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H. M.

New Aspects in Research on Erythrocytes

Specific Transport Mechanisms in the Erythrocyte Membrane

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The crossing of rivers usually occurs by way of bridges. A more primitive form is the ferry-boat which has a much more limited capacity but is simpler and less expensive. The crossing of molecules through cellular and possibly subcellular membranes also appears to use, in a somewhat analogous way, two similar principles. The fixed bridge could be compared to water-filled porous channels across membranes while the ferry-boats roughly correspond to the carrier mechanism of membrane transfer. In cell membranes, however, the carrier mechanism appears to be the more recent and more sophisticated mechanism rather than the more primitive one. It appears to be operative mainly for water-soluble and lipid-insoluble substances of particular biological importance. What are the reasons for this development?

Observations in red cells supply a number of informations which have some relevance with respect to this question. A number of transmembrane movements of molecules or ions in red cells are interpreted in terms of carrier transport. The specific properties of erythrocytes which facilitate experimentation (ease of obtaining red cells in relatively large numbers, of separating them from other types of cells as well as from the surrounding medium and finally the relatively low rate of metabolism) have led to extensive studies in this field.

The red cell is a highly specialized type of cell lacking (in mammals) all subcellular structure and having a low rate of (mainly glycolytic) metabolism. Nevertheless, these studies have a general biological significance, due to the origin of red cells from fully developed and highly organized cell types and to the fact that in spite of specialization important general physiological properties of the cells have been maintained, although they might appear dispensible. Actually, some are found only in a small number of species e.g. the sugar transport system, the main object of this report. Groups of biologically important substances whose transfer across the red cell membrane has been assumed to be operated by special transfer mechanism include sugars, amino acids, cations and organic bases (purines and pyrimidines).

The system studied most extensively in red cells is that of sugar transport. The first indication of mechanisms other than free diffusion were surprising structural specificities. KOZAWA[1] in 1914 reported that lipoid insoluble sugar molecules of closely related structures showed surprising differences in their rates of penetration. HÖBER, in whose laboratory these studies were carried out, suggested that the penetration was under the control of insulin (as was later shown to be true for other cell types, mainly muscle cell). A systematic study of species differences in red cell permeability was started 15 years later by JACOBS[2]. He reported for instance that in mammalian red cells urea penetrates many times faster than glycerol while in bird cells the opposite is true. Fish cells are intermediate.

When, furthermore, kinetical studies revealed that in human red cells the penetration of glucose does not follow diffusion laws and that rate constants calculated on the basis of diffusion kinetics varied over a range of about 10,000 when the sugar concentration was varied[3] it became apparent that, at least in this case, mechanisms different from free diffusion (be it across water-filled pores or across lipoid layers) must be assumed. The carrier mechanism shown schematically in Figure 1 was suggested[4–6]. The passage of a substrate S across a membrane occurs in the form of a complex CS, formed by reaction of S with a membrane component C (carrier) which is reversible and follows the mass law. Such a mechanism would allow high structural specificity

[1] S. KOZAWA, Biochem. Z. *60*, 231 (1914).
[2] M. H. JACOBS, Biol. Symp. *3*, 331 (1941).
[3] W. WILBRANDT, E. GUENSBERG and H. LAUENER, Helv. physiol. pharmac. Acta *5*, C20 (1947).
[4] W. F. WIDDAS, J. Physiol., Lond. *118*, 23 (1952).
[5] W. WILBRANDT, Symp. Soc. exp. Biol. *8*, 136 (1954).
[6] W. WILBRANDT and T. ROSENBERG, Helv. physiol. pharmac. Acta *8*, C82 (1950).

and would, as could be shown, be in accordance with observation with respect to the kinetics of penetration. Two parameters would determine the rate: the mobility of the transport complex and the dissociation constant (Michaelis constant) of the complex. Over a wide range of concentrations the same parameters do with reasonable approximation meet experimental observation[6–9].

Similar to enzymatic reaction at high concentrations the rate reaches a saturation level. This is shown in Figure 2 in which the exit rate of D-glucose is plotted against the concentration difference across the membrane from an experiment in which the external concentration was constant.

If, however, both concentrations are varied in a parallel fashion such as to keep their ratio constant a feature of kinetics appears which is not common in enzyme kinetics: at high concentrations now the rate decreases again. Actually this decrease, if a logarithmic concentration scale is used, is as steep as the increase with rising low concentration and a symmetrical rate concentration curve obtains. This is shown in Figure 3 for three sugars. It is due to the fact that the high saturation now has a double effect: it favors the binding of substrate at the side of higher concentration but it also impedes the release of substrate on the other side of the membrane. The system then approaches a situation first discussed by USSING[10] for which the term 'exchange diffusion' is used: if the carrier can only move in the loaded form the system can perform exchange of substrate molecules between the two sides of the membrane but no net transport.

