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The Effects of Phlorizin, 2,4-dinitrophenol, and Oxygen Deprivation on the Intestinal Transport of D-glucose

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Abstract. The effect of various concentrations of phlorizin and 2,4-dinitrophenol, plus the effect of oxygen deprivation on intestinal absorption of D-glucose was investigated. Paired segments of turtle (*Chrysemys picta*) small intestine *in vitro* were used. Preparations were incubated for one hour in a shaking water bath at 30°C in Krebs-Ringer-bicarbonate, with or without inhibitor.

Phlorizin, in all concentrations used, was found to prevent uptake of glucose by the mucosal epithelium. Inhibition also occurred, in a lesser degree, with 2,4-dinitrophenol, and when the system was flushed with nitrogen instead of air.

The absorption of some sugars from the small intestine of warm-blooded animals has been shown to be an energy-requiring process by which the sugar is transported across the mucosal epithelium in a direction opposing the concentration gradient. In recent years this has been a busy field of research and considerable data now exist bearing on the biochemical specificity of the active transport process, its rates of reaction, and effects of a variety of factors on the transport system in mammals (Crane, 1960; Wilson, 1962). Concomitant data for cold-blooded forms, however, do not exist, for only a few workers have investigated intestinal transport in fish, amphibians, or reptiles (Csaky and Fernald, 1960; Fox, 1961; Musacchia, 1960). Recent work at Clarke College has been concerned with transport phenomena in the painted turtle, *Chrysemys picta.* It has been demonstrated that at least three sugars — D-glucose, D-galactose, and 3-methylglucose — are moved across the intestine of this animal against an apparent concentration gradient. (Fox, 1962).

Since active transport, by definition, is dependent upon energy derived from cell respiration, it would appear that compounds which poison metabolic processes should inhibit sugar transport. Studies with warm-blooded animals have justified this assumption. Phlorizin, 2,4-dinitrophenol, cyanide, azide, iodoacetate, and many other compounds cause partial or complete inhibition of the transport process in rats, hamsters, and guinea pigs (Ponz and Lluch, 1955; Darlington and Quastel, 1953). In this report, the effects of various concen-

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trations of phlorizin and 2, 4-dinitrophenol plus the effect of oxygen deprivation on absorption by the turtle are discussed.

Materials and Methods

Living specimens of the painted turtle *Chrysemys picta* were obtained from a supply house. For each experiment two 6-cm segments of the upper part of the intestine were removed, everted and suspended from a narrow glass tube in a 25 ml Erlenmeyer flask. One ml of fluid was placed within the sac (serosal side). The mucosal side was immersed in 10 ml of the same fluid, with or without aeration depending on the experiment. The preparations were incubated in a shaking water bath at 30°C in Krebs-Ringer-bicarbonate without glucose for 30 minutes. These were then transferred to the experimental solution containing 4.5 mg% D-glucose (2.5×10^{-4} M). One segment of each pair served as a control, while known amounts of inhibitor were added to the solution around the second segment. Both segments were incubated for 60 minutes. At the end of this time samples of the mucosal and serosal solutions were analyzed for their sugar concentration by use of the Nelson colorimetric technique or the glucose oxidase enzymatic method (via Worthington Co.)

Concentrations of phlorizin used ranged from 10^{-2}M to 10^{-4}M; 2,4-Dinitrophenol concentrations were from 10^{-1} to 10^{-3}M. In experiments to test the effect of oxygen deprivation, 100% nitrogen instead of air was bubbled through the mucosal fluid.

Results

Table I contrasts sugar transport in control segments with that obtained when different concentrations of phlorizin were added to the media. $\triangle$S and $\triangle$M represent the changes in sugar concentration in the solutions bathing the serosal and mucosal sides respectively. The S/M ratio is the relationship between the sugar concentrations (in mg%) on either side of the intestinal wall at the end of the incubation period. This ration represents the terminal concentration gradient which was established by intestinal transport.

The S/M ratios of the phlorizin experimental controls were relatively low (around 2.0), perhaps due to the fact that these experiments were run during the coldest part of the winter. The fact that number of these animals were parasitized by an intestinal roundworm might be another explanation, since it has been shown that nematode parasitism reduces glucose absorption (Symons and Fairbairn 1963). However, it may be seen in Table 1 that none of the ratios of the inhibited segments reached even this twofold value, and at 10^{-3} M phlorizin

the final serosal value dropped below that of the mucosal, giving an S/M ratio of less than 1.0.

