A MUCOPOLYSACCHARIDE COMPONENT OF THE PEPSINOGEN GRANULES IN THE CELLS OF THE GASTRIC MUCOSA

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Pepsinogen is formed in the principal cells of the gastric mucous membrane. This has been established by histophysiological and histochemical investigations of the stomach [5, 6, 7, 9, 10]. It has recently been suggested on physiological grounds that pepsin, being a protein, is secreted by the fundal glands of the stomach in some form of combination with glycoprotein and, perhaps, with other proteins [1, 16]. The problem of the combination of pepsin with other proteins or polysaccharides has not yet been solved.

EXPERIMENTAL METHOD

To determine the chemical nature of the substances secreted by the principal cells of the fundal glands, a histochemical investigation was made of the gastric mucosa of dogs using the stains toludine blue [13], Moruzzi's alcian blue [12], Shuboch's acid solution of basic brown [4], and Shabadash's modification of the PAS reaction [3]. Staining of the sections in a 0.1% solution of toluidine blue was carried out in buffer solutions in a pH range of 0.5-6.0 (at pH intervals of 0.5). To obtain more accurate information regarding the chemical composition of the substances secreted, further investigations were made using the analytical scheme of Spicer and Lillie [15], including methylation, dimethylation, and control treatment with alkali.

Success in the detection of the different tissues components is largely dependent on the method of fixation. Preliminary investigations using different conditions showed that pepsinogen granules were best demonstrated after fixation in Hamperl's fluid [8, 14] (formalin 33 ml, ethanol 80° 66 ml, potassium acetate 3 g). The material was also fixed in 10% neutral formalin, in Shabadash's neutral fixative, and in Carnoy's fluid.

EXPERIMENTAL RESULTS

At low pH values, toluidine blue did not stain the histological structures of the gastric mucosa. Starting at pH 1.5 (fixation of material by Hamperl's method) a light blue staining of the basal protions of the cytoplasm of the principal cells appeared. The nuclei of the principal cells, situated in the basal portion, were translucent, vesicular in form, and possessed a well defined nuclear membrane and nucleoli. At pH 3 a red staining of the secretory granules of the principal cells—Michaelis's γ-metachromasia [11]—appeared in addition to the blue (Fig. 1). The staining of the secretory granules was intensified when the pH of the dye solution was 4.0.

The secretory granules of the principal cells were clearly distinguished after staining with an acid solution of basic brown. The granules were an intense brown in color; filling the cytoplasm of the cells, they were more densely packed in the apical portions (Fig. 2). At the periphery of each granule there was a light rim of unstained cytoplasm. The oxyntic cells were not stained with basic brown and appeared as clear areas, round in shape.

Staining with alcian blue and Shabadash's modification of the PAS reaction, the pepsinogen granules, like the other substances of the principal cells, remained unstained, whereas the secretory granules of the parietal cells were intensively stained.

Because of the difference in the degree of dissociation of the carboxyl and sulfate groups of the polysaccharides, reactions with metachromatic dyes can be used to detect sulfated acid polysaccharides [2]. It is also ac-

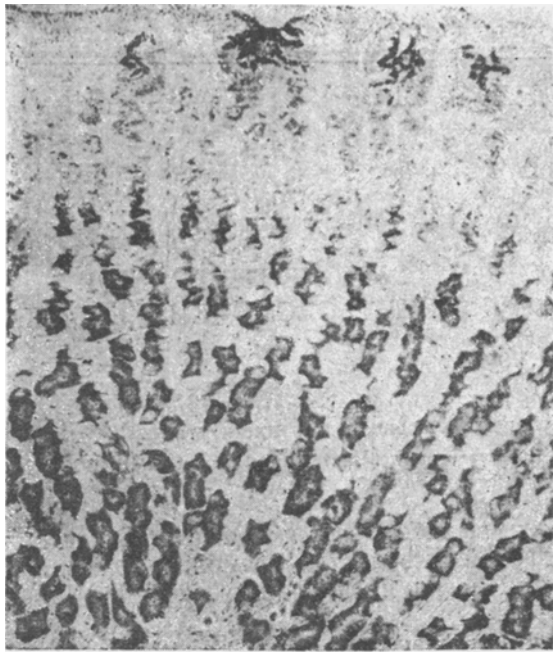

Fig. 1. Gastric mucosa of a dog. Photomicrograph. Stained with toludine blue at pH 4.5. Objective 10×, ocular K-5.

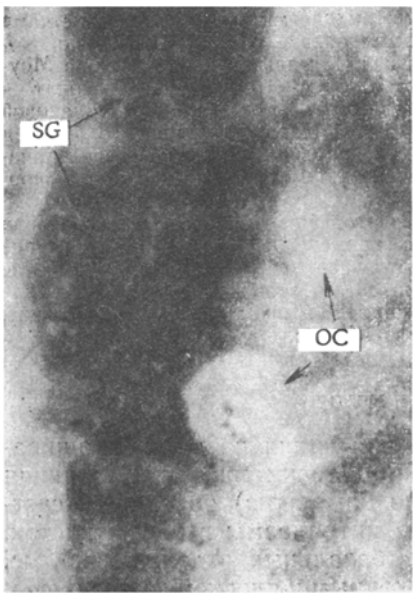

Fig. 2. Part of a fundal gland of a dog's stomach. Secretory granules (SG) of the principal cells stained with basic brown. The oxyntic cells are unstained (OC). Photomicrograph. Objective 90×, ocular K-5.

cepted that the well marked γ-metachromasia of sections of animal tissues may be explained by the presence of esters of sulfuric acid or of substances containing other acid groups [13].

The presence of acid sulfated polysaccharides in the secretory granules of the principal cells was confirmed by experiments in which the sections were methylated and demethylated before being stained with toluidine blue and basic brown. Methylation completely suppressed the staining of the granules, and it was not restored by demethylation. After control treatment with alkali a very slight decrease was observed both in the metachromasia and in the staining with basic brown. When preceding staining with alcian blue and by the PAS reaction, methylation and demethylation did not alter the properties and histochemical reactions of the principal cells: in every case no staining occurred.

During methylation of Spicer and Lillie's method an irreversible detachment of a sulfate group and esterification of carboxyl groups take place, so that the staining of these substances is prevented and the metachromasia disappears. During demethylation ester bonds are broken and carboxyl groups are liberated, which leads to restoration of the basophilia and metachromasia, for which these groups are responsible. Demethylation does not restore the basophilia and metachromasia inherent in sulfate groups.

The absence of staining of the granules of the principal cells in the PAS reaction may be attributed to the fact that acid polysaccharides are PAS-negative [2]. Alcian blue, by Pearse's method [13], stains the acid mucopolysaccharides of the mucins of epithelial and connective tissue, but does not stain most nucleoproteins.

Hence, the presence of acid sulfated polysaccharides in the secretory granules of the principal cells is confirmed by the metachromasia during staining of sections of the gastric mucosa with toluidine blue, by the demonstration of granules with an acid solution of basic brown, by methylation and demethylation of the sections and subsequent staining, and also by the negative results of staining by the PAS reaction and alcian blue.

It is concluded that pepsinogen is present in the secretory granules of the principal cells of the fundal glands in the form of a mucoprotein complex, the carbohydrate component of which is an acid sulfated polysaccharide.

SUMMARY

The presence of acid sulfated polysaccharides was revealed in the composition of secretory granules of principal cells in the fundal glands of the dog's stomach. The latter were demonstrated by staining with toluidine blue, basic brown, alcian blue and the PAS reaction and by Spicer and Lillie's analytical scheme.

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