Proceedings of the Iowa Academy of Science

Volume 69 | Annual Issue Article 91

1962

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Active Transport of D-galactose and 3-0-methylglucose by Turtle Intestine[1]

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Abstract. Isolated segments of the intestine of turtle (*Chrysemys picta*) were found to transport D-galactose and 3-0-methyglucose against a concentration gradient *in vitro* at 30° C. Uptake of both sugars by the mucosal epithelium was directly dependent upon the concentration of sugar in the external fluid. Evidence indicated that 3-methylglucose is not metabolized by turtle intestinal tissue while D-galactose is. Both sugars were absorbed well by upper or middle intestinal segments, but 3-0-methyglucose was absorbed at a faster rate than D-galactose. It was concluded that neither substitution of the hydroxyl group at the third Carbon of the ring by a methyl group, nor reversal of hydroxyl and hydrogen groups at the fourth position, will block the active transport of molecules by the mucosal epithelium of this species.

The absorption of foods by the small intestine has been found to be due in large part to a process of active transport. It has been shown by many investigators since the experiments of Barany and Sperber (1939) that some sugars are absorbed from the intestinal lumen against a concentration difference. Active transport has been reported as operative in the intestinal absorption of some 14 different sugars with rats or hamsters as the experimental animals (Crane, 1960; Wilson and Landau, 1960). Recent work on cold-blooded forms has shown that, in these animals as well, active transport is a mode of sugar absorption (Casky and Fernald, 1960; Fox, 1961a,b).

In previous work isolated intestinal segments of the painted turtle *Chrysemys picta* were used to demonstrate *in vitro* absorption of D-glucose against a concentration difference (Fox, 1961b). When these preparations were incubated with equal concentrations of sugar on either side of the wall, the concentration of glucose on the mucosal side dropped while the serosal concentration rose. A similar study with the use of C^{14}-D-glucose established without doubt the movement of sugar molecules from the mucosal side to the serosal side against a concentration difference (Fox, 1961a).

In preliminary investigations it was found that the system was complicated by two factors: (1) "leakage" of glucose from the tissues into the surrounding medium, and (2) very low recovery

[1] Supported by grant project PX 215 of the Iowa Academy of Science (via AAAS) and a grant from the Society of the Sigma XI.
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of sugar at the end of the incubation period (less than 10%). The first problem was solved by a modification of the procedure (described below). The second difficulty was met by using sugars which are less readily utilized by the cells of the intestinal wall. Two such compounds are D-galactose and 3-0-methylglucose.

The present study was designed to investigate the effect of concentration on the active transport of these sugars. The location of the transport process was also studied, since a number of workers (Fisher and Parsons, 1953; Crane and Mandelstam, 1960) have reported maximal absorption of D-galactose by the mid-portion of hamster intestine.

Materials and Methods

Specimens of the painted turtle *Chrysemys picta* (6-inch shell length) were obtained from the Lemberger Co., Oshkosh, Wis. The method for preparation of intestinal segments *in vitro* was essentially that described by Crane and Wilson (1958), which makes use of everted sacs. One end of a 5-6 cm segment was tied and the other was attached to the tip of a short pipette. The preparation was immersed in 10 ml of the desired sugar solution in a 25 ml Erlenmeyer flask. A known volume (0.6 ml) of the same solution was pipetted into the "serosal lumen." This type of arrangement tests for active transport since there is no net movement of sugar molecules across the intestinal wall in either direction unless active transport processes are in operation.

A stream of air was pumped into the mucosal solution during the entire time of incubation. This ensured the mucosal epithelium of adequate oxygenation.

As mentioned above, an endogenous sugar source caused "leakage" of sugar into the flask medium. In order to minimize this source of error, all preparations were incubated for 15 minutes in plain Krebs-Ringer-bicarbonate, then this fluid was drained and replaced with the test-sugar solution. A significant amount of glucose was found in the Krebs-Ringer solution upon analysis, and 15 minutes was shown to be a sufficient time to remove all measurable amounts of glucose from the intestinal wall.

The test-sugar solutions were made by adding aliquots of a stock sugar solution to known volumes of Krebs-Ringer-bicarbonate having a sodium chloride concentration of 0.7% and a pH of 7.4. All preparations were run for 60 minutes in the sugar solution, while being shaken gently in a Dubnoff water bath at 30°C. Concentrations of 5×10^{-4} M, 2.5×10^{-4}, and 1.25×10^{-4} M were used for these experiments. They are referred to below in terms of mg%, i.e. milligrams of sugar per 100 ml. This range of concentrations was chosen because optimal transport of D-glucose by

similar preparations had been shown with the use of 4.5 mg% D-glucose (Fox, 1961b).

Samples of 0.5 ml taken from the mucosal and serosal fluids before and after incubation were analyzed for total reducing sugars by the Nelson-Somogyi colorimetric technique (Nelson, 1944). Samples at random were checked for the presence of glucose, using a glucose oxidase method which is specific for that sugar (Worthington Co.).

