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The inhibition and mechanism of intestinal absorption

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In studying the mechanisms by which substances presented to the intestine are transferred across the mucosal epithelial cell use has been made of a wide range of inhibitors. These may be classified as (1) inhibitors which reduce transfer by blocking specific sites or carriers involved in the movement of substances across the mucosal epithelial cell, (2) compounds affecting specific metabolic pathways concerned with providing energy for active transport, (3) inhibitors which, although not directly interfering with the breakdown of metabolizable substrate to provide energy, dissociate this process from active transport. In this paper several inhibitors are considered and their values demonstrated in understanding the mechanisms of intestinal absorption.

Phlorrhizin

The movement across the small intestine of actively transported sugars, e.g. glucose and galactose, is specificially inhibited by the glycoside phlorrhizin (Fig. 1). Many other inhibitors have been found to reduce this movement but none to exhibit so definite an effect at such low concentrations. Since the initial observation of Nakazawa (1922) the attention of many workers has been directed to the problem of how phlorrhizin inhibits sugar transfer. Glucose absorption in the small intestine and the functionally similar kidney was considered to involve phosphorylation and dephosphorylation in the absorbing cells (see Verzár & McDougall, 1936) and, since phlorrhizin was known to inhibit phosphorylation, Lundsgaard (1933) suggested that this effect might explain the observed reduction in glucose movement. Lundsgaard (1935) later rejected this possibility as higher concentrations of phlorrhizin were required to inhibit phosphorylation than were required to inhibit renal tubular glucose reabsorption. Convincing evidence that the reduction of intestinal glucose absorption is not due to inhibition of alkaline phosphatase was provided by Jervis, Johnson, Sheff & Smyth (1956). Using a phlorrhizin concentration about 1000

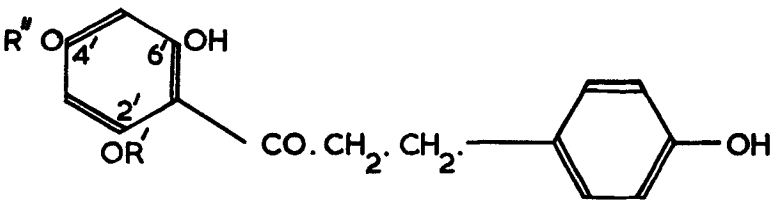

Fig. 1. Structure of phloretin, phlorrhizin and prepared analogues used by Newey, Sanford, Smyth & Williams (1963).

Phloretin	$R' = R'' = H$
Phlorrhizin (phloretin-2′-glucoside)	$R' = \beta$-D-glucosyl, $R'' = H$
Phloretin-4′-glucoside	$R'' = \beta$-D-glucosyl, $R' = H$
Phloretin-2′-rhamnoside	$R' =$ L-rhamnosyl, $R'' = H$

times less than that required to inhibit this enzyme they found a reduction in glucose absorption. Hence the effect of phlorrhizin in inhibiting glucose absorption was well established, but its mode of action remained unknown.

There has been a considerable difference of opinion as to whether phlorrhizin acts as a primary or a secondary inhibitor, in the terminology used by Wilbrandt (1954), i.e. does phlorrhizin directly affect a sugar absorption process or is the effect due to an inhibition of metabolism with a consequent reduction in the amount of energy available for absorption. A considerable amount of information has been published to show the low specificity of phlorrhizin as regards many enzyme systems and this has excellently been reviewed by Lotspeich (1960–1). However, Newey, Parsons & Smyth (1959) found that phlorrhizin inhibits glucose transfer at concentrations having no effect on either the metabolism of endogenous substrate or glucose present initially in the fluid bathing the serosa. It was concluded that phlorrhizin inhibits glucose absorption by acting on a mechanism responsible for glucose movement across the membrane on the luminal side of the mucosal epithelial cell, i.e. phlorrhizin acts as a primary inhibitor.

Recently Diedrich (1963) in the kidney, and Newey, Sanford, Smyth & Williams (1963) in the intestine have made a further approach to the problem of how phlorrhizin acts. This involved a study of the part of the molecule involved in the inhibitory process. In the case of the phlorrhizin molecule the glucosyl moiety was of obvious interest. Jervis *et al.* (1956) had previously shown that the aglycone phloretin is a much less effective inhibitor of intestinal glucose absorption and Larralde, Giraldez & Ron-Noya (1961) had confirmed this observation and extended it to include phloretin phosphate, phloretic acid, phloroglucinol and phlorin, other phloretin derivatives devoid of glucosyl components. Newey, Sanford, Smyth & Williams (1963) prepared several analogues of phlorrhizin in one of which glucose was replaced by rhamnose (shown by Wilson & Crane (1958) not to be actively transported) and in another phloretin-4′-glucoside, glucose was not replaced but attached at a different position on the phloretin group (Fig. 1). The results showed that 5×10^{-5}M and 2×10^{-3}M phlorrhizin inhibited glucose absorption from the mucosal fluid by 64% and 96% respectively while the higher concentration of phloretin-4′-glucoside caused only 60% and of the rhamnoside only 21%

