REVIEW ARTICLE

PERMEABILITY AND TRANSPORT SYSTEMS IN LIVING CELLS*

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Active and passive Transfer

THE interest in permeability was originally more concerned with morphology than with physiology. The question was asked: What is the structure of the cell membrane? Is it a homogeneous layer as the lipid theory postulates or a porous structure with or without electrical charges? Soon, however, new problems arose. Particularly puzzling to earlier workers was the question of how foodstuffs penetrated into living cells. Using methods current at that time the surprising observation was made that the cell seemed to be impermeable to sugars and amino acids, that is to necessary foodstuffs, while appearing highly permeable for a variety of toxic substances including narcotics, alkaloids and other lipid-soluble compounds.

Overton[1] about 50 years ago suggested that sugar is transformed into a lipid-soluble compound, for instance by methylation. Höber[2] in a more general way postulated devices in the cell membrane enabling the cell to regulate the exchange of metabolites at will, a concept that he termed "physiological permeability" as opposed to "physical permeability" and which comes very close to what is called "active transport" today.

Looking back, these two views must now be considered to be prophetic. The past decades have brought not only a large number of observations on secretion across cell layers like intestinal, kidney or gastric epithelia, but have shown that secretory transport of certain substances, particularly ions, appears to be more or less a general property of living cells and that some cells make a highly specialised use of this property. As we know, for example, from the brillant work on the physiology of excitation, carried out mainly in England[3], ion transports in both directions along or against the gradients, and characteristic temporary changes of permeability with a time course of milliseconds are essential parts of the excitation recovery cycle.

In connection with these manifold observations the term "active transport" has been introduced. But the exact meaning that should be assigned to this term has not yet been agreed. When biologists interested in transports come together, they are usually quickly engaged in a discussion of this question.

There is a growing trend to call transports "active" if they occur against the gradient of chemical or of electrochemical activity, or sometimes by a shorter term "uphill". This definition doubtless has

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shortcomings but one definite advantage: it provides an experimental test by which an unequivocal decision can be reached whether a transport system falls within the definition.

The Concept of Carrier Transport

Modified views have been introduced also about the mechanisms of penetration. One of these assumes that molecules or ions may pass the membrane in the form of a complex with a membrane constituent or with a metabolite serving as a carrier; this complex is thought to be formed by special reactions on one side and broken on the other side of the membrane; thereafter the carrier molecule moves back.

This mechanism was for the first time suggested in some detail by Osterhout about 30 years ago[4]. Since that time it has been used more and more in the interpretation of various biological transports. One of the reasons is the fact that it explains a number of characteristics of transports which do not fit into the classical views. Biological transports in contrast to diffusion processes may show a limited transport capacity. In diffusion any increase of the concentration leads to a proportional increase in the rate of translocation. In many biological transports this holds for only low concentrations whereas with higher concentrations a saturation level of the rate is reached which cannot further be increased by raising the concentration. This is true for many absorption and reabsorption processes. It is for instance known to be true for the absorption of sugars and amino acids from the intestine as well as for many reabsorption processes in the kidney where the term of T_m has been introduced[5] to denote maximal transport capacity. It is also applicable to transport mechanisms in single cells like the glucose induced potassium transfer into red cells as shown by Glynn[6]. For more details see reference 7 and 8.

Another observation indicating limited transport capacity is the fact that the presence of one transported substance may inhibit the transport of a second one, in other words phenomena of competition. This also has been early observed for the absorption of sugars from the intestine. In the kidney too, many observations have shown a number of groups of substances to show competition within the same group but not with members of other groups. For details see again references 7 and 8.

In competition experiments some molecular species appear to be more powerful than others. A powerful molecule will be able to reduce markedly the transport rate of a weaker molecule but the reverse is usually untrue. Such observations point to the existence of graded affinities to the transport mechanism.

The carrier mechanism is capable of explaining such observations. If the membrane contains a certain number of carrier molecules the transport will show a limited capacity and molecules differing in their affinity for the carrier will be expected to compete for the mechanism according to their affinity. However, there are certainly other possibilities which might account for a limited capacity and for graded affinity. For instance obligatory enzymatic transformation into a membrane-soluble

molecule without reaction with a carrier would show both the phenomena of competition and of saturation. It appeared useful and necessary therefore to study the corollaries of the carrier concept in more detail in order to appraise its merits and its shortcomings. Today a considerable number of observations have been collected to provide some elements of what may be called carrier transport physiology and the beginning of carrier transport pharmacology.

Most of the experimental observations mentioned so far have been made on active transport passages across the intestinal and the kidney epithelia. It might be assumed therefore that active transports are frequently or always carrier transports and vice versa. This conclusion would certainly not be justified. The carrier mechanism is just one of a number of mechanisms of passage across a membrane. It is certainly a mechanism that may be used for active transportation, but is by no means limited to this type. On the other hand it is a pertinent question to ask whether carrier mechanisms are in some way particularly suited to active transportation and to consider the conditions in which carrier transportation becomes active. These questions will be touched upon later.

