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Active Transport of Sugars by the Intestine of Snail (*Cryptomphalus hortensis* Müller) *

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Sugar transport by sacs of everted intestine of snail have been measured *in vitro* at 30° C. D-galactose, D-glucose and 3-O-methylglucose were actively transported against a concentration gradient from the mucosal to the serosal compartment. The transport of these sugars was inhibited by 5×10^{-8} to 10^{-6} M phlorizin. L-arabinose was also accumulated in the serosal compartment against a concentration gradient; in this case, transport was not affected by phlorizin. The snail intestine did not show any ability for D-fructose active transport but there was a clear uptake of this sugar by the tissue. The O_2 uptake of the snail intestine was not significantly affected by the presence of either sugars or phlorizin.

Active transport of sugars by the intestine appears to be a common feature in vertebrates (4, 12); it has also been demonstrated in some invertebrates (12). However, very few references are found in the literature regarding intestinal absorption in mollusca. It is usually assumed that intestinal absorption in these animals takes place in the digestive gland (6, 17), with exception pointed out by BIDDER (3) in four species of cephalopods which carry out the absorption in the coecal sac and intestine.

LAWRENCE and LAWRENCE (8), have demonstrated that active transport of sugars takes place in the intestine of the chiton, *Cryptochiton stelleri*. Also TRITAR and PERES (14) have revealed that the intestine of the cephalopod *Eledone moschata* absorbed some aminoacids against a concentration gradient.

In this paper the capacity for active transport of sugars by snail intestine (*C. hortensis*, Gastropod, Pulmonate) has been demonstrated, using the everted sac method *in vitro*. This transport exhibits very interesting features.

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Materials and Methods

Snail specimens (*Cryptomphalus hortensis* Müller) weighing approximately 10 g including the shell, were used in all the experiments. They were reared in cages on a diet of lettuce.

After removing the shell the visceral mass was separated from the head and foot. By very careful dissection the intestine was isolated from the digestive gland to the rectum and it was washed with saline solution and everted with the help of a thin glass rod following the method of WILSON and WISEMAN for mammals (18). Only one sac from each intestine was prepared having a length of 3.5 cm and a volume of 0.045 ml approximately.

MENG's saline medium for snails (9), was used in all the experiments, substituting $NaHCO_3$ by NaCl maintaining osmolarity. It was buffered with Tris-HCl (10) at *p*H 7.4. When used, sugars and phlorizin were dissolved in this medium.

Sacs, filled with the solution, were then introduced in 1 ml of the same medium in a small Warburg's flask (7 ml) at 30° C, 100 shakes per minute and 3 cm of amplitude, in O_2 atmosphere for 1 hour. Sacs were weighed empty and full, before and at the end of the incubation. The different analyses were performed in aliquots taken from the serosal or mucosal side mediums.

O_2 uptake was determined by the direct method of WARBURG (16) after UMBREIT *et al.* (15).

Sugars were determined by the method of NELSON-SOMOGYI (11, 13). Glucose was usually determined by an enzymatic method using glucose-oxidase (7).

The ability for active transport has been expressed as a Sf/Mf ratio, Sf and Mf being the final concentration of the sugar in the serosal and mucosal side mediums respectively.

The histological observations by optical microscopy were made after Carnoy's fixation in 5 μ thick slices stained with hematoxylin-eosine.

Results

Histological observations. The intestinal structure (fig. 1) was not very different from that of other invertebrates. The inner (luminal) side is covered with the cylindrical epithelium which increases in thickness and pseudostratification in the distal tract. This epithelium is held in a thin connective layer, and coated with the muscular layer. The epithelial cells of the proximal tract possess cillia and some cells have apical vacuoles. In the mid-tract there are cells with apical vacuoles, other cells with a striated plate, and some mucosecretory cells. In the distal tract microcrypts may be observed showing cells with plain striated plates which could be microvilli, and the rest of the epithelium is very abundant in secretory cells with apical vacuoles.

In the proximal tract the muscular layer is thin and discountinuous and at the end it is well developed with longitudinal and circular layers.

Sugar transport. The results for different sugars are shown in table I.

