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## SPECIFIC SECRETORY INCLUSIONS IN THE PYLORIC PART OF THE MOUSE STOMACH

I. N. Borisov and A. L. Zashikhin

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Specific granules were found in the epithelium of the pyloric part of the mouse stomach on staining with aldehyde-fuchsin. They were PAS-negative and did not stain with lead hematoxylin. Their production in intact mice is limited to a small zone of the pylorus, with the appearance of a circumscribed spot.  $\alpha$ -Adrenergic stimulation caused widening of the zone in which these granules were found. Combined treatment with Inderal and general cooling led to a sharp increase in the zone of distribution of the cells containing these granules.

**KEY WORDS:** epithelium of pylorus; secretory granules; adrenergic stimulation; exposure to low temperatures.

Inclusions of mucoid and mucin and inclusions containing enterohormones are found in the epithelial cells of the gastrointestinal tract.

This paper gives the results of experiments showing the presence of special and hitherto undescribed inclusions in the stomach, revealed by staining with aldehyde-fuchsin. In intact animals these inclusions are found only in a narrow zone of the pylorus. In other parts of the stomach and in the intestine, no such inclusions are found and, for that reason, they are called specific inclusions.

### EXPERIMENTAL METHOD

The pyloric part of the stomach of albino mice was investigated. Experiments were carried out on 15 mice, of which six were intact. Seven mice received injections of adrenoblockers for 1 week: Two animals received Inderal (propranolol), 3 received dihydroergotoxin, and 2 received both adrenoblockers simultaneously. The other two mice received Inderal and exposed to general cooling at 5°C. Material from the experimental animals was fixed by Bouin's method and that from the control animals in Bouin's or Carnoy's fluid or formalin and embedded in paraffin wax. Serial sections were cut transversely and longitudinally to the pylorus and stained with aldehyde-fuchsin (preliminary oxidation with permanganate, counterstaining with hematoxylin-orange), and lead hematoxylin and by the PAS reaction.

### EXPERIMENTAL RESULTS

On staining the epithelial cells with aldehyde-fuchsin, the apices of the cells containing mucoid stained diffusely. In addition, in a small proportion of cells bright dark violet granules could be seen. They had distinct outlines and were distributed throughout the cytoplasm. The concentration of these granules was not predominantly high in the apical or basal parts of the cells (Fig. 1). Sometimes the granules were concentrated around the nucleus (Fig. 2). In intact mice cells with granules of this type were found in a small zone of the pylorus, close to its junction with the duodenum. The zone was oval in shape and contained 20 to 30 pits. The

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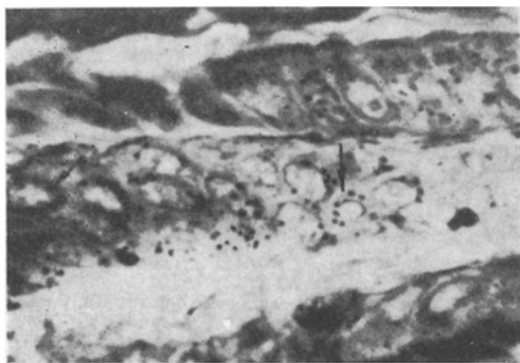


Fig. 1

Fig. 1. Epithelial cells of gastric pit from pylorus of mouse. Arrow indicates cell containing specific inclusions in different parts of cytoplasm. Stained with aldehyde-fuchsin, alum-hematoxylin and orange. Magnification 600  $\times$ .

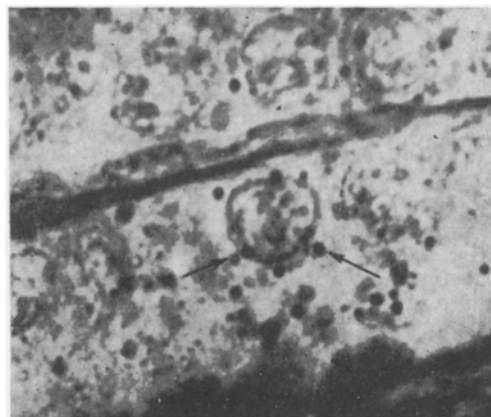


Fig. 2

Fig. 2. Distribution of specific inclusions (black granules) in cytoplasm of cells lining gastric pit. Arrow indicates region of concentration of granules around karyolemma. Stained with aldehyde-fuchsin and alum-hematoxylin. Magnification 900  $\times$ .

upper two-thirds of these gastric pits was lined with epithelium containing the granules described above. Sometimes these cells also emerged on the surface of the stomach. These distinctive features of localization suggested that the granules stained with aldehyde-fuchsin were not atypical collections of mucoids. Verifications by the PAS reaction confirmed this hypothesis: Unlike mucoids these granules were PAS negative. In animals receiving the  $\alpha$ - and  $\beta$ -adrenoblockers simultaneously, the number and distribution of the granules described above did not differ from the control. In mice receiving the  $\alpha$ -adrenoblocker, specific granules were not found whereas mucoid was present in the epithelium. Blocking of the  $\beta$ -adrenoreceptors led to widening of the zone (up to 100 pits) in which the specific granules were found. Cells with granules of this sort were concentrated in this case in the outer (near the surface of the mucosa) parts of the gastric pits.

$\alpha$ -Adrenoreceptors in glands of entodermal origin are known to inhibit, whereas  $\beta$ -adrenoreceptors facilitate the liberation of secretion. The specific granules behaved in the same fashion, confirming their secretory nature. Granules were particularly abundant after a combination of Inderal and general cooling (which is how they were first discovered). The granules were present in all cells of the epithelium of the pits — from the surface to the pyloric glands, and the zone lined by this type of epithelium commenced about 10 pits short of the boundary between the stomach and duodenum and it occupied more than half of the pylorus. In other parts of the stomach the granules described above were never seen.

The whole epithelium lining the pyloric part of the stomach can thus synthesize a specific secretion which stains with aldehyde-fuchsin. Under normal conditions, however, the granules of this secretion accumulate only in cells of a very small zone of the mucosa. If the contents of the specific inclusions are discharged into the lumen of the stomach, the concentration of this substance in the lumen of the stomach must be low. Since the granules were not concentrated in the apical parts of the cells, this suggested that they are discharged into the internal milieu of the body and contain some sort of enterohormone. Endocrine cells, scattered among the integumentary cells, and cells of the exocrine glands are known to be present in the epithelium of the gastrointestinal tract. From 3 to 6 types of such cells has been identified in the stomach [1-5]. In light microscopy they can be demonstrated by silver impregnation or staining with lead hematoxylin, and also during the detection of "chromaffin" substances. To test this hypothesis, sections through the pylorus were stained with lead hematoxylin [2]. The granules described above were not revealed. The secretion staining with aldehyde-fuchsin differs from the secretion of the enterochromaffin cells also in that it is not found in specialized cells but in ordinary integumentary cells, distinguishable morphologically from the other cells of the gastric epithelium. The chemical composition and physiological role of the specific secretory granules described above require further study.

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