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Mechanisms in intestinal transfer[1]

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When a physiologist speaks at a meeting of more clinical interest, he is sometimes expected to provide the 'fundamentals' of the subject on which the clinical phenomena are based. This expectation is based on a dangerous fallacy. Our approaches to problems vary in being made at different levels of biological organization, but none of these is more fundamental than another. Study of biological processes at the molecular level can often be made in more precise terms than study of the human body as a whole. Both, however, are equally important and mutually complementary. A knowledge of human behaviour deduced from irreversible thermodynamics or *x*-ray crystallography would be a very incomplete basis for medical practice. Physiology originally grew out of clinical observation, and is still stimulated and benefited by contact with clinical problems. For that reason I am glad to be here today to learn something about the more clinical aspects of the intestine. My assignment is to talk to you for twenty minutes on mechanisms of intestinal absorption. Instead of regarding this as the 'fundamentals' of absorption, I would prefer to offer you some generalizations which could be made about the absorptive processes in the intestine, and as it is a rather big subject for such a short time there must necessarily be a certain amount of oversimplification.

One basic problem of all living tissues is how the cells obtain and use energy for the work which they do. The energy is made available as the result of metabolic activity. In the case of the intestine the work is transfer of nutritional substances, and the cell is acting as a transducer to convert metabolic energy into osmotic work. The researches of the past 20 years have revealed that a very large number of substances are transferred by the intestinal epithelial cell and by some kind of special mechanism, often involving what is loosely called 'active transport'. This is sometimes defined as movement of a substance against its electrochemical potential, and for non-electrolytes like hexoses it means movement against a concentration gradient. In biological conditions, however, the transfer of a single substance in isolation rarely if ever occurs. There are nearly always a number of components in the system involved in transfer activities and for multicomponent systems the definition of active transfer as movement against a concentration gradient is not satisfactory.

In analysing transfer processes we attempt to describe the processes in physico-chemical terms, eg, diffusion gradients, electrical forces, etc. A discussion of the forces has recently been given by Newey and Smyth (1969). Since the intestine participates actively in the transfer of a large number of substances the interesting question arises, whether we have to discuss the details of a large number of different processes in the intestine or is it possible to show that there are one or two basic mechanisms. This introduces the concept of coupling. By this we mean that movement of one substance can be coupled to movement of another, and in such a coupling we distinguish between the active component and the passive component. The passive component may appear to move 'uphill', ie, against a concentration gradient, but it only does so because of its coupling to a powered component, ie, one which has its own energy source. Thus, in a complex multicomponent system like the alimentary tract, where a number of substances are being transferred simultaneously, three useful questions can be asked. (1) Is it possible to explain the working of the system on the basis of an active process for one component only with others coupled to it? (2) If so, which of the components involves active transfer? (3) How is the movement

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of the other components coupled to the movement of the active one?

It is convenient to begin with the last question and look at the possible types of coupling between different components in a system, and, expressed in very general terms, these are shown in Table I.

1	Movement of solute coupled to movement of solvent.
2	Movement of solvent coupled to movement of solute.
3	Movement of one solute coupled to movement of another solute.
4	Movement of solute coupled to metabolism.

Table I *Types of coupling which may occur in multicomponent transfer systems*

Coupling of Solute and Solvent

The first of these, solute movement coupled with solvent movement, means that a stream of fluid carries with it substances in solution. Fluid movement is the active process, and solute movement the passive. This process is called 'solvent drag', and probably does not play a major part in absorption from the intestine although it may play some role. The second form of coupling is when the solute transfer is the active process and solvent moves passively with it. This process can be called 'solute drag', and plays an important part in intestinal activity. Imagine a system shown in Fig. 1a where there are two solutions separated by a biological membrane. To one compartment (Fig. 1b) is added a solute to which the membrane is not permeable. The result is an increase in osmotic pressure on one side and this will cause movement of fluid towards that side. Suppose, instead of the substance being added by some outside agency, the membrane itself is able to remove solute from one side and transfer it to the other (Fig. 1c). This will likewise cause a difference in osmotic pressure, as a result of which fluid will move in the same direction as the solute moves. In both cases an osmotic gradient has been created and as a result fluid has moved. But there is a very important difference in these. In the first place the osmotic gradient was created by the addition of solute from outside, ie, the energy for the process was supplied by forces entirely outside the membrane. In the second case the energy for movement is derived from the membrane itself, and there is in the membrane a pump of some kind which is able to move solute. It is useful to distinguish between these processes. The first can be called 'classical' osmosis or 'exogenous' osmosis, and in contrast the second is 'local' osmosis or 'endogenous' osmosis (Smyth, 1965). When fluid movement in the intestine is affected by adding magnesium sulphate to the lumen of the intestine, then the effect is by classical osmosis. But more interesting at the moment is the fluid movement which could follow from a solute pump in the membrane