Advantages of the Red Blood Cell as a Cell Model

For the study of the elements of a carrier transport physiology a transport system which is not active will have the advantage of simplicity. An object having this advantage and in addition a number of others is the red cell. The erythrocyte is a peculiar kind of cell. Looked at under the microscope it appears as a bag filled with haemoglobin, showing only remote resemblance to a living cell. The red cell, however, is like an old man who has seen better days and who still has a number of memories of those old times. Some of these memories may be vague and if they are reproduced the performance is certainly much less dramatic than that which happens in vital cells. However, if one asks the red cell intelligent questions it gives a number of interesting answers. Parallel and independent studies on sugar transports in red cells have been made particularly by Widdas in England[9–11], LeFèvre in U.S.A.[12–14] and in our laboratory in collaboration with Rosenberg[7,8,15–17,18–28].

Sugar Transport in Red Cells

The erythrocyte (to carry on our analogy) of man and ape is the only adult mammalian red cell with vivid memories of glucose transport[29]. It takes up or releases various sugars according to existing gradients ending with equal concentrations inside and outside the cell. No active transport is observed under normal conditions. Widdas has made the interesting observation that in foetal red cells of species whose adult cells are impermeable to glucose, very effective sugar transport systems are found.

Nevertheless the red cell shows phenomena of limited capacity and graded affinity similar to those in the epithelia of intestine and kidney. The rate of entry is not proportional to the external concentration as would be expected from diffusion processes but falls off with higher concentrations. Plotting the rate against the concentration yields curves

of the saturation type[23]. Such a curve is shown in Figure 1. Limited capacity is shown by the fact that the total amount of sugar entering from a mixture of three sugars in equal concentrations falls much below the calculated sum for the individual penetration rates to be expected from

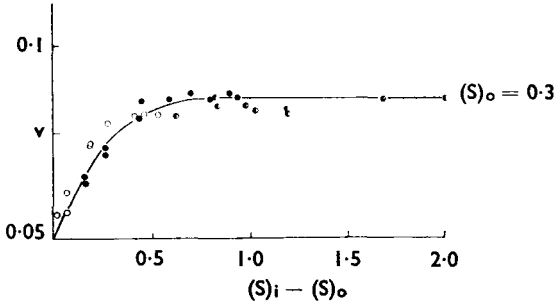


FIG. 1. Dependence of the transport rate v on the internal sugar concentration $(S)_i$ in an experiment on sugar exit from human red cells. Ordinate in units of transport rate v; abscissa in concentration difference.

a consideration of rates of independent penetration[23]. If two sugars are added successively rather than simultaneously they show graded effectiveness: the penetration of glucose in the presence of sorbose is practically unchanged whereas that of sorbose in the presence of glucose is strongly inhibited[13].

Transport Kinetics

A closer study reveals unexpected peculiarities. A striking one is the strong effect of the concentration on the side of the membrane towards which penetration proceeds and which has been termed the trans-side. Variation of the trans-concentration would be expected to affect the rate very little, if it is low compared with the cis-concentration. Actually the rate of exit of glucose from red cells depends largely on the low external concentration into which the sugar enters[8,11,14]. Plotting from such experiments the rate against the internal, that is, the cis-concentration (Fig. 2) one obtains curves of the saturation type in which the maximum rate largely depends on the trans-concentration[23].

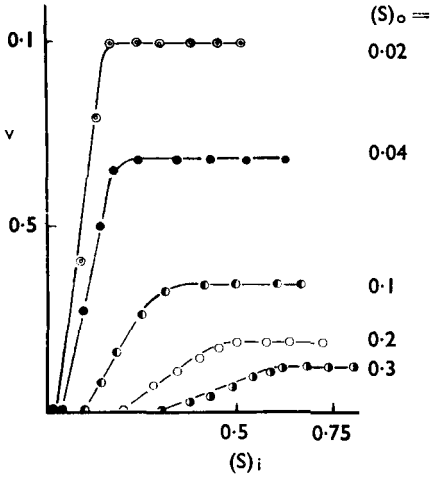


FIG. 2. Dependence of rate of transport on cis-concentration $(S)_i$ in exit experiments in human red cells for different trans-concentrations $(s)_o$. Ordinate: rate of transport, abscissa: cis-concentration $(S)_i$.

