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Dr S P R Rose (*Imperial College, London*), referring to the detection of bound and free intermediates in the membrane system, asked whether these systems were extracted directly or first precipitated with trichloroacetic acid, and whether Professor Widdas looked for incorporation into fractions other than lipid fractions.

Professor Widdas replied that the answer to the first question was no, their method was to extract straight with the lipid solvent. The answer to the second question was also no. He had not done any experiments looking at protein fractions, but this had been done by Stein (1964) at Manchester. Stein had tried to label inhibited cells differentially by different concentrations of isotopically-labelled DNFB and had looked at where the label appeared, and he had looked at the protein fractions.

Dr F J Danielli (*Buffalo, USA*) said that Stein had brought forward some evidence of the carrier combined with two glucose molecules rather than one, and asked for comments on this. He also asked what contribution the entropy term made to the transition state in determining the rate at which passage across the membrane occurred. The activation energies quoted had been fairly high.

Professor Widdas replied that the question whether one or possibly two molecules of glucose reacted with the component had been started off by Stein, but Wilbrandt & Kotyk (1964, *Arch. exp. Path. Pharmak.* **249,** 279) had also done experiments in which they claimed to have evidence for this. Professor Widdas himself kept an open mind about it; he felt it was one of the things which isolation of the complex might help to solve.

Regarding the second point, Professor Widdas thought that the entropy terms must be considerable because, although the dissociation of the complex was given a high activation energy, it must be assumed that it was going on at a fast rate at 37°C, and therefore it could be presumed that there must be some entropy factor which was helping it to work at this rate. But there were difficulties in that they had not been able yet to measure the rate constants by a more direct method. If that could be done, it would be a great help.

Dr Hugh Davson (*University College, London*) asked whether there was evidence of an optimum temperature in any of the studies on the effect of temperature. He felt that the carriers behaved rather like enzymes which have quite well-defined optimum temperatures and pHs.

Professor Widdas replied that they had studied the pH effects between values of about 6 and 8. There was a small change with pH but it did not amount to a definite maximum. Regarding temperature, it depended to some extent on what one was looking for. If one was simply transferring sugar across a cell membrane as a substrate, it was ideal to work somewhere near the half-saturation constant, which at 37°C was about 72 mg/100 ml; this was not very far removed from the blood sugar concentration. One could feel, therefore, that transfer would be efficient at 37°C.

In the fœtal guinea-pig, where this optimal value of the half-saturation constant was maintained down to a much lower temperature than in human red cells, it was not known whether it was related possibly to the adaptation of these animals to withstand subnormal temperatures e.g. immediately after birth.

Professor D H Smyth
(*Department of Physiology, The University, Sheffield*)

Electrical Activity in Relation to Intestinal Transport

Three different kinds of electrical activity can be observed in the epithelium of rat small intestine: (1) Diffusion potentials. (2) Streaming potentials. (3) Transfer potentials.

Diffusion potentials result from alteration of the ionic content of the fluid in contact with the epithelium without necessarily altering the osmolarity. They can be observed by replacing the NaCl in the bathing solution by mannitol, and are presumably due to differences in the concentration of ions on the two sides of the luminal membrane and differences in the permeability of that membrane to different ions.

Streaming potentials, shown in the intestine by Smyth & Wright (1966), are the result of changes in osmolarity of the fluid bathing the epithelium and are related to flow of fluid through the epithelium. On account of the small diameter of the pores through which fluid moves, these streaming potentials are not necessarily analogous to the streaming potentials associated with the Helmholtz double layer, and it would be perhaps better to call them osmotically induced potentials until we know more about their precise causation. Dr P Kohn (personal communication) has suggested that a possible origin might be changes in intracellular concentration of ions due to the fluid movement.

Transfer potentials are the main subject of this paper. They were observed (Barry *et al.* 1961, Barry *et al.* 1964) *in vivo* in the rat intestine when glucose was added to the fluid in the intestinal lumen and *in vitro* when glucose was added to fluid bathing the epithelium (the mucosal fluid). The magnitude of the potential is about 10 mV, the serosal side of the intestine being positive to

the mucosal. Baillien & Schoffeniels (1962) have since observed potentials in the intestine of the tortoise in the presence of certain amino acids. The interesting problem is the cause of these transfer potentials, and at first sight this seems obvious. It is well known that glucose increases the transfer of fluid by the intestine and, as the fluid transfer is roughly isotonic, glucose increases sodium transfer. It is also known that reduction of sodium concentration in the mucosal fluid reduces glucose transfer. Hence there is a reciprocal relationship between sodium transfer and glucose transfer, and the most obvious explanation of the potential is sodium transfer associated with glucose transfer.

