REVISTA ESPAÑOLA DE FISIOLOGIA, 33, 227-232. 1977

Intestinal Absorption of Sugars and Amino Acids in the Earthworm

A. Berjón, R. Jordana and F. Ponz

Departamento de Investigaciones Fisiológicas C.S.I.C.
Universidad de Navarra
Pamplona (España)

(Received on November 24, 1976)

A. BERJON, R. JORDANA and F. PONZ. *Intestinal Absorption of Sugars and Amino Acids In the Earthworm.* Rev. esp. Fisiol., 33, 227-232. 1977.

Absorption of glucose, galactose, fructose and leucine from luminal to coelomic compartments across the intestinal wall of *Scherotheca sp.* seems to be a simple diffusion process and not a product of active transport or facilitated diffusion. A concentration gradient never developed from identical initial concentrations of the ^{14}C-labelled substrates on either side of *in vitro* everted intestinal sacs. The rates of net passage of glucose and galactose were in linear function with the respective sugar concentration gradient. A competition between these two sugars was not observed. The diffusion rate of glucose was higher from mucosal to serosal than in reverse direction. Intestinal tissues synthesized glycogen from glucose in the medium, this process being strongly inhibited by dinitrophenol.

Sugar and aminoacid transport by the intestine has been profusely studied *in vivo* and *in vitro* in mammals (3, 4, 16), and lately also in lower animals (2, 8, 12). In most species, active transport of these substrates has been evidenced, but its universality cannot be affirmed since it is lacking in several cases (8, 14).

In spite of frequent use of earthworms for biological studies, the intestinal absorption physiology of these animals is very scarcely known, and references on the mechanisms for sugar and aminoacid transfer through their intestinal wall. A study, therefore of this physiological aspect of *Scherotheca sp.*, a species apparently unable to transport actively these substrates, was considered highly interesting.

Materials and Methods

Specimens of *Scherotheca sp.* L., collected near Pamplona, were kept in the laboratory on trays provided with a layer of earth taken from the same places inhabited by the animals. Four days before the experiment, they were transferred to another tray with just damped filter paper on the bottom to eliminate most intestinal contents.

After longitudinal incision, the digestive tract was exposed and rinsed with saline,

the intestinal portion excised and everted with the aid of a glass rod of a convenient diameter, and placed on saline solution (9) at 4° C, buffered at 6.9 pH with Tris-ClH. This pH can be accepted as that of the intestine (15). Sacs of everted intestine of about 2.5 cm long were prepared in a similar way to the WILSON and WISEMAN method (19), filled with saline solution (serosal) and then suspended in 2 ml of the same solution (mucosal) in Warburg flasks, for 60 minutes, at 20 or 30° C and 100 oscillations per minute. Substrates were present in serosal, mucosal or both solutions as indicated in the experiments. When their concentration was 10 mM or higher, the adequate dilution of other solutes to keep osmolarity was made. The weight of the empty and filled sacs was determined before and after incubation.

No passage of Evans blue was considered as a test for integrity of the epithelial barrier after manipulation.

In the experiments for measurement of diffusion rates, the sacs were suspended in a 5 ml solution volume and the incubation time lasted 10 minutes. Both the exterior sugar concentration and the diffusion rate did not practically change under these conditions.

Estimations of ^{14}C-labelled D-glucose, D-galactose, D-fructose and D-leucine (Radiochemical Center, Amersham) were made by a liquid scintillation counter with PPO, POPOP in alcohol-toluene (1, 7). S_f and M_f are the final substrate concentrations in the serosal or mucosal compartments respectively.

Synthesis of glycogen was measured by incubating intestinal wall fragments in 2 ml saline solution with 2 mM glucose in Warburg flasks at 30° C. After 60 minutes incubation, the total glycogen was extracted and isolated after SEIFTER *et al.* (17), and its radioactivity counted to measure the amount of the incorporated ^{14}C-glucose.

Oxygen uptake was determined by Warburg direct method (18).

Results

Absence of active transport. Incubation of intestine sacs of *Scherotheca sp.* in the presence of glucose, galactose, fructose or leucine at the same initial concentration in both mucosal and serosal compartments did not develop any gradient of substrate concentration in 60 minutes. The S_f/M_f quotient remained always 1, whatever the initial concentration or temperature (20-30° C) (Table I). In a few cases the incubation was extended up to 120 or 150 minutes, and similar results were obtained.

Some experiments were made by incubating intestinal fragments in 2 mM glucose at 30° C for 60 minutes and then determining the total sugar present in the tissues. If a homogeneous distribution of glucose in the total water of the tissue is assumed, the sugar concentration did not exceed in any case 1.7 mM, insufficient for active transport.

