ENZYMES OF THE SMALL INTESTINAL MUCUS

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All substances transported from the intestinal lumen into the internal medium of the body must pass through the layer of mucous deposits covering the wall of the small intestine [2, 10]. This layer of mucous deposits is formed by the mucus proper, secreted by the goblet cells, and by fragments of the epithelium and villi, desquamated during life and retained in the layer by the viscous mucous secretion [4, 5], included in the bulk of the layer. Continuous secretion of mucus and the desquamated cells of the structures of the intestinal wall, as well as the continuously renewed glycocalyx [7], cause growth of the layer of mucous deposits from the side of the surface of the intestinal wall. The luminal surface of the layer is exposed to the action of the chemically aggressive medium of the intestinal lumen and the mechanical action of the flow of chyme. As a result of these influences the luminal surface of the layer of deposits is destroyed and its components pass into the lumen. On the surface of the small intestine there is thus a continuously renewed heterogeneous layer, which participates in the transport of materials into the internal medium.

In the investigation described below an attempt was made to study the possible role of the layer of mucous deposits in degradation of materials transported through it. For this purpose, activity of enzymes synthesized by the epitheliocytes was determined in the material of the layer by histochemical methods; these enzymes included alkaline phosphatase (AlP), leucineaminopeptidase (LAP), dipeptidyl-aminopeptidase IV (DAP), lactase, and saccharase; activity of the pancreatic enzymes α-amylase and trypsin in the layer also was determined biochemically.

EXPERIMENTAL METHOD

Histochemical determination of activity of enzymes synthesized by the epitheliocytes was undertaken in biopsy specimens from the small intestine of dogs and rats. The biopsy material was frozen in pentane and cooled with a mixture of dry ice and acetone. Enzyme activity was determined as follows in frozen sections 10-12 μ thick: alkaline phosphatase by the method in [1] (naphthol-As-BY-phosphate was used as the substrate), LAP by the method in [11] (leucyl-4-methoxy-β-naphthylamide as the substrate), DAP by the method in [3] (glycine-proline-4-methoxy-β-naphthylamide as the substrate), saccharase by the method in [9] (6-bromo-2-naphthyl-α-D-glycopyranoside as the substrate), and lactase by the method in [3] (α-naphthal-β-glucoside as the substrate).

To prevent diffusion of the reaction product during the histochemical investigation, semipermeable membranes on which the specimen was mounted were used.

Activity of the pancreatic enzymes was studied in chronic experiments on healthy dogs with several fistulas: free-flowing fasting juice was collected through a duodenal fistula and biopsy specimens were taken from the intestinal mucosa. The layer of mucous deposits was separated under a binocular loupe and the integrity of the brush border and glycocalyx of the epitheliocytes was verified under the electron microscope. Biochemical measurements of α-amylase activity in samples of juice and mucus were made by the method in [6] and trypsin activity was measured by the method in [8].

