SOME COMMENTS ON THE COUPLING BETWEEN INTESTINAL ABSORPTION OF GLUCOSE AND OF SODIUM

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1. Introduction

From the results of *in vitro* investigations Crane developed the hypothesis of co-transport or symport for intestinal sugar absorption [1, 2], which assumes that the carrier forms a ternary complex with the nonelectrolytes to be transported and with the sodium ion (for details see: [1–3]). According to this hypothesis the uphill transport of a nonelectrolyte is effected by the simultaneous downhill transport of sodium. Application of this carrier model to intestinal glucose absorption requires that at least two conditions should be fulfilled:

i) For uphill transport of glucose out of the intestinal lumen an opposite downhill concentration gradient for sodium is required.
ii) There should be a stoichiometric coupling between glucose uptake and sodium uptake by the intestinal epithelium.

To test these minimal requirements investigations *in vivo* would be required. However, until now no direct evidence for the sodium gradient hypothesis has been obtained by *in vivo* investigations [4, 5]. Therefore, additional *in vitro* investigations performed with an intestinal preparation under optimal conditions were desirable.

2. Methods

The experiments were performed with male Sprague-Dawley rats weighting 280–320 g, fasted for 24 hr. A jejunal segment of 10 cm length without blood supply was used for the perfusion studies by a method similar to that of Fisher and Parsons [6]. The intestinal segment was prepared in situ with the blood supply kept intact during preparation. A frit was inserted into the aboral part of the intestinal segment chosen. Then a second glass tube was inserted on the upper part of the intestinal segment. The time between cutting of the blood vessels and incubation in the chamber was less than 60 sec. The mucosal solution measured 7.0 ml, the serosal solution 90 ml. Gassing of the mucosal solution with carbogen (95% O_2, 5% CO_2) was accomplished by way of the frit immediately after filling with the mucosal solution. Defoaming of the solution was effected by using siliconized wire. It was assumed that the anoxia period between cutting of the blood vessels and the beginning of gassing of the mucosal solution was minimal (less than 60 sec). Additionally the serosal solution was gassed with carbogen. Incubation was at 37°.

The serosal solution contained Krebs-Ringer bicarbonate solution with glucose. The mucosal solution contained sodium chloride or potassium chloride or xylitol with additions of the various sugars used. The osmotic pressure of the solution was 280 mosmol–320 mosmol/ℓ as determined by freezing-point depression (Knauer-Osmometer). In most of the experiments ^{14}C-labelled sugars were used on the mucosal side. The total activity was 20 μCi/10 ml solution. The radioactivity was measured using a Tricarb scintillation counter (Packard). Glucose was determined with hexokinase–glucose-6-phosphate dehydrogenase. Fructose was analyzed in the same test by addition of hexoseisomerase [7]. In the figures the means of the results of 8–12 experiments are indicated.

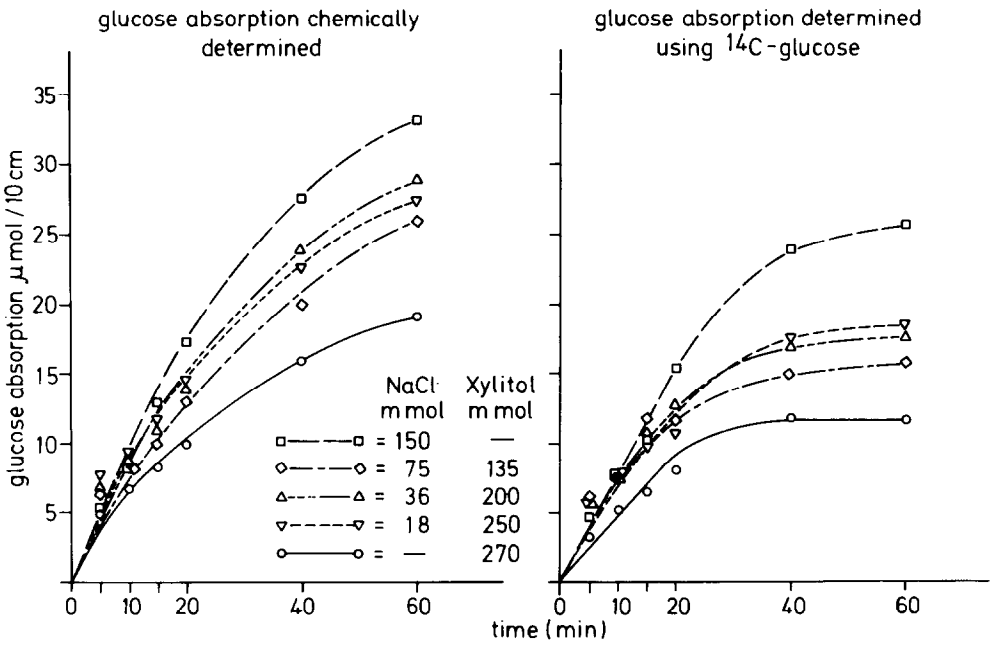

Fig. 1. Glucose absorption *in vitro* as influenced by gradual substitution of NaCl on the mucosal side of Xylitol (glucose concentration: 5.6 mmole/ℓ).

