LOCALIZATION OF THE INVERTASE ACTIVITY IN THE CELLS OF THE SMALL INTESTINES OF ALBINO RATS

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The existing representations of the localization of the concluding stages of hydrolysis are extremely contradictory. A number of authors believe that the peptides and oligosaccharides are cleaved intracellularly [5-10]. On the other hand, there are data [2], indicating that the hydrolysis of the oligosaccharides takes place on the outer surface of the cells of the intestinal epithelium, after which the monomers are actively transported through the membrane.

To resolve the question of the localization of the concluding stages of hydrolysis, the following principle scheme of the experiment seemed advisable: blocking of the external surface of the intestinal cells makes impossible the contact of the substrates with the enzymes, localized both on the outer surface of the brush border, and intracellularly (Fig. 1A). Thus, not only digestion near the wall, but also intracellular digestion was eliminated. The localization of the enzymes could be differentiated after breakdown of the structure of the cells, the brush border of which was blocked. Actually, under these conditions the contact of the substrates with the enzymes of the outer surface of the membrane will be impossible, as before, while the activity of the intracellular enzymes of the broken-down cells should be almost entirely manifested (Fig. 1B).

Such experiments were carried out, and their results are presented below.

EXPERIMENTAL PROCEDURE

We investigated the localization of invertase in the intestinal epithelium of white rats. The pores of the brush border were blocked by treatment of the mucous membrane of the surface of the small intestine with 1% $Pb(NO_3)_2$ solution. The Pb^{++} ions, just like other multiply charged ions of the heavy metals, penetrate extremely slowly through the cellular membranes. It is no less important that a deposit of lead salts was formed between the microvilli of the brush border, on account of the sparingly soluble chloride, carbonates, practically insoluble phosphates, basic chlorides, and possibly other compounds as well. The formation of conglomerates of insoluble lead salts occurred as a result of the diffusion of Pb^{++} ions into the pores of the brush border from a solution of $Pb(NO_3)_2$ (in which the intestines were preliminarily incubated) and simultaneous diffusion of the Cl^-, CHO_3^-, and $H_2PO_4^-$ ions from the intestinal cells outward. The localization of the lead conglomerates* was monitored by electron microscopy.

For the electron microscopic investigations, pieces of small intestines were fixed for 1-2 h in a 2% OsO_4 solution in a veronal acetate buffer (pH 7.4) after preliminary incubation in a 1% $Pb(NO_3)_2$ solution or a 0.4% $NaNO_3$ solution. Part of the material was fixed in liquid oxygen, ground in a mortar, and then stained in a 2% solution of OsO_4. The specimen was passed through alcohols of increasing (by 10°) concentrations, beginning with 40° and continuing up to absolute. The material was contrasted with phosphotungstic acid in absolute alcohol. Then the pieces of intestines were set in a mixture of methyl and butyl methacrylates (methyl to butyl ratio 6 : 1). Polymerization

*General formula Pb_mA_n.

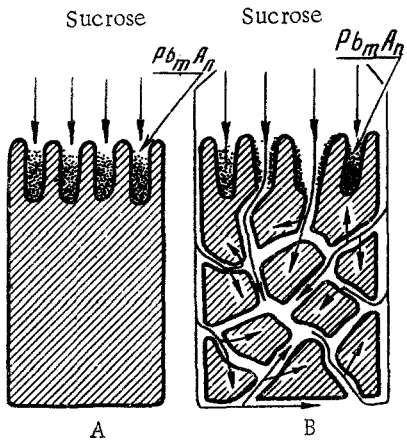

Fig. 1. Principle scheme of experiment. Conditions of contact of substrates with enzymes in undecomposed (A) and decomposed (B) intestinal cells, treated with isotonic $Pb(NO_3)_2$ solution.

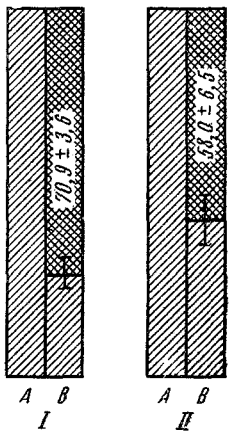

Fig. 2. Decrease in the activity (numbers on the black column) of undecomposed (I) and homogenized (II) pieces of small intestine after treatment with isotonic lead nitrate solution (IB and IIB) in comparison with control (IA and IIA), the activity level of which is taken as 100%.

was conducted in a thermostat at 55-60°. Ultrathin slices, produced on the UMT-2 ultramicrotome, were investigated in the JEM-5 g electron microscope at 80 kV.