It can be shown that this behaviour is a kinetic consequence of the carrier mechanism under special conditions[11,16,21]. If the reaction between

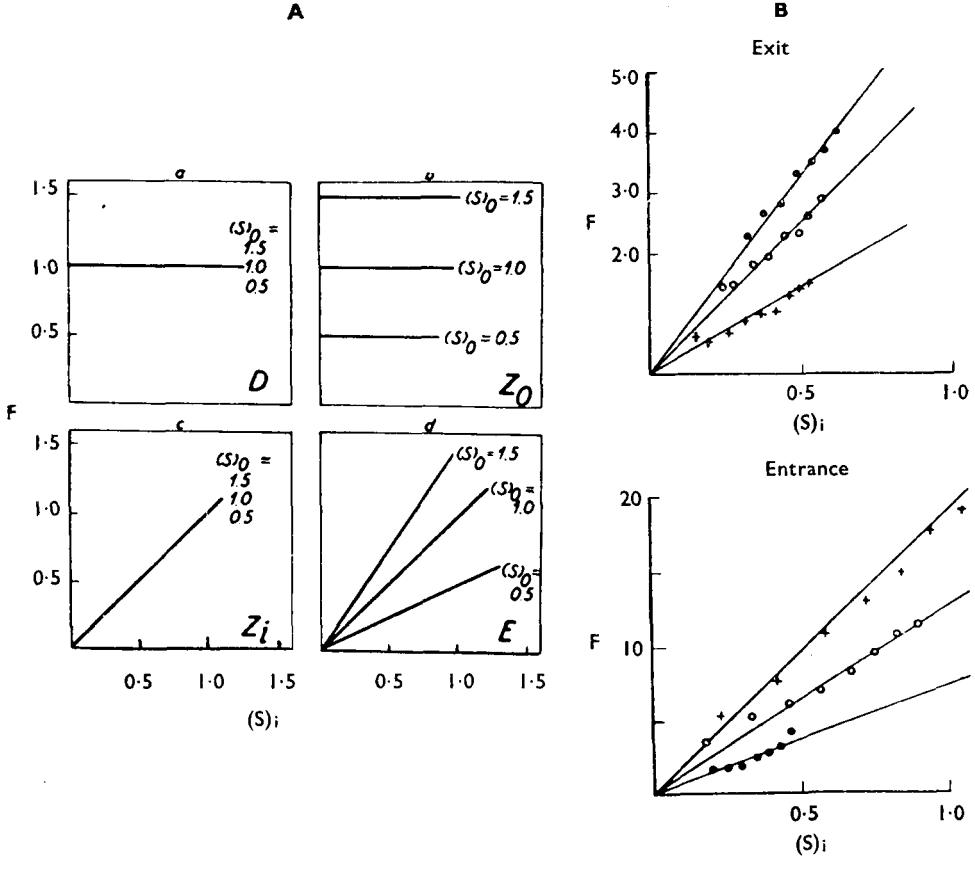


FIG. 3. F-test for E-kinetics.

A. Theoretical plots of the penetration resistance F against the internal glucose concentration $(S)_I$ for the different kinetic types, calculated. The constants K_D, Kz_o, Kz_i and K_E have arbitrarily been chosen as unity. Curves are given for $(S)_o = 0{\cdot}5$, 1·0 and 1·5.

B. Plot of the observed transport resistance F against internal glucose concentration $(S)_I$ for various values of $(S)_o$.
The plots only agree with E-kinetics.

Exit $(S)_o = 0{\cdot}3$: ●; 0·2: ○; 0·1: +.
Entrance $(S)_o = 0{\cdot}5$: ●; 1·0: ○; 1·5: +.

carrier and substrate is rapid so that equilibrium is established on both sides of the membrane the rate will be proportional to the difference between two Michaelis-Menten terms,

$$\frac{dS}{dt} = v = D'(C)_T \left\{ \frac{(S)_I}{(S)_I + K_S} - \frac{(S)_{II}}{(S)_{II} + K_S} \right\} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

(with $\frac{dS}{dt}$ = amount of substrate penetrating unit area per unit time v = transport velocity, D′ = diffusion coefficient of the complex in the membrane, divided by the thickness of the membrane, $(S)_I$ and $(S)_{II}$ = substrate concentrations on the two sides of the membrane, K_s = Michaelis constant of the carrier substrate complex).

The kinetics depend on the degree of saturation of the carrier. Far from saturation, the kinetics will be of the diffusion type:

$$v = \frac{D'(C)_T}{K_S}\left\{(S)_I - (S)_{II}\right\} \qquad (2)$$

the rate being proportional to the difference of the two substrate concentrations.

Near saturation a special type of kinetics emerges which has been termed E-kinetics and in which the rate is proportional to the difference of the reciprocals of the concentrations rather than of the concentrations themselves:

$$v = D'(C)_T K_S\left\{\frac{1}{(S)_{II}} - \frac{1}{(S)_I}\right\} \qquad (3)$$

Since under these conditions the trans-concentration will be dominating over a wide range the strong influence of this concentration now becomes understandable.

