STUDIES ON THE MECHANISM OF INTESTINAL ABSORPTION OF SUGARS

IV. LOCALIZATION OF GALACTOSE CONCENTRATIONS WITHIN THE INTESTINAL WALL DURING ACTIVE TRANSPORT, *IN VITRO**

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SUMMARY

The concentrations of actively transported D-galactose in various parts of frozen, dried sections of hamster intestine have been measured. The relationships of these concentrations strongly indicate that active transport of sugars by the small intestine is mediated by a process which lies in or near the luminal or brush-border end of the epithelial cells.

INTRODUCTION

The mechanism of the process of intestinal active transport which accounts for the absorption of sugars against an apparent concentration gradient is unknown. Recent *in vitro* studies of the chemical aspects of this process, embracing its substrate specificity[1–3] and kinetics[4] and the absence of oxygen exchange reactions between transported sugar and water[5], have eliminated a variety of possible endergonic reactions of sugars as participants. Since they have also failed to uncover direct evidence implicating any specific reaction or reaction sequence[5], there is still no sound basis on which to erect a hypothesis of the chemistry of intestinal active transport of sugars.

There is hope, however, that studies along different lines will yield some insight into the mechanism of the process. For example, with a knowledge of its precise location within the cell, an apparent interdependence or linking of active sugar transport with other localized cellular functions or enzymes could be evaluated on the basis of whether both are contained in the same or different portions of the cell. Since its chemistry is unknown, the process cannot be visualized by the usual histochemical approach. However, as an alternative, the relative concentrations of actively trans-

Abbreviations: DPN, DPNH, oxydized and reduced diphosphopyridine nucleotide.

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ported sugar in the various cell layers of the intestinal wall can be measured and the probable location of the active transport process can be deduced from their relationships. The present report describes experiments of this kind and provides a partial answer to the question of the localization of the process of active sugar transport. The histologically-distinct cell layers of frozen-dried sections of hamster intestine were analysed by a specific enzymic assay for D-galactose[6] which was sensitive enough to measure quantitatively as little as 10^{-11} moles of sugar contained in a tissue sample weighing 0.1 μg or less. The sugar accumulated during incubation with D-galactose, *in vitro*, was found to be present, at the earliest time periods and at the highest concentrations, within the epithelial cell layer.

MATERIALS AND METHODS

Intact strips of hamster intestine were prepared and incubated in the presence of D-galactose by methods that have been previously described[7]. The concentration of galactose was 5 mM; the temperature of incubation was 37°. At intervals during incubation, sample strips were removed from the flasks. Some of them were treated and analysed, *in toto*, as previously described[7]. Others were blotted gently and plunged into dichlorodifluoromethane at its freezing point (—160°). From the latter, frozen-dried sections, 15 μ to 40 μ in thickness, were prepared by the methods described by LOWRY[8]. The various cell layers of these sections were easily distinguished under low magnification with an ordinary microscope, as shown in Plate 1. Selected

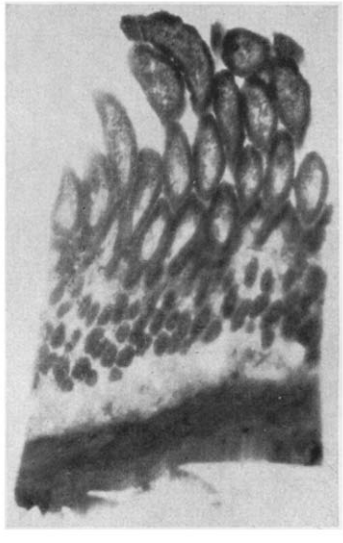


Plate 1. Photomicrograph of frozen, dried section of hamster intestine. Total magnification 60 ×.

ortions of the epithelial cell layer, the tunica propria ("core") and the underlying tissue layers were dissected and weighed, and their volume was determined[8]. The crypt epithelium was not used and only that portion of the tunica propria which extends from the level of the orifices of the crypts of Lieberkuhn to the tips of the villi was used. The weight of the tissue samples used ranged from 0.02 to 0.14 μg, corresponding to volumes ranging from 10^{-4} to 10^{-3} μl. In order to calculate tissue

water from the measured dry weight and tissue volume, the density of the frozen-dried material was assumed to be 1.3*.

D-galactose present in the frozen, dried tissue samples was measured by means of the reaction catalyzed by the galactose dehydrogenase of *Pseudomonas saccharophilia*[9]. This enzyme, which was used in a partially purified form (30–40 fold**), catalyses a DPN-dependent oxidation of D-galactose to D-galactonolactone, with the stoichiometric reduction of DPN[9]. The DPNH formed during incubation of the tissue samples with the enzyme and 0.05 m*M* DPN for 2 h under conditons described elsewhere[6] was measured fluorometrically[11]. Control analyses showed that 10 $\mu\mu$moles of galactose produced consistently 90 to 100 % of the theoretical amount of DPNH. The techniques and precautions used followed the general methods for analysis at this level as described by LOWRY *et al.*[12,13].