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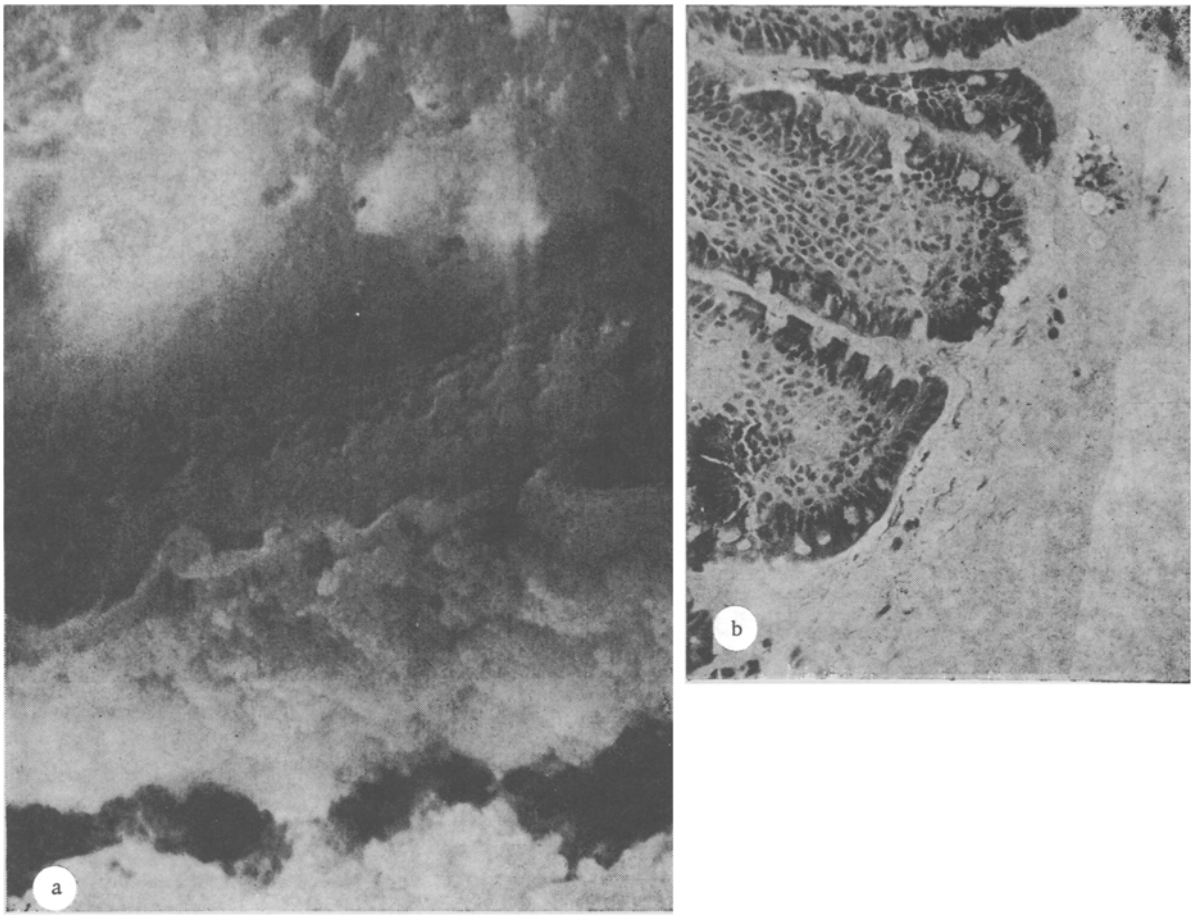

Fig. 1. Sections of small intestine with intact layer of mucus. a) Appearance of mucus on surface of section; b) appearance of section with villi cut longitudinally.

EXPERIMENTAL RESULTS

The results of the morphological investigations confirmed continuity of the layer of mucus covering the apical parts of the villi over the whole surface of the small intestine, and with a thickness of up to 1.5 mm in dogs and up to 1.0 mm in rats. Continuity of the layer of mucous deposits was demonstrated by the use of special methods of fixation of the sections for microscopic examination (Fig. 1). This result confirms the hypothesis [10] that the layer of mucus always participates in the transport of materials from the lumen of the intestine into the blood stream.

Histochemical and biochemical determination of enzyme activity in the layer of mucus showed that all enzymes tested are present in the layer in an active state. Histochemical staining revealed products of the reaction for saccharase and lactase only in the brush border and membranes of the epithelial cells contained in the layer of mucus. This indicates a constant connection between the above-mentioned enzymes and epithelial cells. Products of the reaction for AlP, LAP, and DAP were found both in the immediate vicinity of the epithliocyte membranes and in the space surrounding desquamated epithelial cells in the layer of mucus deposits (Figs. 2 and 3). This distribution of the stain is evidence of the spread of molecules of these enzymes in material of the layer of mucus surrounding the cells and that the layer itself acquires corresponding hydrolytic activity. During biochemical determinations of activity of pancreatic α-amylase and trypsin, an uneven distribution of these enzymes also was found in the thickness of the layer. In thin layers of mucus their relative activity was higher than in thick layers. Dependence of specific activity of the enzyme on the weight of the samples is exponential in character. Comparison of α-amylase and trypsin activity in samples of mucus and in the juice bathing it showed that the mucus has relative amylase activity 38 ± 9 times greater than that of the juice, whereas the corresponding ratio for trypsin activity was only 2.0 ± 0.7.