More stringent tests to see whether E-kinetics hold for glucose penetration can be performed in several ways. One is to plot the "transport resistance" ($F \equiv \frac{(S)_I - (S)_{II}}{v}$) against $(S)_I$ which for the two types of kinetics in question (D and E) should yield the results shown in Figure 3A. The plot from experimental data agrees well with the kinetics type E as shown in Figure 3B.

Another possible test is the comparison of observed penetration curves with theoretical curves obtained by integration of the equations (2) and (3) for the conditions of the penetration experiment. This can best be done by plotting a suitable function of the experimental parameters against time, which should give a straight line if the differential equation chosen is correct. The fit is good with E kinetics for glucose in higher concentrations, whereas for sorbose a similar plot for D-kinetics rather than for E-kinetics yields a good fit[11]. Since sorbose has a low affinity it is expected to be far from saturation in concentrations in which glucose is near saturation. For glucose in low concentrations apparently D-kinetics is approached, as can be seen, from a plot of "permeability constants" calculated on the basis of diffusion against sugar concentration[23]. Whereas in the range of high concentrations the "constants" are far from constant (indicating that the transfer does not follow diffusion kinetics), in the range of low concentrations the "constants" tend to actually become constant indicating an approach towards diffusion kinetics. The prediction of D-kinetics in the range of low saturation thus agrees with several observations.

Enzymic Reaction Between Carrier and Substrate

If the reaction between carrier and substrate is enzymatic the general pattern of the kinetics does not chage markedly. However, under these circumstances two additional sites of possible saturation appear in the two enzymes on the two sides of the membrane. E-kinetics then emerge under conditions under which at least two sites are near saturation, either the carriers on the two sides of the membrane or the carrier on one side of the membrane and the enzyme on the other side; but not if the two enzymes are near saturation[16].

There are a number of reasons for assuming enzymatic reactions to be involved. One is the surprisingly large structural specifity for sugar penetration. For instance under certain conditions (+)-xylose penetrates readily, where (−)-xylose does not and (−)-arabinose penetrates quickly, whereas (+)-arabinose does not[19]. This type and degree of stereospecificity is well-known in enzymatic reactions.

Another indication for enzymatic reactions is the effect of enzyme inhibitors which may block the transport partially or in many cases completely[21,23]. They include phlorizine and related substances, mercury and various mercury compounds, lachrymators, narcotics, tannic acid, formaldehyde and a number of other substances. None of these inhibitors is sufficiently specific to indicate the participation of an individual enzyme.

Since the sugar transport is not uphill no energy yielding metabolism would be expected to be required. This makes it probable that the inhibited enzymes are involved in the transport mechanism itself. Inhibitors interfering with glycolysis like iodoacetate do not inhibit glucose penetration. Shapiro's view that phlorizine inhibition of glucose reabsorption in the kidney is due to inhibition of dehydrogenases[30], would hardly be applicable to the sugar transport in the red cell.

The fact that enzyme inhibitors may block transportation completely has some bearing on the possible mechanisms involved. It seems to indicate that unless the enzymatic part of the mechanism works, the membrane is impermeable to the sugar molecule. Any role for an enzyme which is accelerating only, like a trapping mechanism maintaining steep gradients, would therefore appear to be improbable.

Affinity to the Transport System

With respect to the affinity of various sugars to the transport mechanism, an order has been established from experiments on competitive inhibition by LeFèvre. According to him the order of decreasing affinity is glucose, mannose, xylose, galactose, sorbose. The determination of numerical values for the dissociation constants of the sugar carrier meets with the difficulty that the equation for the rate of transport contains two further unknown quantities besides the dissociation constant: the total concentration of the carrier and its diffusion coefficient in the membrane. Several ways of eliminating these unknowns have been suggested. Widdas determined the constant from experiments in which glucose inhibited the penetration of sorbose[11]. Assuming that the two sugars use the same

mechanism, he arrived at a figure for the constant for glucose of about 10 mM.at 37°.

Transport Rate and Affinity

An interesting question is the relation between affinity and rate of transport. One might expect proportionality. There are, however, observations which would rather point to a reciprocal relationship. Forster and others[31] found in experiments on the transport of dyestuffs across kidney tubules that powerful competitors are slowly transported and vice versa. The same observation was made by Wiseman in experiments on amino acids transport across intestinal cells[32].

A kinetic analysis shows that the relationship to be expected depends on the saturation conditions. Under conditions of low saturation, under which D-kinetics hold, the rate becomes proportional to the affinity, that is, inversely proportional to the dissociation constant as shown by equation (2). Near saturation, however, the rate is proportional to the dissociation constant, that is, inversely proportional to the affinity as shown by equation (3). This result leads to the prediction that the order of rates for different substrates should depend on the conditions of saturation. Near saturation the transport of the substrate with the highest affinity should be the slowest, far from saturation, the fastest one.

