THE MECHANISM OF GLUCOSE TRANSFER INTO AND OUT OF THE HUMAN RED CELL

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(Received for publication, December 31, 1951)

For many years there has been accumulating evidence of certain peculiarities in the kinetics of the process by which glucose penetrates the human erythrocyte (for discussion, see LeFevre, 1948). There seems to be even distribution of normal blood sugar between the water of cells and plasma; and small increments of extra glucose are also rapidly distributed evenly. But the ease of permeation decreases progressively during the process, and is markedly depressed at high glucose concentrations, eventually to the point of apparently complete impermeability.

Previous reports from this laboratory (LeFevre, 1946, 1947, 1948) indicated that an active process at the cell surface is responsible for the unusually rapid penetration of human red cells by glucose and kindred substances. One aspect of this work, paralleling that of Guensberg (1947), was an investigation of the osmotic changes in cell volume accompanying glucose uptake from glucose-saline mixtures (method of Ørskov, 1935). The pattern of these volume changes suggested that there might be a fixed "limiting concentration" for glucose intracellularly, deviation from which governs the rate of glucose uptake (LeFevre, 1948).

However, quantitative considerations make it very difficult to accept any hypothetical "carrier" system consistent with this interpretation. Also, with any appropriate system of this type, there is no accounting for the fact that experimentally no movement against a gradient occurs. Further difficulties arise in application of this hypothesis to newer experiments, reported below, concerned with *outward* movements of glucose, from the cell into the medium. In addition to these general theoretical difficulties, there were previously reported minor, but definite, characteristic differences between the hypothetical and experimental curves, with the higher glucose concentrations. But no proper evaluation of these quantitative deviations could be considered until a particular technical uncertainty was resolved. This concerned the influence on the records of a known, but uncalibrated, action of glucose on the light transmission of the suspensions. Clarification of this matter will therefore first be given brief consideration below.

Correction for the Optical Aberration

The disturbance in question was first noted by Meldahl and Ørskov (1940), in whose experiments it took the following form: the direct light transmission of human red cell suspensions, directly following addition of the slow penetrants, glucose or mannitol, was greater than following addition of sodium chloride solutions with the same freezing point. At the same time, it was shown by use of the hematocrit method (on denser cell suspensions) that the actual cell volume did not differ measurably in equiosmolar solutions of each of these substances (added to the saline medium). Thus some factor other than cell volume changes contributed to the deflection in the light transmission. Because of the limitation of their interest to the estimation of "permeability constants,"[1] Meldahl and Ørskov were able to arrange their procedure so as to dispense with any correction for this factor; but such correction is inevitable here, since an analysis of the complete sequence of events in glucose-saline mixtures is required.

The disturbance may be illustrated in the form mentioned by Meldahl and Ørskov, as it affected preliminary tests of the recording system used in the present work. In these experiments, salt or sugar in varying concentrations was added to red cells in a saline medium. Fig. 1 shows the extent of the discrepancy in question. The original purpose of these tests was to determine whether recorded deflections varied linearly with the cell volume. Note that with addition of either salt or a sugar there is satisfactory linearity; but that, with the latter, the slopes are decreased (there is less change in the recorded deflection for a given change in volume). On the basis of the results with added NaCl, the apparent shrinkage with dextrose is only about 82 per cent, and with sucrose only about 68 per cent, of the calculated shrinkage at each concentration. These figures are reproduced regularly, with little variation, in such tests. As in Meldahl and Ørskov's work, concurrent hematocrit determinations here showed that the actual cell volumes were essentially identical in salt solutions and in sucrose-salt mixtures calculated to have equal osmotic pressures.

Several physical considerations which might account for this alteration of the optical properties of the cell suspensions have been suggested, but these have not been susceptible to direct analysis permitting experimental test.

[1] In addition to the improper equation noted previously, a systematic arithmetical error is evident in all Meldahl and Ørskov's tabulated values for the permeability constants. This error leads to a significant underestimation of the progressive increase in this constant for substances other than glucose, and slight exaggeration of the progressive decrease in the case of glucose; the general conclusions are not altered. They directly disagree, however, with Schönheyder's (1934) statement that the red cell permeability constant for malonamide is not affected by the total tonicity.

