the equilibration period: 13.6 mmoles/liter. Unless expressly stated otherwise, the measurements of unidirectional fluxes presented in this paper are based on such experiments. Calculation of the flux and the permeability constant was performed as described by Gardos *et al.* (1969). *Ordinate:* radioactivity (cpm) in the supernatant. *Abscissa:* time in minutes

Fig. 2. Relationship between erythritol flux and concentration in the medium. *Filled circles:* net fluxes as measured after mixing of the cells with isotonic saline containing ^{14}C-labeled erythritol at varying concentrations. *Open circles:* unidirectional fluxes as measured after the cells were equilibrated with isotonic saline containing unlabelled erythritol at varying concentrations and subsequent addition of trace amounts of ^{14}C-erythritol (t=0). The flux measurements were performed at 20 °C. Hematocrit: 40 vol%. *Ordinate:* flux in moles/cm^3/min. *Abscissa:* erythritol concentration in mmoles/liter

constant for erythritol is 30 to 40% of the original value observed in the absence of glucose. Phlorizin, an inhibitor of the glucose transport system, also inhibits erythritol movements, reducing the permeability constant to the same extent as glucose, i.e., to approximately 30 to 40% of the original value (Fig. 4). The effects of glucose and phlorizin are not additive: the presence of both compounds, in concentrations at which each exerts maximal effect, does not further reduce erythritol permeability (Fig. 5).

Effects of two irreversible inhibitors of the glucose transport system −DNFB and $HgCl_2$ −on erythritol back-exchange from erythritol-loaded cells are reproduced in Figs. 6 and 7.

DNFB inhibits erythritol efflux. The relationship between DNFB concentration and effect is nearly linear up to the highest concentrations tested in our experiments. It is not known whether the curve levels out at complete inhibition of the phlorizin-glucose-sensitive fraction of erythritol flux,

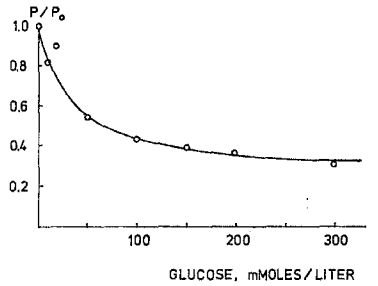

Fig. 3

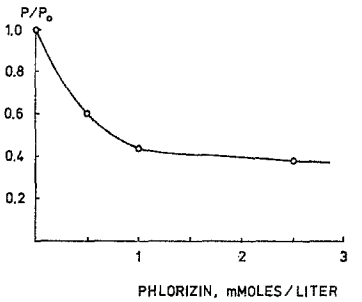

Fig. 4

Fig. 3. Effects of varying concentrations of glucose on unidirectional erythritol fluxes. The cells were equilibrated with glucose and erythritol (in isotonic saline) at 37 °C for 2 hr. At the end of the incubation period, the glucose concentrations indicated on the abscissa were obtained. Subsequently, the suspension was transferred to 20 °C, a trace of ^{14}C-erythritol was added, and the permeability constant was determined. Erythritol concentration in the medium: 20 mmoles/liter. Hematocrit: 40 vol%. *Ordinate:* ratio of flux at glucose concentration indicated on abscissa to flux at zero glucose concentration. *Abscissa:* glucose concentration in the medium

Fig. 4. Effects of varying concentrations of phlorizin on unidirectional erythritol fluxes. Experimental arrangement as in Fig. 3 except that phlorizin was used in place of glucose. *Ordinate:* ratio of flux at phlorizin concentration indicated on abscissa to flux at zero phlorizin concentration

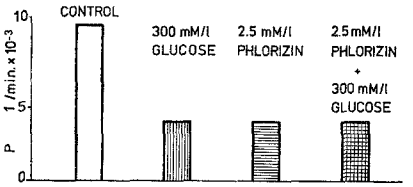

Fig. 5. Effects of maximally inhibitory concentrations of glucose and phlorizin, and a combination of the two substances on erythritol permeability. 25 °C. *Ordinate:* erythritol permeability constant in min^{-1}

or if, at higher ratios of DNFB/cells, inhibition may also encompass that fraction of erythritol flux which is insensitive to glucose and phlorizin.