Experiments on sugar transports in red cells bear out this prediction[22]. In high concentrations the order of increasing penetration rates is glucose, mannose, galactose, arabinose, and sorbose; in low concentrations, however, it is completely reversed: sorbose, arabinose, galactose, mannose, and glucose.

The experiments by Forster and colleagues have been carried out in a range of dyestuff concentrations in which a further increase of concentration did not affect the rate of penetration perceptibly, that is they were near saturation. Under these conditions Forster's observations agree well with those of carrier kinetics.

Lipid-soluble Sugar Complexes

If, as concluded from the effect of enzyme inhibitors, the membrane is impermeable to the unchanged substrate molecule, what type of reaction then will be suitable to change the molecule into a transport form capable of penetration? One possibility appears to be the transformation into a lipid-soluble molecule, as suggested by Overton as early as 1902.

As a model of this type of glucose complex, a neutral lipid-soluble ester, glucose benzoate, has been synthesised and tested experimentally[15]. If glucose penetrates in the form of a lipid-soluble complex the fact that some red cells are impermeable to glucose probably will not be due to their impermeability to this complex because lipid solubility is a general condition for easy penetration. More likely the difference will lie in the ability to perform the substrate carrier reaction. The lipid-soluble glucose complex then will be expected to penetrate not only human cells but also cells which are impermeable to glucose. If, furthermore, inhibitors affect the reaction between substrate and carrier they will not

be expected to inhibit the penetration of the lipid-soluble complex. Actually glucose benzoate was found to penetrate human cells as well as beef cells and all other cells that have been tested, and not to be affected by phlorizine.

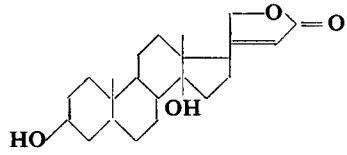
The Possible Chemical Nature of Carrier Molecules

The question of the possible chemical structure of the supposed carrier molecules is unanswered as yet, although suggestions have been made. With respect to sugar transport in red cells at present only a negative statement appears justified: it is highly improbable that the carrier is inorganic phosphate as it is assumed to be in the widely discussed phosphorylation theory of sugar absorption from the intestine and in the kidney tubules.

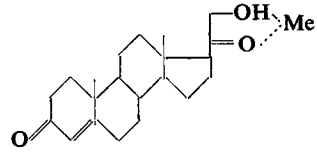
Neither glucose-6-phosphate nor glucose-1-phosphate nor other hexosephosphate esters which were tested are capable of penetrating the red cell membrane[15].

Steroids as Possible Carrier Molecules for Ions

With respect to ion transports a suggestion has been made recently that may be mentioned briefly: the possibility that corticosteroids might act as carriers for cations.



Digitoxigenin



Supposed steroid chelate

FIG. 4. Molecular structures of digitoxigenin and of a supposed chelate of desoxycorticosterone.

The role of corticosteroids in ion transports is well known particularly from the physiology of renal excretion. The chemical structure of corticosteroids contains in the side chain a configuration from which the ability of chelate formation may be expected. The comparison between a supposed steroid chelate and the genin of a cardiac glycoside (Fig. 4) reveals a certain degree of structural similarity[33]. Thus the possibility of a competitive antagonism between the two molecular species seemed worth a test. This test was first performed by Schatzmann[34] on cold-stored red cells which on rewarming in the presence of glucose perform an active transport of sodium and potassium across the membranes (another example of their "memories from old times"). Schatzmann found an

inhibition of the transport by cardiac glycosides which has since been confirmed and extended in a number of laboratories[35,36,37]. The inhibition does not appear to be related to the energy yielding metabolism since glycolysis[34] and the production of ATP[38] are unimpaired, in contrast to to the inhibition by iodoacetate and the like. Glycoside thus seems to act on the transport mechanism, possibly on the carrier.

Schatzmann in 1954[39] found no enhancing effect of corticosteroids in the absence of glycosides. On the contrary, desoxycorticosterone in very high concentrations showed some inhibition. It appeared, however, that cells of adrenalectomized animals transported slightly less actively than did normal cells.

These experiments have recently been taken up again by Sulser and Kunz. Schatzmann's observation of the lower transport activity in cells of adrenalectomised rats was confirmed and extended[40]. Taking the hematocrit reading as a measure of adrenal insufficiency and plotting transport rate against this figure a clear negative correlation was seen: the higher the degree of insufficiency the slower the transport.

With respect to the supposed antagonism between corticoids and cardiac-glycosides it appeared possible that the action of corticoids could only be observed in the presence of glycoside. Actually Sulser and Wilbrandt[33] found that desoxycorticosterone as well as other corticosteroids under certain conditions do show an effect opposite to that of cardiac glycoside but only in the presence of an inhibitory concentration of glycoside. This result would be expected if the cells were saturated with corticosteroids (a condition that may explain the ineffectiveness of steroids in other cases as well).