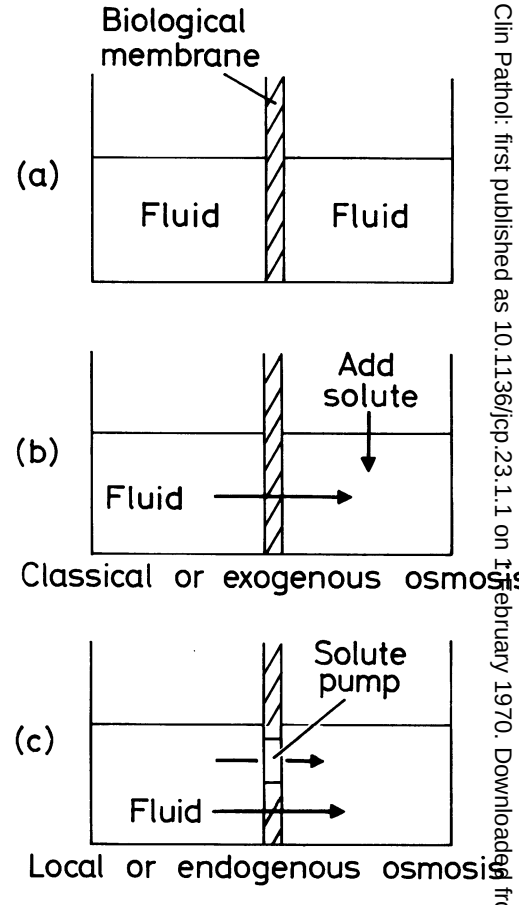

Fig. 1 *Two ways in which solute can affect movement of solvent. In* (a) *there are two compartments containing fluid separated by a biological membrane. In* (b) *solvent is added to one compartment and as a result fluid moves into the compartment with the added solute. In* (c) *the membrane contains a solute pump and is able to move solute from one side to the other. Fluid movement follows in the same direction as the solute movement.*

and this is local osmosis or endogenous osmosis. So, provided that we have a solute pump in the epithelium it is possible to have fluid movement and the fluid will move in the same direction as the solute. This mechanism has been further elaborated by Curran (1960) into a model of a compartment bounded by two membranes with different permeabilities (Fig. 2). The membrane on one side has pores through which solute cannot pass

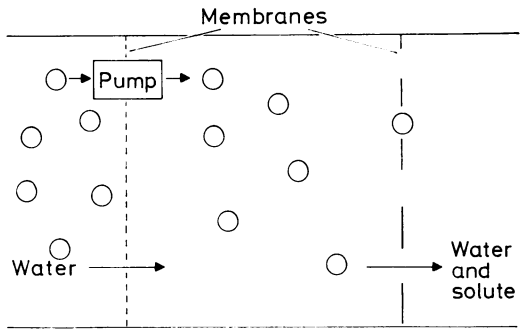

Fig. 2 *Double membrane system of Curran showing how the solute pump can cause movement of solvent.*

but through which fluid can pass. On the other side are pores which both fluid and sodium can pass, and it is easy to see that this process will result in a movement of fluid in the same direction as the solute pump. The solute itself will be carried away in the fluid which is drawn into the compartment. An important problem is which solutes are important in intestinal transfer of fluids, and the answer is any solute which can be transferred, eg, Na, hexoses, amino acids, etc. The most important quantitatively will be that involving the greatest number of moles transferred, and for this reason Na must receive special consideration.