In our experiments, we compared the invertase activity of unwound pieces of small intestine, treated with a 1% $Pb(NO_3)_2$ solution (experimental) and treated with an isotonic $NaNO_3$ solution (control), which made it possible to establish the degree of blockage of the surface of the brush border. In the same experiments we compared the invertase activity of homogenates from the experimental and control pieces of intestine.

The work was conducted on white rats of the Wistar line, 150-200 g in weight. After the animals had been killed, a portion of the small intestine 10-20 cm long, bounded by the duodenum, was rapidly extracted and washed with a 0.4% solution of $NaNO_3$. Segments of the small intestine, unwound and placed over glass rods, were placed in a 1% solution of $Pb(NO_3)_2$ or in a solution of $NaNO_3$ isoosmotic to it (control) for preliminary 10 min incubation on a shaker. After washing (twice for 3 min in 0.4% $NaNO_3$ solution), the pieces were divided into equal 2 cm portions. Part of the experimental and control pieces were ground in a mortar in the presence of liquid O_2.

The homogenates* and whole pieces of intestines were incubated in Ringer's solution, containing 1% sucrose, for 10 min at 37-38° in a Warburg apparatus with constant mixing. The enzymatic reaction was stopped by rapid chilling after the basic incubation.

The invertase activity was determined according to the increase in the reducing sugars (Nelson method in the modification of A. M. Ugolev).

In addition, a supplementary series of experiments was conducted with preliminary grinding of the pieces of small intestine in a porcelain mortar with glass. In the experimental sample, the homogenates were washed out of the mortar with 0.2 ml of a 1% $Pb(NO_3)_2$ solution with 2.8 ml of Ringer's solution; in the control samples—with 0.2 ml of 0.4% $NaNO_3$ solution with 2.8 ml of Ringer's solution. To each sample we added 3 ml of a 1% sucrose solution, after which the basic incubation was conducted according to the scheme described above (Fig. 1).

RESULTS

As is shown in Fig. 2 (IA and IB), hydrolysis of sucrose by pieces of small intestine treated with 1% $Pb(NO_3)_2$ solution is approximately 3 times slower than that of the untreated pieces.† The substantial weakening of the invertase activity, detected in these experiments, may depend either on blockage of the pores of the brush border of the intestinal epithelium, or on the inhibiting action of Pb^{++} ions on the enzyme molecules.

In the electron microscopy, it was evident that the conglomerates of lead salts were arranged at different heights between the villi of the brush border of the intestinal epithelium, sometimes covering them (Fig. 3b). Lead penetrated all the way to the base of the microvilli, forming "stoppers."

On the transverse section, it was evident that lead salts formed a honeycombed structure around the microvilli (Fig. 3c).

*Henceforth, the term "homogenate" will be used to mean the detritus obtained upon grinding, which, as can be seen in electron microscopy, represents fine, irregularly shaped fragments of cells.

† In control experiments it was shown that preliminary incubation of the intestines in isotonic $NaNO_3$ solution does not influence their invertase activity.

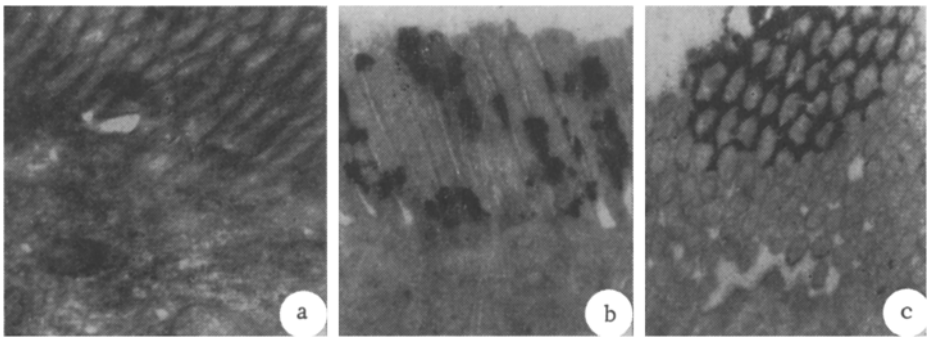

Fig. 3. Localization of conglomerates of lead in intestinal cells. a) Control, pieces of intestine treated with isotonic $NaNO_3$ solution. Brush border and apical portion of the cell. Magnification 30,000×; b, c) experimental: pieces of intestine treated with isotonic $Pb(NO_3)_2$ solution. Longitudinal (b) and transverse (c) sections. Conglomerates of Pb_mA_n, surrounding the microvilli at various levels, are visible. Magnification 75,000× (b) and 38,000× (c).