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inhibition. From these results it follows that the strong inhibitory effect of phlorrhizin depends on both the presence and position of the glucosyl unit. It was suggested that phlorrhizin inhibits glucose absorption by competing for the glucose sites of the absorption mechanism and that the configuration required for attachment of the hexose part of the molecule to these sites is similar to that of the hexoses themselves. The concept that phlorrhizin competes with actively transported sugars for absorption sites and the inhibition observed with low concentrations of phlorrhizin suggest that the phloretin part of the phlorrhizin molecule enhances the affinity of the inhibitor for the sugar absorption sites. Two possibilities were suggested. The first was that the phloretin group might bind the glucose part of the molecule more firmly to the glucose absorption site. A further possibility was that because of the different aqueous and lipid solubilities of phloretin and glucose the phloretin part of the molecule might bring about a definite orientation of the glucosyl moiety in relation to the glucose absorption site. The latter possibility gained some support from the comparatively small inhibitions obtained with phloretin-4′-glucoside in which the position of the glucosyl moiety was different to that of phlorrhizin. It is conceivable that the different orientation of glucose to the glucose absorption sites renders the phlorrhizin analogue a less effective inhibitor.

Diedrich (1963) independently synthesized a number of phlorrhizin analogues and compared these compounds with phlorrhizin in their ability to reduce renal glucose reabsorption. He reached similar conclusions to Newey, Sanford, Smyth & Williams (1963). It was found by replacing the glucose part of the phlorrhizin molecule with galactose or 3-o-methylglucose that the inhibitory effect was markedly reduced. Interaction of the glycosidic moiety with the membrane sugar absorption site was visualized, and Diedrich (1963) suggested that at least the hydroxyl groups at C_3 and C_4 of the sugar molecule were involved He suggested that the most stable interaction occurred when the hydroxyl groups were situated in the chemically more reactive equatorial positions. In retrospect it would have been interesting to study the effect of the phloretin-2′-galactoside or phloretin-2′-3-methylglucoside on galactose or 3-methylglucose reabsorption as these analogues would presumably be more inhibitory on the transfer of these hexoses than on glucose.

The view that phlorrhizin competes with sugars for sugar absorption sites is consistent with the observations of Alvarado & Crane (1962) who concluded from kinetic studies that phlorrhizin behaved as a competitive inhibitor of the intestinal absorption of 1,5-anhydro-D-glucitol and of 6-deoxy-D-glucose.

Using the knowledge that phlorrhizin acts by preventing entry at the luminal side of the mucosal epithelial cell, Newey, Sanford & Smyth (1963) studied the spatial and functional relationships of enzyme systems involved in carbohydrate metabolism. Although the ultimate aim in the study of absorption of substances by cells is the identification of cellular activities with definite cytological structures, an important and necessary preliminary stage is to separate spatially different processes within the cell. This approach Newey, Sanford & Smyth (1963) have termed 'functional topography'. The principle of the method was to use intestinal fluid transfer as evidence that glucose either present initially in the fluid bathing

the intestine, or produced by intracellular disaccharide hydrolysis could reach the mechanism on which fluid transfer depends. When glucose or an equimolar concentration of maltose was present initially in the serosal fluid the fluid transfer was stimulated to a similar extent. In the presence of phlorrhizin the fluid transfer stimulated by serosal glucose was not significantly reduced but that stimulated by serosal maltose was abolished. The distribution of glucose formed from maltose in the mucosal fluid, serosal fluid and gut wall showed that phlorrhizin did not interfere with the access of maltose to maltase, but prevented the glucose formed being transferred to the serosal fluid or to the sites of metabolism, and hence the glucose formed was unable to support fluid transfer. From these observations it was concluded that there are three distinct zones of activity arranged in order from the luminal side of the epithelial cell: (1) a discrete compartment of maltase activity, (2) a phlorrhizin-sensitive glucose entry mechanism and (3) a glucose-dependent fluid transfer mechanism and the site of metabolism on which it depends. These conclusions are in agreement with those of Miller & Crane (1961a,b) who, using a different approach, reported that the hydrolysis of disaccharides is predominantly by an intracellular process located in the brush border.