The concentration with maximum transport rate in Figure 3 is different for the three sugars used: the higher the affinity of the sugar to the system (as concluded for instance from competition experiments) the higher the concentration with maximum rate. This shows that the decrease of rate is not initiated by a certain critical concentration as such but, as in the above discussion, by a certain critical value of saturation. Somewhat related is another effect of high saturation: while at low concentration the rate increases with the affinity of the sugar, at high concentration it decreases, again due to the fact that at high concentration the release of substrate on the second side of the membrane is impeded if the affinity is high. Figure 4 shows experiments bearing out this relation: in a series of different sugars at low concentrations the sugar with the highest affinity (glucose) is the fastest, while at high concentration it is the slowest and vice versa.

Other peculiarities of the system appear if more than one substrate is present. We then have, again partly in analogy to enzyme kinetics and partly not, a number of consequences of the fact that the substrates then compete for the carrier molecules.

The simplest case is that of competitive inhibition, analogous to the situation in enzyme kinetics. Figure 5 shows entry experiments with three sugars, alone and

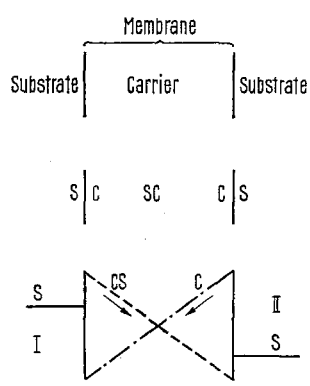

Fig. 1. Scheme of the equilibrating carrier system. The substrate S combines reversibly with the carrier C and, as long as $S_I > S_{II}$, the complex CS moves along its gradient transporting S from the side I to the side II. No uphill movement ensues (from WILBRANDT[5]).

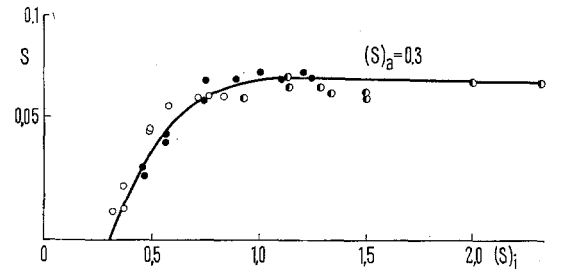

Fig. 2. Exit of glucose from human red cells at 37 °C. Rate of exit plotted against the internal concentration. At high concentrations a saturation level of the rate is reached (from WILBRANDT[22]).

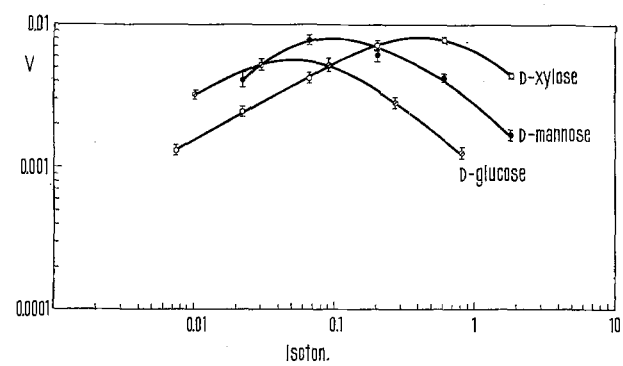

Fig. 3. Initial exit rates of 3 sugars from human red cells at 10 °C. The concentration ratio inside:outside was constant (5:1), the internal concentration (abscissa) varied. The points give average figures from 10–14 experiments ± S.E. of the mean. Ordinate: rate. Abscissa: concentration. Method: isotope-millipore filter procedure. Note the decrease of rate at high concentrations (from WILBRANDT and BECKER[23]).

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[10] H. H. USSING, Nature *160*, 262 (1947).

together. It can be seen that the total rate of the simultaneous penetration of the three sugars is much less than the sum of their penetration rates when each of them is present alone. Thus, their penetration is not independent, but they affect each other in a competitive way. This mutual inhibition, of course, depends on the affinity, the high affinity sugars showing a more pronounced effect on low affinity sugars than vice versa. From his experiments LEFEVRE[11] who was the first to observe competition has concluded that the order of decreasing affinity is D-glucose, D-galactose, D-xylose, L-arabinose, D-arabinose, L-xylose.