Crane *et al.* (1961) put forward a scheme to explain the sodium/glucose relationship, in which they postulated a carrier responsible for both glucose and sodium movement into the epithelial cell from the intestinal lumen. Associated with this was a sodium pump which could transfer sodium from the cell into the intestinal lumen. As the result of this a low sodium concentration in the cell was maintained, sodium moved into the cell on the carrier and, as glucose was linked with this carrier, glucose moved against its concentration gradient. The concept of an interaction between sodium and glucose on a common carrier mechanism is an interesting one and has been further developed by Crane and his school. The scheme outlined above, however, fails to explain why the glucose stimulates sodium movement, since the sodium pump is operating in the reverse direction to glucose movement. To overcome this difficulty Schultz & Zalusky (1964*a*) modified the scheme so that the sodium pump is at the other side of the cell and pumps sodium towards the serosal side. They also concluded that there was a stoichiometric relationship between glucose and sodium movement and further found that the short-circuit current accounted for the amount of sodium transferred. All these results presented a reasonable case for the cause of the potentials being due to sodium movement. Schultz & Zalusky further postulated that a similar carrier mechanism might transfer amino acids also and hence explain the amino acid potential.

There are a number of observations which are difficult to reconcile with this explanation and other facts must also be considered. Barry *et al.* (1964) have studied a number of sugars and found that those which caused a potential could be transferred against a gradient, i.e. glucose, galactose, 3-methyl glucose and α-methyl glucoside. These all have the common structure which Crane (1960) has defined as being essential for active transfer, i.e. a pyranose ring with OH at C2 in the same stereochemical position as D-glucose and with another C attached to C5. If, however, we examine these sugars in relation to fluid transfer we find that the position is rather different. Glucose stimulates fluid transfer, but galactose, 3-methyl glucose and α-methyl glucoside do not. On the other hand there are sugars which cause stimulation of fluid transfer but cannot be transferred against a concentration gradient, e.g. fructose. Furthermore, Duerdoth *et al.* (1965) have shown that mannose, which is not able to enter the epithelial cell from the intestinal lumen and therefore cannot be transferred against a gradient, is able to stimulate fluid transfer if present initially on the serosal side of the intestine, from which it can readily enter the epithelial cell and be metabolized. The position has been discussed at some length by Smyth (1965) and it appears that there are two specificities in the intestine in relation to sugars. Sugars with the 'Crane' specificity can use the phlorrhizin-sensitive entry mechanism and be transferred against a concentration gradient. They also cause an electrical potential. Sugars with the hexokinase specificity can be metabolized by the intestine and can stimulate fluid transfer. They do not necessarily cause an electrical potential. Glucose happens to possess both specificities and therefore can stimulate fluid transfer and cause an electrical potential.

Barry *et al.* (1963) also investigated the short-circuit current. They found that in the presence of glucose the short-circuit current could be accounted for by net sodium transfer and this finding was confirmed by Schultz & Zalusky (1964*b*). However, in the presence of galactose the short-circuit current was greater than could be accounted for by net sodium transfer, while in the presence of fructose it was smaller. It is therefore difficult to argue that in the case of glucose the potential is caused by net sodium transfer, unless we assume that the mechanism of causing the potential is different in the case of glucose from that in the other sugars.

It is difficult at the moment to offer any simple explanation of the experimental findings, but Barry *et al.* (1965) have put forward a tentative scheme. This assumes that fluid transfer is caused by solute pumps, and it is suggested that there are two separate pumps which contribute to the local osmotic change. There is first an electrically neutral sodium pump which transfers sodium with an accompanying anion. This pump depends on the metabolism of the intestine. The endogenous metabolism cannot operate it at maximum rate, but it can be stimulated by sugars with the hexokinase specificity. Such sugars therefore increase fluid transfer without necessarily causing an electrical potential. There is also a quite independent hexose pump. How this pump functions we do not know, but it certainly is

associated with ion movement of some kind involving sodium, although it may involve other ions as well, including movement of anions towards the mucosal side as well as moving cations to the serosal side. This hexose pump operates for sugars with the 'Crane' specificity and the associated ionic movement is responsible for the electrical potential.

