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Influence of the Na^+ concentration on the in vivo intestinal absorption of sugars*

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In several laboratories has been stated that active transport of sugars *in vitro* through the intestine depends on the Na^+ presence at the mucosal side, without possibility of substitution of this ion by any other (1, 6, 13, 16, 17). The accumulation level of sugar inside the cell as a result of the active transport, increases with the Na^+ concentration in the medium. *In vivo,* the glucose absorption is also dependent on the Na^+ presence at the intestinal lumen (7). In a previous paper (12) we have tested a very strict dependence of the galactose absorption *in vivo* on the Na^+ concentrations (from 0 to 154 meq Na^+/l).

In vitro was found that this Na^+-dependence is observed too, when sugars enter into the cell in anaerobiosis, so without possibility of accumulation, but only when one is working with actively transportable sugars (4)

In the present paper we study *in vivo* the different behaviour of the intestinal absorption of sugars in respect to the Na^+ concentration, according to its capacity to be or not actively transported.

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Material and Methods

White rats, 130-200 g weight, have been used, under urethane anesthesia, by the Sols and Ponz method of successive absorptions *in vivo* (19), with 10 ml volumes of solutions to be absorbed and a repletion pressure of 8 cm water. Four sucessive periods of absorption were performed with the same canulated segment of intestine in each animal, and the rectal temperature was controlled as not to change more than ± 0.4° C.

Solutions to be absorbed were 0.3 M D-glucose, 2.77 mM D-glucose, 2.77 mM D-fructose or 3.33 mM L-arabinose, with variable concentrations of NaCl and mannitol (Merck) to get isotony.

Sugars have been determined by the Somogyi method (18). Na^+, by flame photometry with a Standard Lange model. Absorptions are given as disappeared sugar, in μM per cm of intestine (20). The tables only show the mean values with their standard errors (15).

Results **

1. *Absorption of 0.3 M glucose*

First was tested the influence of Na^+ concentration on the absorption of isotonic glucose solutions.

TABLE I

Absorption of 0.3 M d-glucose through the rat intestine in vivo. *Influence of the NaCl presence in the washing solutions. Absorption time, 30 min. Inhibitions referred to the first absorption (control).*

Animals N.°	Abs. control µM/cm (previous washing with NaCl 9 °/₀₀)	Successive Absorptions			
		NaCl in the washing solutions °/₀₀	Variations °/₀		
			2nd Abs.	3rd Abs.	4th Abs.
5	40 ± 2.0	9	+ 1 ± 1.7	— 1 ± 0.5	— 2 ±1.0
7	42 ± 3.0	0.0	—9 ± 7.0	—21 ± 9.0	—26 ± 9.0

Currently, on working with the Sols and Ponz technique, we used for washing the intestine before and after each absorption period, saline solution (NaCl 0.9 %). By puting the sugar solution in the intestinal lumen, becomes slightly mixed with the residual saline, and Na^+ remains at a final concentration of about 13 ± 3 meq/l. Under these conditions, a long series of successive 0.3 M glucose absorptions gives constant values of absorbed sugar.

But if instead of 0.9 % NaCl, solutions lower in Na^+ or even distilled water is used for washing, the initial Na^+ concentrations at the beginning (3 min.) of each absorption period will be correspondently lower. With bidist. water, several determinations gave values from 3 to 4.5 meq Na^+/l, which at the end of the 30 min. period reached up to 30-35, showing a net exit of Na^+ from the blood or tissues to the intestinal lumen.

The Table I gives the results of absorption experiments obtained with 0.3 M glucose but using for washing the intestine, 0.9 % NaCl, or bidist water. In all of them, the first absorption which is taken as control, was made after washing the intestine with NaCl 0.9 %, so in pressence of about 13 meq Na^+/l. Sugar absorption decreases as the Na^+ present in the solution diminishes. This decrease goes on progressively along the successive absorptions.

2. *Absorption of 2.77 mM glucose.*

When using 0.3 M glucose, a concentration much higher than in blood, the absorption may be strongly favoured by the gradient. So, it seemed better to analize the relation between sugar absorption and Na^+ concentration with 2.77 mM (500 mg/l) glucose solutions, a level lower than in blood, condition requiring an active transport.

The solutions were made isotonic by NaCl at different concentrations (from 0 to 154 mM) and the required mannitol 0.3 M to 0). The washing of the intestine was made with saline except the immediately one preceeding to the introduction of the solution to be absorbed, which was carried out with bidist water. Periods of absorption lasted 20 min.

As Table II points out, the glucose absorption becomes markedly inhibited as the initial Na^+ concentration of the solutions decreases, evidencing the Na-dependence of the active transport.

In some cases, the absorption was tested at still higher levels of Na^+ (231 meq/l). The mucosal solutions were then somewhat hypertonic. The glucose absorption increased about 10-20 % in respect to the absorption with 154 meq/l.