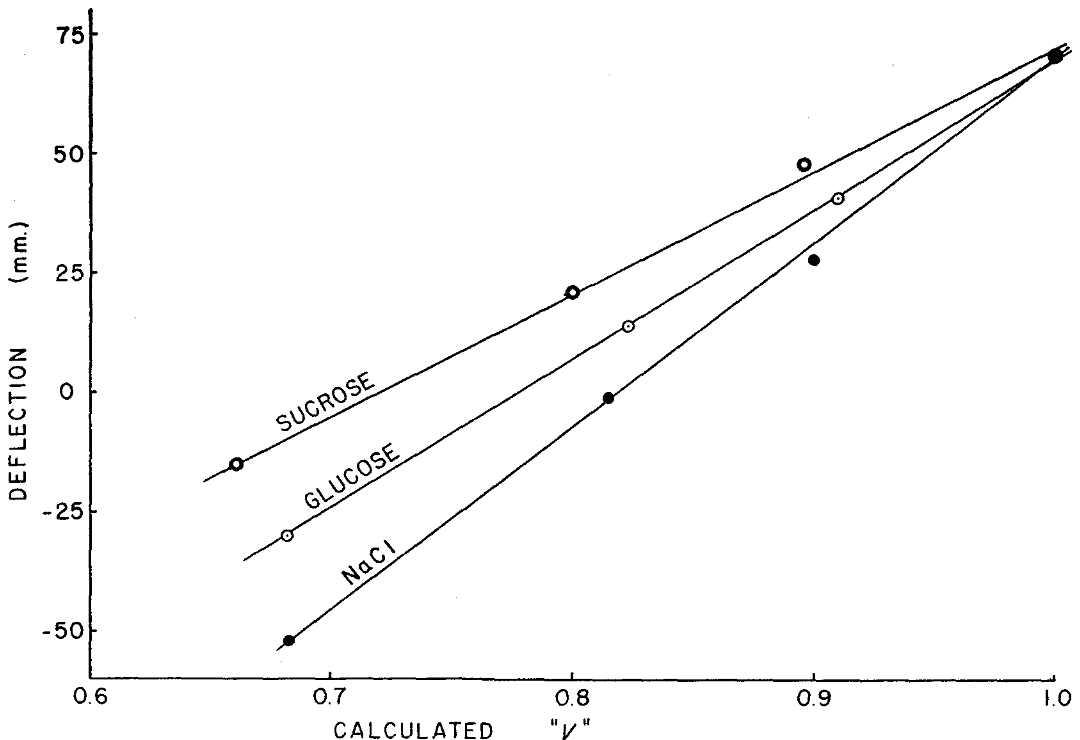


FIG. 1. Optical aberration in presence of non-electrolytes. 2 ml. isotonic saline medium, with extra NaCl, sucrose, or glucose, at varying concentration, added to 10 ml. cell suspension in saline medium. Deflection is resultant of (1) dilution of suspension, (2) change in cell volume, (3) optical disturbance from change in nature of medium. Initial recorded deflection is plotted against cell volume calculated from total tonicity of medium.

In a variety of measurements in several directly contrasting sets of circumstances, however, a simple function of the primary variables was found to apply satisfactorily as a corrective factor. This relation is

$$L = L_o(1 + 0.17\, C_s - 0.08\, S/V),$$

in which L is the direct light transmission of the suspension; L_o, the transmission of a suspension of cells at the same density and volume, in the absence of sugar; C_s, the extracellular sugar concentration; S, the intracellular quantity of sugar; and V, the effective volume of the cell water.[2] Fig. 2 shows the degree to which the aberration under a variety of circumstances can be defined by this relation; the suitability shown here led to the use of this function as a general correction, without consideration of its physical basis. All theoretical curves in later figures are modified in accordance with the relation as stated above.

In arriving at this form of the corrective equation, the following facts were taken into account:

1. If $S/V = 0$, at any fixed C_s the direct light transmission of the suspensions is essentially linear with V, as V is varied over the range 0.4 to 1.8 times the isotonic value.[3]
2. Increase of C_s from zero to a finite value, if $S/V = 0$, increases the light transmission by approximately the same factor at all values of V.
3. The value of this factor increases approximately in direct proportion to C_s.
4. When, through equilibration, C_s and S/V become equal, the light transmission at any V remains only slightly higher than in the absence of sugar.[4] This elevation is further reduced and finally reversed, as S/V is increased above C_s by decreasing V by means of hypertonicity.

This correction, though too large to ignore, is not so large as to alter *radically* the form of the osmotic volume changes in the unmodified records. In application to experiments of the type represented by Fig. 8(a) of the earlier report (LeFevre, 1948), the correction improves the fit of the initial deflections with the calculated relations, decreases the expected curvatures in the records, and

[2] Symbols used throughout this paper correspond, wherever possible, to those of Wilbrandt (1938) and Guensberg (1947).

[3] At higher tonicities, this relation breaks down and even reverses. Passow and Eggers (1950) have investigated this matter thoroughly in the extremely hypertonic range; their observations seem to indicate that the useful range for experiments of the present type corresponds to the range in which the cells are nearly all finely crenated (*Stechapfelform*); at higher tonicities, the appearance of coarse crenation (*Warzenform*) seriously alters the optical properties of the suspension.