The range of affinities, according to LEFEVRE and MARSHALL[12], is more than 1:1000. These authors provided strong evidence for the view that the property determining the affinity is the stability of the Cl conformation of the molecule (Figure 6): the stabler this conformation, the higher the affinity.

Again due to the fact that not only binding at the side 1 but also release on the side 2 affects the transfer rate other interdependences between competing sugars do not correspond to similar phenomena in enzyme kinetics: the phenomena of counter transport and of competitive acceleration which are intimately related. Their interaction might be termed carrier substrate coupling. Figure 7 shows schematically a cell in three situations: I, with a substrate A in equilibrium (equal inside and outside concentration); II, a substrate B present only outside and finally III, both substrates present.

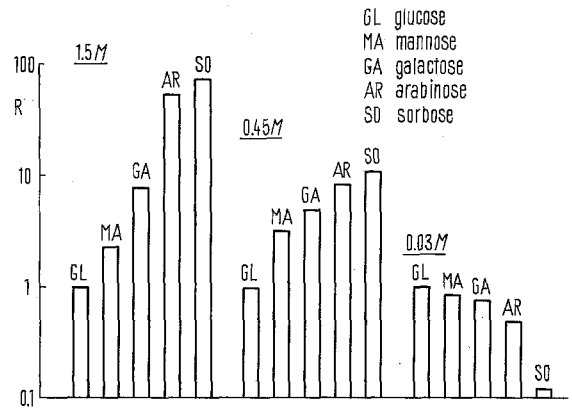

Fig. 4. Dependence of penetration rate order on concentration and affinity: the order of penetration rate of 5 sugars (highest affinity: glucose, lowest affinity: sorbose) is reversed when the concentration is raised from 0.03 to 1.5 *M*. R: penetration rate in arbitrary units, logarithmic scale. Experiments on exit of sugar from human red cells at 37 °C (from WILBRANDT[5]).

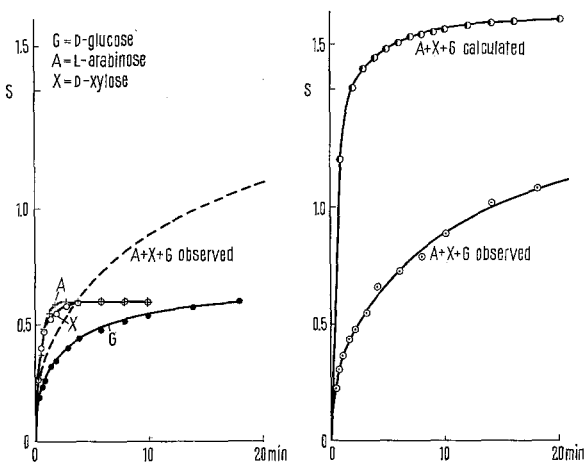

Fig. 5. Competition between 3 sugars for entry into human red cells at 37 °C. Left: individual entry of the 3 sugars. Right: total rate of the 3 sugars, when added simultaneously, observed (lower curve) and calculated from addition of the curves on the left hand (upper curve) (from WILBRANDT[22]).

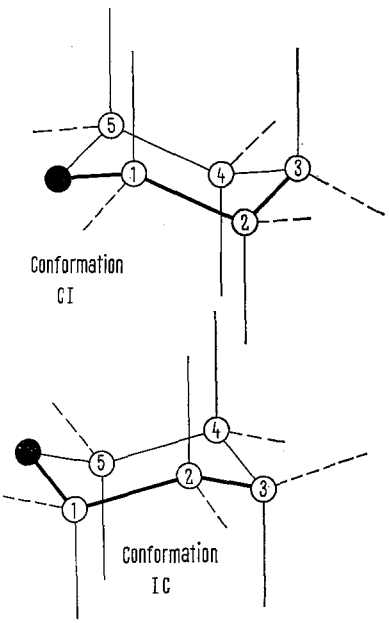

Fig. 6. Molecular conformations C I and I C of sugar molecules. The authors showed that the order of increasing affinity goes parallel with the order of increasing stability of conformation C I (from LEFÈVRE and MARSHALL[12]).

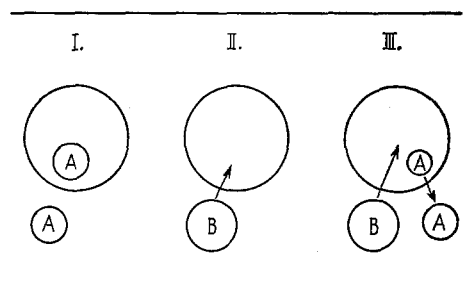

Fig. 7. Schematic representation of the situation leading to counter transport and to competitive acceleration (condition III). Details see text.