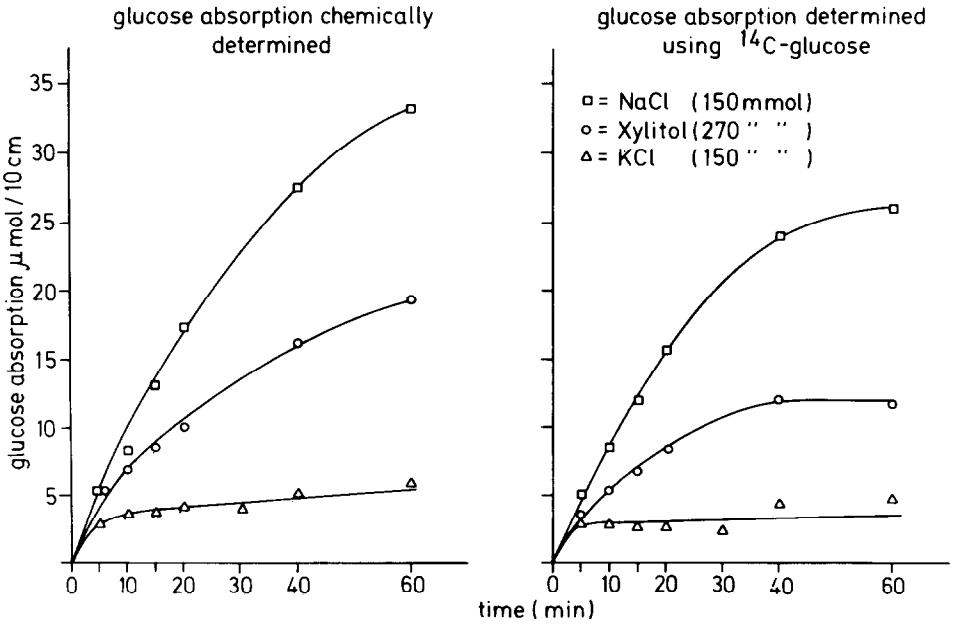

Fig. 2. Glucose absorption *in vitro* as influenced by substitution of NaCl on the mucosal side by KCl or Xylitol (glucose concentration: 5.6 mmole/ℓ).

3. Results

As shown in fig. 1 glucose absorption occurred in the absence of sodium from the mucosal solution. During the first 10 min of the experiment no significant differences were seen at the very different sodium concentrations used. In the course of the experiment a relative decrease of glucose absorption was seen in the experiments performed where a part of sodium chloride on the mucosal side was substituted by iso-osmolar xylitol. On the right side of the figure is shown that the uptake of label was not identical with the uptake of glucose. Obviously part of the glucose was metabolized by the mucosa and the metabolites diffused back to the mucosal solution. When the NaCl was substituted by KCl, uptake of glucose ceased within 5 min (fig. 2). In contrast to the results when xylitol substituted for NaCl, KCl substitution had not only a sodium depleting effect, but led also to potassium poisoning of the cells. It is known, that high potassium is lethal to most animal cells. Moreover, metabolism of labelled substances also ceased (fig. 2). Sodium excretion into the sodium-free incubation medium (sodium chloride substituted by xylitol) is decreased with increasing glucose concentration and, therefore, with increased glucose uptake (fig. 3). However, the same is true for fructose. As fructose is not actively transported by the glucose carrier mechanism but is metabolized by the intestinal epithelium this effect seems to be related to the metabolism of the epithial cells and not to active transport. This hypothesis is substantiated by the experiments shown in fig. 4, galactose and 3-*O*-methylglucose were without significant effect on sodium excretion in spite of active transport of these substances; whereas addition of glucose and fructose led to a significant decrease of sodium excretion.

4. Discussion

As shown earlier, intestinal uphill transport of sugars *in vivo* is accomplished without an opposite sodium concentration gradient. Generally, under *in vivo* conditions a sodium dependency of glucose absorption is not found [4, 5, 8]. Therefore, the existence of a microclimate is needed to maintain the ternary carrier (the sodium gradient) hypothesis of Crane.