An effect of this inhibitor is apparent when we observe uptake by the mucosal epithelium. None of the phlorizin-inhibited segments showed a net drop in sugar concentration on the mucosal side, whereas in control segments, this decrease in concentration was the prime evidence that transport was taking place. All phlorizinized segments showed an increase in sugar concentration in the mucosal fluid at the end of the incubation period.

Results of transport inhibition by three concentrations of 2,4-dinitrophenol are shown in table 2. Control segments in this series developed an S/M ratio of 3.8, which is near-maximal for this species in one hour. There was increase of sugar on the serosal side in all cases, though it was highest in the control segments. A concentration of 10^{-3} M DNP was found to inhibit sugar uptake by the mucosal epithelium by about 20%; 10^{-2}M DNP gave an 80% inhibition, and at 10^{-1}M DNP no net mucosal uptake could be demonstrated by the methods used. S/M ratios of all DNP-inhibited segments were well below those of the controls.

The effect of lack of oxygen was studied in a third series of experiments as shown in table 3. Again, the mucosal uptake was altered in the experimental segments. The techniques used showed only that, when oxygen was in short supply, the net sugar concentration increased rather than decreased on the mucosal side. Thus, if any uptake did take place, it was masked by loss of reducing sugar from the tissues of the mucosal layer into the adjacent fluid.

Discussion

It has been known since 1932 (Jolliffe et al., 1932) that phlorizin is a potent inhibitor of glucose transport systems. Fisher and Parsons (1950) noted that phlorizin depressed by 84% glucose disappearance from the mucosal fluid of rat intestinal preparations. These workers concluded that both utilization and transfer of glucose were inhibited. Parsons, Smyth, Taylor in 1958 suggested that phlorizin exerts two inhibitory actions: (1) it prevents entry of glucose into the cells on the mucosal side of the intestine, and (2) it interferes with cellular oxidation of glucose. The first effect appeared at concentrations as low as 10^{-6}M while the second was not found until a concentration of 10^{-3} M phlorizin was reached. This agreed with the earlier findings of Jervis et. al. in 1956, and with Newey, Parsons and Smyth in 1959. Further evidence that phlorizin acts primarily to inhibit the entry of sugar into mucosal cells has been presented more recntly by Crane, Lots-

peich and many others (Ponz and Lluch, 1955; Krane and Crane, 1959; Alvorado and Crane, 1962; Lotspeich, 1961).

In the studies described here significant inhibitory effects of phlorizin on glucose absorption were observed at 10^{-4}M. Since no net uptake of sugar by the mucosal epithelium was detected in any segments exposed to phlorizin, it may be postulated that the entry of sugar into the mucosal cells was prevented to such a degree that "leakage" of sugar from the tissue into the mucosal fluid proceeded at a greater rate than did uptake. An alternate hypothesis may also be suggested. Since the phlorizin effect was maximal at 10^{-3}M in these preparations, only the second effect, that is, the inhibition of cell respiration may be operative. Shapiro (1947) stated that phlorizin slowed the rate of lactate formation from glucose and the aerobic oxidation of pyruvate and citrate. Thus, any process dependent on cellular energy would be affected.

Phloretin, the agucone of phlorizin, has been reported as being less active than the glycoside itself as an inhibitor of glucose absorption (Jervis et al., 1956; Chan and Lotspeich, 1962). Studies are now in progress in our laboratory to observe the effect of this compound on sugar transport in *C. picta*. In this way, information as to the molecular structure critical for inhibition may be obtained.

The effects of 2,4-dinitrophenol provide evidence that active absorption of glucose in the turtle as well as in warm-blooded forms, is an energy-requiring process. DNP acts to uncouple the respiratory activity of cell metabolism from oxidative phosphorylation (Loomis and Lipmann, 1948), thus bringing about a deficiency of ATP in the cell. An 80% inhibition of uptake by turtle intestine was obtained at 10^{-2}M DNP, and no mucosal uptake could be demonstrated at 10^{-1}M DNP. It may be concluded, therefore, that glucose transport here depends upon the energy resident in ATP. It may be noted, however, that rather high concentrations of inhibitor were required for near-total blockage of the transport process. This may indicate that pathways other than oxidative phosphorylation may be utilized by this animal to drive its transport mechanism.

Further evidence that an oxidative pathway is utilized was the finding that mucosal uptake of glucose by turtle intestine was at least partially inhibited by a lack of oxygen in the system. Similar effect on rat and hamster sugar transport have been observed by Wilson and Wiseman (1954) and others (Wilson and Vincent, 1955; Darlington and Quastel, 1953).

In conclusion, it may be stated that glucose absorption by the turtle is a phlorizin-sensitive process, in which at least one

component is energy-requiring and normally dependent upon an intact oxidative metabolism.

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