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Sodium-Hexose Interactions

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Sodium–hexose interactions

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Hexose and sodium can interact in the columnar epithelial cells of the intestine at various levels (*a*) in changing availability of energy, (*b*) at transport sites, (*c*) at the coupling mechanism between metabolism and transport or the coupling between Na transport and hexose transport. Glucose differs from other hexoses in being both transported and metabolized, and interactions with Na and glucose are therefore likely to be more complex than with other hexoses. The most interesting aspect of Na–hexose interaction is probably the mechanism of the coupling between Na and hexose transport. The Na gradient has been and remains still an attractive hypothesis, but other possibilities must be considered including the role of Na-sensitive ATPases.

The subject of this Symposium is the movement of salt and water in living tissues. The most important ion in this context is probably sodium, and this paper is a discussion of some ways in which Na and hexoses can interact with each other. The problem is complicated by the fact that such interaction can differ in different tissues, and it is difficult to generalize. The tissues which have been most studied in this respect are the erythrocyte, the intestine and the kidney. Only the intestine will be discussed here, but as it has highly specific mechanisms for hexose transfer and in addition has a high capacity for Na transfer, it is likely that in dealing with the intestine the main problems of Na–hexose interaction will be touched upon.

In looking at this problem the following requirements of a transport system should be remembered: (1) specific sites to which the transported substances can attach themselves; (2) availability of energy for transfer which depends ultimately on metabolism of the cell; (3) some method of coupling metabolism to transport. In this connexion it is useful to make a distinction between primary active transport and secondary active transport (Smyth 1969). Primary active transfer is the transfer of any substance which is directly coupled to metabolism, while secondary active transfer implies that the transfer of one substance is coupled to the transfer of another, which in turn is coupled to metabolism. Of the various substances which have claims to be regarded as being involved in primary active transfer Na has some priority. In contrast, hexose movement, according to some current theories, could be regarded as secondary active transfer, and this is immediately relevant to the topic under discussion as the hexose movement may be secondary to a primary transfer of Na. In discussing the interactions of hexose and Na it is useful to think of these in terms of the three requisites of a transport system defined above, i.e. specific sites, availability of energy, and coupling between metabolism and transport or between transport of one substance and that of another.

Availability of energy

Hexoses and Na can interact by either of them increasing or decreasing the energy available for the transport of the other, and indeed also the energy available for themselves. Newey &

Smyth (1964) put forward the concept of substances participating in transport or metabolic activities in cells being either users or suppliers of energy, and this has been further developed by Smyth (1970). This also involves the idea that in the columnar epithelial cell, the amount of

