Section of Experimental Medicine and Therapeutics

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Papers

Intestinal Absorption

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A good deal of progress in the investigation of intestinal absorption in the last decade has depended on the use of *in vitro* techniques, introduced by Fisher & Parsons (1949). By *in vitro* techniques in absorption, we mean the use of segments of intestine completely deprived of their normal blood supply. At first sight these preparations seem highly unphysiological, and some of them even absurdly so, but in spite of this they have shown their value, and have provided a great stimulus to the physiology of intestinal absorption. One of the most useful, and apparently one of the least physiological, is the everted sac of small intestine, which was developed in the Physiology Department in Sheffield by Wilson & Wiseman (1954). It is being extensively used in this country and America at present and offers a very simple experimental approach to problems of absorption. A rat is anæsthetized, and the intestine quickly removed by pulling it away from the mesentery. The intestine is then everted on a glass rod. The everted intestine is now divided into segments of various sizes and from these sacs are made. The sacs are filled with physiological saline, suspended in physiological saline in conical flasks and shaken for 30 minutes, one hour or longer. At the end of the experiment the fluid in which the sac is shaken can be measured and analysed, and also the fluid inside the sac and in the intestinal wall, and in this way it can be shown that substances are taken up by the epithelial cells and are transferred to the inside of the sac. The first question that might be asked is what is the relation of this process to physiological absorption, as in physiological absorption the substances absorbed do not pass through the muscle layer of the intestine. Substances absorbed from the lumen of the intestine are taken up by the epithelial cells and transferred to the other side of the cell, where they enter the subepithelial space. Under physiological conditions they pass into the blood and lymph vessels, and are removed by these channels. Under *in vitro* conditions part of the fluid remains in the subepithelial space, but part passes through the muscle layer. This passage is in fact partly by the physiological route through the cut blood and lymph vessels and this is shown by the fact that the first fluid that comes through is blood stained. A certain amount of fluid remains in the subepithelial space. What we obtain inside the sac is therefore not quite so unphysiological as appears at first sight.

In the last six years we have done a great many experiments of this kind and we have taken care to control these with experiments with various types of *in vivo* preparations, i.e. where the intestine has its normal blood supply. The *in vitro* intestine undoubtedly carries out very similar activities to those which occur *in vivo*, and furthermore it does so in a way which makes it far more amenable to experimental investigation.

The general result of work of this kind is to suggest that the mechanism of transport of many substances is much more complex than once seemed likely, and often involves active participation by the intestinal cells. Absorption is, in fact, often an active process. There are various ways of defining what is meant by an active process, but here I would take the definition that the forces for absorption are created by the epithelial cells, the energy being obtained from their metabolic activity. The meaning of this can be appreciated by considering an example. If a substance is taken into the alimentary tract and digested, a concentration of various products is produced in the lumen of the intestine, and this concentration may be sufficient to enable diffusion to take place across the intestinal epithelium. In this case the force necessary for diffusion is produced by the high concentration, and is not created by the epithelial cells themselves. If, however, a substance is present in the lumen of the intestine in smaller concentration than in the blood, then energy must be provided by the epithelial cells for movement and in this sense the

process is active. We have now clear evidence that active processes of this kind involve at least some of the products of digestion of proteins, fats and carbohydrates, and also fluid and some inorganic salts, and a variety of mechanisms exist in the epithelial cells for causing movement of these substances.

But the *in vitro* preparation enables us to go further than simply say whether the epithelial cells participate in active transport. We are now beginning to analyse the processes involved, and although not much progress has been made, in some cases we know that at least several stages take place in the mechanism and we have also some idea about the localization of these in the cells and their relation to cellular metabolism. I believe that advances in the physiology of absorption are going to be made chiefly in this direction, i.e. separation of the various stages in movement and relation of these to definite parts of the cell, definite structures in the cell, or definite enzyme systems in the cells. I shall illustrate some of these points by reference to the absorption of protein and glucose.

