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Competitive Kinetics of Sugar Active Transport in Snail Intestine

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Everted intestinal rings of the snail *Cryptomphalus hortensis* accumulate labelled sugars against a concentration gradient. The active transport of D-galactose (K_T = 3.6 mM) is competitively inhibited by D-glucose (K_i = 8.2 mM) and by 3-0-methylglucose (K_i = 24 mM), but it is not affected by L-arabinose. D-fructose, L-arabinose and D-mannitol penetrate into the tissue at the same rate, they do not develop accumulation gradient, and all of them follow the kinetics of a diffusion process. D-glucose, on the contrary, like galactose, penetrates much more quickly and accumulates against a gradient.

Intestinal active transport of sugars from mucosal to serosal in snail (*Cryptomphalus hortensis* Müller) was previously observed (1, 2, 3). It was also reported (1) that phlorizin inhibited D-glucose, 3-0-methylglucose, and D-galactose transport, which suggested that the three sugars used the same transport system.

In this paper a competitive inhibition among these three sugars has been proved, and the kinetics parameters of D-galactose accumulation in the tissue are given.

Materials and Methods

Everted sacs and intestinal rings from *Cryptomphalus hortensis* Müller were used. Techniques described in a former paper (1) have been used for the experiments with everted sacs. Saline solution filled sacs (11), to which glucose had been added, were incubated in 1 ml medium of identical composition in Warburg flasks at 30° C in O_2 atmosphere. After incubation time, D-glucose concentration in serosal and mucosal (Sf and Mf) was determined enzymatically (8). The results are given as Sf/Mf ratio.

To prepare the intestinal rings, the technique of CRANE and MANDELSTAM (7), appropriately adapted, was followed. Four portions («rings») 5-8 mm long were obtained from each everted intestine. For incubation, four rings were suspended in 4 ml medium in Erlenmeyer flasks. Rings from different animals and

from different intestinal positions were combined in each flask in order to balance out individual and position differences. The medium was MENG's solution (11) to which the labelled substrate (^{14}C) had been added. After 15 minutes at 30° C, with constant stirring and carbogen bubbling, the rings were taken out, dried in wet filter paper, and their wet weight determined. Then they were digested in 1 ml KOH at 30 % and the radioactivity was measured with a liquid scintillation gage-counter (Nuclear Chicago). The radioactivity was taken as a measure of the quantity of sugar accumulated in the rings. Results are expressed as net transference rates (micromoles of substrate per 100 mg wet weight in 15 minutes).

Substrates D-[u-^{14}C] glucose (3 mCi/mmol), D-[1-^{14}C] galactose (3 mCi/mmol), D-[1-^{14}C] mannitol (32.2 mCi/mmol) and D-[u-^{14}C] fructose (3 mCi/mmol) were supplied by Radiochemical Centre Amersham, and L-[u-^{14}C] arabinose (176.5 mCi/mmol) by the Centre d'Etudes Nucléaires Saclay.

Results

Effect of D-galactose, 3-0-methylglucose, and L-arabinose on D-glucose transport in everted sacs. Table I shows the effect of 10 or 20 mM D-galactose, 3-0-methylglucose and L-arabinose on the transport of 1 mM D-glucose. The glucose accumulation gradient (Sf/Mf) decreases significantly when D-galactose or 3-0-methylglucose is present, but it does not change with L-arabinose. The effects are more pronounced in 30 minutes experiments than in 60 minutes ones. Glucose, galactose and 3-0-methylglucose seem to compete for the same transport system.

Effect of D-glucose, 3-0-methylglucose, and L-arabinose on D-[1-^{14}C] galactose accumulated in intestinal rings. Initial trials with 0,5 mM of D-galactose, in incubations up to 60 min, showed that after 15 min, concentrations somewhat higher in total tissue water than in medium were already reached. Thus, this 15 min period was adopted as the experimental time. The net transference of galactose to the tissue from various initial concentrations in the incubating medium, in the absence or presence of 20 mM D-glucose, 3-0-methylglucose, or L-arabinose, was measured.

Results in table II show that intake rate of D-galactose in tissue decreases significantly when D-glucose or 3-0-methylglucose are present, but it is unchanged with L-arabinose. The LINEWEAVER-BURK (10) plot (fig. 1), shows that D-glu-

Table I. *Effect of various sugars on the snail intestinal active transport of D-glucose in everted sacs.*
Mean values with their standard error and statistical significance of differences after Student's test. Number of experiments in parenthesis.