Results

Results of these studies show that both D-galactose and 3-methyl-glucose are actively transported across the intestinal wall by *in vitro* preparations of *Chrysemys picta* small intestine.

Transport of D-galactose. Figure 1 illustrates the active transport of D-galactose when a concentration of 4.5 mg% (2.5×10^{-4} M) was used. It shows that with segments taken from both the upper and mid-portions of the GI tract, the mucosal fluid concentration of galactose fell and the serosal fluid concentration rose during the incubation time.

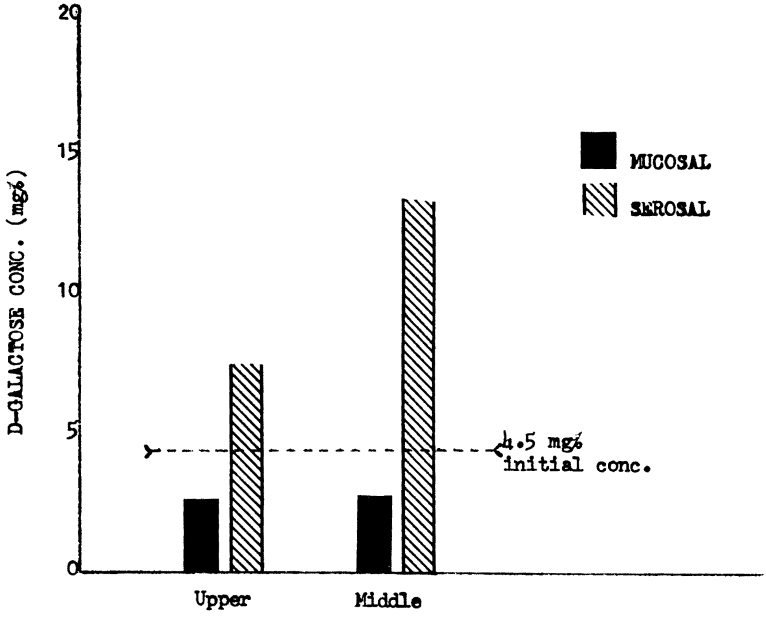

Figure 1. D-galactose transport by everted sacs of turtle intestine from upper and middle regions of GI tract. Incubated 60 min. at 30° C. (average of 6 sacs).

Table 1 shows data for absorption of three galactose concentrations. As the initial concentrations increased, more sugar was absorbed by segments of upper small intestine, while the amount

of sugar appearing in the serosal medium was not significantly different at the three concentrations. In segments taken from the mid-part of the small intestine, uptake was not significantly different from the upper segments except in the case of the 9.0 mg% solution. Increase on the serosal side appeared to be higher, but this may be explained by the thinner wall of the segments in this region of the tract.

Table 1. Transport of D-galactose by everted segments of turtle intestine from upper and middle GI tract. Segments were incubated 1 hr. at 30° C.

Galactose	Mucosal Uptake μM/gm/hr.		Serosal Increase μM/gm/hr.		Serosal/Mucosal Ratio	
conc. mg%	Upper	Middle	Upper	Middle	Upper	Middle
2.25	±0.169[1] 0.42 (8)[2]	±0.022 0.35 (6)	0.13 (10) ±0.024	0.40 (7) ±0.061	4.1 (10) ±0.180	**8.7 (7) ±0.744**
4.5	1.03 (6) ±0.252	1.05 (4) ±0.370	0.10 (6) ±0.018	0.30 (4) ±0.013	2.8 (10) ±0.323	**5.0 (7) ±0.536**
9.0	1.30 (8) ±0.184	0.65 (4) ±0.210	0.15 (8) ±0.040	0.25 (4) ±0.00	2.0 (10) ±0.118	**2.1 (4) ±0.604**

[1] ± values are the standard errors of the means.
[2] Numbers in parentheses refer to the number of segments.

Table 1 also shows S/M ratios. These are the ratios between serosal and mucosal solutions at the end of the run. It is observed that segments from the mid-gut established higher S/M ratios in one hour than did segments from the upper intestine.

