# ACTIVE TRANSPORT INTO THE HUMAN ERYTHROCYTE: EVIDENCE FROM COMPARATIVE KINETICS AND COMPETITION AMONG MONOSACCHARIDES

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The process of penetration of glucose into the human erythrocyte exhibits certain anomalous characteristics to which several investigators have called attention. Klinghoffer (1935) showed that the glucose within the red cell rapidly attains the extracellular concentration, provided this concentration is fairly small; but that the intake is limited so that the intracellular level does not exceed about 2 per cent even though the extracellular concentration be raised considerably. Thus osmotic hemolysis fails to occur in isosmotic glucose solutions. Bang and Ørskov (1937) reported that the "permeability constant" for glucose in this cell varies inversely with the applied concentration, in the vicinity of M/20; these observations were extended over a much wider range by Wilbrandt, Guensberg, and Lauener (1947), who found the "constant" varying by a factor of over 1000, according to the concentration of glucose used. Meldahl and Ørskov (1940) also made quantitative estimates of the progressive decrease in the "constant" during the passage of glucose into the cell, as had been qualitatively noted by several earlier observers.

Previous reports from this laboratory (LeFevre, 1947, 1948) and that of Guensberg (1947) have been concerned with a more specific analysis of the course of volume changes (swelling) of human red cells in glucose-saline mixtures; this has been treated in terms of a supposed transport mechanism for glucose, residing in the cell "membrane" or "cortex." The pattern of the swelling in relation to glucose concentration, together with the action of various inhibiting agents, has led to the suggestion that some surface constituent of the cell (probably enzymatic) temporarily combines with, or otherwise reacts upon, the glucose, facilitating its passage through the barrier of the cell surface.

Any such system involves at least two obvious steps: combination of glucose with the active constituent in penetrating the membrane, and disintegration of this complex with the release of the glucose into the protoplasm. The experimentally observed patterns of swelling, in regard to the dependence of rate on concentration, may be reasonably fitted to theoretical patterns derived from this scheme, provided appropriate relations between the various rate

constants be assumed. However, certain further complications in the observed patterns require additional factors in the hypothetical mechanism (LeFevre, 1948); further investigation of these is in progress, and detailed discussion of the kinetics of glucose movements will be deferred to a later report. The present experiments concern the comparative uptake by human erythrocytes (as indicated by their swelling) of various molecules related to glucose; and the pattern of competition between such molecules for the transport mechanism when more than one type is present. A selectivity is observed which differs from that seen in the process of absorption from the rat gut (Verzár, 1935); and this selectivity correlates suggestively with the comparative kinetics of the several molecular species, as interpreted by the carrier-combination hypothesis. A high degree of specificity has previously been indicated by Wilbrandt (1947), who reported that, while *d*-xylose and *l*-arabinose enter the human red cell fairly readily, *l*-xylose and *d*-arabinose are essentially unable to penetrate.

## *Materials and Methods*

Human blood was drawn from the arm vein and either citrated or heparinized; the cells were washed by several successive centrifugations in relatively large volumes of the suspension medium and stored at about 5°C. until used. Cells were used as late as 6 days following their withdrawal, but were rewashed and resuspended shortly before use.

Cell volume changes were followed by the photometric method developed by Ørskov and Parpart. The light source was a single straight coil filament headlight bulb operated from a 6.3 volt storage battery or Sola constant voltage transformer. The light passed through a filter of $CaCl_2$ solution, and thence through a flat sided Amphenol vessel of 13 ml. capacity (containing the cell suspension), the central beam falling on the cathode of a photocell (RCA type 925). The Amphenol vessel was enclosed below and laterally, except for the path of the light beam, by circulating water at 37°C., in a small brass tank.

The photocell response was recorded on paper on a moving drum, by an ink-writer carried on the movable contact of a voltage divider; this voltage divider contact was driven by a shaded-pole motor activated by any deviation from some fixed potential at the contact itself, so that the deviation would be nullified. The output from the photocell, after one stage of amplification, was applied across this potential divider. Some such mechanical means of recording was desired because of the technical difficulties in the usual photographic recording occasioned by the fact that the rates of penetration to be dealt with were so very slow. There is also an obvious advantage in seeing the entire record as it progresses over a period of 15 to 60 minutes, as commonly required in these experiments. The feedback characteristics of this recorder were adjusted so that a slight "hunting" about the null point was maintained, at about one cycle every 1.5 seconds (see figures for typical behavior). The speed of response in the recording was limited to the maximum indicated in the most abrupt shifts in the records (at the time of addition of the experimental solutions); so that the very rapid volume adjustments cannot be followed by this means.