Experimental condition	Gradient Sf/Mf	
	30 minutes	60 minutes
D-glucose 1 mM (controls)	3.00±0.27 (10)	4.33±0.29 (36)
+D-galactose 10 mM	—	3.07±0.30 (15) $p < 0.01$
+D-galactose 20 mM	1.93±0.12 (9) $p < 0.01$	2.14±0.20 (13) $p < 0.001$
+3-0-methyl glucose 10 mM	—	3.28±0.47 (14) $p < 0.05$
+3-0-methyl-glucose 20 mM	1.75±0.13 (7) $p < 0.01$	3.08±0.42 (12) $p < 0.05$
+L-arabinose 10 mM	—	3.69±0.42 (4) N.S.
+L-arabinose 20 mM	2.98±0.31 (13) N.S.	3.90±0.48 (12) N.S.

N.S. = Non significant.

Table II. *Accumulation of D-[1-^{14}C] galactose in snail intestinal rings (μmoles/100 mg w.w./15 min)*

Effects of 20 mM D-glucose, 3-0-methylglucose, or L-arabinose. Mean values with their standard error. Number of experiments in parenthesis. Statistical significance of differences according to Student.

Experimental condition	[D-galactose] in the medium (mM)			
	0.5	1	2.5	10
Galactose (control)	0.067±0.004 (5)	0.123±0.009 (2)	0.233±0.007 (2)	0.430±0.014 (4)
+ Glucose	0.025±0.003 (4) $p < 0.001$	0.047±0.004 (4) $p < 0.01$	0.100±0.018 (4) $p < 0.02$	0.252±0.017 (4) $p < 0.001$
+ Methylglucose	0.044±0.005 (4) $p < 0.02$	0.075±0.003 (4) $p < 0.01$	0.146±0.016 (4) $p < 0.05$	0.363±0.011 (4) $p < 0.02$
+ Arabinose	0.071±0.008 (5) N.S.*	0.131±0.014 (4) N.S.	0.184±0.016 (3) N.S.	0.488±0.019 (4) N.S.

* N.S. = Non significant.

cose and 3-0-methylglucose behave as competitive transport inhibitors of D-galactose, which strongly suggests that the three sugars use the same transport system. The calculated maximum intake rate (V_{max}) for galactose was 0.57 micromoles of sugar/100 mg wet weight/15 minutes, and the transport constant (K_T) 3.63 mM. The corresponding K_i for 3-0-methylglucose could be estimated as 23.9 mM and as 8.18 mM for glucose. This suggests that the carrier presents much greater affinity for D-glucose than for 3-0-methylglucose.

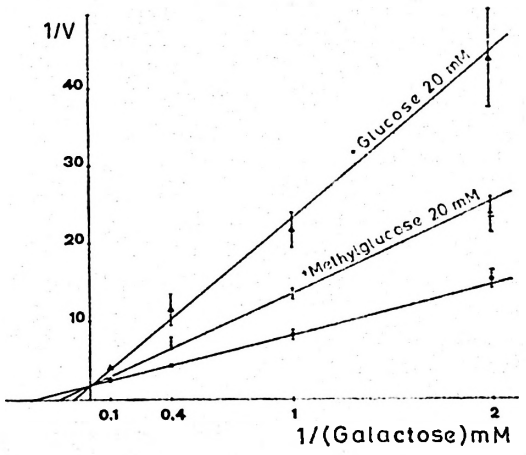

Fig. 1. *Kinetics of D-galactose accumulation by snail intestinal rings.* Competitive inhibition by 20 mM D-Glucose or 3-0-Methylglucose.

With initial concentrations of 0.5, 1, 2.5, and 10 mM D-galactose in the medium, the concentrations in tissue after 15 min reached 0.84, 1.53, 2.92, and 5.38 respectively. So, leaving aside the 10 mM initial concentration, galactose accumulates inside the cells againts a gradiens.

Penetration kinetics of D-mannitol, L-arabinose, D-fructose and D-glucose. Table III shows the penetration kinetics of these compounds with concentrations ranging from 0.5 to 10 mM.