Since a release of glucose from the tissues takes place during incubation, mistakes could be made if sugars were deter-

Table I. *Non ability of* Scherotheca sp. *Intestine for active transport of sugars and leucine.*

Everted intestine sacs were incubated for 60 minutes with labelled substrates. Mean values ±S.E. for final serosal/mucosal quotient (S_f/M_f) and oxygen consumption are given. Number of experiments are in parentheses.

Substrate	mM	T °C	Sf/Mf	μl O_2/100 mg w.w.
D-galactose	2.0 (13)	30	1.01±0.02	23.77±1.59
	0.5 (8)	30	0.98±0.01	22.55±1.75
	0.5 (8)	20	0.99±0.01	—
D-glucose	0.5 (10)	30	1.02±0.02	21.72±1.28
	0.5 (10)	20	1.00±0.01	15.92±0.74
D-fructose	0.5 (9)	30	0.98±0.01	25.22±0.55
	1.0 (6)	30	0.99±0.01	22.99±0.91
L-leucine	1.0 (10)	30	1.01±0.01	23.60±1.21

mined by reduction chemical methods. This glucose release amounted 0.30±0.04 mg/g w.w./hr at 30° C (14 experiments) and 0.18 ± 0.01 at 20° C (9 experiments), and it ought to be enzymatically derived from glycogen stores. This was the chief reason for using labelled substrates.

Oxygen consumption. Oxygen consumption by intestinal sacs throughout incubations in the presence of different substrates was not affected by the nature and concentration of the compound actually present in the medium (Table I). With 0.5 mM galactose, at 30° C, oxygen uptake was 22.5 μl O_2/100 mg w.w./60 minutes, and very similar, nonstatistically different, values were found for 2 mM galactose or other substrates.

Temperature, however, clearly influenced oxygen consumption. In the presence of 0.5 mM glucose, it rose about 36 per cent by passing from 20 to 30° C.

Simple diffusion of sugars through the intestinal wall. As active transport of hexoses is excluded, it had to be decided whether sugars passed down a concentration gradient through the intestinal wall of *Scheroteca* using a specific transport system or by simple diffusion. Two approaches were adopted to solve this question: possibility of competition between analogues and diffusion kinetics.

For competition experiments, sugars were initially present in only the mucosal compartment, and the net movement to the other side was measured after 10 minutes of incubation at 30° C. As Table II shows, diffusion of 2 mM ^{14}C-glucose towards serosal side was not modified by the presence of 20 mM inert galactose, nor that of 2 mM ^{14}C-galactose by 20 mM inert glucose.

Diffusion kinetics for glucose and galactose was separately followed in the concentration range of 2 up to 100 mM sugar. Diffusion rates of these hexoses linearly increased with exterior sugar concentration, and saturation kinetics was never observed (Fig. 1).

Both approaches strongly suggest that sugar passage from luminal to the coelomic side through the intestinal wall of the earthworm *Scherotheca sp.* is a simple diffusion process.

Table II. *No competition between galactose and glucose diffusion through the intestinal wall of* Scherotheca sp.
Everted intestine sacs were incubated at 30° C for 10 minutes. Mean values ±S.E. are given. Number of experiments, in parentheses.

Substrate in mucosal	Sugar diffused to serosal µg/100 mg w.w.
2 mM ^{14}C-galactose	18.9 ± 0.9 (9)
2 mM ^{14}C-galactose + 20 mM glucose	20.2 ± 0.6 (8)
2 mM ^{14}C-glucose	12.1 ± 0.5 (12)
2 mM ^{14}C-glucose ± 20 mM galactose	12.9 ± 0.7 (12)

Intestine asymmetry for diffusion. To test the symmetry of intestinal wall in

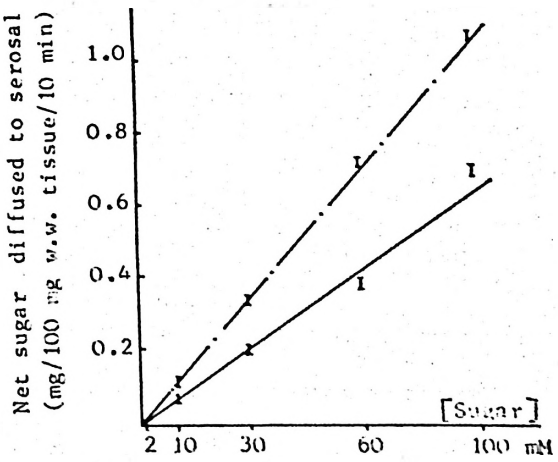

Fig. 1. *Plot of diffusion rates of galactose (—·—) and glucose (—) from mucosal to serosal compartments in everted intestinal sacs of* Scherotheca sp., *against sugar concentrations.*

Table III. *Preferential diffusion of glucose from mucosal to serosal across the intestinal wall of* Scherotheca.
Sacs of everted or non-everted intestine were incubated for 10 minutes with 30 mM ^{14}C-glucose in a single compartment. Net glucose diffusion was from outside to inside the sacs (x) or in reverse direction (+).