Coupling of Movement of Two Solutes

The most interesting form of coupling in some ways is the coupling of movement of one solute to movement of another. The epithelial cell is bounded on its luminal side by a membrane called the brush border, and this has various important properties. It appears to be the site where selectivity is exerted. We know that certain substances cannot pass through this membrane, although they may be able to enter the cell from the other side, and there is good reason for locating in this membrane some mechanisms concerned with intestinal absorption. Three important problems in absorption are: (1) movement of hydrophilic substances through a lipid barrier; (2) the high degree of selectivity which is exerted; and (3) how the movement of substances can take place against a concentration gradient. The most plausible way at the moment of explaining the first two is to locate in the brush border a process called facilitated diffusion, and by an extension of this attempts have also been made to explain movement against a concentration gradient. The

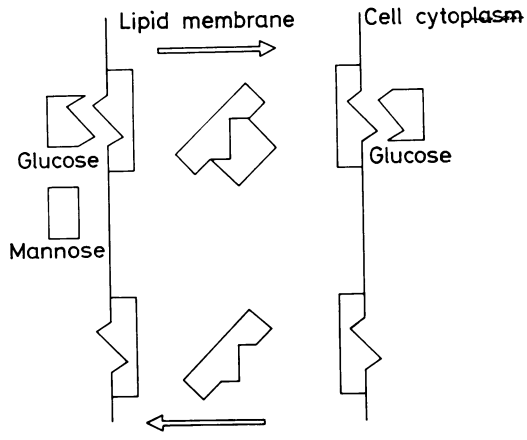

Fig. 3 *Facilitated diffusion. The lipid membrane contains carriers which have sites for attachment of solute molecules. The specificity of these sites is shown by the different geometrical shapes so that glucose fits the site while mannose does not. The carrier together with the solute is able to cross the membrane. The solute leaves the carrier site at the other side and the unoccupied carrier returns and is available for further transfer.*

mechanism of facilitated diffusion is shown in Figure 3. There is postulated in the membrane a carrier substance which is able to combine with the substance transferred and the combined complex is able to move through the lipid membrane. This movement depends only on thermal agitation and does not require metabolic energy. This process explains how the hydrophilic solutions move through the lipid barrier, and at the same time explains selectivity, because the sites on the carrier for attachment of substances transferred have a certain chemical configuration, which facilitates or makes difficult attachment of other chemical groupings; for example, glucose is readily attached and mannose scarcely at all. Having got across the lipid barrier in combination with the carrier, the substance transferred is able to detach itself from the carrier, which now returns empty to the other side of the membrane and is available for further transfer. It is evident that such a process can explain specificity and also the passage of hydrophilic substances through a lipid membrane.

Such a mechanism can also be used for linking movement of one substance to that of another. Suppose the carrier is made a little more complicated, as suggested by Crane (1962), so that there are two sites with different specificities, eg, one for sodium and one for hexose. In addition to this a sodium pump is postulated at the other

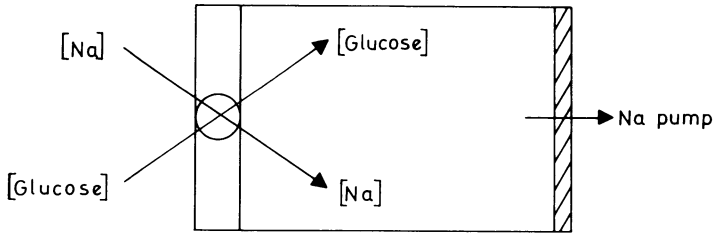

Fig. 4 *Scheme suggested by Schultz and Zalusky to explain how glucose transfer is coupled to sodium transfer. A sodium pump at the serosal side pumps sodium out of the cell. As a result there is a Na concentration difference which provides a gradient for sodium movement across the brush border into the cell. Since glucose moves on the same carrier, glucose is moved against its gradient so as to produce a high intracellular concentration.*

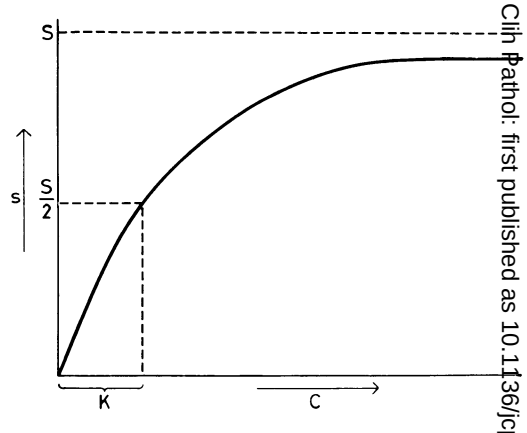

Fig. 5 *Relation between the concentration (C) of a substance and the amount of carrier which is occupied by the substance. The total carrier available is S, and the ordinate shows the amount of carrier (s) occupied. K is the concentration which will give half saturation of the carrier and is called the affinity constant.*