Recently the effects of disaccharides and dipeptides have been studied by Kohn *et al.* (1966). Maltose caused a potential very similar to glucose with about the same time relation, sucrose caused a potential coming on more slowly, lactose caused a small potential in the opposite direction. This is interpreted as meaning that maltose is rapidly hydrolysed in the brush border and the potential generating site is close to the brush border. Sucrose is hydrolysed more slowly and the limiting factor in potential development is hydrolysis of sucrose. Lactose is not hydrolysed at all, in fact it does not leave the lumen of the intestine and the potential is in the reverse direction, i.e. it is a streaming potential. Experiments with glycine showed that the potential due to glycine developed more slowly than that due to hexoses, the time-lag being of the order of a few minutes instead of seconds. The potential is also very much smaller than in the case of glucose (a few mV), and furthermore glycyl-glycine can produce a very much higher potential than glycine. The time relation suggests that the site of generation of the potential in the case of glycine lies further from the luminal surface of the cell than does the site in the case of glucose. The high potential shown by glycyl-glycine is probably due to the high concentration of glycine produced intracellularly by hydrolysis of glycyl-glycine.

Since the presence of sodium is important for glucose movement it was of interest to study the effect of change in sodium concentration on the hexose potential. Barry *et al.* (1966) have shown that if sodium is replaced with mannitol a diffusion potential is produced but until the concentration of sodium is quite low the hexose potential can be obtained. On the other hand, if sodium is replaced by potassium then the hexose potential is reduced and abolished when the sodium concentration falls to 50 mM. It is not therefore only a matter of sodium concentration. It should be remembered that sodium produces many effects in the cell (e.g. on the ATP-ases), and it appears to us to be unwise to attribute all the effects of sodium to its effect on the affinity of the glucose with the carrier. The origin of the transfer potentials is still not explained, but it would appear to be a very promising line of approach to elucidate the mechanism for hexose and amino acid transfer.

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Dr C D Holdsworth (*Royal Free Hospital, London*) asked whether, unless there were some marked species variation, the difference between the fluid transfer associated with glucose and that associated with galactose might have been exaggerated. It could be essentially an artifact of the *in vitro* preparation, because of its need of a substrate for energy production, water absorption involving an energy-coupled mechanism. Certainly in man, *in vivo*, galactose stimulated water absorption to precisely the same extent as glucose, whilst fructose, although well metabolized, and quite well absorbed, did not stimulate fluid transfer at all (Holdsworth C D & Dawson A M, 1964, *Clin. Sci.* **27**, 371).

Professor Smyth, in reply, agreed that this was correct. The intestine *in vitro* had to get all its metabolic fuel from added substrates whereas *in vivo* it obtained it from the blood stream. Hexoses present *in vitro* could participate in two activities, i.e. they could either be actively transferred or be metabolized. Those which were metabolized supplied energy for transfer processes, those which were transferred required energy. As glucose participated in both activities it was both supplier and user of energy, but since it could stimulate other transfer processes the balance was in favour of supplying energy. Furthermore, any solutes transferred could probably take fluid with them. Hence *in vitro* glucose could cause fluid transfer in two ways either by simultaneous movement of water with glucose or by stimulating a fluid pump by being metabolized. In this way the effects of glucose and galactose could be quite different *in vitro* but could be very similar *in vivo*. This competition for energy by transfer processes could be demonstrated more easily if substances other than fluid were considered, e.g. amino acid transfer was stimulated by glucose and inhibited by galactose, and this could be reasonably interpreted as competition between galactose and amino acids for cellular energy.

Mr P L Bradley (*Nicholas Research Institute, Wexham, Slough*) said he had often wondered what effect the hydration capacities of various ions might have on the entry of water into and exit out of the cell. For example, sodium had a much higher hydration capacity than the potassium ion and if there were a

preponderance of sodium in the cell, he wondered whether the large hydrated sodium ion would actually cause the cell to swell, thereby altering the structural associations inside it.

Professor Smyth agreed that this was not an easy question; not enough was known about the hydration of different ions to give any adequate reply. In all the measurements done in trying to estimate pore radius the problem of hydration had not always been adequately considered.