With D-galactose the Sf/Mf ratio was greater than one. This indicates that the sugar was accumulated in the serosal side against a concentration gradient; therefore an active transport may be assumed. With initial sugar concentration of 5 mM the gradient developed in sixty minutes (1.61) was greater than in thirty minutes (1.20) and it did not change when the incubation time was prolonged to ninety minutes (1.57). Therefore, a sixty minutes time was adopted for the experiments. The smaller the initial concentration the higher was the final gradient. It reached values of up to 3.39 when the sacs were incubated with 1 mM galactose.

Also 3-O-methylglucose was actively transported to the serosal, but the final

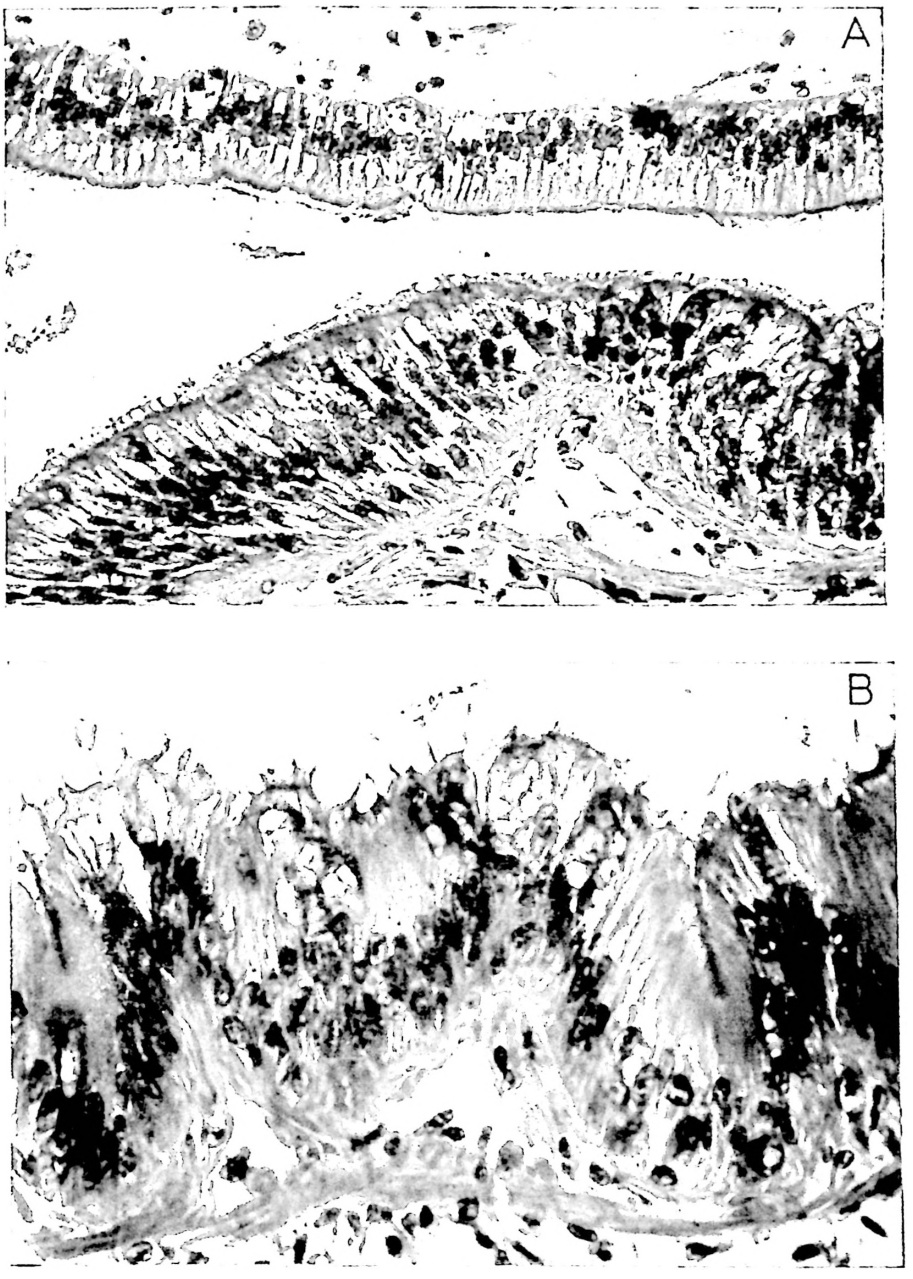

Fig. 1. *Cross-section of snail intestine of* Cryptomphalus hortensis *Müller (5 μ slices, Hematoxylin-Eosine).*
A) Proximal tract, ×200. B) Distal tract, ×320.