Commonly, 10 ml. of a ⅛ or ½ per cent cell suspension (previously brought to 37°C. in a constant temperature bath) were added to the vessel, and the effects of addition of 1 or 2 ml. of the experimental solution (similarly prewarmed) were recorded; this involved a fixed increase of light transmission due to the simple dilution of the suspension, plus the appropriate shift in either direction resulting from the adjustment of cell volume to the osmotic changes. The saline medium used was a balanced mixture of NaCl, $CaCl_2$, KCl, and $MgCl_2$, with the cations approximately at plasma concentrations, buffered at pH 7.1 with M/50 sodium phosphates, or at pH 7.4 with M/50 sodium maleinate (or, in specific cases noted, sodium barbital). The phosphate, used in all earlier work, predisposed the cells to agglutination by the higher concentrations of certain of the sugars used; for this and other technical reasons, the phosphate buffer is not recommended, and maleinate is used exclusively in current work (see Smits, 1947).

## RESULTS

The related compounds tested included the hexoses *d*-dextrose, *d*-levulose, *d*-galactose, *l*-sorbose, and *d*-mannose, the pentoses *d*-xylose and *l*-arabinose, and the polyhydric alcohols adonitol, *d*-mannitol, and *i*-inositol. The alcohols, though of dimensions similar to those of the related monosaccharides, did not penetrate the red cells at all (or did so at rates so very slow as to be undemonstrable by this method). All the sugars penetrated the cells at measurable rates (the order of magnitude of which is best appreciated by reference to examples in the figures). These results are entirely in accord with the early observations of Kozawa (1914), who studied by hematocrit and direct chemical analytic methods the uptake of all the substances in question except inositol. Kozawa's arrangement of the sugars as regards rate of penetration from approximately isosmotic solutions at room temperature is as follows, from fastest to slowest:

Arabinose, xylose > galactose, mannose, sorbose > dextrose > levulose

However, Wilbrandt (1938) presents a graph of the penetration, at 38°C., as calculated from the progressive susceptibility to hemolysis in diluted saline solutions, giving the series:

Xylose, arabinose > mannose > galactose > dextrose > sorbose >> levulose

The experiments reported below indicate that the discrepancies apparent between these two series are not due to the difference in temperature employed but rather to differences in concentrations of sugars used. Wilbrandt gives no indication of the concentration used in the particular experiment presented, but the nature of his method would seem to require something in the neighborhood of $\frac{1}{10}$ isosmotic (in a salt solution about $\frac{1}{2}$ isosmotic). The present experiments would indicate, at this concentration, such a series as Wilbrandt gives, while agreeing completely with Kozawa's series for concentrations near the isosmotic (about 0.3 M).

This reconciliation of the two sets of data results from the fact that a distinct dichotomy in behavior is apparent in this array of sugars, with regard to the dependence of the rate of penetration on the sugar concentration. This is evident in the form of families of curves such as in Fig. 1; these curves trace the progress of swelling of the cells (following the immediate shrinkage) upon the addition of various amounts of the sugars to the saline medium. The behavior of galactose, as exemplified in Fig. 1 *b*, is typical of all the aldoses

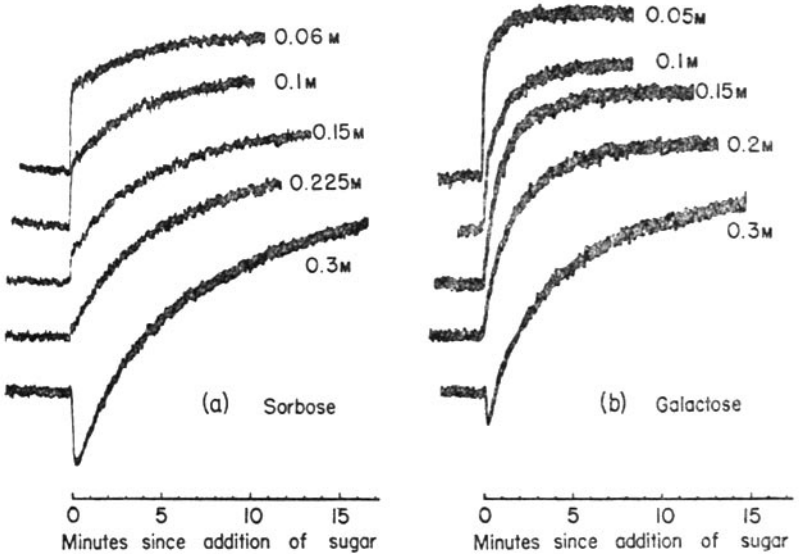


FIG. 1. Kinetics of swelling in sugar-saline mixtures. At zero time, 2 ml. saline medium with sugar at 6 times final concentration indicated added to 10 ml. cell suspension in saline medium. Downward deflection indicates cell shrinkage; upward deflection indicates swelling or, at instant of additional volume, dilution of the suspension.