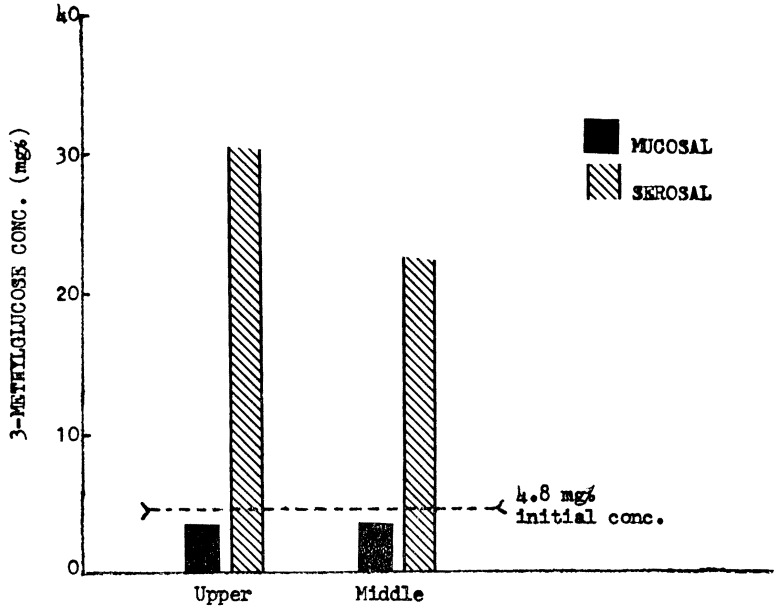

Figure 2. 3-0-methylglucose transport by everted sacs of turtle intestine from upper and middle regions of GI tract. Incubated 60 min. at 30° C. (average of 6 sacs).

Transport of 3-0-methylglucose. Figure 2 demonstrates the active transport of 3-methylglucose by the preparations. The mucosal

concentrations dropped and the serosal concentrations rose above the initial level.

Table 2 gives data for this series of experiments. Here one may observe increased uptake in both upper and middle segments as higher concentrations of the sugar were used. Segments from both parts of the tract absorbed the sugar at an equally rapid rate. The concentration of the serosal fluid also showed greater increase with higher initial concentrations in all average values except one.

Table 2. Transport of 3-0-methylglucose by everted segments of turtle intestine from upper and middle GI tract. Segments were incubated 1 hr. at 30° C.

3-0-methyl-glucose	Mucosal Uptake μM/gm/hr.		Serosal Increase μM/gm/hr.		Serosal/Mucosal Ratio	
conc. mg%	Upper	Middle	Upper	Middle	Upper	Middle
2.4	0.35 (6)[2] ±0.013[1]	0.40 (4) ±0.186	0.35 (6) ±0.132	0.30 (4) ±0.083	7.0 (5) ±0.568	7.7 (4) ±0.771
4.8	0.80 (5) ±0.134	0.78 (4) ±0.177	0.85 (6) ±0.143	0.56 (4) ±0.071	9.2 (6) ±0.974	6.8 (4) ±0.701
9.7	2.0 (6) ±0.214	1.58 (4) ±0.543	1.7 (6) ±0.214	0.45 (4) ±0.065	11.1 (6) ±0.580	3.6 (4) ±0.850

[1] ± values are the standard errors of the means.
[2] Numbers in parentheses refer to the number of segments.

Serosal-mucosal ratios after 60 minutes were high; the serosal fluid concentrations rose to eleven times that of the mucosal fluid.

Discussion

Movement of D-galactose and 3-0-methylglucose across the intestinal wall (*in vitro*) against a concentration difference has been demonstrated in the turtle by these investigations. This, by definition, indicates that active transport is the mechanism involved (Rosenberg, 1954).

Active transport of D-galactose has been reported by several workers who used intestinal segments and cell preparations from the rat and hamster (Wilson and Vincent, 1955; Crane and Mandelstam, 1960). The active transport of 3-0-methylglucose by warm-blooded animals was described by Campbell and Davson (1948) and by Wilson and Vincent (1955), and a similar finding was made by Csaky and Fernald (1960) using the frog *Rana pipiens*. The last two workers demonstrated that transport of this sugar is a temperature-dependent process in the frog and is effected by the initial intestinal concentration (*in vivo*).

It may be noted that the percentage of recovery of 3-methylglucose in the serosal fluid was very high—up to 100% (table 2), while recovery of D-galactose was about 10%, a value comparable to D-glucose recovery. This finding provides indirect evidence that 3-methylglucose is not metabolized by turtle intestinal tissues, while D-galactose is. Csaky and Glenn (1957) analyzed 3-methylglucose urinary excretion by rats and concluded that this molecule "does not undergo any metabolic change during its

passage across the mucosa" (p. 161). If this is true, it becomes quite apparent that active transport of a molecule does not depend upon tissue metabolism of the absorbed compound, and the energy for the transport process must come from another source.

The work reported here does not give evidence that D-galactose is taken up better by the middle than the upper part of the small intestine. Similarly, 3-0-methylglucose appears to be absorbed equally well by upper or middle intestinal segments.

A comparison of rates of absorption of these two sugars shows that 3-methylglucose is absorbed faster than D-galactose by turtle intestine (in *vitro*) and a steeper concentration gradient is established in 60 minutes.

Finally, we may conclude that neither substitution of the hydroxyl group at the third Carbon of the ring by a methyl group, nor reversal of the hydroxyl and hydrogen groups at the fourth position, will block active transport of molecules by the mucosal epithelium of this species.

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