If added to cells which are simultaneously exposed to maximally inhibitory concentrations of phlorizin, DNFB exhibits no measurable effects, even at the highest concentrations employed in the experiments described above. This shows that the effects of DNFB and phlorizin are not additive and suggests that both agents act on the same rather than parallel erythritol pathways.

Like DNFB, $HgCl_2$ inhibits erythritol flux. However, with increasing $HgCl_2$ concentration, the inhibitory effect passes through a maximum. The maximum is reached before the maximal inhibition by glucose or phlorizin

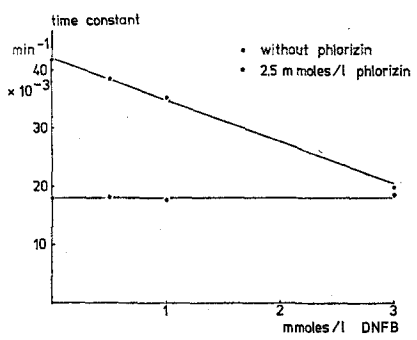

Fig. 6

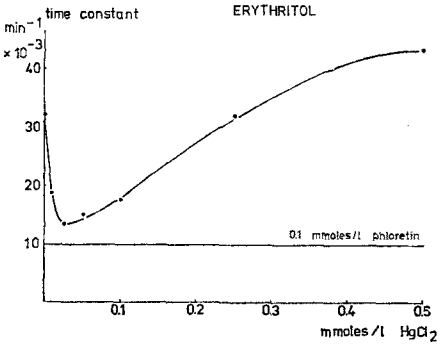

Fig. 7

Fig. 6. Effect of dinitrophenylation of human erythrocytes on erythritol flux as measured in the presence and absence of phlorizin. *Ordinate:* time constant for erythritol exchange in min^{-1}. *Abscissa:* DNFB concentration at which the cells (10 vol%) were dinitrophenylated (37 °C, 60 min) prior to the flux measurements. After unreacted DNFB was removed, the flux measurements were performed at a cell density of 2.5 vol% in the presence or absence of 2.5 mmoles/liter phlorizin in the medium. 25 °C. For details, *see* Materials and Methods

Fig. 7. Effect of exposure to $HgCl_2$ on erythritol flux, as measured in the presence and absence of phloretin. *Ordinate:* time constant for erythritol exchange in min^{-1}. *Abscissa:* $HgCl_2$ concentration to which the erythrocytes (10 vol%) were exposed (4 °C, 5 min prior to the flux measurements. After unreacted $HgCl_2$ was removed, the flux measurements were performed at a cell density of 2.5 vol% in the presence or absence of phloretin (0.1 mmoles/liter) in the medium. 25 °C. For details, *see* Materials and Methods

is attained. The effects of Hg on sorbose penetration follow the same pattern, although the reversal of the action of $HgCl_2$ is not quite as pronounced as is the case with erythritol permeation (note the differences of the scales of the abscissas in Figs. 7 & 8). Similar observations have been made by Weed, Eber and Rothstein (1962) in their studies of the effects of $HgCl_2$ on glucose permeability of human red blood cells.

It is tempting to assume that the remaining portion or erythritol penetration in the presence of high concentrations of glucose and inhibitory quantities of phlorizin might be accomplished through the glycerol transport system. There is, in fact, some 30 to 40% inhibition of erythritol movement by glycerol at 2 moles/liter. However, in the presence of maximally inhibitory concentrations of glucose or phlorizin, the addition of 2 moles/liter glycerol had only a small insignificant effect (Fig. 9). Perhaps, at extremely high concentrations, glycerol can compete with erythritol for the glucose transport system, but erythritol is incapable of competing with glycerol for the glycerol transport system. Thus, in the human erythrocyte, the glucose system may be available for both erythritol and glycerol, a fact