Gradient direction	Net glucose diffusion (μg/100 mg w.w.) Everted	Non everted
Mucosal to serosal	151.9 x	107.8 +
Serosal to mucosal	15.1 +	10.9 x

respect to sugar diffusion net passage of sugar from mucosal to serosal compartment, and viceversa, was first measured with 30 mM glucose initial concentration either in the mucosal or in the serosal solutions in everted intestinal sacs. The surprising result was (Table III) a sugar net passage from mucosal to serosal compartment about ten times higher than in the reverse direction, i. e., the intestinal wall showed a very strong functional asymmetry for sugar diffusion.

Similar experiments were then carried out with sacs of non-everted intestine, and again a higher net sugar diffusion rate from mucosal to serosal was observed, although the differences between diffusion rates in both directions were not so great as with everted intestine. Manipulation of intestine on eversion procedure may be responsible for such differences. At any rate there is a very clear preference for diffusion of glucose from mucosal to serosal.

Glycogen synthesis by intestinal tissues and DPN inhibition. Fragments of intestinal wall were able to synthesize glycogen from glucose added to the incubation solution (Table IV). Before incubation, glycogen content in the intestinal wall was approximately 1.34 ± 0.12 mg/g w.w. Starting with 2 mM (^{14}C) glucose in the medium, the labelled sugar incorporated into glycogen after 60 minutes was 22.8 ± 0.88 μg/g w.w./h, about a 10 per cent of the total radioactivity recovered from the tissues, the rest being mainly free glucose. Glycogen increase was nearly 1.7 % of the initial amount stored in the intestine.

Addition of 0.5 mM 2,4-dinitrophenol to the medium substantially diminished the total radioactivity found in the tissues and recovered as glycogen (71 per cent), whereas it did not change oxygen uptake.

Discussion

The results reveal that sacs of everted intestine of *Scherotheca sp.* do not show any ability to transport glucose, galactose, fructose of leucine from the lumi-

Table IV. *Effect of DNP on synthesis of glycogen, from glucose in the tissues and O_2 uptake.*
Sacs were incubated with or without DNP (5×10^{-4} M) in the presence of 2 mM ^{14}C-glucose for 60 minutes at 30° C. Mean values ± S.E. Number of experiments in parentheses. Statistical signification, after Student's method.

	Control	DNP 5×10^{-4} M	Inhib. %	P
Synthesized glycogen (μg/g w.w./h)	22.80 ± 0.88 (22)	4.42 ± 0.86 (6)	80.6	0.001
O_2 uptake (μl/100 mg w.w./h)	20.81 ± 1.47	8.08 ± 0.52	61.2	0.001
Glucose in the tissue (μg/100 mg w.w./h)	24.02 ± 1.54 (6)	26.28 ± 1.83 (6)	—	N.S.*

* N. S. not significant.

nal to the coelomic compartment against a concentration gradient. Sac experiments do not completely exclude the possibility of active transport of the same substrates from lumen into cells, but they offer strong support for this exclusion. Moreover, experiments made by incubating intestinal wall fragments following the CRANE and MANDELSTAM technique (5) confirm the absence of active transport.

In spite of the inherent uncertainty in correlating experimental data from kinetics with actual transfer mechanism (10), the available evidence does not favour the existence of a facilitated diffusion process for the transfer of sugars through the intestinal wall in earthworm. Actually, there was no competition between glucose and galactose diffusion, nor was a saturation kinetics observed for the passage of these sugars.

The linear dependence of sugar movement on the concentration gradient along a large range (2-100 mM) is in greater accord with a simple diffusion process.

The absence of sugar active transport in earthworm intestine may be related to morphological characteristics of the epithelial cells which lack (11) the typical microvilli present in other animal species capable of non-electrolyte active transport.

Non active transport for glucose, fructose, galactose, 3-o-methylglucose and xylose has also been reported in the intestine of other lower animals such as the trematodes *Fasciola hepatica, Philophtalmus megalurus* and *Haematoloechus medio-pleens* (12, 14). However, facilitated diffusion of sugars seems to take place in these cases, and so competition between analogous substrates sharing a common transport system has been observed. In *F. hepatica*, D-xylose crosses intestinal wall by simple diffusion (8).