In the control experiments, no granules were detected among the microvilli (Fig. 3a).

It is most important that the lead conglomerates are always localized extracellularly. We never observed lead granules either in the cytoplasm of the cells of the intestinal epithelium or in the microvilli. This probably occurs both as a result of the fact that polynuclear ions penetrate poorly through the cellular membranes and as a result of the unique "anionic barrier," arising as a result of diffusion from the cells of the anions that form insoluble salts with Pb^{++}. Part of the brush border remains free, which makes the conservation of a certain fraction of the enzymatic activity comprehensible. No correlation could be detected among the number and the localization of the lead conglomerates detected in electron microscopy, and the degree of inhibition of the enzyme activity.

The degree of decomposition of the cells during the grinding process was monitored by electron microscopy. It was found that the detritus consists of fine, irregularly shaped cell fragments.

Characterizing the principle of the method, we noted that the decrease in the enzyme activity as a result of blockage of the pores of the brush border of intact cells does not provide the possibility of judging the localization of the concluding stages of hydrolysis. The resolution of this question may be approached by studying the influence of breakdown of the cellular structures on the enzyme activity. If hydrolysis occurs intracellularly, the activities of the treated and untreated homogenates should be approximately the same. On the contrary, in the case of hydrolysis near the wall, inhibition of the enzyme activity of homogenates preliminarly treated with an isotonic $Pb(NO_3)_2$ solution, in comparison with the untreated homogenates, should be approximately the same as the inhibition of the enzymatic activity of treated pieces of intestines in comparison with the untreated. As it follows from the data presented in Fig. 3, the experimental results correspond to the second hypothesis.

A certain weakening (statistically unreliable in our experiments) of the inhibitory effect may depend on the fact that during the process of grinding, part of the lead is removed from the pores of the brush border, which is confirmed by the data of electron microscopy.

It is important to emphasize that the hypothesis assuming inhibition of the intracellular invertase by Pb^{++} ions, localized in the pores of the brush border, was verified experimentally and proved incorrect. In a series of experiments, lead, precipitated in Ringer solution, in an amount one to two orders of magnitude greater than that detected in the brush border, was added to intestinal cells. In this case, the invertase activity was equal to 49.2 ± 4.4 mg%, in comparison with the activity in the control—44.1 ± 4.05 mg%. The difference between these values proved statistically unreliable.

Thus, the results obtained permit us to conclude that the concluding stages of sucrose hydrolysis take place near the wall and contradict the hypothesis of the intracellular accomplishment of this process.

After this work had been sent to press, we conducted analogous experiments to elucidate the influence of Ag^{+} and Cu^{++} ions on the invertase activity. It was found that these cations, which practically do not penetrate through

the cellular membranes during short periods of time, almost entirely inhibit the invertase activity. These data may be considered as a new argument in favor of the hypothesis of localization of invertase on the outer surface of the cellular membrane.

As is well known, the hydrolysis of sucrose is accomplished not only by invertase, but also by certain maltases [3]. Thus, the conclusion that the concluding stages of hydrolysis are carried out in the zone near the walls, and not the zone of intracellular digestion, should pertain not only directly to invertase, but also to some of the intestinal maltases.

There is information permitting the assumption of the presence of a wall mechanism of hydrolysis for a whole series of other substances [2].

The previous data indicating that breakdown of the structure of the intestinal cells leads not to an increase (as we should have expected in the case of intracellular digestion) but to a decrease in the invertase activity [1, 8] possible in hydrolysis on the surface, agree with the results described in this work.

In conclusion, it seems important to note the numerous data on diseases of the digestive system, due to the absence of various disaccharidases in the intestinal cells, in particular, those that cleave sucrose [4, 11, 12]. These diseases, encountered especially often in children, should evidently be treated as diseases of parietal digestion.

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