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[12] P. G. LEFÈVRE and J. K. MARSHALL, Am. J. Physiol. *194*, 333 (1958).

In the case I, of course, no net movement ensues. In the case II B will enter the cell. In the combined situation III, however, the behaviour of both sugars will be changed: A moves out of equilibrium against its own concentration gradient. This is termed counter transport[4,13]. B will again enter the cell but not with the same rate as in the absence of A. The rate may of course be decreased by competitive inhibition. However, under certain conditions it may be increased rather than decreased. This was termed competitive acceleration[14–16].

Figure 8 shows an example of counter transport. In Figure 9 experiments demonstrating competitive acceleration are presented.

All features of carrier transport discussed so far are predicted by calculated kinetics, and experimental observations are in good harmony with prediction. The experiments reported so far are all taken from the sugar system.

One characteristic feature of sugar penetration in red cells is that although the mechanism differs from that of diffusion the end result is the same: equal concentrations on the two sides of the membrane. Particular interest has been aroused in recent decades by observations showing uphill movement of substrates (against gradients of concentrations, activities or, in the case of ions, electrochemical activities). The question, then, arises: Can these uphill movements also be accounted for by carrier mechanisms, and, if so, what are the additional features leading to the uphill movement?

Actually many uphill transport observations are currently interpreted in terms of carrier mechanisms. Reasons are partly the same as in the case of sugar movement in red cells: high structural specificity, kinetics differing from diffusion kinetics, competition between substrates, etc. Additionally two reasons can be named which perhaps are more fundamental. The first is that the various phenomena discussed above as characteristic for carrier systems (decrease of transport rate at high concentrations, inverse relationship between affinity and rate at high concentrations, competitive acceleration and others) have been observed in numerous cases of uphill movement. Second: obviously uphill movements require energy which apparently is drawn from metabolic reactions as evidenced by the fact that many metabolic inhibitors are also inhibitors for uphill movements. Therefore, some sort of coupling must be assumed between metabolic reactions and the transport system. Now a carrier system is particularly suited for coupling, for instance in the form of reactions between metabolites and parts of the system, i.e. the carrier, the substrate or the transport complex. Two examples of uphill movement observed in red cell and interpreted in terms of coupled carrier systems may be shortly discussed: the movement of potassium and sodium and the movement of amino acids.

The unequal distribution of sodium and potassium, at one time interpreted by the assumption of cation impermeable membranes, today is accounted for by the existence of a transport system, shortly termed 'pump', moving potassium inward and sodium outward

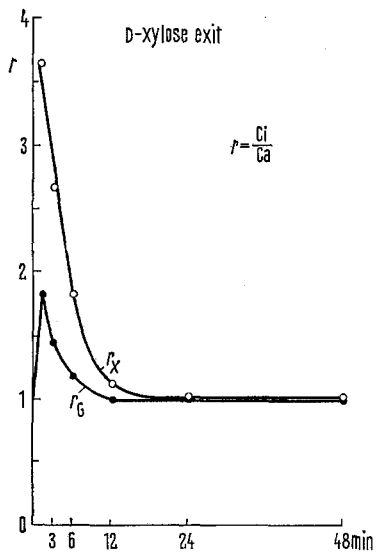

Fig. 8. Counter transport of D-glucose (originally at equilibrium) induced by exit of D-xylose from human red cells at 24 °C. The movement of glucose out of equilibrium against the gradient (up to $r = 1.8$) subsides with decreasing gradient of xylose (from WILBRANDT, FREI and BECKER[24]).

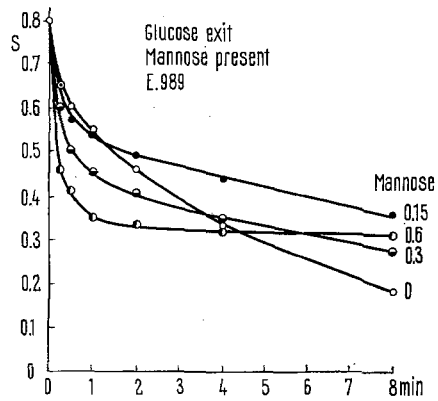

Fig. 9. Competitive acceleration. Exit of glucose from human red cells at 37 °C in the absence and in the presence of mannose in 3 concentrations (equal inside and outside initially). S, amount of intracellular glucose in cell units according to JACOBS[2]. The initial exit rate of glucose is increased in the presence of mannose. Later the increase turns into decrease due to re-distribution of mannose such that competitive inhibition ensues (from WILBRANDT, FREI and BECKER[24] and WILBRANDT[25]).