Tests of the specificity of the enzyme showed it to the adequate for the present purpose. The preparation showed moderate activity toward L-arabinose, but none toward D-arabinose, D-ribose, L-galactose, D-glucose and D-mannose. In tests with D-galactosamine** and D-galactose 1-phosphate***, an amount of DPNH equivalent to 3 % of the former and 6 % of the latter was formed during two hours of incubation.

EXPERIMENTAL

The results of these studies are presented in Table I and Fig. 1. The analyses in Table I were made of tissue that had been incubated for the single time period of 15 min. After incubation, the tissue was recovered and processed as described in the preceding section. Assays for galactose were made on the total tissue and on four readily distinguished portions of the frozen, dried sections; the epithelial cell layer, the tunica propria or "core" of the villi, the submucosa and the muscularis (see Plate 1). In Expt. 1, Table I, the data in the column to the far right show that the highest concentration of galactose was present in the epithelial cell layer. In Expt. 2, Table I, analytical data are given for epithelium and for "peeled" epithelium. This experiment was done to provide assurance that the higher concentration found in the epithelial cell layer was not caused by the presence of dried, galactose-containing medium on the surface of the tissue. When the outermost or luminal 5 to 10 μ portion of the epithelial layer was cut off before analysis, the concentration found was somewhat less than when this operation was not done. However, the concentration in the "peeled" epithelium was still much higher than the concentration in the "core". Expt. 3, Table I, was carried out for much the same purpose. In this experiment, samples of tissue were taken at the end of 15 min incubation and processed directly. Other samples were taken and transferred to a galactose-free medium for an additional one minute period of incubation. These were then recovered and processed. The concentration difference between the epithelial cell layer and the "core" was maintained during this "washing" period.

The curves in Fig. 1 show the results of analyses performed on tissue recovered

* Based on an assumed tissue content of fat = 5 % of wet weight, density, 0.9; protein = 15 %, density, 1.35; and salts = 1.5 %, density, 2.34 (for KH_2PO_4).

** We are indebted to Dr. M. DOUDOROFF for a sample of the enzyme, some of the organisms, and for instructions for preparing the enzyme[10], in advance of publication.

*** We are indebted to Dr. J. L. STROMINGER for samples of these compounds. Free galactose was a possible small contaminant of both.

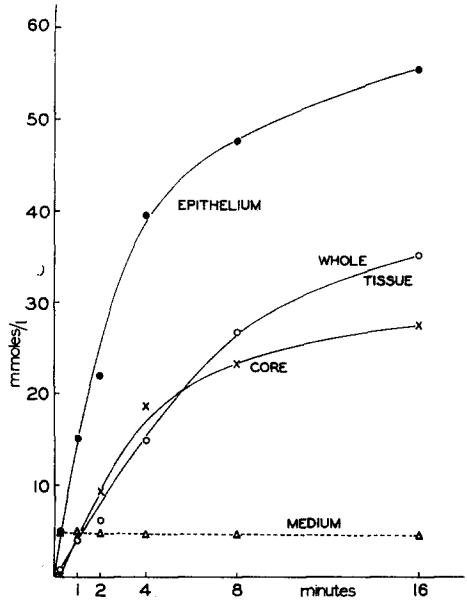


Fig. 1. Time course of the accumulation of D-galactose in intact strips of hamster intestine during incubation at 37°.

TABLE I

CONCENTRATIONS OF D-GALACTOSE FOUND IN VARIOUS PARTS OF INTACT STRIPS OF HAMSTER INTESTINE

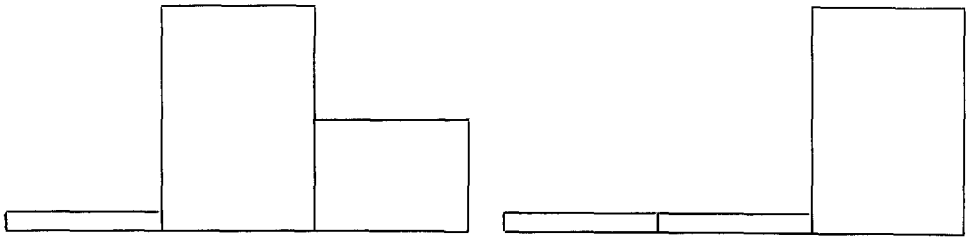
Incubated for 15 min at 37° in 5 m*M* galactose. The numbers in parentheses indicate the number of tissue samples assayed. Standard errors are indicated. The concentration in the total tissue was determined by methods previously described[7]. The concentrations in the various tissue layers were determined on frozen, dried material as described in the present paper. The convention of reporting tissue analyses on a kg basis originates with LOWRY[8]. For further details consult the text.