Protein

The classical view of protein absorption is that digestion of amino acids occurs in the intestine and the amino acids are then absorbed. This view was challenged by Fisher (1954) who suggested that aggregates larger than amino acids might be absorbed and possibly even whole proteins. We undertook a series of investigations to attempt to clarify this problem, but we very soon realized that the question we were trying to answer was not adequately framed. There were in fact not one question but two questions. The first was the form in which protein leaves the lumen of the alimentary tract, and the second the form in which it enters the blood stream. The answers to both these questions are now quite definite. Protein can leave the alimentary tract in products larger than amino acids, but the protein enters the blood stream almost exclusively in the form of amino acids. (I refer here to the amounts of protein related to nutritional quantities, because it is quite certain that very small amounts of whole proteins can pass through the intestinal barrier.) In coming to these conclusions we have used both *in vivo* and *in vitro* techniques, but I will choose the *in vitro* as an illustration. An everted sac is shaken for 30 minutes in a solution containing a peptide, and at the end of this time, a sample of the fluid is taken for analysis; the wall of the intestine and the fluid inside the sac are also analysed. A certain amount of amino acid is formed. This might be explained by hydrolysis by peptidases in the luminal fluid (i.e. the fluid in which the sacs are shaken), or it might be by

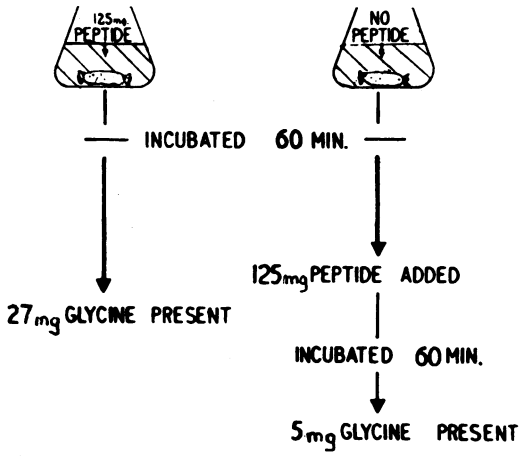

Fig 1 *Experiment showing that most of hydrolysis of glycyl-glycine must take place after it has entered the mucosal cell, and not by peptidases in the fluid in which sacs are suspended*

hydrolysis inside the intestine after peptide has entered the cells. We can easily distinguish between these by incubating peptide in the luminal fluid (Fig 1), without the presence of the sac, and this shows clearly that the luminal fluid peptidases could only account for a small amount of the total peptide hydrolysed (Newey & Smyth 1960). There seems little doubt therefore that peptide must enter the epithelial cells as such and undergo intracellular hydrolysis. What we understand by protein digestion may therefore take place not only in the lumen of the intestine but also intracellularly. This was in fact guessed at by many of the older workers but not explicitly demonstrated.

One of the arguments about the form of protein absorption is the rate at which digestion occurs, as it has been suggested that breakdown to amino acids could not take place at a sufficiently rapid rate. Our experiments show that luminal breakdown to amino acids is not essential in order that amino acids should enter the blood stream. Crane & Neuberger (1960) have recently published results suggesting that protein digestion may in fact be very rapid, as the difference in time of appearance of amino acids in the blood stream after giving a protein hydrolysate and the unhydrolysed protein may be as little as 15 minutes.

At present we are trying to find out more about the intracellular mechanisms for transport of protein and amino acids. If we carry out experiments with amino acids it can be shown that a number of amino acids are transferred by the intestine against a concentration gradient (Wiseman 1953, 1955). There is also competition between different amino acids, and methionine can compete effectively with most other amino acids for the mechanism. In trying to localize such

a mechanism one approach is to ask whether the mechanism is located at the entrance to the cell, or whether it is an exit mechanism dealing with amino acids which have entered by diffusion. We can use the intracellular hydrolysis of peptide as a method of getting amino acids into the cell, without the amino acid, as such, having to enter the cell. We can also, however, use it to study entry of peptide as distinct from entry of amino acids, and by experiments of this kind we can get some information about the localization of mechanisms inside the cell.