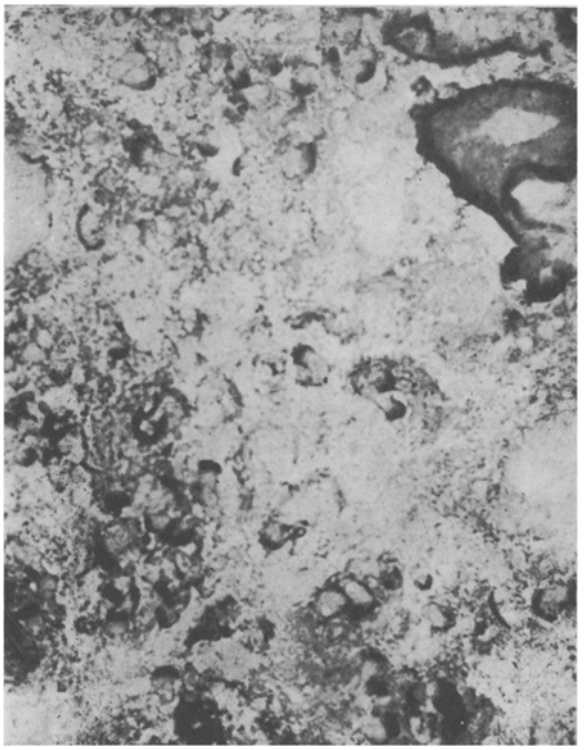

Fig. 2. Alkaline phosphatase in mucus of dog's small intestine. Sections cut parallel to surface of small intestine. 140 ×.

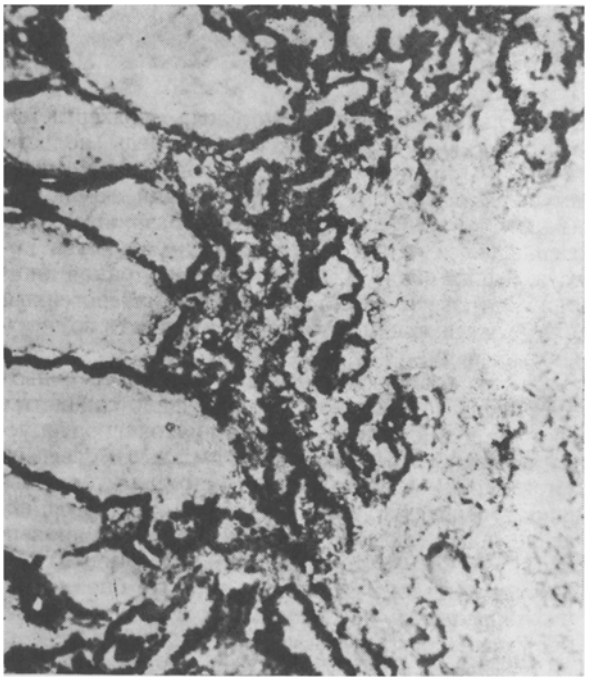

Fig. 3. DAP in dog's small intestine. Reaction product visible in mucus and near membranes of epitheliocyte. 100 ×.

On the basis of these results the following hypothesis can be put forward: The surface of the small intestine is completely covered by a layer of mucous deposits containing active epithelial and pancreatic enzymes. All substances transported from the intestinal lumen into the blood stream pass through the layer of mucous deposits where they are exposed to the action of enzymes contained in it.

Polymer materials and products of luminal hydrolysis, transported in the layer of mucus toward the absorbing surface of the cell, meet active enzymes — endo- and exohydrolases, and undergo hydrolysis to a certain degree. The productivity of this hydrolytic zone requires further study. At this stage we can indicate a number of facts that point to its being highly effective. First, the layer of mucous deposits is richer in certain pancreatic enzymes than the intestinal medium and, consequently, hydrolysis may be more effective in it. Second, the layer accumulates in its substance epitheliocytes which are constantly being produced, and the enzymes synthesized by them. The distribution of pancreatic enzymes and of enzymes synthesized by epitheliocytes in the layer corresponds to the distribution of substrates of different molecular weight in the thickness of the layer: Pancreatic enzymes are located in the luminal part of the layer, into which polymer substrates enter and are retained, the juxtamural part of the layer contains enzymes of epitheliocytes, substrates for which are products of the action of pancreatic enzymes. The observed distribution of enzymes in the layer and the changing pattern of enzyme—substrate relations evidently facilitate a more profound degree of hydrolysis on account of the sequential, conveyor type of processing of substrates entering the layer by enzymes of the appropriate specificity.

Degradation of polymers in the mucus is important for the more rapid transport of materials through the viscous layer. Hydrolysis products, with a smaller molecular weight, are transported more rapidly in the material of the layer than the original substrates. This acceleration is due to an increase in the coefficient of diffusion, with an increase in the molecular weight of the diffusant. In addition, the appearance of an increased concentration of products in the layer in the immediate vicinity of the absorption surface, due to hydrolysis, increases the concentration gradient and, correspondingly, the flow of materials toward the intestinal wall.

When defining the physiological role of the degradation of materials in the mucus, it must be noted that, on the one hand, this is a protective process, for it prevents the entry of immunoactive substances into the blood stream and, on the other hand, it is a digestive process, for most of the materials degraded in the layer are nutrient. By analogy with luminal and contact digestion, the process of degradation of materials in the mucus may be called mucous digestion.

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