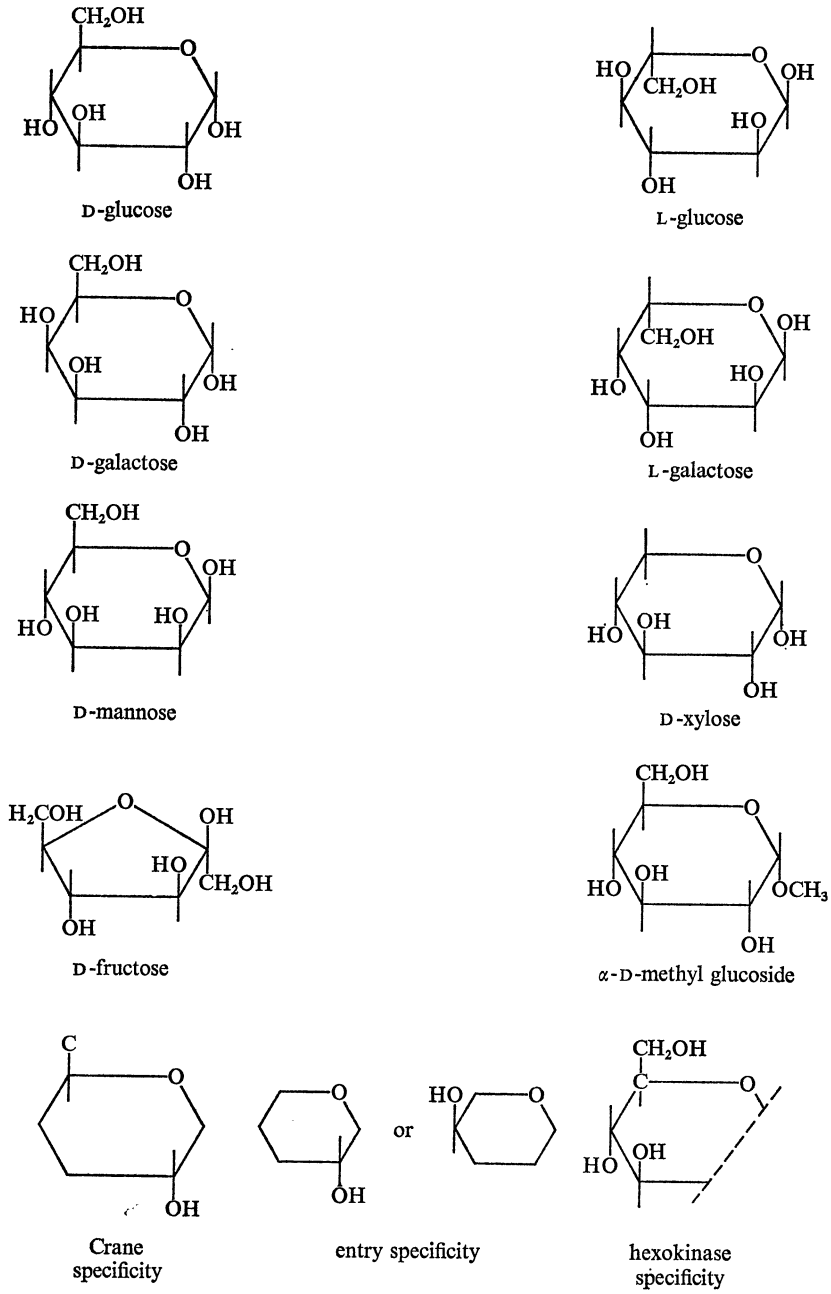

FIGURE 1. The structure of some hexoses and the specificities of transport and metabolism. Of the hexoses shown here the Crane specificity includes D-glucose, D-galactose and α-D-methyl glucoside; the entry specificity includes the above sugars together with L-glucose and D-xylose; the hexokinase specificity includes D-glucose, D-mannose and D-fructose.

energy available for transport processes is not sufficient to enable all of these to operate at maximum capacity, and there may be competition by different systems for the available energy. Substances which are transferred, and these include Na and some hexoses, are users of energy.

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For this reason they make less energy available for other transport systems and may cause reduction in the activity of these. Suppliers of energy are substances which make more energy available, and these may be expected to stimulate transfer systems. Some hexoses fall into this class because they are metabolized, and Na may also fall into this class, as it may control availability of energy through Na-sensitive ATPases.

The classification of hexoses as users or suppliers of energy can be made on chemical structure. Figure 1 shows some hexose structures and some basic configurations related to transport and metabolism. The first, called for convenience the 'Crane' specificity, as it was first defined by Crane in 1960, is present in hexoses which are moved against a concentration gradient in *in vitro* intestinal preparations. Substances with this specificity are therefore energy users. They include D-glucose, D-galactose, α-methyl glucoside and 3-*O*-methyl glucose. It now appears that other substances without this specificity may use at least the entry stage of the hexose transfer mechanisms; e.g. L-glucose, which can be moved against a concentration gradient in certain conditions (Neale & Wiseman 1968) and the pentose D-xylose which can use the glucose entry mechanism (Alvarado 1967). It would seem that the C attached to C5 is not an absolute requirement for using the carrier. As regards L-glucose Caspary & Crane (1968) pointed out that OH at C4 in L-glucose will produce a configuration similar to the OH at C2 in D-glucose. This specificity can best be expressed in the terminology of Cahn, Ingold & Prelog (1966) as a tetrahydro-pyran with an OH at C3 in the R configuration. In this terminology with the numbering of the ring starting at oxygen, then OH at C3 in the R configuration includes both the configuration at C2 in D-glucose and at C4 in L-glucose. This configuration can be called the 'entry' specificity, and although it includes the Crane specificity it still seemed useful to recognize this in addition. L-glucose and D-xylose are transferred in smaller quantities than glucose or galactose, they do not give a significant electric potential, and it would seem that the transfer is less 'active' than that of substances with the Crane specificity. We are certainly on safer ground if we regard as energy users substances with the Crane specificity, rather than those with the entry specificity, although we would not say that the latter are not energy users. The third specificity shown in figure 1 is the hexokinase specificity and it will be evident that this includes D-glucose, D-mannose and D-fructose. All of these substances are metabolized and are energy suppliers.