The following are some preliminary conclusions we have reached: Peptides enter the cells mainly by diffusion but not entirely so, because the amount of peptide entering is reduced by anaerobic conditions. Since, however, this reduces water absorption it is possible that peptide entry depends partly on solvent drag, i.e. the peptide is transported in an active water stream. Once the amino acid is formed inside the cell it may either diffuse back into the lumen of the intestine or it may be transferred across the cell to the subepithelial space, and by studying various conditions we can find effects which modify these two processes. The most important condition affecting this process is anoxia, and in this condition most of the amino acid diffuses back instead of being transferred forward.

Our concept of peptide and amino acid transfer is thus as follows: Peptides may enter the cell as such, and amino acids are formed by intracellular hydrolysis. Part of the amino acid formed attaches itself to a carrier in the cell, and part diffuses back again out into the lumen (Fig 2). This carrier requires the presence of aerobic energy and in anaerobic conditions or in the presence of DNP most of the amino acid formed inside the cell diffuses back into the lumen. There is thus a carrier mechanism inside the cell as distinct from a carrier mechanism at the luminal border of the cell. Amino acids in the lumen of the intestine may use the same carrier mechanism, but there is certainly another mechanism involved, i.e. at entry

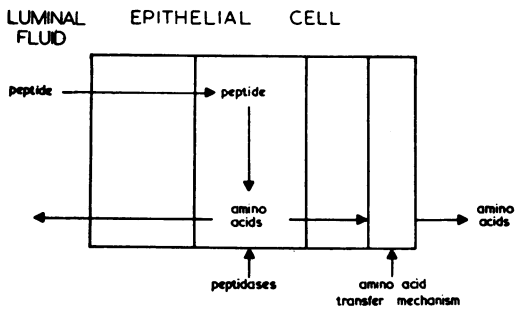

Fig 2 *Fate of amino acids formed by intracellular peptidase activity*

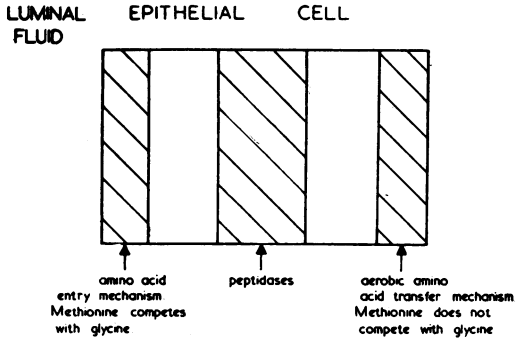

Fig 3 *Possible intracellular location of various processes involved in peptide hydrolysis and amino acid transfer*

into the cell. This can be shown by the study of competition between amino acids (Newey & Smyth 1961). Methionine inhibits the entry of glycine into the cell but it inhibits much less the exit of glycine formed intracellularly. There are thus two stages in glycine transfer, an entry mechanism and an exit mechanism. The entry mechanism is where competition with methionine occurs, the exit mechanism does not involve methionine competition but requires aerobic energy (Fig 3). Another interesting possibility is that these two mechanisms may have different specificities for different amino acids, and this may explain some of the curious results which have been obtained in relation to the specificity of amino acid transfer.

Glucose

The question of glucose transfer has attracted great interest for a long time and has been very well reviewed by Crane (1960). The first question that arises in this connexion is the status of the phosphorylation theory. According to this theory glucose is phosphorylated at the mucosal border of the cell, is carried across the cell as a phosphorylated compound and is subsequently dephosphorylated. Since first put forward by Wilbrandt & Laszt (1933), this theory has persisted and still keeps its place in many modern textbooks. It is not widely appreciated that those who put it forward soon withdrew their support; there is no real evidence in favour of the phosphorylation theory and there is a good deal of evidence against it. In fairness to the authors of the theory, it should be stated that it was put forward at a time when the part played by phosphorylating reactions in metabolism was not so widely known as it is now, and it was not recognized that substances which interfere with phosphorylation might produce effects by interfering with metabolism. There are two major types of evidence against the phos-

phorylation theory: (1) Many sugars can be actively transported which cannot be phosphorylated. (2) By using labelled glucose and galactose it has been shown that the glucose transported by the intestine has not passed through the glucose-6-phosphate pool. These are very serious objections to the phosphorylation theory of glucose absorption (for references *see* Crane 1960).