The assumption that cardiac glycosides compete with steroid chelates has kinetic consequences that can be tested. The competitive antagonism in this instance should not only depend on the concentration of glycoside and steroid but also on that of the ions to be chelated. The antagonistic action of glycosides should be diminished by high concentrations of these ions. This is what Glynn found experimentally for the inhibition of K influx in red cells by cardiac glycoside. Kinetical equations for this influence of cation concentrations can be derived. The result is that for a given concentration of steroid the ratio between cation concentrations with equal transport rates in the presence and the absence of cardiac glycosides should be constant. Glynn's experiments have born out this quantitative prediction in a very satisfactory way. A qualitatively similar dependence on the potassium concentration was observed by Caviezel[42] for the glycoside action on heart muscle.

One of the main sites of physiological action of corticosteroids is the kidney. In this organ a definite antagonism between cardiac glycosides and steroids was found in the adrenalectomised rat[43]. While desoxy-corticosterone induces the well-known retention of sodium, cardiac glycoside causes a considerable increase in the excretion of sodium which is antagonised by desoxycorticosterone very effectively. In normal (not adrenalectomised) animals the same type of antagonism can be demonstrated clearly after the administration of adrenocorticotrophic hormone,

ACTH. The sodium retention induced by ACTH is antagonised by intravenous injections of K-strophanthoside in a very rapid response. With high doses of strophanthoside the effect is dramatic, but transient. It can be repeated at short intervals. In heart muscle comparable antagonisms have been observed by several authors and under different conditions. Sulser and Kunz[41] have found that the potassium loss induced by cardiac glycoside in the perfused guinea pig heart is completely antagonised by aldosterone. Pöldre and Taeschler[44] described a considerable increase of the toxicity of cardiac glycosides in adrenalectomised animals, that was counteracted by corticosteroids. Similar effects have been observed by Greeff[45].

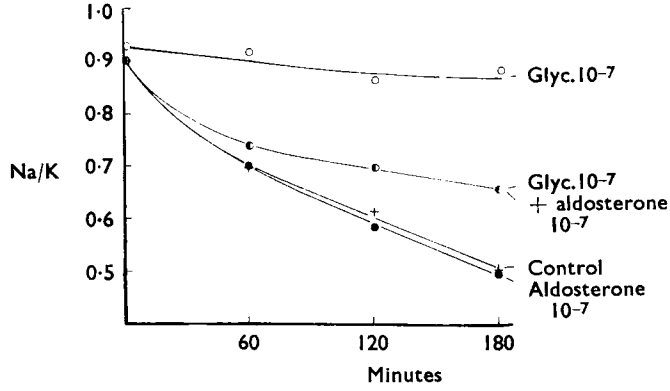


FIG. 5. Antagonism between K-strophanthoside (Glyc.) and aldosterone with respect to their influence on active transport of Na and K across the red cell membrane. Ordinate: intracellular concentration ratio Na/K of cold stored cells, incubated at 37° in the presence of glucose.
The ratio decreases due to active transport of K into and Na out of the cells. The transport is inhibited by glycoside and reactivated by aldosterone.

In summary, some experimental support for the suggestion of steroids acting as carriers in ion transports is available. Up to now, however, the evidence, while suggestive, is indirect and further experimental analysis is to be awaited.

Uphill Transfer by Counter-Transport

Returning to the general features of carrier transports the previously raised question as to which special features distinguish the uphill transport may now be taken up again. A discussion of this question leads back to the sugar transporting systems.

The sugar transport across the red cell membrane is not an uphill transport. In other cells and cell layers, particularly in the intestine and in the kidney it is or may be active. What is the essential difference?

Rosenberg[46] has given a most valuable discussion of the thermodynamics of active transports. He pointed out that an uphill transport is one special case of a movement of a thermodynamic quantity from a lower to a higher level of thermodynamic potential, and that two thermodynamic

conditions exist for such a movement. One is the simultaneous movement of another thermodynamic quantity from higher to lower level of thermodynamic potential, the second is a coupling link between the two transports. The second thermodynamic quantity may for instance be electricity moving between two levels of electrical potential, or entropy between two temperatures or again molecules moving from higher chemical potential to lower.

In the case of red cells there appear to be several sugars using the same transport mechanism which then could serve as the coupling link. Thus a first sugar moving in one direction across the cell membrane should be able to induce an active transport of a second sugar in the opposite direction. The experimental test was carried out with labelled glucose and with unlabelled mannose[17]. A concentrated cell suspension was equilibrated with labelled glucose and then mannose was added to the external solution in high concentration. A temporary rise of activity in the external solution indicated the movement of labelled glucose from the cells into the external solution against the gradient, an effect that was reversible with a time course corresponding to the penetration of mannose. Unlabelled glucose produced the same effect (Fig. 6). A similar experiment was performed by Park and others[47] using glucose and xylose.