side of the cell (Schultz and Zalusky, 1964) as shown in Figure 4. This pump moves sodium from the inside of the cell. As a result the concentration of sodium in the cell is lowered, and a gradient is created down which sodium can pass across the brush border into the cell. It can, however, only pass down this gradient by using the carrier. As this carrier also moves hexose, the hexose is moved against its concentration gradient while sodium moves down its concentration gradient. Thus facilitated diffusion provides a possible mechanism for linking the active transfer of sodium with passive transfer of another substance. This arrangement is called the 'ternary complex', and in this case the three constituents of the complex are the carrier molecule, Na, and hexose. A ternary complex involving Na and amino acids has also been suggested.

Once we accept this concept, some care is necessary in the terms we use. While the overall process can be described as 'active' transfer of hexose, there is really a 'passive' transfer of hexose linked to an active movement of sodium. There is a case for distinguishing two kinds of active transfer: primary active transfer and secondary active transfer (Smyth, 1969). In primary active transfer the transfer is coupled directly to metabolism, in secondary active transfer it is linked to the transfer of another substance.

There are, however, some difficulties about this ternary complex, and the main one is to explain how the glucose gets off the carrier on the other side. The point will be clear from an elementary consideration of carrier kinetics. The usual concept of carriers is that the amount of substance attached to the carrier depends on the concentration in contact with the carrier. It obeys the same rules as adsorption or attachment of a substrate to an enzyme, a system often called 'Michaelis-Menten kinetics'. With increasing concentration more of the substance is attached to the carrier and ultimately the carrier becomes saturated. The relationship between concentration and saturation of the carrier is shown in Figure 5. This also shows how the affinity of the carrier can be defined in terms of the concentration which causes half saturation of the carrier. The curve in Fig. 5 can be represented by the equation:

$$s = \frac{CS}{C + K} \quad \ldots \quad \ldots \quad \ldots \quad (1),$$

where s is the amount of carrier occupied, S the total carrier present, C the concentration of glucose, and K the affinity constant, often called the Michaelis constant from the analogy with enzymology. It is evident that the degree of saturation of the carrier is s/S, and this is obtained from the equation

$$\frac{s}{S} = \frac{C}{C + K} \quad \ldots \quad \ldots \quad \ldots \quad (2).$$

The determination of K becomes a matter of some importance in discussing the mechanisms of absorption, and this is best done by means of the Lineweaver-Burk plot of the reciprocals of C and S (Fig. 6). This follows from a rearrangement of (1) into the form

$$\frac{1}{s} = \frac{K}{S} \cdot \frac{1}{C} + \frac{1}{S} \quad \ldots \quad \ldots \quad (3)$$

from which it is clear that the relation between 1/C and 1/s is linear and the intercept on the abscissa is the negative reciprocal of K.

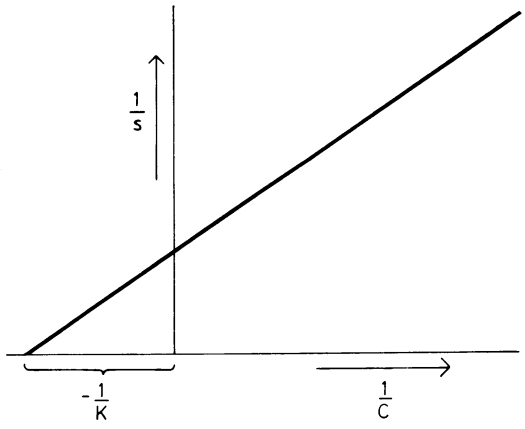

Fig. 6 *Lineweaver-Burk plot showing the linear relation between* $\frac{1}{s}$ *and* $\frac{1}{C}$. *The intercept on the abscissa is* $-\frac{1}{K}$.

