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IN VIVO AND IN VITRO SUGAR TRANSPORT IN FROG INTESTINE

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In the frog intestine, both in vitro and in vivo, experiments were carried out in order to increase knowledge of the mechanism of sugar exit across the basolateral membrane of the enterocyte. The frog intestine was chosen because it lacks crypt cells and, consequently, any external fluid circuit mechanism during sugar transport can be avoided. Therefore, the sugar concentration in the absorbate collected on the serosal side is likely to be similar to that present underneath the basolateral membrane of the enterocyte. Under this condition, cell and absorbate sugar concentrations are similar; yet there is a concomitant net transintestinal sugar transport. Moreover, in in vivo experiments a net transintestinal sugar transport takes place even against a concentration difference. These results suggest that sugar exit across the basolateral membrane is not simply due to a chemically facilitated diffusion.

The transport of sugars across the intestine has been widely studied both in homotherms and poikilotherms, but the mechanisms underlying this transport still present some uncertainties. This is due, most probably, to animal species differences and/or to the different experimental approaches used. During their transport, monosaccharides cross two membranes in series, i.e., the brush border and the basolateral membrane of the absorbing cell. The apical membrane has been well characterized as far as its structure, chemical and enzymatic composition and transport mechanisms are concerned. It is known, for instance, that sugar entry across the apical membrane is a carrier-mediated, Na^+-dependent and phlorizin-sensitive mechanism [1,2], while sugar exit across the contraluminal membrane is a carrier-mediated, Na^+-independent and phloretin-sensitive process [3–6]. Moreover, the sugar specificity for the carrier in the luminal membrane is different from that present in the basolateral membrane [3]. As to sugar accumulation within the cell, some differences have been found depending on experimental conditions, i.e., whether in vitro or in vivo. In mammalian intestine, for instance, several authors could not find sugar accumulation during in vivo experiments [7,8]. This fact led to a reconsideration of the mechanism of sugar exit across the basolateral membrane. Sugar accumulation is not always evident in in vitro conditions as it depends on animal species [8–10]. Hamster enterocyte, for instance, accumulates sugar much more than rat enterocyte; yet net transepithelial sugar transport always takes place in both animal species. Sugar concentration in the fluid emerging directly from the serosal side of the everted rat intestine (both under normal and hyperglycemic conditions [11]) has been found to be higher than in the enterocyte. If in vivo and in vitro results are considered together, one might conclude that an active extrusion mechanism for sugars is present in the basolateral membrane of the enterocyte. Similar conclusions have been drawn for L-leucine transport in the vascularly perfused small intestine of anurans [12]. However, two main objections can be raised. Firstly, the determination of cell sugar concentration is an

average one (absorbing and non-absorbing cells), although radioautographic studies have shown that the majority of villus cells are absorbing cells [13] (crypt cells are not involved in our previous experiments because, after scraping, they remain in the underlying tissue, as histologically demonstrated [14]). Moreover, by using the same methodology, in vivo experiments show a low cell sugar concentration while in vitro experiments show a higher cell sugar concentration [7,9]. Secondly, if one accepts the hypotheses of an external fluid circuit [1,15], sugar concentration in the emerging fluid (absorbate) is likely to be higher than that present at the base of the absorbing cells (i.e., at their serosal side) (see Fig. 1). To rule out the latter objection, experiments have been performed using animals lacking crypts (where the external fluid circulation takes place), such as amphibia. Interestingly, in another poikilotherm species (the winter flounder) which lacks crypts as well, the absorbing cells appear similar both at the tip and at the bottom of mucosal folds [16].

