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Active Transport of Sugars in Tortoise Intestine

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The tortoise intestine capability for active transport of sugars has been studied *in vitro* at 30° C, using labelled sugars. A release of glucose from the glycogen stores of the intestinal wall to the medium took place throughout the incubation period of the sacs. An active transport of ^{14}C-D-glucose against a concentration gradient from the mucosal to the serosal compartment was evident, whereas no such activity could be detected for ^{14}C-D-galactose. The tissue oxygen uptake was 36 % higher with glucose than with galactose in the medium.

Active transport of sugars by the intestine has been well demonstrated in mammals (3, 4, 14, 15) and other animals both *in vitro* and *in vivo*. Few references are found, however, in reptile intestinal absorption literature. Fox (5, 6) has proved the existence of active transport of glucose, galactose and 3-O-methylglucose in the intestinal wall of the turtle *Chrysemys picta*. GIORDANA *et al.* (7, 8) observed Cl^- and Na^+ transport in the intestinal epithelium of *Testudo graeca*. BAILLIEN *et al.* (1) measured transmucosal and transerosal potentials in the small intestine epithelium of *Testudo hermanni hermanni* Gmelin.

The results reported in this paper show that the intestine of the tortoise (*Testudo hermanni robertmertensi* Wermuth) exhibits its ability for the active transport of glucose, but not for that of galactose.

Materials and Methods

Specimens of the tortoise *Testudo hermanni robertmertensi* Wermuth, with 10-17 cm in shell lengths and variable weights, were used, kept for a few days on lettuce diet.

After decapitation, the shell was opened and the small intestine excised, washed with saline solution and everted with the help of a glass rod after WILSON and WISEMAN's method for mammals (20) Four of five sacs of about 4 cm were prepared from the intestine of each specimen.

All the experiments were conducted in

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a modified Krebs-Ringer saline medium (18) with a sodium chloride concentration of 7 g/l, and with Tris-ClH (12) at pH 7.4, as a substitute of the phosphate buffer. Sugars were then dissolved in this Krebs-Ringer-Tris medium (KRT).

Intestinal sacs were filled with sugar solution, and then suspended in 4 ml of the same medium in Warburg's flasks at 30° C for 1 hour. Sacs were weighed empty and full, before and after incubation time.

O_2 uptake was measured by the direct method of WARBURG (19).

Natural sugars were determined by the method of NELSON-SOMOGYI (13, 17); glucose was further determined by the use of glucose-oxidase (10); ^{14}C-sugars, by a liquid scintillation counter (PPO, POPOP and toluene) (2, 9) and glycogen from the intestinal wall samples, by the anthrone reagent (16).

The ability for active transport has been expressed as the S_f/M_f ratio, S_f and M_f being the final sugar concentrations in the serosal and mucosal compartments.

Results

Intestinal glycogen. A large glycogen reserve was found in the wall of the tortoise small intestine, ranging from 2-5 mg/g w.w. in the various segments, cut and chemically digested immediately upon removal from the animal. The amount of glycogen depended on the weight of the animal and on the intestinal region: greater in proximal than in distal. The glycogen distribution contents along the intestine of a tortoise specimen is shown in figure 1.

Glucose released by the intestinal tissue. After incubation of intestinal fragments in sugar-free KRT medium, a reducing substance released from the tissues was detected and identified as D-glucose through paper chromatography. The amount of released glucose depended on the incubation time, reaching ater 15 and 75 minutes values of 0.487 ± 0.11 and 0.933 ± 0.17 mg/g w.w. tissue respectively (fig. 2).

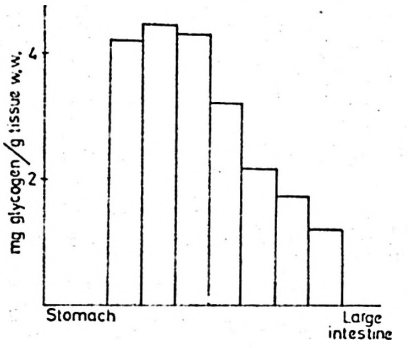

Fig. 1. *Glycogen reserves along the small intestine of the tortoise* T. hermanni robertmertensi *Wermuth.*
The small intestine of a single specimen was cut in seven adjacent fragments and the glycogen content from each was separately determined.

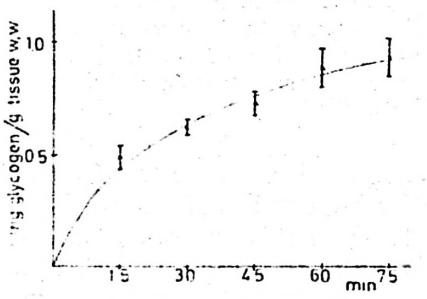

Fig. 2. *Glucose release from the intestinal tissues of the* T. hermanni robertmertensi *Wermut during Incubation.*

Transport of D-galactose. Former experiments on D-galactose transport resulted in S_f/M_f values higher than 1, suggesting an active transport of this sugar from mucosal to serosal (table I, without preincubation). However, a miscalculation due to the released glucose could be expected, since only the Nelson-Somogyi method was used.