Figure 2 is a diagrammatical representation of the location of two of these specificities in the columnar cell. Substances with the Crane specificity, if present in the luminal or mucosal fluid, can enter the cell and be transported against a concentration gradient. Substances with the hexokinase specificity can be metabolized provided they can enter the cell. All sugars appear to be able to enter the cell from the serosal side, and hence mannose can be metabolized if present in the serosal fluid but not if present in the mucosal fluid. Fructose is a rather anomalous sugar in this respect. It does not possess the Crane specificity and cannot be transported against a concentration gradient. However, if present in the mucosal fluid it can enter the cell (presumably by a different route from that used by glucose and galactose) and be metabolized.

It will be evident from the above considerations that glucose is probably not the best hexose to use for studying Na-hexose reactions, and some of the difficulties can be avoided by using mannose, which in rat intestine is metabolized but not transferred, and galactose which is transferred but not metabolized.

As energy users hexoses and Na take part in transport processes, and it is convenient to describe these as hexose pumps and Na pumps respectively. The plural is used advisedly, as there may be more than one. It is useful to begin by looking at these pumps in the columnar cell.

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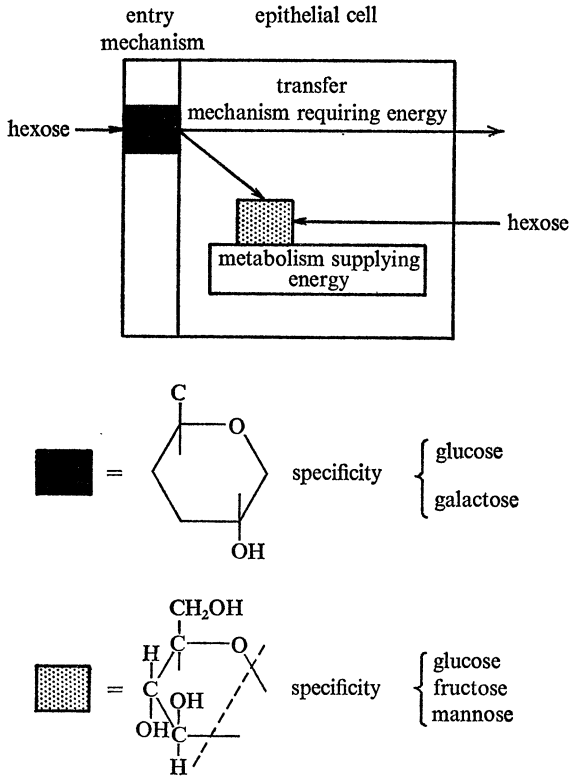

FIGURE 2. Location of two hexose specificities in the columnar epithelial cell related to transport and metabolism. Hexoses with the hexokinase specificity may be metabolized if present initially in the serosal fluid, and those with both specificities may be metabolized if present initially in the mucosal fluid. This means that the only hexose metabolized under all conditions is D-glucose. (From Smyth, D. H. 1970).