Dr A M Dawson (*St Bartholomew's Hospital, London*) agreed that Professor Smyth had demonstrated that glucose seemed to be important in stimulating net fluid transport. This had been assumed to be due to glucose acting as a source of energy in the *in vitro* systems and Professor Smyth had pointed out that *in vivo* this would be supplied by the blood system. This did not seem to be the explanation in man for, surprisingly, there was very little net fluid transport from isotonic saline placed in the jejunum, but small concentrations of glucose seemed to trigger off the process.

Professor Smyth, in reply, said he had no immediate answer to the question of glucose triggering off fluid transfer. As he had just mentioned, glucose could cause fluid transfer either by being metabolized or by taking fluid with it. It was not always easy to separate these two fractions of fluid transfer.

Mr K R L Mansford (*Imperial College, London*) said that Professor Smyth had drawn attention to the fact that galactose generated a similar potential to glucose, and he asked whether it would be expected that fructose would have a similar effect in a species such as the hamster, where there was substantial conversion to glucose in the epithelial cell.

Professor Smyth thought that this would depend on how much glucose was being formed from fructose. He had done experiments in which glucose was liberated from various disaccharides. In the case of maltose a large potential was produced. In the case of sucrose a smaller potential was produced while in the case of lactose there was no potential at all. It appeared to be a question of the concentration of glucose produced at the potential-generating site.

Dr A E M McLean (*MRC Toxicology Research Unit, Carshalton*) asked how the fluid transfers were measured and what kinds of deviation were expected. He also asked whether ATP levels had been measured inside the preparations.

Professor Smyth in reply to the first question said this was done by weighing the intestine. There was a considerable variation, but differences reported under different conditions were always based on statistical treatment of the results. The question regarding ATP was very interesting. They had not measured ATP levels in Sheffield but had just started to do so. However, it had been measured elsewhere, and Saunders & Isselbacher (1965, *Biochim. biophys. Acta* **102,** 39) had found that ATP level was increased in the presence of glucose and decreased in the presence of galactose. These findings fitted in with the view expressed that glucose increased availability of energy and galactose decreased it. Saunders & Isselbacher did not find stimulation of amino acid transfer by glucose, but this discrepancy seemed to depend on the parameters used for assessment of transport capacity. If one considered the total amount of amino acid moved as an index of transfer then glucose undoubtedly stimulated.

Dr S L Hart (*Guy's Hospital, London*), referring to the models shown of the absorbing cell, asked Professor Smyth if he thought the carriers were confined to the membrane or whether they were moving through the cell.

Professor Smyth said that although we knew that a good deal of activity in transfer was close to the luminal border of the cell there was also reason to believe that there might be more than one stage in transfer so that the second stage might be more intracellular while the first stage might be at the luminal membrane. For example, when two amino acids competed with each other it seemed likely that this was for a carrier mechanism near the luminal border of the cell. However, when galactose and glycine competed with each other it was unlikely to be a competition for a carrier in the sense that Professor Widdas had mentioned but more likely to be a more non-specific competition for cellular energy. Competition between glucose and galactose was only observed when they were both entering the cell from the luminal side and was not present if the glucose was initially present on the serosal side of the cells.

Dr T Hastings Wilson
(*Harvard Medical School, Boston, USA*)

Transport in Bacteria

Bacteria have been the subject for permeability studies for well over sixty years. In 1903 Alfred Fisher published a monograph describing his studies on the osmotic properties of bacteria. He showed that when bacteria were suspended in concentrated solutions of non-penetrating substances (such as sucrose) water was withdrawn from the interior of the cell and the cytoplasm with its surrounding plasma membrane pulled away from the rigid cell wall leaving a distinct space between the two. This phenomenon, also found in plant cells, was called plasmolysis. Concentrated solutions of penetrating solutes resulted in a transient plasmolysis (due to rapid water movement) followed by the return to normal (as solute and water entered the interior of the cell). With this technique Fisher found that Gram-negative bacteria were largely impermeable to sucrose and most electrolytes but permeable to chloral hydrate and other lipid soluble substances. Besides establishing the permeability properties of these cells this work provided strong evidence for the existence of a plasma membrane separating cytoplasm from the cell wall.

The direct visual demonstration of cell wall and plasma membrane remained for the electron microscope. The cell wall, which contains both