What in fact do we now know about glucose transfer, and is there any modern alternative to the phosphorylation theory? No precise scheme has been put forward to explain glucose transfer, simply because no precise scheme has yet been put forward which adequately explains transfer of any substances across biological membranes. Modern views tend to think of carrier mechanisms, the carrier being probably of a protein nature, and it would seem likely that the transfer of glucose by the intestine involves a carrier mechanism of this kind. The following facts are well established: (1) Glucose can be moved against a concentration gradient. (2) The movement of glucose depends on aerobic energy and does not happen in anaerobic conditions. (3) It shares a mechanism with at least some other sugars, e.g. galactose. (4) It is inhibited specifically by phlorrhizin. It is also inhibited by a great many other substances, but none of these have the specificity of phlorrhizin, nor do they act in anything like as small a concentration. Phlorrhizin is able to affect glucose transfer in a concentration as low as 10^{-6}M.

The action of phlorrhizin has given some interesting clues as to the nature of the mechanism for glucose transfer (Newey *et al.* 1959). For example it is possible to study the effect of phlorrhizin on the entry of glucose into the cell as distinct from its transfer. This can be done by using ^{14}C-labelled glucose and collecting from the intestine the metabolic CO_2 (Fig 4). If the CO_2 is labelled it is a reasonable conclusion that the labelled glucose must have entered the cell. If experiments are done in which labelled glucose is present on either the mucosal or the serosal side of the cell it can be shown that phlorrhizin will affect the amount of labelled CO_2 formed, and two different effects are produced. If we have a high concentration of phlorrhizin (10^{-3}M) we find that production of CO_2 from glucose on either side of the intestine is greatly reduced, and this suggests that phlorrhizin acts on the metabolism of glucose inside the cell. If, however, we use a much smaller concentration of phlorrhizin, we can show that the effect of phlorrhizin is to prevent metabolism of glucose initially present on the luminal side of the intestine but not on the other side (Fig 5). There must therefore be an entry mechanism which is inhibited by phlorrhizin. We can get some further information about the mechanism of glucose transfer by comparing the effects of phlorrhizin, anaerobic conditions and 2:4-dinitrophenol (DNP) on glucose transfer. All these abolish the active movement of glucose by the intestine. In contrast to this, anaerobic conditions and DNP do not prevent the entry of glucose into the cells and from this it follows that there are at least two stages in the transfer of mechanism of glucose: (1) An entry mechanism

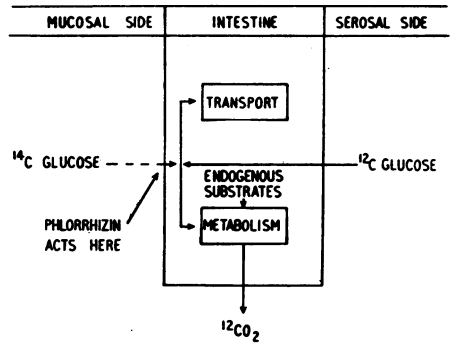

Fig 5 *Site of action of phlorrhizin in inhibiting intestinal transfer of glucose*

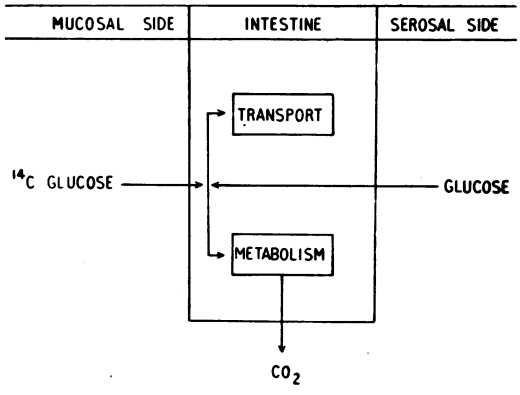

Fig 4 *Fate of glucose initially present on either mucosal or serosal side of the intestine*