It was surprising that in a simple diffusion process, the diffusion rate for galactose should be 1.8 times higher than for glucose, in as much as both hexoses share equal molecular size and very similar hydrophilic characters. There remains also to be explained the greater diffusion rate of glucose from lumen to coelomic cavity than that from the opposite direction.

The values of glycogen contents found in the intestinal wall of earthworm are not very different from those reported for other earthworm species, as for visceral tissues from *Eisaenia fetida* (13) and for chloragocytes from *Allobophora calliginosa* (6). In the present experiments, 50 per cent higher level of glycogen was found in isolated typhlosolis (1.86 $\pm$ 0.16 mg/g w.w.) than in intestinal wall previously almost deprived of chloragogen tissue (1.21 $\pm$ 0.08).

Intestinal tissues of *Scherotheca* are able to synthesize glycogen from the glucose in the medium. This process may perhaps favour glucose absorption by lowering the intracellular level of the hexose.

5×10^{-4} M dinitrophenol inhibits glycogen synthesis and also the diffusion of sugar into intestinal tissues, but it do not affect oxygen consumption. These effects migth be attributed to its uncoupling action on oxidative phosphorylation and to other less known DNP induced changes in metabolic activities or membrane permeability.

Resumen

La absorción de glucosa, galactosa, fructosa y leucina desde la luz del intestino de la lombriz de tierra *(Scherotheca)* hacia el lado celómico, parece realizarse por un proceso de simple difusión, sin intervención de transporte activo ni difusión facilitada. Con sacos de intestino evertido, partiendo de concentraciones iniciales iguales a ambos lados, nunca se desarrolló un gradiente de concentración de los sustratos marcados con C^{14}. Tampoco pudo observarse acumulación de glucosa en el tejido. Las velocidades de paso de glucosa y de galactosa a favor de gradiente a través de la pared del intestino eran función lineal de la concentración del azúcar. No se ha observado competencia entre ambos azúcares. A igualdad de

gradiente, la difusión de glucosa era mayor de mucosal a serosal que en sentido contrario. Los tejidos de la pared intestinal son capaces de sintetizar glucógeno a partir de glucosa presente en el medio y el dinitrofenol inhibe fuertemente este proceso.

References

1. BELL, C. G. and HAYES, F. N.: Liquid Scintillation Counting. Pergamon Press, Inc., New York, 1958.
2. BARBER, A., JORDANA, R. and PONZ, F.: *Rev. esp. Fisiol.*, **31**, 271-276, 1975.
3. CRANE, R. K.: *Physiol. Rev.*, **40**, 789-825, 1960.
4. CRANE, R. K.: In «Handbook of Physiology», Sect. 6: Alimentary Canal, vol. 3, *Amer. Physiol. Soc.*, Washington, 1968, p. 1323.
5. CRANE, R. K. and MANDELSTAM, P.: *Biochim. Biophys. Acta*, **45**, 460-476, 1960.
6. GANSEN, P. S. VAN: *Enzymologia*, **20**, 98-108, 1959.
7. HAYES, F. N., OTT, D. G., KERR, V. N. and ROGERS, B. S.:*Nucleonics*, **13**, 38-50, 1955.
8. ISSEROFF, H., and CLARK, P. R.: *Comp. Biochem. Physiol.*, **398**, 1017-1021, 1971.
9. KAMEMOTO, F. I., SPALDING, A. E. and KEISTER, S. M.: *Biol. Bull.*, **122**, 228-231, 1962.
10. LEFEVRE, P. G.: *Current Topics Membrane Transport*, **7**, 109-215, 1975.
11. MILLOT, N.: *Proc. Roy. Soc. London*, **131**, 271-295, 1943.
12. NOLLEN, P. M.: *J Parasit.*, **54**, 295-304, 1968.
13. O'BRIEN, B. R.: *Aust. J. exp. Biol. Med. Sci.*, **35**, 83-92, 1957.
14. PARKENING, T. A. and JONHSON, A. D.: *Exp. Parasitol.*, **25**, 358-367, 1969.
15. ROBERTSON, J. D.: *J. exp. Biol.*, **13**, 279-297, 1936.
16. SCHULTZ, S. G.: In «Biological Membranes» (R. M. Dowben, ed.). J. A. Churchill Ltd., London, 1969, p. 59.
17. SEIFTER, S., SEIMOUR, S., NOBIC, B. and WUNTWYLER, E.: *Arch. Biochim.*, **25**, 141-152, 1950.
18. WARBURG, D.: *Biochem. Z.*, **152**, 51, 1924.
19. WILSON, T. H. and WISEMAN, G.: *J. Physiol.*, London, **123**, 116-125, 1954.