[4] Meldahl and Ørskov state that the deviation becomes zero in these circumstances; it does become rather small in the case of glucose, but this is not at all general.

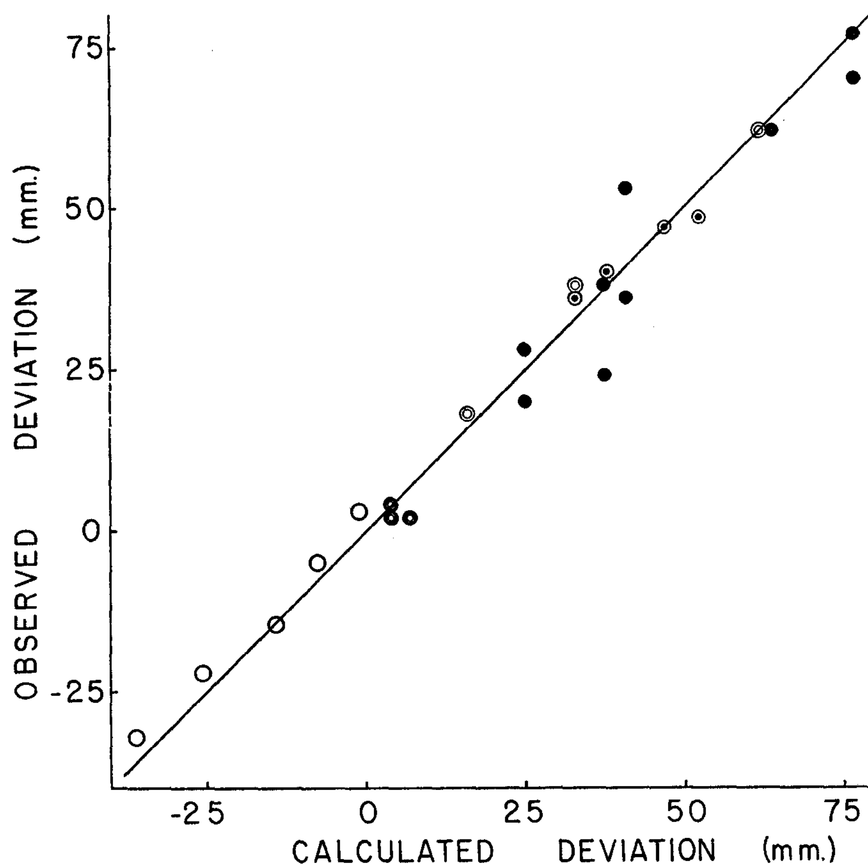


FIG. 2. Applicability of empirical corrective factor for glucose optical aberration. Observed deviation of recorded deflection from that corresponding, in saline medium, to calculated cell volume, is plotted against deviation calculated from corrective equation in text.

Solid circles, initial $C_s = 0$; final C_s fixed; C_m varied.

Open circles, initial and final C_s fixed; C_m fixed; S/V varied.

Open circles, with solid centers, initial $C_s = 0$; final C_s and C_m concurrently varied.

Double circles, initial $C_s = 0$; C_m fixed, final C_s varied.

Triple circles, initial C_s = final C_s; initial C_m = final C_m.

(Text explains these symbols.)

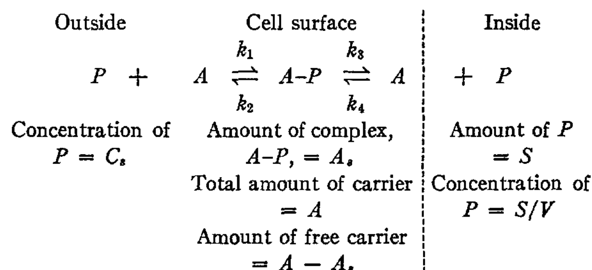
increases the expected contrasts in the recorded rates of swelling at the higher glucose concentrations.[5] All these alterations tend to eliminate the small

[5] Reason for changes in the directions indicated may be appreciated by consideration that the initial deflections will be raised as C_s is raised, but that this elevation will become less and less as the glucose enters the cells.

quantitative deviations of the records from the theoretical patterns previously developed (LeFevre, 1948). But, as has been mentioned above, the 1948 hypothesis is not translatable into a reasonable physicochemical "carrier" system, and does not provide for newer observations. It was therefore felt advisable to attempt an alternative explanation for the observations, beginning with a consideration of the applicability of the simplest carrier systems.