Table I. *Active transport of sugars by the intestine of snail* (C. hortensis *Müller*). The intestinal sacs were incubated at 30° C in O_2 atmosphere for one hour. The initial concentration of sugars in the mucosal and serosal sides were equal. Mean values with their S.E. are given.

Sugar [mM]	No of animals	Sf/Mf
D-galactose		
5	118	1.61±0.03
2.5	28	2.01±0.15
1	8	3.39±0.39
3-O-methylglucose		
5	4	1.31±0.15
2.5	12	1.79±0.24
L-arabinose		
5	12	1.26±0.08
1	10	3.39±0.27
D-fructose		
5	4	0.45±0.03
2.5	7	0.52±0.05
D-glucose		
5	19	0.91±0.04
2.5	25	1.17±0.05
1	9	4.96±0.46
D-glucose (+ D-fructose 10 mM)		
5	23	1.38±0.05

gradients were smaller than with D-galactose. With 5 mM L-arabinose, the gradient was small, but when the initial concentration was 1 mM manifest gradients were developed.

With 5 and 2.5 mM D-fructose, active transport was not observed. The serosal concentration did not increase througout the experiments, but on the contrary it dropped to about 50 %. This suggests a utilization of the sugar by the tissue from the serosal side. This uptake seems to be greater when the initial concentration of fructose is increased.

With D-glucose the experiments were carried out with 5, 2.5 and 1 mM initial concentrations. With 1 mM concentration the Sf/Mf ratio reached values as high as 5, revealing an active transport of glucose. However, with 2.5 mM concentration the final gradient was smaller, and with that of 5 mM no gradient was produced and even the glucose serosal concentration decreased. These results led us to think that this sugar was perhaps metabolized by the tissue masking thus the active transport. For this reason in some experiments the sacs were incubated in a medium with 5 mM glucose and 10 mM fructose. Under these conditions a clear glucose gradient appeared with an accumulation of the sugar in the serosal side.

Oxygen uptake. The mean value of the oxygen uptake for 147 intestinal sacs, incubated in a saline medium with 5 mM galactose was 55.33±1.62 μl O_2/100 mg w.w. (3.20±0.10 μl O_2/mg dry w.), the tissue water amounting 82.71 % approximately. There were no significant differences between this value and those measured on the different conditions corresponding to the experiments showed in table I. There were also no differences in O_2 uptake when intestinal sacs were incubated in the absence of sugars.

Phlorizin inhibition. Table II shows the results obtained when phlorizin, a well know inhibitor of the active transport of sugars in other animals, was present in the medium at various concentrations. Starting with 5×10^{-8} M or 10^{-7} M concentrations, phlorizin was a strong inhibitor of active transport of 5 mM galactose. Moreover, at a 10^{-6} M concentration it also abolished active transport of 1 mM glucose. The inhibitory effect on the transport of 2.5 mM 3-O-methylglucose was rather low. On the contrary, 10^{-5} M phlorizin did not affect at all L-arabinose transport.

The presence of phlorizin did not affect significantly oxygen consumption by the intestine.

Discussion

The results show that intestine of the *C. hortensis* Müller carries out active trans-

Table II. *Effect of phlorizin on the active transport of sugars by the intestine of snail* (C. hortensis *Müller*).

The intestinal sacs were incubated for one hour at 30° C in O_2 atmosphere. Initial concentrations of phlorizin and sugars were equal on both sides. Mean values with their S.E. are given. Number of experiments are given in parenthesis. Differences with respect to the control value analyzed according to the Student's *t* method.