HEXOSE PUMPS

The hexose pump is a term we will use for the whole mechanism of hexose transfer, without defining its nature, and the basic evidence for its existence is that certain hexoses; i.e. those with the Crane specificity, can be moved against a concentration gradient. The intestinal hexose pump was shown by Barry, Dikstein, Matthews, & Smyth (1960) and Barry, Matthews, Smyth & Wright (1961) to be associated with a phlorhidzin-sensitive electrical potential. Phlorhidzin prevents entry of glucose into the cell from the mucosal side but not from the serosal side (Parsons, Smyth & Taylor 1958; Newey, Parsons & Smyth 1959) and its effect on the hexose potential is therefore due to stopping the entry of hexose into the cell. Barry *et al.* (1964) elaborated on this, but did not postulate any specific mechanism for the potential. They said it involved Na but could also involve other cations moving in the same direction as the hexose, or anions moving in the opposite direction. It also seems likely from the work of Barry *et al.* (1964) and Detheridge, Matthews & Smyth (1966) that the energy for hexose transfer and associated potential can come either from the endogenous metabolism of the cell by the citric acid cycle or from the glycolysis of the hexose which is being transferred or of some other metabolizable hexose present. These facts are shown in figure 3. This figure does not attempt to show the mechanism of the pump as this must still be regarded as a matter of some uncertainty.

The first attempt in what could be called the modern era of transport to describe a mechanism for the hexose pump was that of Crane and his co-workers (Crane, Miller & Bihler 1961), who placed a pump at the brush border which removed Na from the cell and transferred it to the intestinal lumen. As a result a gradient of Na across the cell membrane was caused. This gradient could be utilized by a ternary carrier, on which both Na and hexose could move, and in this way the Na gradient provided the energy for hexose movement. Schultz & Zalusky (1964) moved

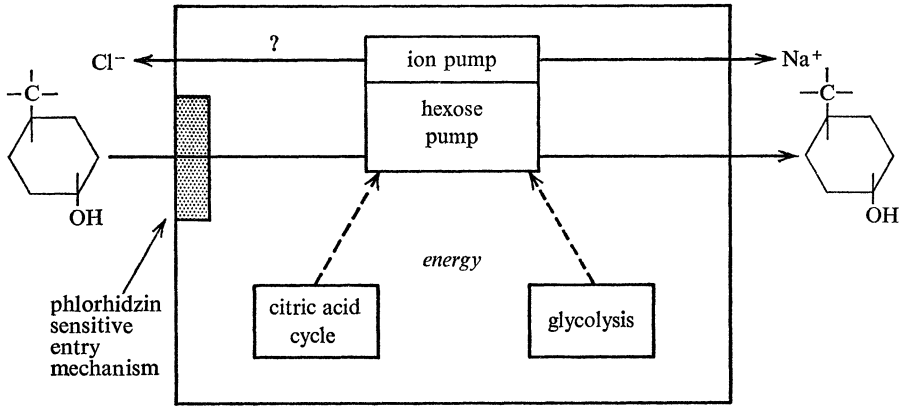

FIGURE 3. The hexose pump in the columnar epithelial cell. The diagram shows that in addition to movement of hexoses with the Crane specificity there is a movement of ions, which includes sodium towards the serosal side but may also include anions (e.g. chloride) towards the mucosal side. The diagram is meant to indicate the sources of energy for the mechanism as a whole and not to suggest any particular relationship between the ion pump and the hexose pump.

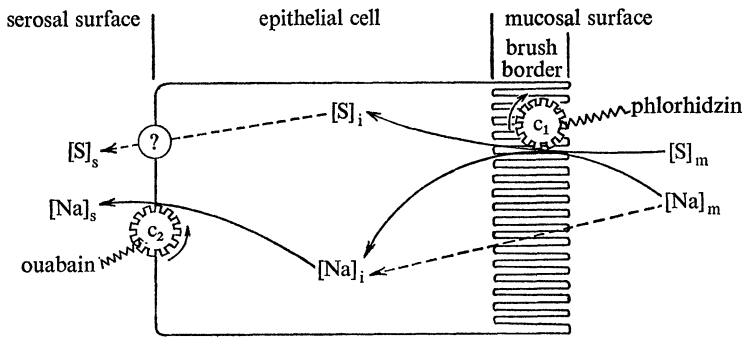

FIGURE 4. The Schultz–Zalusky model for coupling of hexose and Na movement. (From Schultz & Zalusky 1964.)