Expt.	*Tissue sample*	*Water content l/kg dry wt.*	*Galactose concentrations*	
			mmole/kg dry wt.	*mmole/l H_2O*
I	Epithelium (8)	4.30 ± 0.26 (6)	211 ± 8	49.1 ± 2.1
	Core (10)	7.03 ± 0.36	199 ± 13	28.3 ± 1.9
	Submucosa (7)	10.52 ± 0.62	292 ± 20	27.8 ± 1.9
	Muscularis (8)	3.69 ± 0.12 (7)	72.7 ± 3.0	19.6 ± 0.91
	Total tissue			38.4
II	Epithelium (9)	3.26 ± 0.8 (8)	112.4 ± 5.7	34.5 ± 1.8
	Epithelium (peeled) (4)	—	90.0 ± 3.1	27.6 ± 0.95
	Core (8)	4.76 ± 0.29	83.2 ± 1.7	17.5 ± 0.36
III		Before washing		
	Epithelium (8)	5.95 (7)	160	28.0 ± 2.3
	Core (8)	7.51 (6)	123	14.4 ± 1.8
	Total tissue			18.9
		After washing		
	Epithelium (10)	5.84 (6)	150	26.5 ± 2.7
	Core (8)	7.39 (5)	128	17.5 ± 1.9
	Total tissue			17.9

at various time intervals during incubation. At the earliest time period, 15 sec., the epithelial cells contained galactose at a concentration at least equal to that of the medium. At the 1-min time period, the epithelial cells contained three times the medium concentration. At these same time intervals, on the other hand, the concentration in the "core" of the villi lagged greatly behind the concentration in the epithelial cells. At the 1-min period, for example, the concentration in the epithelial cells was 3 times the concentration in the "core". Throughout the entire 16 min of observations, a large concentration difference between epithelial cells and "core" was maintained, indicating that the rate of active transport into the epithelial cells from the medium was much faster than diffusion into the underlying tissues. The initial rate of galactose active transport extrapolated from the value at the 15 sec interval was 1080 μmoles/g wet wt./h (4.2 moles/kg dry wt./h).

DISCUSSION

The major possibilities with respect to the relative concentrations of galactose present in the medium, the epithelial cells and the "core" of the villi after incubation are shown in the following diagrams.



The relationship depicted in diagram A is what would be expected if the process mediating active sugar transport lies in or near the luminal or brush-border pole of the epithelial cells and if most of the cells have this activity. If, on the other hand, the process were localized at the basal pole of the epithelial cells and most of the cells were active, the relationship shown in diagram B would be expected. If only a few of the cells were active in transporting galactose or if the rate of diffusion of sugar between the epithelial cells and the "core" were as fast or faster than the rate of active transport, one would expect the concentrations in the epithelial cells and the "core" to be nearly equal.

The relationship of galactose concentrations shown in diagram A was found in the present study. Galactose was accumulated against an apparent concentration gradient by the epithelial cells and at all time periods during incubation the concentration of galactose in the epithelial cells was markedly higher than in the immediately adjacent "core" of the villi. This is strong support for the conclusion that active transport of sugars by the small intestine is mediated by a process which lies in or near the luminal pole of the epithelial cells. It is also evidence contrary to the suggestion made several years ago[14] that active transport of sugars is a property of a special kind of epithelial cell which is present in relatively small numbers.

Whether the galactose accumulated within the epithelial cells is in a "free" state cannot be decided from the data available. The manipulations of the tissue prior to and during analysis for galactose do not include any procedures which could be expected to rupture covalent bonds. Thus, if binding of galactose to an intracellular component occurs, it is most likely a freely reversible association. Assuming galactose to be in the "free" state within the cells, the concentration relationships between the epithelial cells and the medium are such as to suggest either that there is a large osmotic pressure difference between the two, that another solute has leaked or been "pumped" from the epithelial cells into the medium to prevent this pressure difference, or that water has entered the cells with the sugar. The recent observations that active transport of sugars requires the presence of Na^+ in the medium[15,16] and that the steady state level of sugar accumulation is dependent upon Na^+ concentration* suggest that ion "pumping" is directly involved in sugar transport. Also our data suggest that some water moves into the cells during sugar transport. However, these data are inadequate to show the exact change in cell volume and are not included in the present paper. In the absence of solute exchange between the intracellular and extracellular compartments, osmotic equilibrium at the 16-min interval in Fig. 1 would require an increase in cell volume of 20 %.

With respect to the various methods used to measure active transport of sugars by the intestine, *in vitro*[17–19] in which the transported sugar must diffuse through the entire intestinal wall in order to be measured, the present data indicate that what may actually be measured in this way is not active transport, *per se*, but the downhill diffusion of sugar from a high concentration in the epithelial cells to a lower concentration in the serosal medium. Thus, kinetic measurements by these techniques may be based more on diffusion rates than on rates of active transport.

In a recent study[20] the histochemical localization of glucose-6-phosphatase was employed as added evidence for the participation of this enzyme in the active transport of sugars by the small intestine. It was found to be localized near the basal pole of the epithelial cells from which point the sugar released from glucose-6-phosphate or other primary phosphate esters[21] could then diffuse into the core of the villus and into the blood stream. The present data indicate that this enzymic activity is inappropriately localized for participation in the events of active sugar transport.

ACKNOWLEDGEMENTS

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