[13] T. ROSENBERG and W. WILBRANDT, J. gen. Physiol. *41*, 289 (1957).
[14] T. ROSENBERG and W. WILBRANDT, J. theor. Biol. *5*, 288 (1963).
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of the cell against gradients[17,18]. This view has replaced the previous impermeability notion because isotope studies showed that the membranes actually are permeable both for sodium and potassium. The movements of the two cations appear to be rather rigidly coupled as evident for instance by the fact that the outward movement of sodium in a number of cases has been shown to cease in the absence of external potassium and therefore of inward potassium movement.

An interpretation of this sodium potassium exchange pump (as it has been termed occasionally) using carrier notion is shown in Figure 10. It was suggested by SHAW[19]. Two forms of carrier are assumed, X with high affinity to potassium and Y with high affinity to sodium. Metabolic reactions transform X into Y at the inside, spontaneous reactions Y into X at the outside of the membrane. This leads to low potassium saturation inside in spite of high potassium concentration and low sodium saturation outside in spite of high sodium concentration. Thus, the unequal distribution of K and Na is maintained. Recent observations on the so-called membrane ATPase have led to the assumption that the inside reaction is a phosphorylation of the carrier performed by ATP.

A similar system has been worked out kinetically by ROSENBERG[14], differing from that of Figure 10 only in the fact that only one substrate is involved. The system was termed CZ-system, C and Z being the carrier forms with high or low affinity respectively. An important result of the kinetical treatment was that the equation for the transport rate is nearly identical with that for an equilibrating system (for instance the sugar system) except that the equivalents of Michaelis constants in the saturation terms for the two sides of the membrane are not identical as in the equilibrating system but are different. (They involve not only dissociation constants but also concentrations of reactants and concentration ratios characterizing the metabolic effect.)

The formal identity of the rate equation implies that all the kinetical characteristics derived for the equilibrating system are, in slightly modified form, to be expected in the pump system as well. As mentioned above actually numerous observations show such features in uphill transport systems.

The amino acid system is characterized by intracellular accumulation of amino acids, and, as was shown recently, by dependence of the accumulation on the presence of sodium. The interpretation given of this system by VIDAVER[20], based on suggestions made by CRANE[21] for intestinal absorption (also sodium-dependant and uphill) is a carrier with two binding sites, one for sodium and the other for the amino acid. If, then, all carrier forms have the same mobility but the affinity of amino acids to the carrier is greatly increased when the sodium site is occupied by sodium an uphill movement of amino acid into the cell ensues as long as the internal sodium concentration is lower than the external (as is the case in most cells).

These two examples, then, illustrate that carrier systems with coupling of various kinds (counter transport would be another case) allow interpretations of a variety of membrane pump actions. The carrier mechanism, thus, may be the common element of transport forms both 'active' and 'passive' according to current terminology, the transition from diffusion to carrier transport being more fundamental than from equilibrating to pumping carrier systems.

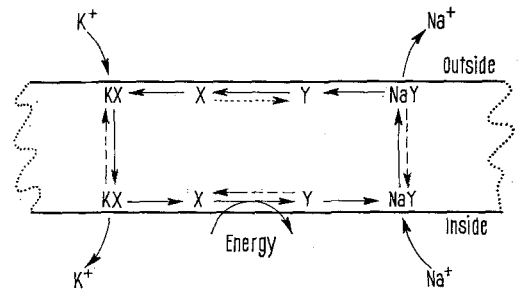

Fig. 10. SHAW's scheme for the mechanism of the sodium potassium exchange pump in the cell membrane. The unequal distribution of sodium and potassium is maintained by metabolic transformation of the carrier form X (with high affinity to potassium) into the carrier form Y (with high affinity for sodium) by reactions with metabolites inside the cell (from SHAW[18]).

Zusammenfassung. Es wird die Entwicklung, die zum Konzept des Trägermechanismus geführt hat, dargestellt. Besonderheiten dieses Transportmechanismus durch die Membran (zum Teil in Analogie zur Enzymkinetik, zum Teil abweichend) werden an Beispielen des Zuckertransports durch die Erythrozytenmembran diskutiert. Schliesslich wird der 'aktive' Transport im Sinne einer Stoffbewegung entgegen dem Aktivitätsgradienten an zwei Beispielen erläutert, die am Erythrozyten studiert wurden: an der Kalium-Natrium-Pumpe und am Aminosäuretransport durch die Membran. In bezug auf ihre Deutung im Sinne von Trägermechanismen wird darauf hingewiesen, dass der einfache Trägertransport durch verschiedene Arten von Kopplung zu einer «Pumpe» mit Aufwärtstransport umgestaltet werden kann.

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