the Na pump to the other side of the cell, and made it also responsible for the phlorhidzin-sensitive electric potential described by Barry *et al.* (1960). Their concept is shown in figure 4. Schultz & Zalusky (1965) extended this mechanism to amino acids, and it still remains the most popular model to explain the relation between Na and non-electrolytes. It can be referred to as the Na gradient hypothesis. If we accept this scheme Na movement is primary active transfer and hexose movement secondary active transfer. This scheme has the great attraction that it offers one primary active transfer system (i.e. the Na pump), to which other transfer systems, e.g. hexose, amino acid, fluid, etc., could be coupled, a problem discussed by Smyth (1969). Another view of the hexose pump was put forward by Czaki (1963), who believed that the part

played by Na in hexose transfer was the activation of ATPases in the brush border. Since the problem is not yet resolved, it is useful to retain the term hexose pump to include the whole process of hexose movement with the associated ion movement and electric potential.

Sodium pumps

At least four Na pumps have been postulated in the intestine which, according to the terminology of Barry, Eggenton & Smyth (1969), can be designated as follows: (1) an endogenous Na pump, (2) an electrogenic Na pump related to hexose transfer, (3) a non-electrogenic Na pump related to hexose metabolism, (4) a non-electrogenic pump related to galactose transfer.

The endogenous sodium pump

This is the mechanism which, *in vitro* in the absence of added hexose, causes Na transfer and may be responsible for the small potential 1 to 2 mV, observed in these conditions. As it is not related to hexoses it is outside the scope of this discussion. It may, however, be the same mechanism referred to below as the non-electrogenic Na pump; as it is possible that the same Na transfer mechanism may be operated by the endogenous metabolism of the cell or by the metabolism of added hexose.

The electrogenic sodium pump

This is an integral part of the hexose transfer mechanism as shown by Barry *et al.* (1960, 1961, 1964, 1965). Its causation may be a Na pump at the serosal side of the cell, which links Na and hexose movement through the ternary carrier (Schultz & Zalusky 1964). It is based on the finding that Na movement and an electric potential are both associated with hexose transfer. The relation between Na movement and electrical activity were studied by means of the short circuit current with some diversity of results. According to the Sheffield Group (Barry, Smyth & Wright 1963; Barry *et al.* 1965) the relation between Na transfer and short circuit current is variable. With glucose the two were roughly equivalent, a result which was considered to be fortuitous, with galactose the short circuit current was greater than the net Na transfer while with fructose the Na transfer was greater than the short circuit current. In contrast Schultz & Zalusky (1964) found that net Na transfer was equivalent to short circuit current, and they even considered that the short circuit current could be used as a measure of Na transfer. Later Taylor, Wright, Schultz & Curran (1968) obtained results confirming those of the Sheffield group, as they found a short circuit current greater than net Na transfer in the presence of galactose. The problem was also studied by measuring both hexose movement and potential when Na in the fluid in contact with the epithelium was replaced with other substances (Clarkson & Rothstein 1960; Bosakova & Crane, 1965; Barry, Eggenton, Smyth & Wright 1967). Replacement of Na with K or Li has a much greater effect on hexose transfer and/or potential, than when mannitol or Tris was used. Barry *et al.* (1967), however, found that there was not a strict relationship between the magnitude of the potential and the amount of hexose transferred. Barry, Smyth & Ude (1969) also studied the effect of applying a potential to the intestine, and found that while this affected net Na transfer, it had little effect on galactose movement. The location of this Na pump at the serosal side of the cell was postulated by Schultz & Zalusky (1964) and support for this came from the finding that the electrical potential is produced at the serosal membrane (Gilles-Baillien & Schoffeniels 1965; Wright 1966) in the tortoise intestine. Recent findings by White & Armstrong (1970) with the bull-frog, and Rose & Schultz (1970) with the rabbit, suggest that in other species the location of the Na pump may be different.