This way of considering the saturation of carriers can be applied to facilitated diffusion, where the carrier can be considered to be in equilibrium with different concentrations at two sides of the membrane. At one side the concentration is C_1 and at the other C_2. The saturation of the carrier at one side will be $\frac{C_1}{C_1 + K}$, and at the other side $\frac{C_2}{C_2 + K}$. It is assumed that the rate (v) at which substance is transferred across the membrane is proportional to the difference in saturation of the carrier of the two sides, and hence can be expressed by the equation

$$v = k\left\{\frac{C_1}{C_1 + K} - \frac{C_2}{C_2 + K}\right\} \quad .. \quad (4).$$

If $C_1 = C_2$ the term in brackets becomes zero, and hence there will be no movement. This shows that facilitated diffusion only takes place from a higher concentration to a lower concentration, ie, it could never cause movement against a concentration gradient. But supposing we had the same concentration on the two sides C_1 but we had different K values. Equation 4 now becomes

$$v = k\left\{\frac{C_1}{C_1 + K_1} - \frac{C_1}{C_1 + K_2}\right\} \quad .. \quad (5).$$

It can readily be seen that if $K_2 > K_1$ the term in the brackets is positive, and hence movement takes place without any concentration difference. If the concentrations are not the same as on the two sides, so that equation 5 becomes

$$v = k\left\{\frac{C_1}{C_1 + K_1} - \frac{C_2}{C_2 + K_2}\right\} \quad .. \quad (6),$$

it is clear that even if $C_2 > C_1$ values of K_1 and K_2 could be chosen which would make v positive, and hence it would be possible to have movement against a concentration gradient.

This mechanism provides an interesting way in which movement of one solute can be coupled with movement of another. It has been seen that if a sodium pump is postulated at the serosal pole of the cell, it could cause a lowered concentration of Na in the cell. If it is further postulated that affinity of the carrier depends on Na concentration (Crane, Forstner, and Eichholz, 1965), it is easy to see how a Na pump could create conditions where equation 6 could describe the activity across the membrane, and as a result hexose could move from a lower concentration in the intestinal lumen to a higher concentration inside the cell. This incidentally solves the problem of how the substance is able to detach itself from the carrier in the presence of a high concentration inside the cell.

Another way in which coupling of solute movement can occur is by electric potentials. In the cases discussed above an Na pump at the serosal border of the cell has been postulated. Na pumps can be either electrogenic or non-electrogenic. In an electrogenic Na pump it is envisaged that the pump moves Na^+, and this will create a positive potential on the side on which Na is moved. In the case of the non-electrogenic pump the mechanism could transfer equal number of Na^+ with some anion, eg, Cl^- or HCO^{3-}, so that no electro-potential is generated. In fact we know that both kinds of pumps are present (Barry, Eggenton, and Smyth, 1969). The effect of the electrogenic Na pump is to create a potential and this could assist in the movement of anions, eg, chloride, bicarbonate, or lactate. The movement of some of these anions could thus be said to be coupled to movement of Na^+.

There is still another interesting way in which movement of solutes can be coupled. It is usually

assumed that the energy for transport of substances is ultimately related to membrane ATPases, as these make the energy from ATP available for transfer processes. These membrane ATPases are sodium sensitive so that the concentration of cellular sodium can affect the action of the ATPase. A Na pump at the serosal pole of the cell would affect the concentration of intracellular Na and this provides another way in which sodium can be related to the transfer of other substances in the intestine.

Coupling of Solute Movement to Metabolism

The last type of coupling shown in Table I is solute movement coupled to metabolism. This is what has already been called primary active transfer, and is the real problem of cellular transfer, ie, how metabolic energy causes osmotic work. The term 'chemi-osmotic coupling' has been used for this (Mitchell, 1967) and expresses very well the basic problem involved. Various interesting schemes have been devised but so far there is no unanimity about how these could work, and I do not therefore want to go into the problems now.

One Basic Mechanism

It will not have escaped your notice that in all the methods discussed here of coupling movement of substances together, whether fluid to solute or solute to solute, Na has played a major role. This prompts the question whether the sodium pump coupled to metabolism is not the central driving force in the intestinal epithelial cell. It has always been attractive to the scientist to reduce the number of hypotheses and to find a basic underlying mechanism. This is well known as Occham's razer: 'Entia non multiplicanda sine necessitate.' During the past decade intestinal absorption has become an increasingly complicated field, with new mechanisms being continuously postulated to deal with different substances. Have we now really reached the stage when simplification is beginning and the basic underlying mechanism is in sight? This is not only a fascinating possibility, but the unifying hypothesis of the Na pump, whether proved true or false, fits the even more important criterion of a good hypothesis—it suggests experiments which can be done. But perhaps it is possible to go too far. It has been said that God does not always shave with Occham's razer and in fact biological processes are usually fairly complicated. Nevertheless, it can be said that the epithelial cell is slowly yielding its secrets and is ceasing to be the magic black box that we had to be content with only a few years ago.

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