The "Carrier" System

The simplest series of reactions imaginable, involving a reversible combination of the penetrant, P, with a cell surface constituent, A, might be represented as follows:—



in which k_1, k_2, k_3, and k_4 are the velocity constants[6] for the indicated reactions, so that the equilibrium constant, K_1, is equal to k_2/k_1 for the exterior reaction, and similarly $K_2 = k_3/k_4$ for the interior reaction.

With such a system,

$$\frac{dS}{dt} = k_3 A_s - k_4 (A - A_s) S/V,$$

and

$$\frac{dA_s}{dt} = k_1 C_s (A - A_s) - k_2 A_s - \frac{dS}{dt}.$$

What special restrictions of this general set of relations can be derived from the observations?

There are two aspects of the osmotic swelling curves which require special explanation as regards their relation to the sugar concentration: (*a*) the relative slope (rate of swelling) in the initial stages, and (*b*) the steady state volumes ultimately attained. At body temperature, the latter presents no difficulty unless the sugar concentration is rather high; below about 3/4 isosmotic, even

[6] In all equations in this paper, each of these constants is considered to include a factor for the volume of the surface phase to which the carrier is restricted; dimensions are therefore as follows: for k_2 and k_3, t^{-1}; for k_1 and k_4, concentration$^{-1}t^{-1}$. K_1 and K_2 therefore have dimensions of concentration.

distribution is attained in a reasonable time, and the cell volume equals that found in an electrolyte solution of the same tonicity as that in the mixture. Discussion will therefore first be limited to this range of concentrations, and problems under item (*a*), above, considered separately.

(*a*) *Kinetics of Volume Changes.*—The earlier observations showed that the rate of entry of glucose is fairly constant over a wide range of extracellular glucose concentrations. This clearly provides one characteristic of the system: the combination of glucose with the cell surface constituent must have a high velocity constant compared with that of the reverse reaction or of the succeeding reaction. For, initially, when S is very small, the back reaction 4 cannot contribute much to the net dS/dt, and this rate will be essentially proportional to A_s. The fact that it is constant, then, indicates a constancy of A_s in the face of variation of C_s. This implies that the reactions at the outer surface are essentially in equilibrium and that K_1 is small compared to the lowest C_s used; since, at equilibrium,

$$A_s = \frac{A}{1 + K_1/C_s},$$

and since experimentally the initial rate of uptake appears not to vary with C_s. Further evidence of such a small K_1 for glucose has been presented earlier (LeFevre and Davies, 1951) in the competitive superiority of glucose in achieving combination with the "carrier" when mixed with other sugars apparently using the same system.

This attribute of the system does not limit the number of factors which may be involved in the process of dissolution of the complex; but it does imply that *this dissolution is the rate-limiting step*, having lower velocity constants than the initial step of complex formation. (This conclusion is also more directly derivable from the form of the dependence of dS/dt on C_s in experiments such as in Fig. 6, below.) This is equivalent to stating that, whatever the reactions of the carrier in the cell surface with the sugar dissolved in the aqueous phases, these reactions are considerably slower on the inner interface than on the outer interface. The simplest working hypothesis would be that these reactions are otherwise the same in both loci, differing only in rate. In order to test the generality of applicability of this simplest of the likely carrier systems, a much wider variety was arranged in the factors related to the kinetics of glucose movements in experiments with osmotic volume changes. For example, the initial cell content of glucose, or the extracellular glucose concentration, or the initial glucose gradient, or the initial cell volume, or the quantity of glucose which need be transferred to attain equilibrium, or other such factors, were each held constant while other factors were varied, and the transfer of glucose recorded in terms of the resultant osmotic volume changes. With glucose concentrations not in excess of about 70 per cent of the isosmotic,

all records thus far obtained have been in satisfactory agreement with the quantitative predictions of the simple system outlined above, regardless of what combination of variable and constant factors was used in the experiment.

With the stipulations indicated above, that $k_1 \gg k_4$ and $k_2 \gg k_3$, then by simple mass action,

$$\frac{dS}{dt} = \frac{Ak_3}{1 + K_1/C_s}\left[\frac{C_iV_i + S\left\{1 - \frac{K_1(C_m + C_s)}{K_2C_s}\right\}}{C_iV_i + S}\right]$$

in which V_i is the volume of the cell water at isotonicity (C_i), and C_m is the concentration of the non-penetrating salts in the medium. Since it is thermodynamically necessary that $K_1 = K_2$ if the intracellular sugar is in free aqueous solution, and since this is evident experimentally in that the glucose becomes evenly distributed at the concentrations under discussion; and since $K_1 \ll C_s$ in the actual experiments, the above equation reduces to

$$\frac{dS}{dt} = Ak_3\left[\frac{C_iV_i - SC_m/C_s}{C_iV_i + S}\right].$$

And, since

$$S = V(C_m + C_s) - C_iV_i,$$

$$\frac{dV}{dt} = \frac{Ak_3(C_iV_i - C_mV)}{C_sV(C_m + C_s)},$$

which by integration gives

$$t = \frac{(C_m + C_s)\,C_s}{Ak_3C_m}\left[V_o - V + \frac{C_iV_i}{C_m}\ln\frac{C_iV_i - C_mV_o}{C_iV_i - C_mV}\right],$$

in which V_o is the cell water volume when $t = 0$.