The experiments on frogs (*Rana esculenta*, 40–60 g body weight, kept in a cold room for 2 weeks and allowed to stand at room temperature for 1 h before the experiment) were carried out during spring and summertime under both in vivo and in vitro conditions. In the latter case the everted sac technique was used, both with the serosal compartment initially empty (in order to collect the absorbate directly, i.e., as fluid emerging from the enterocyte) and with a finite volume of serosal fluid present at the beginning of the experiment. When the serosal compartment was initially empty, the intestine before being everted, was preincubated for 10 min in the solution (see below) in order to let the subepithelial spaces equilibrate with the sugar containing solution. The in vitro experiments were based mainly on the Wilson and Wiseman technique [17]. The incubating fluid was Ringer solution for amphibia. The everted sac was filled with 0.3 ml solution (serosal solution) if this fluid was initially present in the serosal compartment and then incubated in 10 ml of the same solution, aerated and shaked for 90 min at room temperature in a Dubnoff shaker. The initial concentration of the sugar, the 3-*O*-methyl-D-glucose, a monosaccharide which is not metabolized by the animal, was 5.1 mM with a trace amount of 3-*O*-[^{14}C]methyl-D-glucose. The extracellular space was determined by using poly([^{3}H]ethylene glycol) M_r 900 in the incubating fluids. This non-absorbale marker was also utilized to determine the net transintestinal fluid transport. When the serosal side was initially empty, net fluid transport was measured by collecting in a pre-weighed weighing-bottle the serosal fluid obtained by gently squeezing the everted gut, after a rapid blotting of the mucosal side on filter paper. The amount of fluid transported was referred to 1 g dry weight of total intestine and 1 h. At the end of the experiment the mucosa was scraped off immediately at 0°C and diluted with distilled water (2–3 ml), and the intestinal cells were broken by osmotic shock and subsequent freezing at −30°C and thawing [9,18]. After the tissue extract and the mucosal and the serosal fluids had been centrifuged, deproteinized and centrifuged again, samples of each supernatant were taken for the determination of radioactivity, sugar and electrolyte concentration.

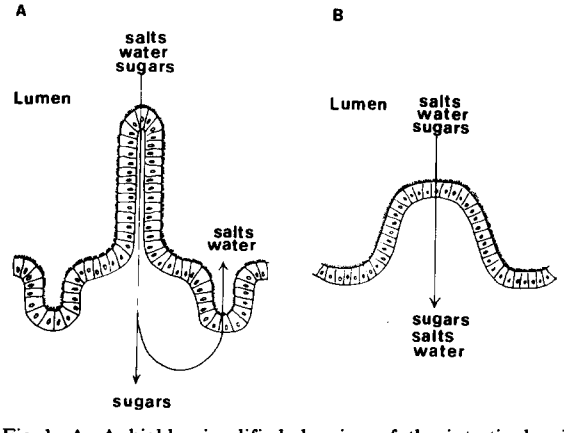

Fig. 1. A. A highly simplified drawing of the intestinal epithelium of a homoiotherm with villus and crypts; possible salt and water recirculation is also represented. B. A highly simplified drawing of the intestinal epithelium of a poikilotherm lacking crypts; in this case, salt and water recirculation should not take place.

The in vivo experiments were carried out as follows. The frog was anaesthetized by placing the animal in 200 ml Ringer's solution for amphibia containing 100 mg tricaine, (MS 222 Sandoz, ethyl-*m*-aminobenzoate methanesulfonate), an anaesthetic for cold-blooded animals, kindly supplied by Sandoz Italiana. After anaesthesia, the

TABLE I

3-*O*-METHYL-D-GLUCOSE (3MG) CONCENTRATION IN THE THREE COMPARTMENTS

3-*O*-Methyl-D-glucose concentration in the three compartments (mucosal side or lumen, cell, serosal side or blood) is given in mmol/litre cell water. Net transintestinal solute and fluid transports are expressed in μmol or ml per g dry total tissue weight per h. *T/M* represents the cell sugar concentration relative to that in the mucosal (or luminal) fluid. Values ± S.E. and number of experiments (in parentheses) are reported.

	Incubating and perfusing solutions	3-*O*-Methyl-D-glucose concentration (mM)			*T/M*	Solute and fluid transport		
		mucosal or lumen	cell	serosal or blood		3MG ($\mu mol \cdot g^{-1} \cdot h^{-1}$)	Na^+ ($\mu mol \cdot g^{-1} \cdot h^{-1}$)	fluid ($ml \cdot g^{-1} \cdot h^{-1}$)
I.	In vitro Ringer + 5.1 mM 3MG (mucosal and serosal) (8)	5.6 ± 0.3	21.3 ± 2.3	8.9 ± 0.9	3.8 ± 0.3	41 ± 8	375 ± 89	2.4 ± 0.6
II.	In vitro (absorbate) Ringer + 5.1 mM 3MG (only mucosal, initially) (4)	5.1 ± 0.1	18.5 ± 4.5	14.7 ± 3.2	3.6 ± 0.9	10.3 ± 0.5	–	0.6 ± 0.1
			$P > 0.5$					
III.	In vivo Ringer + 1 mM 3MG (lumen) Ringer + 5 mM 3MG (blood) (6)	0.87 ± 0.04	3.6 ± 0.5	6.8 ± 1.0	4.1 ± 0.8	24 ± 8	340 ± 86	3.7 ± 0.7
			$P < 0.02$					