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The non-electrogenic sodium pump

This was postulated by Barry *et al.* (1965) to explain the large increase in Na movement which occurred in the presence of metabolized hexoses. Since both glucose and mannose are metabolized in rat intestine, they both operate this pump, but experiments with glucose are confusing because in addition glucose also operates the electrogenic pump. It is therefore convenient to use galactose and mannose, to distinguish between the electrogenic pump and the non-electrogenic pump. In this way Barry *et al.* (1969) showed that in the presence of mannose a large amount of Na transfer takes place but no phlorrhizin sensitive electric potential is produced The small potential produced by mannose is probably an osmotically induced potential of the kind described by Smyth & Wright (1966). We cannot be certain that all the potential has an osmotic origin and for this reason it has been suggested above that the non-electrogenic Na pump may be the same as the endogenous Na pump. The non-electrogenic Na pump associated with hexose metabolism is probably not a specific interaction between Na and metabolized hexose, but is simply due to more energy becoming available for transfer, since hexose metabolism also increases transfer of amino acids (Dawson, Newey & Smyth 1965; Bingham, Newey & Smyth 1966), galactose (Newey, Sanford & Smyth 1966) and lower fatty acids (Barry, Jackson & Smyth, 1966).

The galactose sodium pump

A Na pump associated with galactose transfer was postulated by Taylor *et al.* (1968). This pump is specific for galactose and moves Na in the opposite direction to galactose, and therefore in the opposite direction to the non-electrogenic Na pump described by Barry *et al.* (1965). It resembles this, however, in being non-electrogenic, and some anion, possibly chloride, is moved in the same direction as the Na. This pump was postulated to explain the fact, observed by Barry *et al.* (1965) and confirmed by Taylor *et al.* (1968), that Na transfer is much greater in the presence of glucose than of galactose. The two explanations rest on quite different concepts of Na pumps in the intestine and their relation to hexoses. According to the Sheffield view the Na transferred in the presence of glucose consists of three fractions, that due to the endogenous Na pump, that due to the electrogenic pump associated with hexose transfer and that due to the non-electrogenic pump associated with hexose metabolism. In the presence of galactose not only is the latter absent, but the endogenous pump may be reduced due to the competition for the energy required for galactose transfer. According to the other view (which for convenience may be called the Schultz view) the total Na movement to the serosal side is associated with the transfer of hexoses, i.e. either glucose or galactose, but in addition galactose operates a Na pump in the opposite direction. Schultz believes very firmly that Na movement is completely unrelated to the metabolic fate of hexose, a view which does not seem to take into consideration the effect of mannose on Na movement. One point of interest about this galactose pump is that it is operated by an energy user, which according to the Sheffield view would require energy rather than supply it.

Sodium–hexose relation at carrier level

There are two aspects of this relationship of great interest, i.e. the Na gradient and the differential affinity theory, both developed by Crane and his colleagues. The former had already been referred to. The latter (Crane, Forstner & Eichholz 1965) is a further development of the ternary carrier and implies that on account of the different concentrations of Na on the

two sides of the membrane the affinity of the carrier for hexose is changed and this explains how hexose can be transferred against a concentration gradient. This change in affinity is an allosteric effect due to the occupation of an adjacent transport site by Na.

This process has the same basic mechanism as facilitated diffusion, but this term is no longer applicable as by long usage facilitated diffusion implies carrier-mediated movement down a concentration gradient. The case under discussion is carrier-mediated movement against a concentration gradient, the energy required coming from the unequal concentration of sodium on the two sides of the membrane. It is suggested that the term energized facilitated diffusion might be used to describe this process. One difficulty about this theory is that the experimental demonstration of the effect of Na on affinity for hexose depends on Michaelis–Menten kinetics, and yet the kinetic system in which the differential affinity would operate is energized facilitated diffusion. The system could function only if the affinity inside the cell was infinitely low, when energized facilitated diffusion would be identical with Michaelis–Menten kinetics. A further extension of allosteric effects has been introduced by Alvarado (1966), who believes that hexoses, Na and amino acids all react with the same carrier.