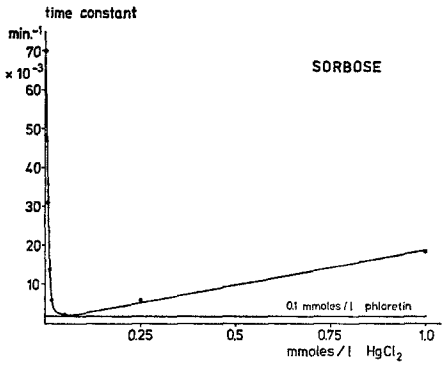

Fig. 8

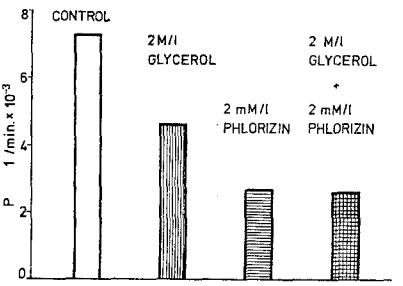

Fig. 9

Fig. 8. Effect of exposure to $HgCl_2$ on sorbose flux as measured in the presence o absence of phloretin. Experimental arrangement as in Fig. 7, except that sorbose wa used in place of erythritol. Ordinate and abscissa are the same as in Fig. 7

Fig. 9. Effects of glycerol and a combination of glycerol and phlorizin on erythrito permeability. 20 °C. Hematocrit: 40 vol%. *Ordinate:* erythritol permeability constan in min^{-1}

which could not have been demonstrated by measuring the competitior between the alcohols and glucose, because of the very high affinity of the glucose system for glucose.

In order to demonstrate further that very little erythritol penetrates via the glycerol system, the effect of Cu and pH on erythritol permeability was tested. Although Cu is known to be a very effective inhibitor of glycerol permeability (Jacobs & Corson, 1934), it had virtually no influence on erythritol transfer. Moreover, variations of pH over the range between pH 6.5 and 8.0, which dramatically affect glycerol movements across the membrane of human red blood cells (Jacobs, Glassman & Parpart, 1935) had no measurable effect on erythritol fluxes (Fig. 10). It was therefore concluded that the 30 to 40% portion of erythritol movement which does not utilize the glucose system also does not move through the glycerol system.

In facilitated diffusion, the temperature coefficient is assumed to be less than that of a simple diffusion process (Stein, 1967). One would anticipate, therefore, that the temperature coefficient of the residual erythritol flux should be higher than the temperature coefficient of the uninhibited flux. Surprisingly, it was found that, within the rather considerable limits of experimental error, no significant difference between the apparent activation energies of total and residual erythritol flux could be observed. In the absence of inhibitors, the apparent activation energy amounted to 22.2

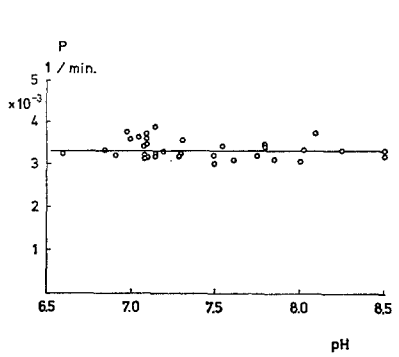

Fig. 10

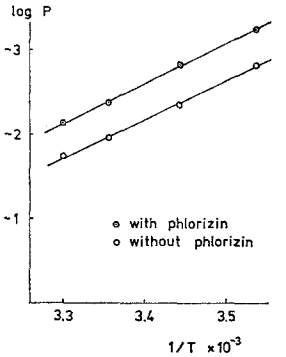

Fig. 11

Fig. 10. Effect of varying the pH in the medium on erythritol flux. pH was adjusted by adding HCl or NaOH to the cell suspension in saline. Erythritol concentration in the medium: 20 mmoles/liter. 20 °C. Hematocrit: 40 vol%. *Ordinate:* permeability constant in min^{-1}. *Abscissa:* pH

Fig. 11. Temperature dependence of erythritol penetration in the absence and presence of a maximally inhibitory phlorizin concentration (0.5 mmoles/liter). *Ordinate:* log of permeability constant (min^{-1}). *Abscissa:* reciprocal of absolute temperature

kcal/mole (four determinations). In the presence of maximally inhibitory concentrations of glucose (400 mmoles/liter), or phlorizin (0.5 mmoles/liter), or a combination of both, the apparent activation energy was about 20.7 kcal/mole (six determinations) which is not significantly different from the uninhibited value (Fig. 11). The similarity of the apparent activation energies is further indicated by the finding that, over the temperature range covered in our experiments, the apparent activation energy was independent of temperature. Such behavior would not be expected to be typical of a system with two or more activation barriers of different sizes in parallel.