In a group of animals the absorption of glucose from solutions with 154 and 8.5 meq Na^+/l was compared, and Na^+ total in lumen was determined at the end

TABLE II

Absorption of 2.77 mM D-glucose through the rat intestine in vivo. *Effect of Na+ concentration in the sugar solution. NaCl substituted by mannitol for isotony. Absorption time, 20 min. Inhibition referred to the first absorption (control).*

Animals N.°	Abs. control (154 meq Na^+/l) µM/cm	Successive Absorptions			
		Na^+ meq/l	Variations %		
			2nd Abs.	3rd Abs.	4th Abs.
10	0.35 ± 0.02	154	— 1 ± 1.0	— 1 ± 2.0	— 2 ± 1.0
5	0.30 ± 0.04	51.3	—16 ± 5.1	—18 ± 0.9	—18 ± 1.0
4	0.34 ± 0.07	25.6	—31 ± 1.2	—31 ± 1.4	—35 ± 1.5
12	0.35 ± 0.06	8.5	—56 ± 5.0	—58 ± 5.1	—60 ± 5.2
10	0.36 ± 0.10	0.0	—70 ± 4.1	—69 ± 3.9	—73 ± 2.4

TABLE III

Absorption of 2.77 mM glucose through rat intestine in vivo *and Na+ luminal contents at the beginning and the end of each absorption period (20 min.), at different initial concentrations of NaCl. Solutions were made isotonic with mannitol. Inhibitions referred to the first absorption (control). The Na+ values are given in parenthesis (initial-final) in mg.*

Animals N.°	Abs. control (154 meq Na^+/l) µM/cm	Successive Absorptions (8.5 meq Na^+/l)		
		Inhibitions °/,		
		2nd Abs.	3rd Abs.	4th Abs.
7	0.37 ± 0.02 (35.36→35.32	—52 ± 0.9 (1.92→7.71)	—51 ± 1.10 (1.92→7.71)	—54 ± 1.3 (1.92→7.77)

of each period of absorption. The initial Na^+ in lumen was obtained from tests with the same animals by removing the solution immediately after its introduction in the intestine. The results (Table III) reveal that a nett movement of Na^+ in the 20 min. is not measured when solution has 154 meq/l (a little higher than in blood), whereas with very low levels of Na^+ in lumen (8.5 meq/l) a net exit of the cation to the intestinal lumen is produced, which is practically constant in the three successive periods of absorption, amounting about 0.25 meq. with intestinal segments of 18-20 cm. However, the Na^+ concentration remains very low in lumen. It appeared also interesting to know if the inhibitory effect of very low levels of Na^+ on the glucose absorption was or not reversible. In a group of animals (Table IV) a first period of absorption was carried out with 154 meq Na^+/l, a second one with 8.5 meq/l and the third and fourth again at 154 meq/l. It is clearly seen that on coming back to high levels of Na^+, the absorption of glucose progressively improves along the two successive periods, but the recuperation is rather slow. The luminal Na^+ at the beginning and the end of each period was determined, and, as is shown in the table, there are not

differences between the Na^+ in the first, third and fourth periods; so the slowness of the recuperation may be not accounted by different levels of Na^+ in lumen.

Absorption of 2.77 mM fructose

The absorption of 2.77 mM fructose, a non actively transportable sugar, is very scarcely affected by the Na^+ concentration in the intestinal lumen (Table V). Only at very low or nul levels of Na^+, a slight inhibition, less than 20 %, may be found.

Absorption of 3.33 mM arabinose

The 4 absorption periods were in this case of 60 minutes. A curious observation was that with this low concentration of arabinose, as it had been previously seen with isotonic solutions (11), the absorption in the second period was lower than in the first one, but it remained thenceforth constant. Therefore, the two first periods were studied with 154 meq Na^+/l and the last two with 8.5, referring the absorption in the third and fourth periods to that of the second one.

As it is seen in Table VI, with arabinose, also a sugar non actively transportable, there is no influence of the Na^+ concentration on the intestinal absorption.

Discussion

As we had previously reported for galactose (12), has been possible to prove

TABLE IV

Absorption of 2.77 mM D-glucose through rat intestine in vivo. *Inhibition by very low concentration of Na+ reversibilized on turning to 154 meq./l. NaCl substituted by mannitol for isotony. Absorption time 20 min. Inhibitions referred to the first absorption (control).*

Animals N.°	Abs. control (154 meq Na^+/l) µM/cm	Successive Absorptions		
		Inhibitions %		
		2nd Abs. (8.5 meq. Na^+/l)	3rd Abs. (154 meq Na^+/l)	4th Abs. (154 meq Na^+/l)
14	0.41 ± 0.02 (35.36→35.31)	— 60 ± 4.7 (1.90 7.0)	— 54 ± 5.1 (35.36→35.33)	— 48 ± 1.6 (35.36→35.40)