animal was laid on its back in a tray partially filled with the same solution to wet the skin. The solution was continuously oxygenated with pure O_2 to oxygenate the animal via the skin even when it was breathing normally. The abdomen was opened and the upper part of the small intestine (about 5 cm long, starting from 3 cm from the pylorus) was cannulated with a cranial and a caudal glass cannula. 5 ml aereated luminal fluid (Ringer's solution for amphibia, containing 1 mM 3-*O*-methyl-D-glucose, a trace amount of ^{14}C-labelled sugar and phenol red, and sometimes also poly([^{3}H]ethylene glycol) 900 to measure water and 3-*O*-methyl-D-glucose absorption) was recirculated by a peristaltic pump at a rate of 25 ml/min for 90 min, after a previous equilibration period of 10 min. Another solution was injected by an infusion pump into the lymphatic sacs of the animal throughout the experiment; this solution was Ringer's solution with an amount of 3-*O*-methyl-D-glucose (and trace amount of 3-*O*-[^{14}C]methyl-D-glucose) such as to reach a constant final sugar concentration in the blood of about 5 mM. The specific radioactivity of [^{14}C]sugar was always the same, in the luminal solution and in the blood. A trace amount of poly([^{3}H]ethylene glycol) 900 was added to this solution in order to determine the serosal extracellular space. The extracellular spaces determined both in vitro and in vivo were utilized for the determination of intracellular solute concentrations of the epithelium. At the end of the experiment the intestine was processed as in in vitro experiments. Histological preparations of scraped mucosa in both sets of experiments showed that the tissue consisted mainly of epithelial cells.

The results are summarized in Table I. In the in vitro experiments (I) the concentration profile of 3-*O*-methyl-D-glucose in the three compartments, i.e., mucosal, cell and serosal side, is similar to that found in hamster jejunum where the cell accumulates the sugar about 4-times as much. It is interesting to note, however, that in the emerging fluid (absorbate, II) the 3-*O*-methyl-D-glucose concentration is similar to that in the cell, yet there is still a net transintestinal 3-*O*-methyl-D-glucose transport. The sugar concentration in this fluid should not be the result of a sugar handling, i.e., of a progressive increase of concentration due to the fluid circuit (see Fig. 1), along the subepithelial tissue (core of the villus and underlying tissues) because frog intestine lacks crypt cells. Therefore, the sugar concentration in the absorbate should be approximately the same as that present in the fluid close to the basolateral membrane, i.e., the serosal side membrane of the enterocyte. In this set of experiments the 3-*O*-methyl-D-glucose transport is rather low but this value is certainly underestimated because it is impossible to recover completely the fluid emerging from the enterocytes.

Another interesting observation comes from in vivo experiments where, although there is still a cell sugar accumulation of 4-times, the movement of 3-*O*-methyl-D-glucose from the cell to the blood takes place against a concentration difference (the blood sugar concentration is about double than that of the cell). There is also a considerable net transintestinal sugar transport, although it is lower than that in the first set of in vitro experiments because the initial in vivo blood sugar concentration is approximately 5-times higher than that in the lumen. Both in vitro and in vivo intestines were functioning well, as shown by the net transintestinal fluid and Na^+ transport. Also, cell water and cell electrolyte concentrations were within a normal range (data not reported in the table).

To conclude, using frog intestines which do not possess crypts, it is possible to determine directly the sugar concentration in the fluid emerging from the epithelial cells, which in turn is presumably similar to that present at the base of the contraluminal membrane of the enterocytes. This concentration is not statistically different from that found in the enterocyte; yet there is still a net transintestinal sugar transport. In addition, sugar was shown in vivo to move from the cell to the blood against a concentration difference. Thus sugar movement through the basolateral membrane is most likely due, not simply to a facilitated diffusion, but to some extent at least, to a metabolic driving force.

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