Uranyl nitrate

At least part of the mechanism by which actively transported sugars are absorbed into the blood stream is located close to the border of the mucosal epithelial cell (Newey *et al.* 1959; McDougal, Little & Crane, 1960). In studying this mechanism, Newey, Sanford & Smyth (1965, 1966) have made use of uranyl ions which Rothstein (1962) has shown to exert a surface action in yeast cells, and Ponz (1952) and Ponz & Lluch (1958) have shown to interfere with intestinal hexose absorption. Newey *et al.* (1965) measured the absorption of glucose and galactose in the presence of uranyl nitrate and observed that concentrations (10^{-5}M—3×10^{-4}M) inhibiting glucose entry into the epithelial cell from the mucosal fluid had no effect on galactose entry. In these concentrations uranyl nitrate also inhibited fluid transfer stimulated by glucose present in the mucosal fluid but not by glucose present initially in the serosal fluid. Experiments in which equimolar concentrations (28 mM) of glucose and galactose were present in the mucosal fluid confirmed the observations of Fisher & Parsons (1953) that galactose transfer was inhibited. However, if the concentration of glucose in the mucosal fluid was reduced (5·6 mM) or a high concentration of glucose (111 mM) was present initially in the serosal fluid there was a marked stimulation of galactose transfer. Uranyl nitrate reduced the increase in galactose transfer caused by the low mucosal glucose concentrations but had no effect on that transfer stimulated by serosal glucose.

Two suggestions have been put forward to explain these observations. In one of these two different routes of entry of sugar into the epithelial cell from the intestinal lumen were postulated. One is available to both glucose and galactose and is not affected by uranyl nitrate. The other is available to glucose only, channeling glucose into metabolism, and is blocked by uranyl nitrate. As uranyl nitrate

inhibited fluid transfer stimulated by glucose in the mucosal fluid the uranyl-sensitive route of entry may channel glucose into metabolic pathways on which fluid transfer depends. The route of entry shared by glucose and galactose is at least part of a transfer mechanism able to utilize energy derived from glucose metabolism. In order to stimulate galactose transfer glucose must reach the sites of metabolism without competing with galactose for the entry mechanism. This explains why high mucosal glucose concentrations, or low mucosal glucose concentrations in the presence of uranyl nitrate, inhibit galactose transfer while low mucosal or high serosal glucose concentrations stimulate this transfer. If this scheme is correct then it must be assumed that both pathways of entry into the mucosal epithelial cell are phlorrhizin-sensitive, as low concentrations of this inhibitor abolish glucose entry from the mucosal fluid.

An alternative explanation is the existence in the mucosal epithelial cell of two different metabolic pathways (either biochemically or topographically or in both ways), both available for glucose utilization and which may have different efficiencies in relation to supplying energy for transfer mechanisms and also different sensitivities to uranyl nitrate. In this scheme it is unnecessary to postulate two different entry mechanisms, uranyl nitrate acting by blocking the more efficient pathway, and diverting glucose to the other.

Metabolic inhibitors—sodium fluoride and sodium fluoroacetate

The observation that fluid transfer in the small intestine could be divided into two fractions, one glucose-dependent and the other glucose-independent was made by Barry, Matthews & Smyth (1961). It was found that the former was more important in the jejunum, the latter in the ileum. This suggested that different sources of energy exist in the intestine, and use has been made of a number of metabolic inhibitors to study the relation between these sources and various transfer systems, e.g. dinitrophenol (Fridhandler & Quastel, 1955) and anaerobiosis (Wilson & Vincent, 1955). Sanford, Smyth & Watling (1965) have recently made use of sodium fluoride, an inhibitor of the glycolytic pathway, and sodium fluoroacetate, indirectly an inhibitor of the citric acid cycle through its synthesis to fluorocitrate (Peters, 1957), to investigate the absorption of glucose and galactose. Both these inhibitors have been shown to reduce sugar absorption in the small intestine, the former by Baker, Searle & Nunn (1961) and the latter by Darlington & Quastel (1953). A concentration of 5×10^{-3}M sodium fluoride was found to inhibit glucose metabolism, glucose transfer and fluid transfer but not to prevent the transfer of glucose against a concentration gradient, nor was the transfer of galactose affected. The glucose-stimulated transfer of galactose (Newey *et al.* 1965) was however abolished by fluoride. Fluoroacetate (10^{-2}M) inhibited the movement of galactose when the sugar was present alone in the mucosal fluid but had no effect on the stimulation of galactose transfer by glucose. To explain these results it was suggested that two energy sources were available for certain transfer mechanisms. Energy derived from one source, the citric acid cycle, could be utilized for galactose transfer. This energy is provided by endogenous substrate metabolism and this

appeared to be non-carbohydrate as fluoride was without effect. The other source, aerobic glycolysis, plays an important part in supplying energy for intestinal transfer systems as is shown by the increases in transfer rates brought about in the presence of glucose, these stimulations being abolished by fluoride and unaffected by fluoroacetate.

Summary

The research presented in this paper shows how with the use of several types of inhibitor the complex processes of intestinal absorption are being elucidated. One of the great problems in using inhibitors is to know the specificity of the compounds involved. Certainly such inhibitors as fluoride (Borei, 1945) and phlorrhizin (Lotspeich, 1960–1) in high concentrations have been shown to be of low specificity and to affect many different enzyme systems. However, with careful use of these compounds, coupled with other approaches to the problem of intestinal absorption, much valuable information is being obtained.

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