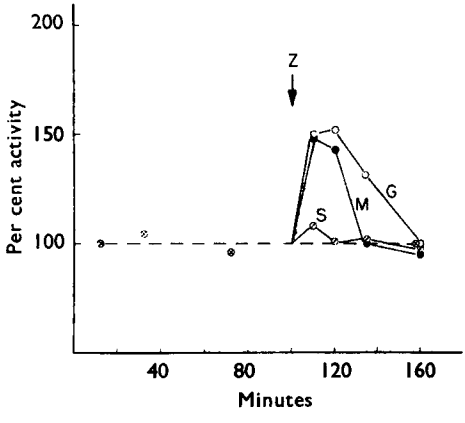


FIG. 6. Countertransport of labelled glucose out of red cells induced by a flow of mannose into the cells.
Ordinate: activity in the external medium of a suspension of red cells, equilibrated with ^{14}C-glucose. Z: addition of mannose (M) or glucose (G) or sucrose (S) in high concentration to the medium to produce a gradient into the cell. The transient rise of external activity induced by M or G indicates a transient uphill contertransport of ^{14}C-glucose from equilibrium out of the cells lasting for the time of mannose (or glucose) penetration into the cells. Correction has been made for activity changes due to dilution.

Thus movements of substrates across a common transport mechanism appears to be one possibility for the mechanism of active carrier transports. If it is a general mechanism the question may be raised what then are the conditions for active transports of high efficiency like the hydrogen ion transport in the stomach which is performed against a gradient of $1 : 10^6$? If the immediate source of energy for transports are gradients, these must be very steep indeed. This, in combination with the high turn-over numbers of some transports appears to make the participation of enzymes indispensable. The free energy of an enzymatic reaction then produces the "pressure" which is necessary to create steep gradients.

Efficiency of Uphill Carrier Transport

Another remark may be made with respect to the question raised above —whether carrier transports have any particular advantages for active transports. One such advantage would appear to be the fact that they act or may act across membranes which are impermeable to the unchanged substrate molecules. If this were not so, back diffusion of the substrate would be considerable. This would mean poor efficiency and the necessity of keeping the machine running continuously at high speed to diminish losses. Apparently this is not so in the case of active sugar transports. In the intestine[48] as well as in the kidney[49], observations have shown that if the active transport of glucose is blocked by phlorizine there is no back diffusion of glucose into the lumen as would be expected if the transport operated across a system with permeable membranes.

Flux Ratios in Carrier Transports

Finally the question of flux ratios may be touched briefly. Ussing[50] has shown that in free membrane diffusion the ratio of influx to outflux equals the ratio of the chemical activities outside to inside, or in the case of ions the ratio of the electrochemical activities. He also pointed out[51] that in cases where the substrate penetrates in combination with a carrier while the carrier cannot move uncombined (for example, from reasons of electric charge), the flux ratio will be equal to one. He introduced the term "exchange diffusion" for such a situation. Exchange diffusion has since been assumed to explain a number of observations, particularly with low flux ratio.

Such interpretations occasionally evoke the feeling that exchange diffusion is visualised as a complicating additional feature, that is, as a second mechanism operating parallel to and independent of the particular transfer in question, be the latter free diffusion or active transport. It seems worth pointing out that in carrier transports of the type discussed here any value of flux ratios between one and the activity ratio may be expected depending on the degree of saturation of the carrier. With very low degrees of saturation the flux ratio will approach the activity ratio, near saturation it will approach unity. Thus it appears quite unnecessary to invoke exchange diffusion as an additional "revolving door" in cases where unexpected values are found for the flux ratios, as long as they are within the range between unity and the activity ratio.

A consideration of the kinetics and the flux ratios to be expected in simple carrier systems might in some systems be helpful.

Conclusion

In summary the concept of a carrier transport appears to have a number of quantitative consequences which have been tested experimentally with positive results. The question of course can be raised whether or not other interpretations may be found to provide an equally good quantitative fit for some of these features. There are certainly other mechanisms that will show similar transport kinetics. E-kinetics will be observed in all systems with a linear relation between rate of transport and the difference

of two Michaelis-Menten terms. They include for instance under certain conditions, systems with two adsorption layers on the two sides of the membrane. Such an "adsorption membrane", however, would not be able to be used for uphill transports induced by counterflow, because this mechanism requires a coupling link with a mobile element as can be shown thermodynamically as well as kinetically[17]. A mechanism which does contain a mobile element is the rotating molecule discussed by Danielli[52].

With respect to alternative mechanisms, however, another remark may be pertinent. What appears to favour the carrier concept is not so much its exclusiveness as, rather the fact that it works with elements none of which requires essentially new assumptions[7]. It is well known that enzymes may be located on the surface of the membrane both within and without the cell. It is equally well known that substrate complexes move from one enzyme protein to the other and longest of all it has been known that lipid-soluble molecules move across cell membranes. Thus, one might be led to wonder how carrier transports could be avoided rather than whether they are probable. Nevertheless it should clearly be kept in mind that although the carrier concept may be a useful hypothesis, still it is but a hypothesis.