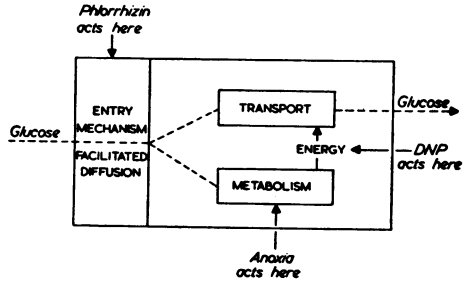

Fig 6 *Possible location of mechanisms involved in intestinal transfer of glucose*

which is inhibited by phlorrhizin. (2) A mechanism which is prevented by anaerobic conditions (Matthews & Smyth 1960). It seems possible that this second mechanism is the one responsible for movement against a concentration gradient. Hence it seems likely that, as in the case of amino acids, the transfer by the intestine is a complex process and involves more than one stage. A scheme showing the possible sites of action of different inhibiting conditions is given in Fig 6. In this it is suggested that the phlorrhizin sensitive entry mechanism may be a facilitated diffusion. This is an interesting speculation but has not yet been proved.

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Motility of the Intestine

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It is known that peristaltic activity is entirely under nervous control, but a few years ago we were able to throw some light on the mechanism of the reflex and on its intrinsic nervous pathway (Bülbring, Lin & Schofield 1958). We obtained evidence that the sensory receptors, which trigger the reflex when the intestine is extended and the filling pressure rises, are situated in the deepest layers or at the base of the mucosal epithelium. The sensory fibres arise mainly from cells in the submucous plexus of Meissner which contains a large number of unipolar or bipolar neurons. Their axons make connexions with multipolar ganglion cells chiefly situated in the myenteric plexus of Auerbach. The submucous plexus appears therefore to be mainly sensory, while the myenteric plexus contains mainly the motor neurons innervating the muscle.

The peristaltic reflex was found to depend on the integrity of the mucosa. If the mucosa was removed, or asphyxiated, or a local anæsthetic was applied to its surface the peristaltic reflex was abolished. More recent experiments by Ginzel (1959) suggest that some sensory receptors might be slightly further removed from the mucosal epithelium, possibly in the muscularis mucosæ. Whatever their exact site, they are situated in close proximity to the enterochromaffin cells which are also found in the deep layers of the mucosa. The release of 5-hydroxytryptamine (5-HT) from these cells exerts an important subsidiary action on peristalsis. 5-HT is a sensory stimulant and it is known to excite sensory endings in many parts of the body. In the intestine, where it is locally produced and released in proportion to the rise of intraluminal pressure, 5-HT lowers the threshold of excitation of the mucosal nerve endings and thereby has a modulating influence on peristalsis (Bülbring & Lin 1958, Bülbring & Crema 1958, 1959).

The peristaltic contractions are produced by the concerted activity of an immense number of very small muscle cells whose properties we are only just beginning to understand. They are very thin and not very long, measuring about 5 μ by 100 μ. It is fascinating to think that these cells are really like a large population of independent beings, like a crowd of unicellular organisms, rather primitive, not so highly specialized as for example skeletal muscle, and capable of several functions, all united in one and the same cell. This cell can be the focus of excitation or it can be excited by a stimulus from elsewhere. It can also behave like a sensory receptor and, in addition, it can contract. Moreover, the intestinal smooth muscle contracts not only in response to a nervous impulse, but it can contract spontaneously, and it does so, rhythmically, like the heart.

Like the heart, intestinal smooth muscle has an intrinsic rhythm, it generates action potentials and each action potential is followed by a small individual contraction. Unlike the heart, these contractions can summate and this is the basis of what is generally known as 'tone' (Bozler 1948, Bülbring 1957). Spontaneous changes in tone are commonly observed and they are entirely the consequence of spontaneous changes in the frequency at which action potentials are discharged. If the frequency is high the individual contractions may fuse, like a tetanus, to a high maintained tone. If the discharge rate is low there is time for relaxation before the next impulse arises and the muscle tone declines.

One peculiar property of intestinal smooth muscle is that the more it is stretched the higher its tone. It endeavours to contract against the extending force. The stimulus is stretch, and the response is the same as the response of a stretch receptor, i.e. a burst of impulses or, if rhythmic discharge was already present, an increased rate of firing. Stretch deformation of the cells leads to a depolarization of the membrane which in turn leads to the firing of impulses. The cell produces the typical response of a sensory organ to its specific stimulus. However, the intestinal smooth muscle cell is not a specific receptor. To every