(galactose, xylose, arabinose, mannose, and, as previously reported, dextrose) in that increase of concentration beyond a certain point results in a decrease in the initial rate of swelling. This has been interpreted in the case of glucose (LeFevre, 1948) as evidence of the participation of an active process in the mechanism of penetration. However, the behavior of sorbose (Fig. 1 *a*) in comparable experiments, and that of the only other ketose tested, levulose, does not show this peculiarity (at least in the range tested, up to 0.4 M). These two hexoses obey the simple Fick's law in their penetration of the human red cell, insofar as there is progressively faster swelling with increased concentration of the sugar. (It is not altogether certain from the experimental curves that no minor deviations from Fick's law occur.) At first sight, then, these

results might seem to indicate that the active process previously postulated for dextrose transport is involved with all the aldoses, but that the ketoses penetrate by simple diffusion.

If such were the case, aldoses might be expected to show competition for the carrier system, while there should be complete independence between the movements of two ketoses, or of a ketose and an aldose. This is far from the actual situation; in mixtures of two sugars, each at 0.15 M (in the usual medium), the rate of swelling was always less than indicated by the appropriate additive treatment of the curves for the separate entry of each sugar. (Compare

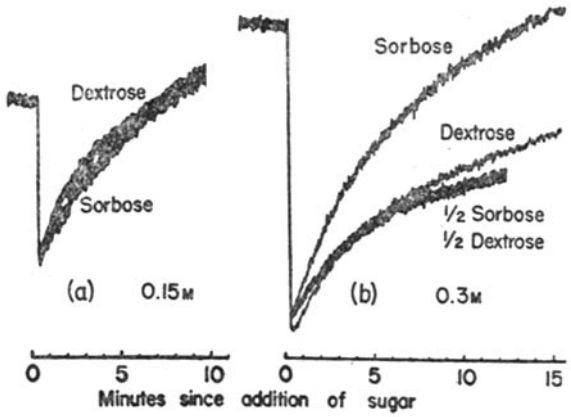


FIG. 2. Competition between sugars during simultaneous entry. At zero time, 2 ml. saline medium with sugar at 6 times final concentration indicated added to 10 ml. cell suspension in saline medium. Downward deflection indicates shrinkage of cells. Saline medium here is made only 60 per cent isotonic strength, so as to render more distinct differences between swelling curves.

curves of Fig. 2 *a* with that of the mixture in Fig. 2 *b*.) With certain of these combinations, as in the example in Fig. 2, the swelling in such a mixture proceeded slightly more slowly than in a solution of *either* ingredient at 0.3 M (the total concentration of the two sugars in the mixture). Wilbrandt (1947) mentions that arabinose and xylose interfere with each other when entering simultaneously, though the nature of the experiment is not indicated.

Partial elucidation of the events in such a situation is provided by a slight change in procedure: addition successively of the two sugars, awaiting equilibration of the cells with the first before adding the second. Fig. 3 shows records of a typical experiment; prior equilibration of cells with sorbose, at about 0.15 M, had only the direct osmotic effect of so much additional solute on the subsequent rate of swelling in glucose, at 0.15 M; whereas, with the reciprocal procedure, the glucose essentially prevented completely any uptake of sorbose.

(Following the immediate shrinkage upon the addition of the sorbose, the slight progressive shrinkage is a reflection of the exit of glucose from the cells as a result of the gradient established by the slight dilution of the extracellular glucose by the added volume, and the increased concentration within the cells because of the shrinkage.) Similar relations were observed with any combination of an aldose and a ketose: the presence of the former inhibited uptake of the latter, but not *vice versa*. Inhibition of the ketose uptake was not in every case so absolute as that of dextrose on sorbose uptake (as in Fig.

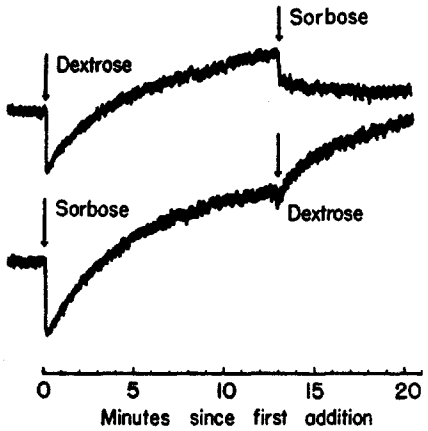


FIG. 3. Unilateral inhibition of uptake between sugars. At zero time, 1 ml. saline medium with sugar indicated, at 1.8 M, added to 10 ml. of cell suspension in medium. About 13 minutes later, at time marked, a second 1 ml. added, with sugar indicated, at same concentration. (Final concentration of each sugar was thus 0.15 M.) Downward deflection indicates cell shrinkage.