To minimize this source of error, other experiments were conducted with empty

Table I. *Active transport of sugars by the tortoise* (T. hermanni robertmertensi *Wermuth*) *intestine.*

The everted intestinal sacs were incubated at 30° C in O_2 atmosphere for one hour with the same initial sugar concentration in the KRT medium at both sides. S_f and M_f, final concentrations of sugar in the serosal (S_f) and mucosal (M_f) compartments. Mean values are given with their standard error. Number of experiments in parenthesis.

[Sugar] mM	Condition	S_f/M_f
D-galactose		
0.5 (16)	Without pre-incubation	1.80±0.28
0.5 (19)	After 15 min pre-incubation in KRT	2.84±0.14
0.5 (32)	After 45 min pre-incubation in KRT	2.37±0.09
5.0 (17)	Discounting glucose released from the tissue	1.37±0.05
2.0 (23)	Discounting glucose released from the tissue	1.53±0.12
0.5 (12)	Discounting glucose released from the tissue	1.80±0.22
D-galactose-1-^{14}C		
0.5 (18)	Without pre-incubation	1.00±0.02
2.0 (12)	Without pre-incubation	0.96±0.01
D-glucose(u)-^{14}C		
0.5 (40)	Without pre-incubation	2.53±0.25
2.0 (13)	Without pre-incubation	1.53±0.09
5.0 (11)	Without pre-incubation	1.54±0.10

sacs pre-incubated for 15 or 45 minutes in sugar-free KRT solution, in order to reduce glucose release before their transfer to the 0.5 mM galactose medium. However, at the end of the experiments the calculated values for S_f and M_f were higher than those of the initial galactose concentration, evincing glucose release, and invalidating the apparently developed gradients.

Active transport of galactose was further suggested when higher values for S_f than for M_f were apparently obtained after released glucose had been enzymatically determined and the results substracted from the Nelson-Somogyi absorbance values. Nevertheless, this procedure was not found sufficiently reliable, and the use of labelled sugars was adopted.

Experiments clearly reveled that ^{14}C-galactose was not actively transported by the tortoise intestine. The S_f and M_f values for 0.5 and 2 mM initial sugar concentrations did not change throughout the experiments, and no serosal/mucosal gradient was observed in any case.

Active transport of D-glucose. Table I shows the data for D-^{14}C-glucose transport with initial concentrations of 0.5, 2 and 5 mM on both compartments. After 60 minutes, S_f/M_f ratios of 2.35, 1.53 and 1.56 were obtained. The developed serosal/mucosal gradient depended almost linearly on the incubation time (0.5 mM glucose, 30-90 min).

Oxygen uptake. The mean values for oxygen uptake when the intestinal sacs were incubated in saline medium with 0.5 mM galactose or 0.5 mM glucose were 14.88 ± 0.54 and 20.35 ± 0.45 μl O_2/100 mg w.w. respectively. Thus, a 36 % increase was observed in the presence of glucose, whereas no significative differences were found in the presence or absence of galactose. The developed glucose gradients for the various tested sugar concentrations were not in simple ratio to the corresponding oxygen uptake.

Discussion

The results have shown that the intestine of *T. hermanni robertmertensi* Wermuth is able to perform active transport of D-glucose but not of D-galactose.

The fact that apparent S_t/M_t values higher than 1 unit were found on using non-radioactive galactose, can be explained as mistakes in the calculations due to glucose released from the tissues into both mucosal and serosal compartments during incubation. The inability of the tortoise intestine for active transport of galactose is an unusual finding if compared with many animal species, especially mammals. However, a specific active transport of glucose, not galactose, has also been referred in the proximal portion of the intestine of *Chryptochiton stelleri* (11).

The values of the S_t/M_t ratio in the experiments with labelled glucose are high enough to prove its transport against a concentration gradient. The highest gradient was found when the initial glucose concentration was lowest, as usually observed in other animal species. Evidently the lower the initial concentration, the greater the final will be if an equal quantity of sugar is transferred to the small serosal compartment.

The oxygen uptake by the intestinal tissues was about 36 % higher in the presence of 0.5 mM glucose than in the presence of 0.5 mM galactose or in the absence of sugars. This difference led to the assumption that a stimulation of tissues respiration was produced as a consequence of the metabolic utilization of glucose, as it is usually observed in animal tissues in the presence of easily metabolizable sugars, or to supply the required energy for the active transport processes.

Resumen

Se ha estudiado la capacidad del intestino de tortuga para el transporte activo de azúcares, *in vitro*, a 30° C. Durante la incubación de los sacos tiene lugar una cesión de glucosa al medio procedente de las reservas de glucógeno de la pared del intestino. Utilizando azúcares marcados, se ha podido evidenciar el transporte activo de C^{14}-D-glucosa contra un gradiente de concentración del compartimento mucosal al serosal. El intestino de tortuga no mostró en cambio capacidad para el transporte activo de C^{14}-D-galactosa. El consumo de oxígeno por el intestino era un 36 % mayor cuando había glucosa en el medio que cuando había galactosa

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