Discussion

The participation of the glucose transport system in the transfer of erythritol across the membrane of human red blood cells is suggested by the finding that glucose as well as a number of typical inhibitors of glucose transport, such as phlorizin, phloretin, DNFB and $HgCl_2$, partially inhibit erythritol movements. Even more significant, the combined actions of maximally inhibitory concentrations either of glucose and phlorizin or of phlorizin and DNFB are not stronger than those of each inhibitor separately. Moreover, the characteristic maximum in the relationship between the concentration of $HgCl_2$ and its effect on permeability is observed not only with glucose or sorbose but also with erythritol.

Certain features of the kinetics of erythritol permeation (Table 1) ca be easily explained by the assumption that the affinity of erythritol to th glucose system is much smaller than the affinity of glucose to that system These features are: the equality of ^{14}C-erythritol fluxes as measured a equilibrium and the net fluxes proceeding from an initial non-equilibriun position to equilibrium, and the absence of saturation even at the highes erythritol concentrations. Some more subtle differences between erythrito and glucose transfer still await explanation. For example, glucose permea tion is slightly pH-dependent (Sen & Widdas, 1962*b*), whereas erythrito permeability is not. In summary, our findings strongly support Bowyer anc Widdas' (1955, 1956) conclusion that the glucose transport system is alsc involved in the transfer of the polyhydric alcohol erythritol. In addition, the demonstrated inhibition of part of the glucose-phlorizin-sensitive frac tion of erythritol permeation by glycerol suggests that even the trihydric alcohol shows some affinity to the glucose transport system. In contrast to former beliefs (Bowyer & Widdas, 1955), there seems to exist no critical

Table 1. *Comparison of various kinetic features of erythritol and glucose permeability*

Comparison of:	Erythritol	Glucose
Isotope vs. net flux	Isotope flux at equilibrium = net flux	Isotope flux at equilibrium ≠ net flux [12]
Saturation kinetics	No	Yes [21]
Inhibition by:		
DNFB	Maximally 60 to 70% inhibition	Virtually complete inhibition [3, 4, 16]
$HgCl_2$	Inhibition passes through a maximum	Inhibition [10], effect passes through a maximum [20]
Phlorizin and phloretin	Maximally 60 to 70% inhibition	Inhibition [15]
Glucose	Maximally 60 to 70% inhibition	—
Temperature dependence	$\Delta H^{\ddagger}$ independent of temperature	$\Delta H^{\ddagger}$ varies with temperature [17]
pH	Independent	Slightly dependent[a] [17]
Glycerol	Slight effect on glucose-sensitive fraction	No effect [3]

[a] Faust [6] observed a strong pH dependence with a minimum at pH 7.0. His results were obtained with citrated blood whereas Sen and Widdas [16, 17] used heparinized blood. According to Wilbrandt, Bolis, and Lully (1969, *unpublished results*), heparin suppresses the pH dependence of glucose permeation. The present experiments with erythritol were performed with citrated blood.

transition between the trihydric and the tetrahydric alcohols with respect to their capacity of interacting with the transport sites of the glucose system.

From the observation that erythritol flux is linearly related to erythritol concentration up to 400 mmoles/liter, one may deduce that the K_m of erythritol flux is at least one order of magnitude higher than this value. Measurements of the relationship between glucose flux and glucose concentration in the absence of glucose net movements (i.e., at equal glucose concentrations within cells and medium) yielded a K_m of about 38 mmoles/liter (Miller, 1968). Hence, the affinity of erythritol to the glucose system is at least two orders of magnitude lower than the affinity of glucose to that system. In view of the considerable differences in affinity, the concentration at which glucose produces half-maximal inhibition of erythritol flux should be nearly identical with the K_m for glucose. In our experiments, the flux measurements were performed by means of ^{14}C-erythritol after the distribution of erythritol and glucose between cells and medium had reached equilibrium. Thus, our data were obtained under the same conditions as Miller's. Our value for half-maximal inhibition of 25 mmoles/liter is lower than Miller's, but still of the same order of magnitude. It is much higher than Miller's value of 1.8 mmoles/liter for net glucose exit.

The glucose-phlorizin-insensitive fraction of erythritol flux across the membrane is rather large (about 30 to 40% at 20 °C). As has been pointed out by Dawson and Widdas (1963) in their discussion of the nature of an N-ethyl maleimide-insensitive component of glucose transport, many possible mechanisms of inhibition could result in a residual permeability. In the present context, only four mechanisms will be considered.