3), but was always very marked; in no case did a ketose exert any apparent effect on an aldose uptake except by virtue of its osmotic pressure.

However, the ketoses and aldoses did not form simply two homogeneous groups in this respect; different degrees of inhibitory efficacy were apparent between the members of each class. Sorbose was very effective in slowing the entry of levulose, whereas no effect was apparent in the reverse case. Among the aldoses, no such strictly unilateral inhibition was seen; each exerted some degree of inhibition on the movement of each of the others. The over-all arrangement with respect to mutual inhibition, at 0.15 M, is summarized in Table I.

Among the hexoses, the relative mutual activities indicated in Table I parallel their comparative effectiveness against the uptake of a given sugar; thus, inhibition of the entry of levulose from a 0.15 M solution is effected by

prior equilibration with dextrose at 0.01 M, to about the same extent as with galactose at 0.025 M, or with sorbose at 0.1 M.

The behavior of the pentoses, shown in Table I, however, does not permit of any simple pattern of relative inhibitory effectiveness as compared with the aldohexoses. For example, at 0.15 M, galactose blocked nearly completely the uptake of sorbose from a 0.15 M solution; while xylose still permitted a slow but definite sorbose uptake. Yet, as shown in the table, xylose slowed the entry of galactose more effectively than galactose slowed the entry of xylose. Such discrepancies are apparent only in contrasting a hexose with a pentose.

TABLE I

*Mutual Inhibition in Uptake of Sugars*

| In presence of | Inhibition of uptake of | | | | | | |
|---|---|---|---|---|---|---|---|
| | Arabinose | Dextrose | Galactose | Levulose | Mannose | Sorbose | Xylose |
| Arabinose | .... | ++ | ++ | +++ | ++ | ++ | + |
| Dextrose | +++ | .... | +++ | ++++ | +++ | ++++ | +++ |
| Galactose | ++ | ++ | .... | ++++ | ++ | +++ | + |
| Levulose | 0 | 0 | 0 | .... | 0 | 0 | 0 |
| Mannose | ++ | +++ | +++ | ++++ | .... | ++++ | ++ |
| Sorbose | 0 | 0 | 0 | +++ | 0 | .... | 0 |
| Xylose | + | ++ | ++ | +++ | ++ | ++ | .... |

0 = no, or doubtful, effect; + = just noticeable inhibition; ++ = moderate inhibition; +++ = very marked inhibition; ++++ = essentially complete block of uptake.
See text for experimental procedure.

This fact called special attention to the observation that the mercuric ion, or the sulfhydryl inhibitor *p*-chloromercuribenzoate (at about $2 \cdot 10^{-5}$ M), completely prevented the uptake of dextrose, mannose, sorbose, or levulose, but had only a partial inhibitory action on galactose or pentose uptake. No inhibitor has as yet been found to stop completely the entry of galactose or the pentoses; this suggests that xylose, arabinose, and galactose may enter only partly by means of the active mechanism, and partly by passive means. Support for this suggestion was seen in the fact that the pattern of the kinetics (such as in Fig. 1) of the residual uptake of these sugars in the presence of *p*-chloromercuribenzoate (at $4 \cdot 10^{-5}$ M) was not of the aldose type, but was shifted toward the ketose type. This provides an explanation of the discrepancy noted above between the relative immunity of the two pentoses to inhibition by the aldohexoses, and their relative ineffectiveness among the aldoses in the inhibition of ketose entry; since the presence of other competitors for the carrier system would not affect that fraction of the pentose uptake which

proceeded by other means, and this would give an exaggerated impression of their competitive prowess in mixtures with the aldohexoses. When this factor is taken into account, a more proper arrangement of the sugars with respect to their "affinity" for the carrier system would be:

Dextrose, mannose > galactose, xylose, arabinose > sorbose > levulose

The uptake of the last members of this series may be selectively hindered with little disturbance of the entry of the others; the temporary use of a barbital buffer in the suspension medium led to accidental demonstration of this. The barbiturate depressed the intake of levulose or sorbose measurably at 0.01 M, and nearly stopped the intake altogether at 0.03 M, with only a moderate interference with arabinose, galactose, or xylose, and almost no effect on dextrose uptake. It seems likely that this is merely another instance of competition for the carrier molecule, since the barbital molecule includes several =CO groupings; (failure of the polyhydric alcohols to penetrate indicates that the aldehyde or ketone group of the sugars is probably the critical configuration in this connection).