Phlorizin [M]		Sf/Mf	P
D-galactose 5 mM			
—	(118)	1.61±0.03	—
10^{-8}	(8)	1.63±0.12	< 0.9 *
5×10^{-8}	(12)	1.10±0.04	< 0.001
10^{-7}	(17)	0.85±0.03	< 0.001
10^{-6}	(18)	1.15±0.05	< 0.001
10^{-5}	(18)	0.90±0.03	< 0.001
10^{-4}	(20)	1.07±0.06	< 0.001
3-O-methylglucose 2.5 mM			
—	(12)	1.79±0.24	—
10^{-6}	(10)	1.17±0.04	< 0.05
D-glucose 1 mM			
—	(9)	4.96±0.46	—
10^{-6}	(8)	0.83±0.09	< 0.001
L-arabinose 5 mM			
—	(12)	1.26±0.08	—
10^{-5}	(4)	1.13±0.05	< 0.5 *
10^{-4}	(11)	1.11±0.06	< 0.2 *
L-arabinose 1 mM			
—	(10)	3.39±0.27	—
10^{-5}	(8)	3.37±0.45	< 0.9 *

* = not significant.

port of sugars. The microscopic morphology reveals the features of an absorbent epithelium.

Among several sugars tested, D-fructose was the only one not actively transported by the snail intestine. The serosal concentration of this ketohexose not only did not increase during incubation, but on the contrary it diminished. Moreover this decrease in fructose concentration inside the sac was not compensated by an increase in the mucosal medium.

Because the volume of water in the tissue was very small, the disappearance of fructose cannot be accounted for its diffusion to the tissue. It may be concluded then that fructose could be metabolized by the cells; the concentration of sugar diminishes in the serosal medium because the volume is far smaller in this compartment. As the quantity of fructose which disappears from serosal is greater when fructose initial concentrations are also greater, it might mean that diffusion and utilization by the tissue is enhanced by an increasing gradient.

In experiments with D-galactose, D-glucose, 3-O-methylglucose and L-arabinose the Sf/Mf ratio is greater than one. This strongly suggests that all these sugars are actively transported by the snail intestine as there was no significant change in serosal water volume. The positive results with L-arabinose, which was also clearly accumulated in serosal, are unusual if compared with the negative ones from many different authors with other species mainly vertebrates (5).

For all the sugars the Sf/Mf ratio, was so much higher when lower the initial concentrations. The same fact has been observed in preparations of everted sacs from other animals. Besides other possible factors the increase of the serosal concentration for the same amount of sugar transported from mucosal to serosal, will be obviously more apparent when lower is the initial concentration.

With D-glucose the Sf/Mf ratio was considerably high (4.96) when initial concentration of 1 mM was used. It was lower with 2.5 mM and less than one with 5 mM. These results could be explained as for fructose by diffusion from the serosal side to the extracellular space followed by sugar uptake by the tissue. If also the glucose uptake is greater at a higher concentration, it may be understood that with 1 mM glucose initial concentration a clear active transport takes place, whereas with that of 2.5 mM, almost no gradient could be observed and with that of 5 mM

the Sf/Mf becomes less than one. The experiments with 5 mM glucose and 10 mM fructose provide evidence for an active transport of glucose against a concentration gradient, because under this condition the fructose may be preferently metabolized.

Phlorizin inhibits active transport of D-galactose by snail intestine. This animal species shows a high sensitivity to the inhibitor. Even at concentrations as low as 5×10^{-8} M, phlorizin completely blocks active transport of galactose. At 10^{-6} M concentration, phlorizin also abolishes the active transport of the glucose and the Sf/Mf ratio for 3-O-methylglucose is greatly diminished. Nevertheless phlorizin even at 10^{-5} M or 10^{-4} M concentrations does not affect L-arabinose active transport.

The O_2 uptake was not affected by phlorizin. The inhibitory effect of this substance on the active transport of sugars cannot be explained through an action on the respiratory metabolism, but to a direct action on the transport system, probably because of the competition between the sugar and phlorizin with respect to the carrier, as it has been shown in other species (1).

Under this hypothesis, it may be proposed two transport systems: one phlorizin sensitive for D-galactose, D-glucose and 3-O-methylglucose and another one phlorizin insensitive, for L-arabinose. A similar coexistence of transport systems for sugars with different properties has been recognized in other invertebrates (2, 8).

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