TABLE V

Absorption of 2.77 mM fructose through rat intestine in vivo. *Effect of Na+ concentration in the sugar solution. NaCl substituted by mannitol for isotony. Absorption time 20 min. Inhibitions referred to the first absorption (control).*

Animals N.°	Abs. control (154 meq. Na^+/l) µM/cm	Successives Absorptions			
		(Na^+) meq/l	Successive Absorptions		
			2nd Abs.	3rd Abs.	4th Abs.
6	0.20 ± 0.02	154	— 1 ± 1.0	+ 1 ± 1.0	+ 2 ± 1.0
6	0.22 ± 0.01	51.3	— 1 ± 1.0	+ 2 ± 0.9	+ 2 ± 0.8
8	0.22 ± 0.01	8.5	— 18 ± 1.2	— 18 ± 1.5	— 19 ± 1.0
6	0.20 ± 0.03	0.0	— 17 ± 2.0	— 18 ± 1.4	— 19 ± 1.3

TABLE VI

Absorption of 3.33 mM arabinose through rat intestine in vivo. Effect of Na+ concentration in the sugar solution. NaCl substituted by mannitol for isotony. Absorption time, 60 min. Inhibitions referred to the second absorption (control).

Animals N.°	1st. Abs. (154 meq. Na^+/l) µM/cm	2nd Abs. control (154 meq. Na^+/l) µM/cm	Successive Absorptions		
			Na^+ meq/l	Variations °/.	
				3:d Abs.	4th Abs.
5	0.10 ± 0.01	0.07 ± 0.003	154	+ 1 ± 1.0	+ 2 ± 0.9
4	0.12 ± 0.02	0.08 ± 0.004	8.5	— 1 ± 0.8	— 1 ± 1.0

in vivo by the Sols and Ponz's method (19) of sucessive absorptions the Na^+-dependence of the intestinal absorption of glucose. This fact is already observed using 0.3 M glucose solution, but has been better studied with 2.77 mM glucose, a concentration requiring active transport for its absorption to the blood. The Na^+-dependence has been confirmed at a wide range of concentrations, from O up to 231 meq Na^+/l, notably extending the previous results of Csaky (7). Within this range, the absorption rises with the increase of Na^+ concentration. (Fig. 1) It is difficult to discuss the kinetic relations between absorption (as glucose dissapeared) and Na^+ concentration, since the absorption should shall be a process resulting from an accumulation of the sugar in the cells and a posterior egress of it to the blood after some metabolic consumption by the tissues, and on the other hand the Na^+ concentration shifts during the absorption with cation levels lower than those of the plasma.

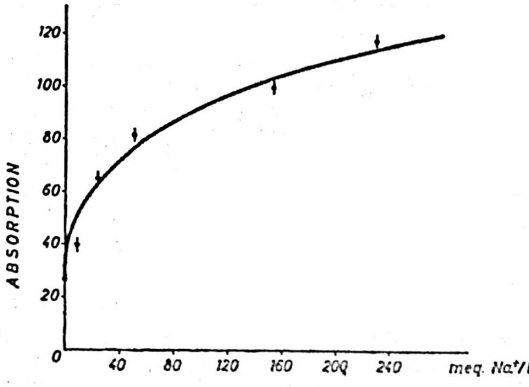

FIG. 1. Absorption of 2.77 mM glucose from the *in vivo* rat intestine, as depending on Na^+ concentration in the lumen. All the solutions were isotonized with mannitol, except that with 231 meq. Na^+/l.

The differences found between the absorption of glucose and galactose and that of fructose and arabinose are very interesting. Absorption of fructose and arabinose may be considered as not depending on Na^+ concentrations, a result that allows us to state that also *in vivo* only the movement of the actively transportable sugars is affected by the Na^+. The slight inhibition of fructose absorption with 8.5 meq/l or without initial Na^+, may be due to the fact of that transference of fructose is facilitated by its partial transformation in the cells (8, 9, 10, 21) and with these very low levels of Na^+ in the lumen a probable alteration of the mucosal cellular metabolism may be developed (14).

In this last sense also speak the experiments of reversibility. By passing from 8.5 meq Na^+/l up to 154 meq/l, the absorption of glucose is not quite restored but remains some grade of inhibition that slowly is going back. That so low level of Na^+ indeed may modify by

any way the functional state of the epithelial cells.

Summary

By the Sols and Ponz method of *in vivo* successive absorptions, the Na^+-dependence of the intestinal absorption of monosacharides has been studied. In the range between O to 231 Na^+/l the absorption of 2.77 mM glucose increases not linearly with the level of Na^+ in the sugar solutions. This dependence is not observed with fructose nor arabinose, and seems to be a propriety of the actively transportable sugars. The inhibition of the glucose absorption by very low levels of Na^+ is reversible, but this reversibility requires a certain time to be complete, revealing some alteration of the functional capacity of the mucosa.

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