No significant differences were observed between mannitol, arabinose, and fructose in any of the used concentrations. The penetration rate of the three compounds, seems to be approximately proportional to the concentrations in the medium, which strongly suggest a passive process of simple diffusion. The final concentrations of the three substrates in the total tissue water were between 33 and 39 % in relation to their corresponding exterior

Table III. *Penetration rates for D-[1-^{14}C] mannitol, L-[u-^{14}C] arabinose, D-[u-^{14}C] fructose, and D-[u-^{14}C] glucose (μmoles/100 mg w.w./15 min) in snail intestinal rings.* Mean values with their standard error. Number of experiments in parenthesis. Statistical significance of differences in relation to D-mannitol according to Student.

Sugar	Initial [sugar] (mM)			
	0.5	1	2.5	10
D-mannitol	0.016±0.001 (6)	0.027±0.003 (6)	0.080±0.007 (6)	0.291±0.014 (6)
L-arabinose	0.015±0.001 (6) N.S.*	0.031±0.003 (6) N.S.	0.063±0.004 (6) N.S.	0.303±0.023 (6) N.S.
D-fructose	0.018±0.000 (2) N.S.	0.029±0.001 (2) N.S.	0.072±0.002 (2) N.S.	0.314±0.029 (2) N.S.
D-glucose	0.078±0.005 (5) $p < 0.001$	0.118±0.010 (6) $p < 0.001$	0.252±0.009 (4) $p < 0.001$	0.597±0.052 (4) $p < 0.001$

* N.S. = Non significant.

concentration. So, there is no intracellular accumulation of these sugars, at least within the first 15 min of incubation.

Similar experiments with D-[u-^{14}C] glucose reveal penetration rates very significantly higher than those of D-mannitol, and perhaps even somewhat higher than its corresponding ones for galactose. On the other hand, the final concentrations of D-glucose in total tissue water reached 0.97, 1.48, 3.14, and 7.45 mM when those in the medium were 0.5, 1, 2.5, and 10 mM respectively, showing that D-glucose is able to penetrate through active transport into the intestinal epithelial cells of snail.

Discussion

Results corroborate that the intestine of snail (*Cryptomphalus hortensis* Müller) is capable of active transport of D-glucose, D-galactose and 3-0-methylglucose against a concentration gradient. Competitive inhibitions are observed among the three sugars, which strongly suggests their use of the same transport system, as foreseen in a previous paper (1). This common system shows the same or greater affinity for D-glucose than for D-galactose, whereas that for 3-0-methylglucose, is lower.

The only similar study within the Phylum Mollusca corresponds to *Cryptochiton stelleri* (Poliplacophora) in which two transport systems seem to have been found; a common one for D-glucose and 3-0-methylglucose, and another one for D-galactose (9).

Intestinal active transport of D-galactose by snail shows a MICHAELIS-MENTEN kinetics (12), with K_T values approaching 3.6 mM, and with a saturation rate of 0.57 micromoles/100 mg wet weight/15 minutes. This D-galactose K_T value is very similar to that estimated by other authors in Mammals (4, 5, 6).

The results obtained for D-fructose and L-arabinose can be explained by a penetration process into the tissue through simple diffusion. They do not differ significantly from those obtained with D-mannitol.

Previous experiments with everted sacs and L-arabinose had pointed to the intestinal active transport of that sugar by snail (1, 3). The present results with labelled sugar clearly allow to reject the

capacity of the snail intestine for such a process. Those misleading results are now to be attributed to glucose released from the tissue to the serosal side of the sacs used in those previous experiments, as the sugar estimation technique based on reducing power (13, 14), could not differentiate glucose from arabinose. The releasing of glucose is in fact particularly important in lengthy experiments with closed sacs. After repeating these last experiments with sacs and 1 mM labelled arabinose on both compartments, no accumulation has been observed on the serosal at any time.

Resumen

Anillos de intestino evertido del caracol *Cryptomphalus hortensis* acumulan azúcares contra gradiente. El transporte activo de D-galactosa muestra una K_T de 3,6 mM, y es inhibido competitivamente por la D-glucosa (K_I=8,2 mM) y por la 3-0-metilglucosa (K_I= 24 mM). La L-arabinosa no afecta al transporte de D-galactosa. La penetración de D-fructosa y L-arabinosa en el tejido es del mismo orden que la del D-manitol, no se desarrolla gradiente de acumulación y sigue la cinética de un proceso de difusión, mientras que la D-glucosa, como la D-galactosa, penetra mucho más rápidamente y se acumula contra gradiente.

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