MAST CELLS AND α-ENDORPHIN-PRODUCING CELLS IN THE ANTRAL MUCOSA

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α-Endorphin, an endogenous morphine-like substance, is formed from its biosynthetic precursor pro-opiomelanocortin. ACTH, melanocyte-stimulating peptides of different types, insulinotrophic hormones, and γ- and γ-endorphins are all formed from pro-opiomelanocortin. Most of these hydrolysis products can be detected in the gastrointestinal tract. For instance, Larsson [6] found ACTH in G cells whereas Polak et al. [9] reported the existence of immunoreactivity of methionine-enkephalin in gastrin cells. The precursor of ACTH and the endorphins has recently been found in rat mast cells (MC) [5]. Previously the peptide hormone VIP was found in MC [4], and on that basis Solov'eva [2] included cells of this type among endocrine cells of the APUD system.

The aim of this investigation was an immunohistochemical study of α-endorphin-producing cells and also a study of MC in the antral mucosa of the human stomach.

EXPERIMENTAL METHOD

Altogether 13 men aged from 18 to 30 years, undergoing in-patient treatment, were studied. During endoscopic investigation diagnostic biopsy was carried out on all patients on the antral portion of the stomach. The biopsy material was fixed in a mixture of a saturated solution of picric acid and neutral formalin (3:1) and embedded in paraffin wax. Serial sections (3-5 μ thick were stained with hematoxylin and eosin, with Bismarck brown (after Shubich), and with alcian blue (pH 1.0) to detect MC; the argyrophilic reaction of Grimelius [14] was carried out, and also immunohistochemical staining with a peroxidase–antiperoxidase complex (from "Miles," England) by Sternberger's method (the PAP test).

Antiserum obtained by immunization of Chinchilla rabbits with conjugates of bovine serum albumin and synthetic α-endorphin (Laboratory of Peptide Synthesis, Head M. I. Titov, All-Union Cardiological Scientific Center, Academy of Medical Sciences of the USSR) was used for the immunomorphologic investigation. The antiserum had high specificity: According to the results of radioimmunoassay, antibodies against α-endorphin did not react with enkephalins, β-endorphin, or the C-terminal fragment of β-endorphin, but had cross reactivity of about 10% with γ-endorphin [1].

Stained cells were counted by means of a special grid; in each preparation 5-10 fields of vision were studied and the result expressed as the number of cells per square millimeter of gastric mucosa. The results were subjected to statistical analysis by Student's t test at a 95% level of significance ($P < 0.05$) and they are given in Table 1.

EXPERIMENTAL RESULTS

MC in the antral portion of the stomach were found both in the lamina propria of the mucosa and in the epithelium of the neck and upper third of the pyloric glands (Fig. 1a). Stromal MC were large, mainly oval in shape with a central nucleus, less frequently elongated, and distributed around the glands. In most cells the granules were ill defined. Interepithelial MC were similar in shape with a central nucleus; cells of this kind also were found beneath