References

1. Overton, *Arch. ges. Physiol.*, 1902, **92**, 115.
2. Höber, *Physikalische Chemie der Zellen und der Gewebe*, Leipzig, 1911, p. **264**.
3. Hodgkin, *Biol. Rev.*, 1951, **26**, 339.
4. Osterhout, *Erg. Physiol.*, 1933, **35**, 967.
5. Smith, *The Kidney*, O.U.P., New York, 1951.
6. Glynn, *J. Physiol.*, 1957, **136**, 148.
7. Rosenberg and Wilbrandt, *Int. Rev. Cytology*, 1952, **1**, 65.
8. Wilbrandt, *Symp. Soc. exp. Biol.*, 1954, **8**, 136.
9. Widdas, *J. Physiol.*, 1952, **118**, 23.
10. Widdas, *ibid.*, 1953, **120**, 23P.
11. Widdas, *ibid.*, 1954, **125**, 163.
12. LeFèvre, *J. gen. Physiol.*, 1948, **31**, 505.
13. LeFèvre and Davies, *ibid.*, 1951, **34**, 515.
14. LeFèvre, *Symp. Soc. exp. Biol.*, 1954, **8**, 118.
15. Rosenberg, Vestergaard-Bogind and Wilbrandt, *Helv. physiol. acta*, 1956, **14**, 334.
16. Rosenberg and Wilbrandt, *Exp. Cell. Res.*, 1955, **9**, 49.
17. Rosenberg and Wilbrandt, *J. gen. Physiol.*, 1957, **41**, 289.
18. Wilbrandt, Guensberg and Lauener, *Helv. physiol. acta*, 1947, **5**, C20.
19. Wilbrandt, *ibid.*, 1947, **5**, C64.
20. Wilbrandt, *Arch. exp. Path. Pharm.*, 1950, **212**, 9.
21. Wilbrandt and Rosenberg, *Helv. physiol. acta*, 1950, **8**, C82.
22. Wilbrandt, *J. cell. comp. Physiol.*, 1956, **47**, 137.
23. Wilbrandt, *Dtsch. med. Wschr.*, 1947, **92**, 1153.
24. Wilbrandt, Unpublished results.
25. Wilbrandt, Frei and Rosenberg, *Exp. Cell. Res.*, 1956, **11**, 59.
26. Wilbrandt and Rosenberg, *Helv. physiol. acta*, 1951, **9**, C86.
27. Wilbrandt, *ibid.*, 1958, **16**, 31.
28. Wilbrandt, *Geigy Kolloquium.* "Ueber den Kohlehydratstoffwechsel im Kindesalter". To appear shortly in *Acta Paediatrica*
29. Kozawa, *Biochem. Z.*, 1914, **60**, 231.
30. Shapiro, *Biochem. J.*, 1947, **41**, 151.
31. Forster, Sperber and Taggart, *J. cell. comp. Physiol.*, 1954, **44**, 1.
32. Wiseman, *XXth Internat. Physiol. Congr.*, 1956.
33. Sulser and Wilbrandt, *Helv. physiol. acta*, 1957, **15**, C37.
34. Schatzmann, *ibid.*, 1953, **11**, 346.

35. Glynn, *J. Physiol.*, 1954, **126**, 35P.
36. Joyce and Weatherall, *ibid.*, 1955, **127**, 33P.
37. Kahn and Acheson, *J. Pharmacol.*, 1955, **115**, 305.
38. Kunz and Sulser, *Experientia*, 1957, **13**, 365.
39. Schatzmann, *ibid.*, 1954, **10**, 189.
40. Kunz and Sulser, *ibid.*, 1958, **14**, 278.
41. Sulser and Kunz, Unpublished results.
42. Caviezel and Wilbrandt, *Helv. physiol. acta*, 1958, **16**, 12.
43. Gantenbein, Sulser and Wilbrandt, *ibid.*, 1957, **15**, C64.
44. Pöldre and Taeschler, *ibid.*, 1956, **14**, C37.
45. Greeff, *Arch. exp. Path. Pharm.*, 1957, **231**, 391.
46. Rosenberg, *Acta chem. scand.*, 1948, **2**, 14.
47. Park, Post, Kalman, Wright, jr., Johnson and Morgan, *Ciba Colloquia Endocrin.*, 1956, **9**, 240.
48. Rummel, W., Personal communication.
49. Höber, *Pflügers Arch.*, 1933, **233**, 181.
50. Ussing, *Acta physiol. scand.*, 1949, **19**, 43.
51. Ussing, *Nature, Lond.*, 1947, **160**, 262.
52. Danielli, *Symp. Soc. exp. Biol.*, 1954, **8**, 502.