(1) There may exist in the red blood cell membrane mobile sites which are identical with the glucose-carrier molecules but inaccessible to glucose and the inhibitors of the glucose transport system. If these sites could be reached by erythritol molecules, then all of the erythritol could permeate by means of "glucose" carriers, but only part of the erythritol flux would be sensitive to glucose and inhibitors of the glucose system. Such an assumption would easily explain why the apparent activation energies of the glucose-phlorizin-sensitive erythritol flux and of the residual flux are virtually identical. However, the inhibitors used in this work (DNFB, phloretin, phlorizin, and glucose) show marked differences in lipid solubility and molecular size. One would anticipate, therefore, that these various agents could react with different numbers of transport sites and hence would produce different degrees of inhibition if applied at maximal doses. This was not observed.

(2) An even less likely explanation of our findings is the assumption that saturating concentrations of the various inhibitors, including glucose reduce the rate of carrier transfer across the membrane to a finite value rather than to zero. The modes of action of DNFB, phloretin, phlorizin and glucose are probably not identical. Hence, it would seem rather far fetched to stipulate that all agents produce virtually the same decrease of V_{max}.

(3) A partial inhibition of erythritol transport by maximally effective concentrations of inhibitors of the glucose transport system could also be explained by the assumption that erythritol not only permeates via the glucose system but also through another, parallel pathway. In view of its chemical similarity to glycerol, it would seem possible that erythritol is capable of moving through the glycerol-transport system. Table 2 summarizes a number of striking differences between glycerol and erythritol permeability. Apparently, the tetrahydric alcohol does not permeate through the same channel as the trihydric alcohol, although, for reasons presented above, it seems likely that some of the glycerol may permeate via the glucose system.

(4) The residual erythritol flux could be caused by simple diffusion of erythritol molecules without the participation of a specific transport system. If so, then the similarity of the apparent activation energies for the glucose-phlorizin-sensitive and -insensitive fractions does not imply that activation energies for carrier diffusion and diffusion of the uncombined erythritol molecules are identical. According to Wilbrandt (1969) and others, the

Table 2. *Comparison of various kinetic features of erythritol and glycerol permeability*

Comparison of:	Erythritol	Glycerol
Inhibition by:		
DNFB	Maximally 60 to 70 % inhibition	Slight [3]
Cu	None	Strong [8, 19]
$HgCl_2$	Maximally 60 %	Strong [10]
H^+	None	Strong [9]
Glycerol	Slight	–
Action of glycerol on phlorizin-glucose-insensitive fraction	None	–
Temperature coefficient	High	Low without Cu, high with Cu [9]

transfer of glucose across the red blood cell membrane can be described by the equation:

$$V = V_{max} \cdot \left[\frac{C_1}{K + C_1} - \frac{C_2}{K + C_2} \right]$$

where V = rate of glucose transfer, V_{max} = maximal rate of glucose transfer at saturation of the transport system, C_1, C_2 = glucose concentration at inside or outside of the membrane, respectively, and K = dissociation constant for the glucose-carrier complex.

This simple equation should be applicable to our experimental conditions where the erythritol flux is determined with ^{14}C-labeled compounds in the absence of net erythritol movements. If unidirectional fluxes are measured, $C_1 \gg C_2$. For erythritol, $C_1 \ll K$, hence,

$$V = \frac{V_{max}}{K} \cdot C_1;$$

$$V_{max} = V_{max\,(0)} \cdot e^{-\frac{E_A}{RT}};$$

and

$$K = K_0 \cdot e^{-\frac{\Delta H}{RT}}$$

$V_{max(0)}$ and K_0 are constants; E_A represents the activation energy for the translocation of the loaded carrier; and ΔH is the enthalpy change associated with the combination between erythritol and carrier.

The temperature dependence of V can be described by combining the expressions for V_{max} and K:

$$V = V_{max\,(0)} \cdot K_0^{-1} \cdot e^{+\frac{\Delta H - E_A}{RT}} \cdot C_1.$$

Hence, under our conditions, where the system is far from saturation, the apparent activation energy for erythritol transport via the glucose system is composed of two terms: the apparent activation energy for the translocation of the loaded carrier, and the enthalpy change associated with the reaction between carrier and free erythritol. For simple diffusion of uncombined erythritol, ΔH should be replaced by a term for the enthalpy change associated with the establishment of the distribution equilibrium between the membrane and the medium. Consequently, it is likely that the similarity of the apparent activation energies for the carrier-mediated and the diffusional components of erythritol flux is purely fortuitous. On the basis of our experiments presented here, it is impossible to decide whether simple diffusion of uncombined erythritol molecules or other

transfer mechanisms account for the glucose-phlorizin-insensitive fraction of erythritol permeability.