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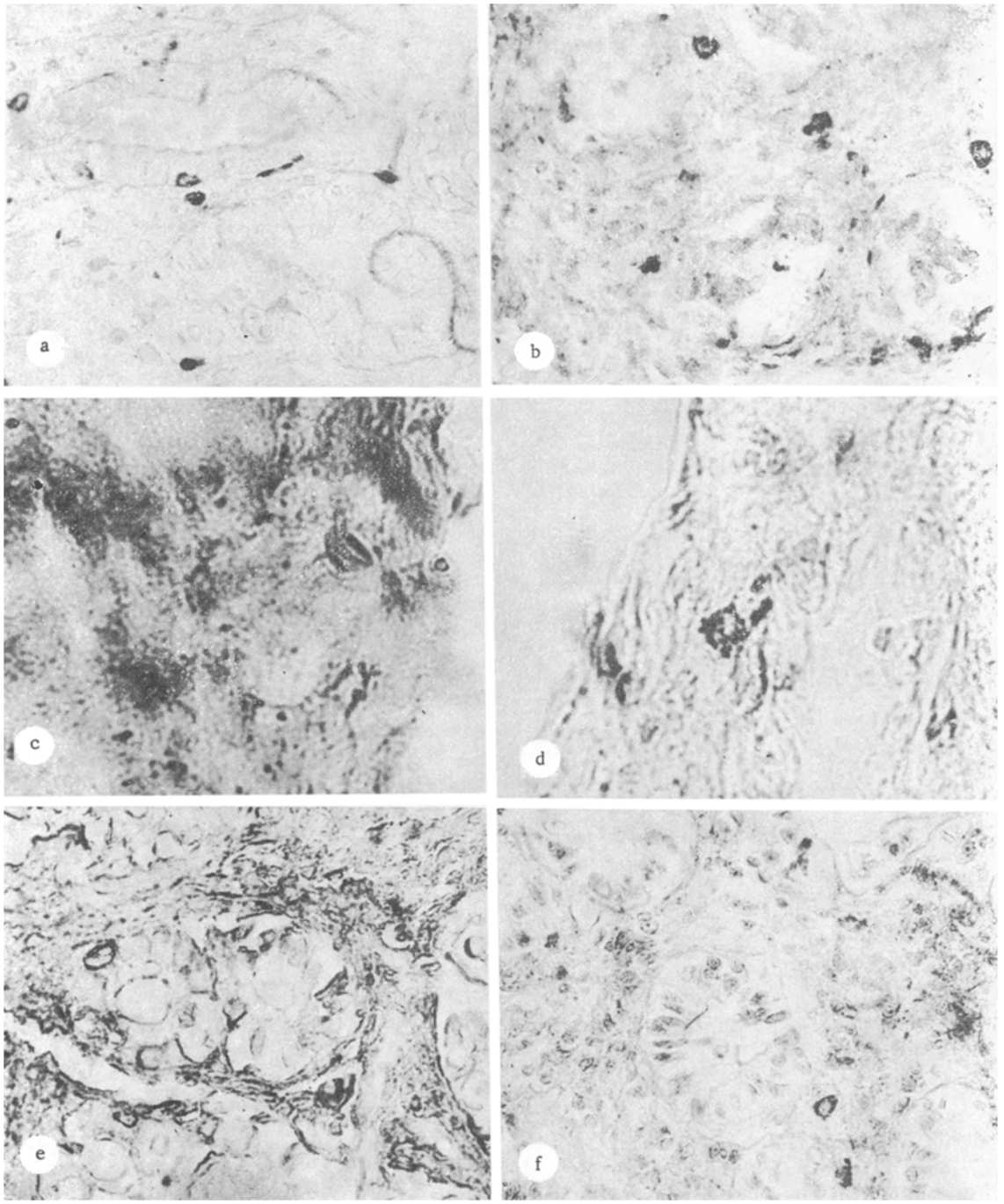

Fig. 1. Antral mucosa. a) MC in epithelium and lamina propria. Bismarck brown (Shubich's method); b) AEP cells in lamina propria. PAP reaction; c) AEP cell in lamina propria, PAP reaction + staining with alcian blue (pH 1.0); d) serial section – the same cell. Stained with Bismarck brown (by Shubich's method); e) AEP cell in epithelium. PAP reaction; f) argyrophilic cells in lamina propria. Impregnation with silver by Grimelius' method. Magnification: a, b, e, f) 375×, c, d) 500×.

the epithelium near the basement membrane; the cytoplasm of some cells stained pale yellow by Shubich's method.

Cells producing α-endorphin (AEP cells), like MC, were uniform in size. Most of them were located in the lamina propria (Fig. 1b), they were oval in shape, and granules in some cells stained intensively. These cells were seven times less numerous than MC (Table 1). The location of the AEP cells was the same as that of MC (Fig. 1c, d). Alcian blue (pH 1.0) stained both MC and AEP cells (Fig. 1c). Small round cells with a large nucleus, in which

TABLE 1. Number of Cells per Square Millimeter of Human Antral Mucosa

Types of cells	Location of cells	
	epithelium	lamina propria
Mast cells	19,7±1,7	194,4±22,0
AEP cells	8,7±2,8	28,7±2,6
Grimelius-positive cells	262,0±25,2	26,3±3,6

the granules were intensively stained, were found less frequently, under high power. Interepithelial and subepithelial AEP cells (Fig. 1e) were similar in shape to MC. Individual cells were located on the basement membrane, apparently between the lamina propria and the epithelial layer.