It may be noted that if the factor C_s is dropped from the right-hand side of this equation, it becomes directly convertible to that derived on the assumption of passive diffusion (by Schiødt, 1931; Wilbrandt, 1938; LeFevre, 1948) for the case of addition of a penetrant to a saline medium.[7] This means that the form of the swelling *in any given solution* is that of passive diffusion; but the dependence on C_s, in comparing a series of different solutions, is, of course, very different from that in the case of passive diffusion.

Figs. 3 to 6 show characteristic patterns relating to the contrast in the kinetics of glucose movements into and out of the cell, in accordance with this equation. In Figs. 3 *a* and 5 *a*, the glucose concentration of the medium, in the critical phase of the experiment, is the same for each test in the series,

[7] Wilbrandt's original statement of this equation differs from the above due to an error in sign, but this error was obviously corrected in his actual use of the equation.

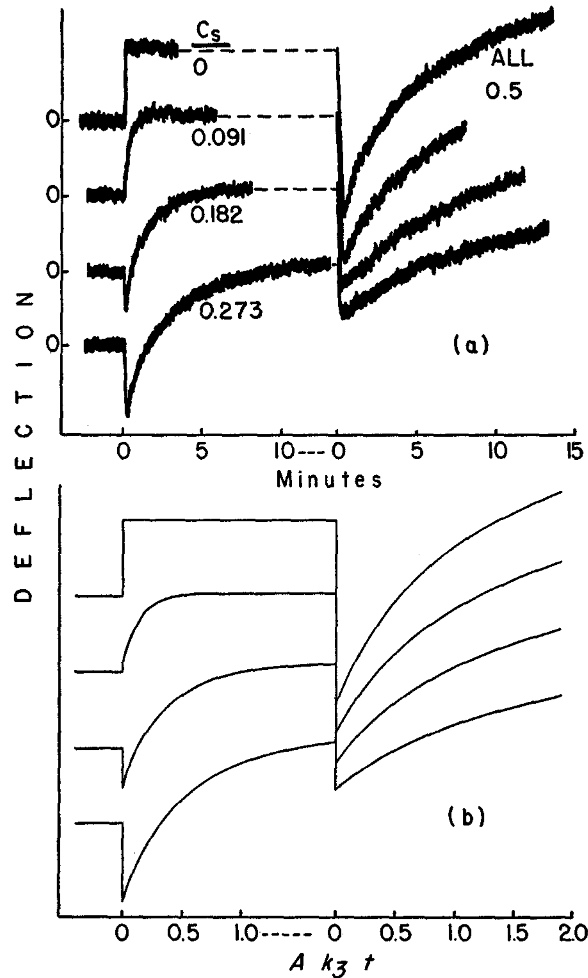


FIG. 3. Glucose entry with fixed C_s, varied initial S/V.

(*a*) At zero time, 1 ml. added to 10 ml. cell suspension to increase glucose concentration from zero to tonicity as labelled. After equilibration, at second "zero" time, 1 ml. added to give final glucose concentration, in each case, of ½ isosmotic. All solutions contained salt mixture at 60 per cent of isotonicity. Temperature = 37.5°C. In all figures, increase in cell volume produces upward deflection. Records here reduced to one-fourth actual size.

(*b*) Pattern for same experiment, on basis of the hypothetical carrier system; scale for deflections matched to fit lowest record in (*a*).

but the initial quantity of intracellular glucose differs, as labelled. In Figs. 4 *a* and 6 *a*, the initial gradient for glucose, in the critical phase of the experiment, is constant or very nearly so, while the extracellular concentration is