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References

1. Berg, H. C., Diamond, J. M., Marfey, P. S. 1965. Erythrocyte membrane: Chemical modification. *Science* **153**:1010.
2. Bowyer, F., Widdas, W. F. 1955. Erythrocyte permeability to erythritol. *J. Physiol.* **129**:7 P.
3. — — 1956. The facilitated transfer of glucose and related compounds across the erythrocyte membrane. *Disc. Faraday Soc.* **21**:251.
4. — — 1958. The action of inhibitors on the facilitated hexose transfer system in erythrocytes. *J. Physiol.* **141**:219.
5. Dawson, A. C., Widdas, W. F. 1963. Inhibition of the glucose permeability of human erythrocytes by N-ethyl maleimide. *J. Physiol.* **168**:644.
6. Faust, R. G. 1960. Monosaccharide penetration into human red blood cells by an altered diffusion mechanism. *J. Cell. Comp. Physiol.* **56**:103.
7. Gardos, G., Hoffman, J. F., Passow, H. 1969. Flux measurements in erythrocytes. *In:* Laboratory Techniques in Membrane Biophysics. Editors, H. Passow and R. Stämpfli. p. 9. Springer-Verlag, Berlin.
8. Jacobs, M. H., Corson, S. A. 1934. The influence of minute traces of copper on certain hemolytic processes. *Biol. Bull.* **67**:325.
9. — Glassman, H. N., Parpart, A. K. 1935. Osmotic properties of the erythrocyte. II. The temperature coefficients of certain hemolytic processes. *J. Cell. Comp. Physiol.* **7**:197.
10. LeFevre, P. G. 1948. Evidence of active transfer of certain non-electrolytes across the human red cell membrane. *J. Gen. Physiol.* **31**:505.
11. — 1961. Sugar transport in the red blood cell: Structure activity relationships in substrates and antagonists. *Pharmacol. Rev.* **13**:39.
12. — McGinniss, G. F. 1960. Tracer exchange *vs* net uptake of glucose through the human red cell surface. *J. Gen. Physiol.* **44**:87.
13. Miller, D. M. The kinetics of selective biological transport. IV. Assessment of three carrier systems using the erythrocyte-monosaccharide transport data. 1968. *Biophys. J.* **8**:1339.
14. Passow, H. 1969. Passive ion permeability of the erythrocyte membrane. *Progr. Biophys. Mol. Biol.* **19**:425.
15. Rosenberg, T., Wilbrandt, W. 1957. Strukturabhängigkeit der Hemmwirkung von Phlorizin und anderen Phloritinderivaten auf den Glukosetransport durch die Erythrozytenmembran. *Helv. Physiol. Acta* **15**:168.
16. Sen, A. K., Widdas, W. F. 1962*a*. Variations of the parameters of glucose transfer across the human erythrocyte membrane in the presence of inhibitors of transfer. *J. Physiol.* **160**:404.
17. — — 1962*b*. Determination of the temperature and pH dependence of glucose transfer across the human erythrocyte membrane measured by glucose exit. *J. Physiol.* **160**:392.

18. Stein, W. D. 1962. The Movement of Molecules Across Cell Membranes. Academic Press, New York.
19. — Danielli, J. F. 1956. Structure and function in red cell permeability. *Disc. Faraday Soc.* **21**:238.
20. Weed, R. I., Eber, J., Rothstein, A. 1962. Interaction of mercury with human erythrocytes. *J. Gen. Physiol.* **45**:395.
21. Widdas, W. F. 1954. Facilitated transfer of hexoses across the human erythrocyte membrane. *J. Physiol.* **125**:163.
22. Wilbrandt, W. 1969. Countertransport in human red blood cells. *In:* Laboratory Techniques in Membrane Biophysics. Editors, H. Passow and R. Stämpfli. p. 28. Springer-Verlag, Berlin.