Argyrophilic cells also were found in the lamina propria of the mucosa (Fig. 1f). They were mainly oval in shape with a central nucleus, around which there were a few intensively stained granules. Less frequently these cells were elongated, with palely stained granules. Their location was the same as that of the AEP cells and MC, and they were about as numerous as AEP cells (Table 1). Under high power small, oval cells with deeply stained granules and a large nucleus could be examined.

The study of serial sections thus showed that AEP cells were similar in shape and location and also in staining properties to MC. However, they were only one-seventh as numerous. The MC population can be divided into two types: MC of the mucosa and MC of connective tissue [10]. They differ not only in size, intensity of staining of the granules, and location, but also in some biochemical characteristics [3]. Recently "granular" lymphocytes have been found, first in the intestinal epithelium of rodents [13], and later in man also [10]. These cells, according to the authors cited, are precursors of MC of the mucous membranes. The possibility cannot be ruled out that the small cells with granules, staining by the argyrophilic reaction and also for α-endorphin, discovered in the present investigation under high power, are in fact, "granular" lymphocytes. However, this is a matter for further study.

In connection with the discovery of the peptide hormone VIP in MC, these cells have recently been included among the endocrine cells of the APUD system [2, 4]. Our results suggest that there exists a small subpopulation (one-seventh of the total) of MC which contains α-endorphin also.

The number of Grimelius-positive cells in the lamina propria of the gastric mucosa was found to be almost the same as the number of AEP cells. These cells corresponded also both in shape and in location. The argyrophilic reaction has been shown to reveal a number of different cells in the lamina propria. Nodular hyperplasia of the endocrine cells close to the pyloric glands has been described [12]. Stromal endocrine-like cells, located mainly around nerve endings and vessels, have been found in the intestine, first of rats [7] and later of man [8]. Similar endocrine-like cells also have been found in the lamina propria of the gastric mucosa of patients with chronic gastritis [11].

These facts suggest that MC of the human gastric mucosa include argyrophilic cells which contain α-endorphin.

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MORPHOMETRIC STUDY OF ULTRASTRUCTURAL RESPONSE OF MICROVILLI OF RAT SMALL INTESTINAL ENTEROCYTES DURING NATURAL FEEDING

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The problem of changes in the microvilli during performance of its basic function of absorption by the intestine has recently come up for discussion. Previously, when functions of the microvilli were reduced simply to enlargement of the absorbing surface of the intestine, they were considered to be stable formations. Recent investigations have shown that microvilli can contract [10, 13, 14]. Immunofluorescence and immunochemical identification of the actomyosin complex in microvilli demonstrated clearly that the microvilli of enterocytes are active dynamic structures [9, 11]. The study of the response of the microvilli during absorption of individual nutrients confirms the lability of these structures, but gives contradictory results [7, 8, 15]. Under natural conditions a wide range of different nutrients undergoes simultaneous absorption from the chyme; the response of the microvilli during activation of digestive processes, however, has not hitherto been described.

The aim of this investigation was to study the dynamics of changes in size of the microvilli of absorptive enterocytes located in the active zone of the villi of the three principal parts of the intestine, during digestion and absorption of a combination of natural food products.

EXPERIMENTAL METHOD

Mature Wistar albino rats weighing about 200 g were used. Before the experiment the animals were kept on the ordinary animal house diet. The rats were deprived of food (water *ad lib.*) for 36 h before the experiment, and kept in cages preventing coprophagy. After starvation, the animals were fed for 15 min on sunflower seeds and white bread soaked in milk, after which the residual food was removed from the cages.

The animals were killed 25, 50, 100, and 200 min and 8 h after the beginning of feeding. Rats deprived of food for 36 h served as the control. Pieces of duodenum, the middle part of the jejunum, and the distal part of the ileum were removed for investigation.

After decapitation of the animals, a 4% solution of paraformaldehyde in Hanks' buffer (pH 7.3) was injected slowly into the intestinal lumen. After 3-5 min pieces of the three parts of the small intestine were excised and immersed in the same fixative as that in which the tissues were kept for 3 h at 4°C. After rinsing three times in buffer, the pieces were fixed in 2% OsO_4 for 30 min, washed in buffer, incubated for 30 min in a 1% aqueous solution of thiocarbohydrazide, washed, and postfixed for 1 h in OsO_4 [6]. After dehydration in ace-

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