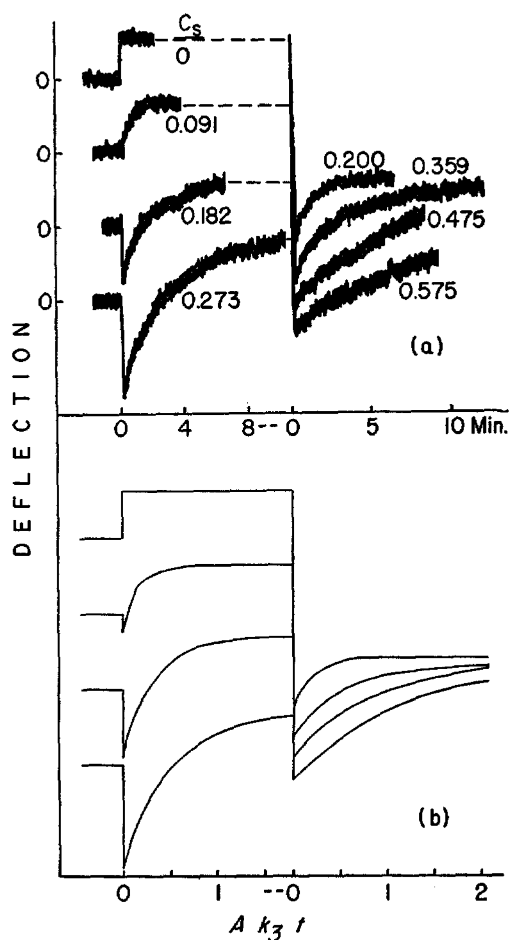


FIG. 4. Glucose entry with fixed initial gradient ($C_s - S/V$), varied C_s.

(*a*) At zero time, 1 ml. added to 10 ml. cell suspension in 60 per cent isotonic salt mixture, to give final glucose tonicity as labelled (final salt tonicity of 63.5 per cent). After equilibration, at second "zero" time, 1 ml. added to give final glucose concentration as labelled, and *total* tonicity of 5/4. This made saline tonicity such that, in each case, immediate glucose gradient at this moment was 1/5 isosmotic concentration ($C_s - S/V = 0.2$; *i.e.*, 0.06 M). Temperature = 37°C. Records here reduced to one-fourth actual size.

(*b*) Pattern for same experiment, on basis of the hypothetical carrier system; scale for deflections matched to fit lowest record in (*a*).

varied. (The figure legends give specific details.) Figs. 3 *b*, 4 *b*, 5 *b*, and 6 *b* show the corresponding curves derived from the above equation, modified slightly by correction for the optical aberration discussed above; the match is evident, and is equally good for all the experimental variations thus far at-

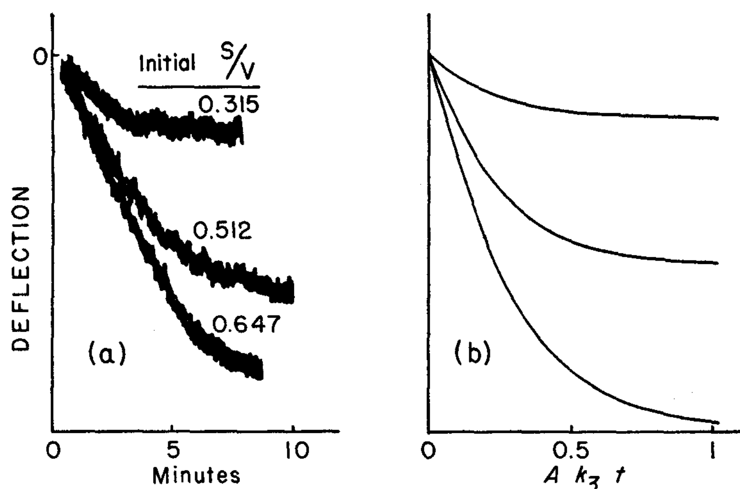


FIG. 5. Glucose exit with fixed C_s, varied initial S/V.

(*a*) Cell suspensions previously equilibrated, in isotonic salt mixture, with three concentrations of glucose: 0.3, 0.6, or 0.9 isosmotic. At zero time, 2 ml. suspension added to 10 ml. salt mixture, at 120 per cent of isotonicity, with glucose such that final sugar concentration was, in each case, 1/5 isosmotic. Temperature = 37°C. Alignment of records (here reduced to three-eighths actual size) is by superimposing position at zero time, by backward extrapolation.

(*b*) Pattern for same experiment, on basis of the hypothetical carrier system; arbitrary scales.

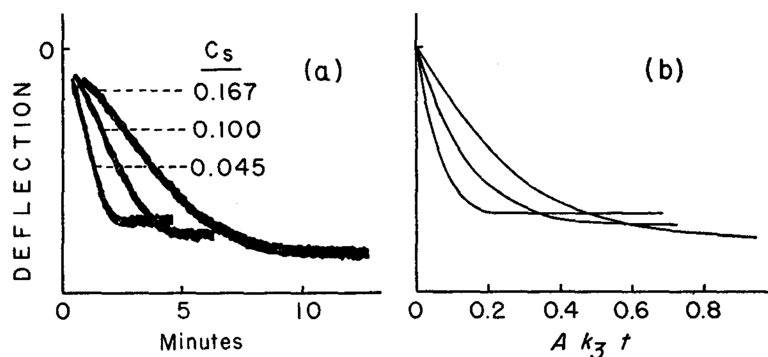


FIG. 6. Glucose exit with varied C_s.