Coupling between energy and transport

One of the central questions of non-electrolyte transfer is how it is coupled to cell metabolism. There have been till recently two main views: (1) the Na gradient and differential affinity discussed above, and (2) the view of Czaki (1963) that hexose movement is not related to a Na gradient, but the action of Na is on Na-sensitive ATPases. A new model has recently been put forward by Kimmich (1970). This makes use of the scheme suggested for ion transport in red cells involving a membrane ATPase (for references see Skou (1965), and Glynn (1968). Membrane bound $[Na^+ + K^+]$ activated ATPase can form a phosphorylated intermediate ($E \sim P$) by a reaction involving Mg, while high levels of Mg lead to a second intermediate $E_2 \sim P$. The hydrolysis of $E_2 \sim P$ takes place in the presence of K^+, and the energy of hydrolysis is used for ion transfer. Kimmich suggests that the energy for hydrolysis of this reaction can also provide energy for transfer of non-electrolytes, e.g. sugars or amino acids. This model is claimed by Kimmich to fit in with the need for Na, and the effects of ouabain and oligomycin on transfer of non-electrolytes. In this scheme different transfer systems can compete for the available energy, and this is fundamentally similar to the concept suggested by Newey & Smyth (1964), although these workers suggested that the common source of energy might be ATP.

In this paper Kimmich (1970) reviews the evidence for the Na gradient theory, and considers his results are incompatible with this. His views certainly make a re-evaluation of the coupling of Na and non-electrolytes essential. At this point, it is difficult not to transgress on the field of Na–amino acid interactions as Kimmich's model applies also to amino acids. It is worth noting that Newey, Rampone & Smyth (1970) have recently studied the relation of both external and internal Na in relation to methionine transfer and have produced evidence that the Na gradient is not essential for methionine entry but the intracellular Na concentration is. Against this should be weighed the very substantial evidence for the Na gradient offered by Eddy (1968) and by Curran, Hajjar & Glynn (1970).

The stoichiometry of hexose and Na movement deserves some comment. It would seem that the Na gradient theory requires a fairly tight stoichiometry if Na and hexose and amino acids move on the same carrier, and Schultz & Zalusky (1964) suggested a 1:1 ratio for Na and glucose.

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Evidence for a fixed stoichiometry has also been obtained by Goldner, Schultz & Curran (1969). Other results suggest a variable stoichiometry (see, for example, Crane *et al.* 1965). Kimmich (1970) points out that his system involves no fixed stoichiometry for Na and amino acid or Na and hexoses, as hexoses and amino acids transport systems could compete for the same source of energy. The system which least requires any fixed stoichiometry is that of Barry *et al.* (1965) because in this system part of the movement of Na is related to metabolism of the glucose entering the cell. The possibility of the absorbed glucose supplying energy for transport does not enter into any of the other schemes. Energy from absorbed glucose certainly plays a role *in vitro*, and probably plays a role *in vivo* also, as Atkinson, Parsons & Smyth (1957) showed that part of the glucose absorbed is metabolized in the intestine.

The scheme put forward by Kimmich (1970) explains the action of ouabain on transport systems as interfering with the available energy and points out the remarkable similarity between the hexose and amino acid transfer systems. In this respect the observation of Newey *et al.* (1968) is of interest that ouabain inhibited amino acid transfer at a concentration which did not affect galactose transfer. It also inhibited mannose stimulation of galactose transfer more easily than endogenous galactose transfer. Several possible explanations were suggested. According to the differential affinity theory the intracellular Na level need not affect the affinities of the carrier for amino acid and hexose equally. Another possibility was a different location of some part of amino acid and hexose transfer mechanism, providing a different accessibility to ouabain. A third possibility is that if two mechanisms are competing for the same source of energy one may be more favourably placed in the cell in relation to this. Yet another possibility is that ouabain affects hexose metabolism at a different concentration from that needed to affect endogenous metabolism. It certainly does not seem possible to ascribe all the effects of ouabain to an action on a Na pump located at the serosal border of the cell.

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