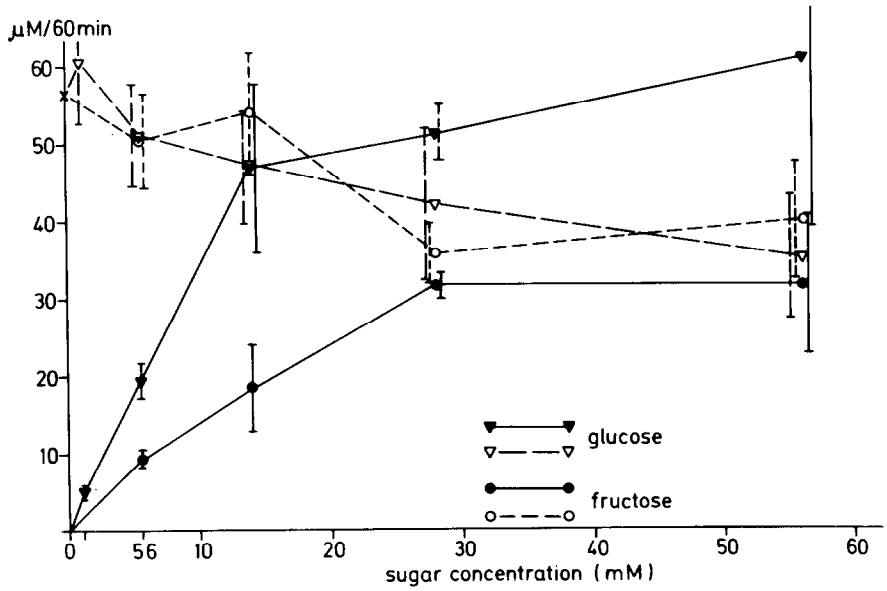

Fig. 3. Absorption of sugar (glucose or fructose) and excretion of sodium by the rat intestine, *in vitro*. The mucosal solution was sodium free initially. Xylitol (270 mM) was substituted for NaCl.

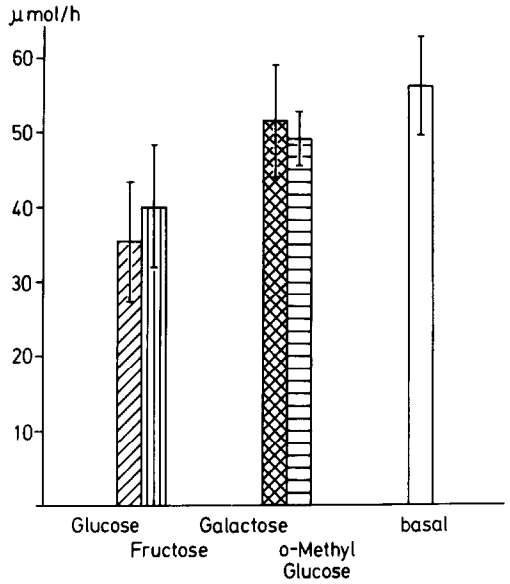

Fig. 4. Sodium excretion following addition of different sugars to sodium free mucosal solution. Addition of Xylitol (270 mM). $\bar{X} \pm$ S.D.

It is assumed, that the sodium ions pumped into the intestinal lumen are captured by the carrier at the brush border and are therefore not found in the intestinal lumen. It is claimed that the sodium concentration at the brush border would be high enough with respect to the intra-epithelial sodium concentration to accomplish an uphill sugar transport [7].

However, using sodium-free solutions iso-osmotic with blood for intestinal perfusion, the uphill transport of glucose is without any effect on the downhill excretion of sodium under *in vivo* conditions [4]. The increase of glucose absorption to tri-fold the rate of sodium excretion did not exert a measurable effect on sodium excretion in *in vivo* experiments [4]. These results constitute conclusive evidence against the existence of a coupling of glucose uptake to sodium uptake. According to the *in vitro* results, reported in this study there is also no evidence for a relation between glucose absorption and sodium uptake. Our results using xylitol for iso-osmotic substitution of sodium chloride differ from the results of Bosackova et al. [10] in that no relation between sodium concentration and glucose uptake is evident. Additionally, glucose uptake in the first period of the experiment was unaltered. Substitution of sodium chloride by potassium chloride led to a termination of glucose uptake due to potassium poisoning. Therefore, experiments performed under similar conditions (e.g. by Riklis and Quastel [11]) are no proof of the essential role of sodium.

In contrast to *in vivo* conditions, under *in vitro* conditions the loss of sodium into the intestinal lumen cannot be substituted by sodium from the blood circulation. Additionally, glucose required as metabolic fuel by the intestine is also not supplied by the blood *in vitro*. Therefore, the slight inhibition of sodium excretion by increasing glucose absorption *in vitro* (when using sodium free mucosal solution) seems to be a metabolic effect. Absorption of fructose, which is not transported actively with the ternary carrier, resulted also in an decrease in sodium excretion. On the other hand, 3-*O*-methylglucose and galactose which are transported by the carrier system did not show this effect. In contrast to 3-*O*-methylglucose and to galactose, fructose is metabolized by the intestinal mucosa to a considerable extent. Therefore it seems to be established that the slight inhibition of sodium excretion by fructose and by glucose is a metabolic effect and has no relation to the active transport of glucose or other sugars.

The validity of the sodium gradient hypothesis has never been adequately evaluated by *in vivo* experiments (see [5]). Recently Saltzman and coworkers [9] using human intestine found no effect on glucose absorption when the intraluminal sodium concentration was reduced from 140 meq/ℓ to as low as 2.4 meq/ℓ. Kimmich using isolated epithelial cells from chicken intestine also found no indication that glucose absorption is dependent on a sodium concentration gradient [12]. The incubation time in his experiments was 1–2 min only. Smulders and Wright performed experiments with hamster intestine clamped between two lucite half chambers [13]. There was no difference whether the sodium concentration was lowered on the mucosal side or on the serosal side. In both cases the galactose absorption was diminished to the same degree. Burdett and Schneider also claimed on the basis of *in vitro* experiments with hamster intestine that sodium is not essential for the active transport of glucose [14].

Acknowledgements

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