(*a*) Cell suspensions, at three densities in ratio 1, 2, 3, previously equilibrated in isotonic salt mixtures with glucose near 60 per cent isosmotic. At zero time, 3, 2, or 1 ml. of suspension added, respectively, to 9, 10, or 11 ml. of glucose-saline mixture, such that final glucose concentrations were as labelled. (Initial gradients, at this moment, were all between 0.63 and 0.66.) Temperature = 35°C. Same recording procedure as in Fig. 5.

(*b*) Pattern for same experiment, on basis of the hypothetical carrier system; arbitrary scales.

tempted. There is therefore no reason to suppose any complication of this most elementary carrier system, in interpreting all results under the limitation of item (*a*).

It may be noticed that, in the examples provided, the most suitable values for Ak_3 vary from about 0.08 to about 0.17 isocontents per minute. In these particular cases, the variation may be partly attributable to the fortuitous differences in the temperature, indicated in the figure legends (see especially Fig. 6); as reported earlier, the temperature coefficient of the over-all process is very high. However, considerable variation was evident from day to day, even at a fixed temperature of 37°C., in the apparent velocity of the critical reaction. Meldahl and Ørskov (1940) found a progressive decrease in the rate of glucose penetration, with storage of the cell suspension; in the present work, this was not systematically investigated, but no obvious correlation between the age of the suspensions and the value of Ak_3 was apparent, and the variability remains unexplained.

It is informative to restate the rate equation above as follows:—

$$\frac{dS}{dt} = \frac{Ak_3}{C_s}(C_s - S/V),$$

or

$$\frac{dS}{dt} = Ak_3\left(1 - \frac{S/V}{C_s}\right).$$

These forms call attention to certain simple relations: at a given C_s, the rate of transfer in either direction is *proportional to the gradient;* while with a given gradient, the rate of transfer in either direction is *inversely* proportional to the C_s. And in any case, the rate of transfer is proportional to the difference from unity in the ratio of intracellular glucose concentration to extracellular glucose concentration. Thus the *inward* transfer rate is limited (can never exceed Ak_3, no matter how great the gradient); while the *outward* transfer rate may be much greater (large negative value for dS/dt if S/V is many times C_s). This is apparent in the experimental records. Wilbrandt and Rosenberg (1950) found that dS/dt varied with the gradient, but more so than by an uncomplicated Fick's law, and that there was a fixed upper limit to the rate of transfer. Wilbrandt and Rosenberg's general conclusions have been so far expressed only qualitatively; a more complete statement of their results would be helpful, as it is at present uncertain whether they corroborate or contradict the scheme presented above.

From his investigations of the inhibitory effects of phloretin and related compounds, Wilbrandt (1950) concluded that the sensitive step in the process is the *exit* of glucose from the cell membrane, whether this be on the outside or the inside of the cell. The fact that the entry into the membrane from the outside and exit into the cell interior may be undisturbed, while the exit to the

outside is blocked by an extracellular inhibitor, is taken as an indication that the individual carrier units do not operate in both directions. This important line of investigation deserves special attention; the present reports of this work are somewhat fragmentary.

The success of the schema above in describing the actual events of glucose transfer across the red cell surface does not, of course, demonstrate that the proposed series of reactions actually occurs. Other systems might well show similar kinetics; the present form is preferred only by reason of its simplicity. It is believed that the complications introduced have been the minimal consistent with the facts.

(*b*) *Terminal Volumes Attained.*—This simplicity disappears when concentrations of glucose appreciably above about 3/4 isosmotic are used. When glucose is initially added to the medium, the rate of swelling is at first approximately as predicted from the final equations above, but this rate rapidly diminishes, as reported earlier, and the swelling is terminated (or slowed to an imperceptible rate) before the cells have attained the volume appropriate to an even distribution of glucose. This has been amply demonstrated also by hematocrit and direct chemical measurements, both in the authors' laboratory and elsewhere.

Several possible explanations are easily suggested: (1) in such hypertonic solutions, particularly with non-electrolytes, the cells may lose an appreciable fraction of their osmotically active constituents; (2) with high intracellular concentrations, particularly of the penetrants, the cell structures may become radically altered so that free osmotic volume changes cannot occur, as in the paracrystalline state (Ponder, 1945); (3) in such solutions, the cell surfaces may become altered so that the carrier system gradually fails to operate; (4) the carrier system may be complicated in such a way that the steady state condition is not necessarily one of equality of concentrations on the two sides of the surface.