The high thermal coefficient of dextrose uptake (Bang and Ørskov, 1937; LeFevre, 1948) is seen also for each of the other sugars concerned in this report; comparison of rates at 20°C. and 37°C. gives $Q_{10}$ ranging from 2.4 to 3.6, with no apparent correlation with other comparative aspects of the relation of the sugars to the proposed transport system.

## INTERPRETATION

The appearance of a kinetic pattern as of passive diffusion, here observed for the ketoses, does not *necessarily* imply that there is no active step involved in the ketose penetration. As Shannon (1938, 1939) has pointed out for the carrier system he proposes for renal tubular reabsorption, the rate of transfer might be either directly proportional to, or essentially entirely independent of, the applied concentration of the transported substance, depending on which of the two steps in the reaction is assumed to be the more rapid (carrier-complex formation or complex disintegration). But the present distinction between the aldoses and ketoses need not involve the contrast in relative rates that Shannon suggests. Even with his proposed pattern of a rapid membrane penetration, essentially at equilibrium, followed by a slower (and thus rate-limiting) exit from the membrane, the two observed types of behavior could still result, merely from differences in the equilibrium constant of the primary (complex-forming) process. With a limited supply of the carrier molecule, a low dissociation constant for the carrier-sugar complex would result in a membrane concentration of the complex independent of the extracellular sugar concentration; while a high dissociation constant would mean that the complex concentration would be essentially proportional to the concentration of the penetrant

externally, over a wide range of concentrations. Thus, if $A$ = the total concentration of carrier available in the membrane, $C_S$ = the external concentration of the sugar, $A_S$ = the concentration of the carrier-sugar complex in the membrane, and $k$ = the dissociation constant of the complex, then

$$kA_s = C_s(A - A_s)$$

or

$$\frac{A_s}{A} = \frac{1}{1 + k/C_s}$$

The fraction of the total carrier which is bound to the sugar therefore does depend on the sugar concentration *unless* this significantly exceeds the constant $k$.

The contrasting behavior of the ketoses and aldoses could then be resolved into a difference between the two classes in this constant; specifically, aldoses might show a markedly lower complex-dissociation at equilibrium. A deduction from this hypothesis is that the rate of uptake of the ketoses should be greatly depressed, while that of the aldoses essentially unaffected, when the two are mixed at equal concentrations. As reported above, this was found to be the actual case in every instance. Further evidence supporting the conclusion that, in spite of the kinetic pattern of passive diffusion, the ketoses do in fact share at least part of the active transport mechanism suggested for the aldoses is found in the equally ready action of the mercurial inhibitors on both groups. The inhibition of ketose uptake by barbital, and the high thermal coefficient of the uptake, are further indications of its active nature.

In terms of the proposed scheme, the final series indicated above for the sugars would correspond to their sequence in regard to the magnitude of their equilibrium constants in reacting with the carrier molecule. That this cannot be the only distinction between them is evident, however, from the fact that this series does not correspond altogether with that based on the relative rates of penetration. This implies similar differences perhaps in the rates of disintegration of the various sugar-carrier complexes, in the second class of the transport reactions.

## SUMMARY

1. Applicability of the previously postulated active transport system for conveying glucose into the human red cell was tested in connection with a number of related substances, comprising 6-carbon aldoses and ketoses, 5-carbon aldoses, and 5- or 6-carbon polyhydric alcohols.

2. The alcohols did not perceptibly penetrate the cells; all the sugars penetrated, the rates differing, but all of the same order of magnitude.

3. All the aldoses penetrated according to the pattern previously reported for glucose, in that the rate of penetration was not directly related to the gradient, but subject to some limiting factor.

4. The ketoses penetrated approximately according to the pattern of passive diffusion.

5. When present in equal concentrations, any aldose prevented or greatly delayed the entrance of a ketose, while the ketose did not perceptibly alter the rate of aldose uptake. Within each class, similar inhibitory relations were observed.

6. Penetration of all the sugars showed a high temperature coefficient and was inhibited by the mercuric ion or *p*-chloromercuribenzoate; certain of the sugars showed a residual degree of penetration not thus inhibitable.

7. Penetration of the ketoses was selectively inhibited by barbital.

8. These observations are interpreted in terms of simple equilibria between the various sugars and a hypothetical carrier molecule in the membrane, with which the sugars form a complex during their passage through the membrane. Comparisons between the sugars in relation to their reactions with the carrier system are indicated.

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