The first of these suggestions was rejected on experimental grounds. If any significant osmotic loss occurs in the experimental solutions with which we are concerned, it should be largely accounted for as a loss of potassium ion, since this is the predominant human red cell cation, and prolytic loss of K^+ into the medium is the most commonly described accompaniment of a variety of adverse conditions. Ege (1919) suggested loss of K^+ as the likely factor in his original description of the anomaly in question, but he did not attempt to test this suggestion. Analyses in the present case show that such a loss does not occur. Flame photometer (Perkin-Elmer Model 18) measurements of the potassium content of the suspension medium, with suitable control measurements of the medium alone, and after complete hemolysis osmotically induced, showed that *less than 5 per cent, if any*, potassium ion was lost from the cells during exposure to the most hypertonic glucose-saline

mixtures used in these experiments. Similarly, the supernatant fluid after centrifugation of such suspensions never showed any coloration indicative of hemolysis. It is commonly observed that erythrocytes lose K^+ in *plain* non-electrolyte solutions; Klinghoffer (1935) has pointed out, however, that the critical factor in slowing the uptake of glucose is the glucose concentration itself, rather than alteration of the relation between electrolyte and non-electrolyte concentrations in a mixture.

Accessory experiments also made untenable the suggestion of cell fixation. Addition of various tonicities of saline, or of isosmotic glycerol or thiourea, to cell suspensions containing the higher concentrations of glucose, resulted in the expected volume changes as calculated on the basis of the apparent cell content of glucose at the moment, estimated from the recorded volume. The inability of the cells to acquire glucose at the high external concentrations does not therefore seem to involve any restrictions on osmotic changes in cell volume. Cells from such suspensions readily hemolyzed when transferred to an appropriately hypotonic saline medium. Klinghoffer (1940) showed also, by comparison of chemical analytic and hematocrit data, that simple osmotic equality on the two sides of the cell surface was maintained throughout the process.

No direct evidence can at present be cited in regard to the last two suggestions enumerated above, or to any comparable alternative. It should be noted that explanation by any complication of the hypothetical carrier model itself must involve introduction of some asymmetry. This can scarcely take the form of a stoichiometric removal of a factor in the chemical chain, since (in addition to several kinetic discrepancies) the quantities involved experimentally sometimes amount to more than the total original number of osmotically active particles in the cell! A physical asymmetry, which could be expressed as an inequality of K_1 and K_2, is equally unsatisfactory; since the *recorded* quantity, the cell volume, is determined by the osmotic pressure (reflecting the thermodynamic *activity* of the intracellular glucose), so that this sort of asymmetry would not be evident even if present.

For these reasons, the best explanation at present is that the high glucose concentrations block the carrier reactions. This, indeed, is the view adopted by Wilbrandt and Rosenberg (1950), who compare the situation to that of enzyme inhibition in the presence of an excess of substrate. If this action occurs here, it must be gradual in its development and readily reversible upon removal of the cells to media with lower glucose levels. Meldahl and Ørskov suggested a gradual adsorption of glucose on pores of the cell membrane, in this connection. Ponder (1950) also reported depression of the potassium-accumulating mechanism in this cell, when the external glucose concentration was considerably above normal blood levels.

Attention should perhaps be drawn to the fact that the supposed carrier system derived from these kinetic experiments does *not* constitute a glucose "pump," and does not perform thermodynamic work in transport of glucose across the red cell surface. It does, however, imply chemical activity on the part of some surface constituent in forming with the penetrant a complex necessary for the process of penetration. Without this special ingredient, which is sensitive to sulfhydryl inhibitors (LeFevre, 1947, 1948), the human red cell would presumably share the impermeability to hexoses characteristic of mammalian red cells generally.

SUMMARY

1. The kinetics of the movements of glucose in both directions across the surface of the human red cell were studied by optical recording (Ørskov method) of resultant cell volume changes.

2. A wide experimental variety was arranged in the relations between the several quantitative factors contributing to the glucose gradient and the volume changes expected, in order to provide a maximum variety of systematic relations between those factors and the rate of glucose transfer.

3. The kinetics were shown to follow the patterns predicted on the basis of a simple carrier system, involving formation of a highly undissociated complex between the sugar and some factor in the cell surface, provided the glucose concentrations used did not exceed about 3/4 isosmotic. Certain simple properties of this system are derived from the data.

4. At very high glucose concentrations, this system apparently gradually fails to operate; this failure is reversible upon lowering of the excessive glucose concentration.

5. An empirical correction was derived for a previously known but uncalibrated optical disturbance complicating the use of the Ørskov method with media containing appreciable concentrations of non-electrolytes.

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