MORPHOLOGICAL FEATURES OF INTESTINAL ENDOCRINE CELLS IN EXPERIMENTAL *Escherichia coli* INFECTION

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UDC 616.98:579.842.11]-07:616.43].45-018.1-031:611.34]-092.9-07

KEY WORDS: *Escherichia coli* infection; intestine; endocrine cells; morphometry; ultrastructure

The endocrine cells of the intestine constitute an important part of the diffuse endocrine system of the stomach, intestine, and pancreas [3, 6, 8]. The gastrointestinal hormones of these cells are involved in activation of the adenylate cyclase of the intestinal epithelium, they act on the motor activity of the gastrointestinal tract and various functions of the intestinal mucosa, and at the same time, they possess immunomodulating activity [5, 10, 11]. This indicates the possibility that cells producing various peptide hormones may exert a pathogenetic action on the course of acute intestinal infections. However, the character of the morphological changes in the endocrine cells of the intestine in acute intestinal infections has virtually not been studied, and accordingly, that was the aim of the present investigation.

EXPERIMENTAL METHOD

Experimental *Escherichia coli* infection was produced by the method described previously [1]. Pieces from different parts of the intestine of BALB/c mice were fixed in 10% neutral formalin and embedded in paraffin wax. For quantitative analysis of the endocrine cells, histological sections 5-7 μ thick were stained with silver nitrate by Grimelius' method and the cells were counted in 1 mm^2 section of the intestine. The statistical analysis was carried out by Student's test. Material for electron-microscopic study was fixed in a mixture of 1% glutaraldehyde and 4% formaldehyde in 0.05 M cacodylate buffer, postfixed in 1% OsO_4 solution, dehydrated, and embedded in Vestopal. The material was taken 15 and 30 min and 1, 3, 6, 12, and 24 h after infection. Ultrathin sections were stained with lead citrate and examined in the JEM-100C electron microscope.

EXPERIMENTAL RESULTS

Altogether 15 types of endocrine cells were found in the mouse intestine, the largest number of them (15 types) being found in the mucosa of the duodenum, 14 types in the jejunum and ileum, five types in all parts of the colon, and six types in the rectum.

As Table 1 shows, in response to peroral administration of the *E. coli* culture, a response developed with unequal intensity in different parts of the intestine. A cellular response was observed in all parts of the small intestine and was characterized mainly by a decrease in the number of endocrine cells, the greatest changes being shown by these cells in the duodenum. In the large intestine different parts gave different kinds of response to this procedure at different times after infection. In the distal parts of the intestine, moreover, the greatest increase in the number of endocrine cells took place.

Laboratory of Infectious Pathology, Research Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. K. Permyakov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 6, pp. 668-672, June, 1991. Original article submitted April 28, 1990.

0007-4888/91/0006-0887$12.50

TABLE 1. Number of Endocrine Cells in 1 mm^2 of Histologic Section

Organs	Number of cells, times of infection in h				
	control	1	6	12	24
Duodenum	243±15	88±6	77±15	90±23	78±13
Jejunum	175±16	72±9	85±11	69±8	95±10
Ileum	87±7	54±10	47±8	44±5	35±7
Ascending colon	33±4	47±8	31±1	25±1	23±3
Transverse colon	39±6	49±4	40±4	47±4	49±6
Descending colon	25±4	23±1	31±4	42±6	35±4
Rectum	53±1	42±1	35±1	68±1	68±4

Analysis of the ultrastructural changes shows that phenomena such as degranulation of a high proportion of endocrine cells occurred quite early, starting 15 min after the procedure. Basically these phenomena affect endocrine cells of the small intestine. In the large intestine, endocrine cells of the ascending colon and rectum were involved in degranulation. The ultrastructural changes in the endocrine cells were uniform in character, the only difference being in the rate of these changes depending on the type of cells and their location. The greatest changes 15 min after infection were found in the G-, D-, and S-cells of the duodenum. In these cells a considerable decrease in the number of secretory granules, slight dilatation of the tubules of the smooth endoplasmic reticulum (SER) and rough endoplasmic reticulum (RER) and its partial degranulation were observed, together with local areas of dilatation of the intercellular spaces. The mitochondria of these cells had a dense matrix and widened intracristal spaces. Cells of the EC type also underwent partial degranulation at this time (Fig. 1). Between 30 and 60 min after infection, the ultrastructural changes in the endocrine cells continued to increase, and at these times the number of types of cells undergoing different kinds of disturbances increased. Similar changes affected cells of the G, EC, D, D_1, L, S, N, and P types. Widening of the perinuclear space and nuclear pores was observed in many of these cells, and granules of secretion, released by exocytosis (Fig. 2), were observed in the perinuclear space and in the widened intercellular space. Processes of a destructive character were intensified at these times. From 3 to 6 h after infection, besides the changes described above, phenomena characteristic of repair and increased secretory activity were observed in individual endocrine cells: many free monosomes and polysomes appeared in the cytoplasm, elements of the Golgi complex underwent hypertrophy and hyperplasia. These processes gradually increased in intensity during subsequent periods after infection (Fig. 3a).

Degranulation in the jejunum and ileum was most marked in cells of EC type. The pathological processes in the endocrine cells of these parts of the intestine follow a milder course than in the duodenum, but they were similar in character. After 10-30 min slight degranulation of the EC cells was observed, and this continued until about 3 h after infection. After 3 h, dilatation of the cisterns of RER and SER was more marked than after 30 min to 1 h (Fig. 3b, c). The packing density of the granules and their secretion increased considerably after their accumulation for 3 h.

In the large intestine processes of degranulation of the endocrine cells were not only milder than in the small intestine, but they were not found in all parts, nor did they involve cells of the PP type. This cell population was more stable in all parts; destruction of organelles was not found in them (Fig. 3d, e). In the ascending colon, degranulation of endocrine cells began relatively late, namely after 6 h, and continued until 24 h. The early periods were marked by an increase in the number of endocrine cells in this part of the intestine. In the transverse colon, degranulation of the endocrine cells could not be found. In the descending colon slight degranulation of endocrine cells was observed 1 h after infection, but in the rest of the large intestine this process was not observed. Widening of RER and SER was found in some endocrine cells, which contained a quite considerable number of secretory granules (Fig. 3f, 9). The most complex processes occurred in the rectum. An important factor was that in this part of the large intestine exoendocrine cells were found at different times of the experiment (Fig. 3h). Degranulation of endocrine cells in the rectum was observed during the first 6 h after infection, after which secretory granules began to accumulate and repair processes were activated in cells of the EC_1, D_1, L, X, and P types. Phenomena connected with destruction of organelles were observed in these cells mainly from 1 to 6 h after infection. These included dilatation of the cisterns of the RER, their partial degranulation, slight dilatation of the cisterns of the SER and reduction of the Golgi complex (Fig. 3i), widening of the perinuclear space and

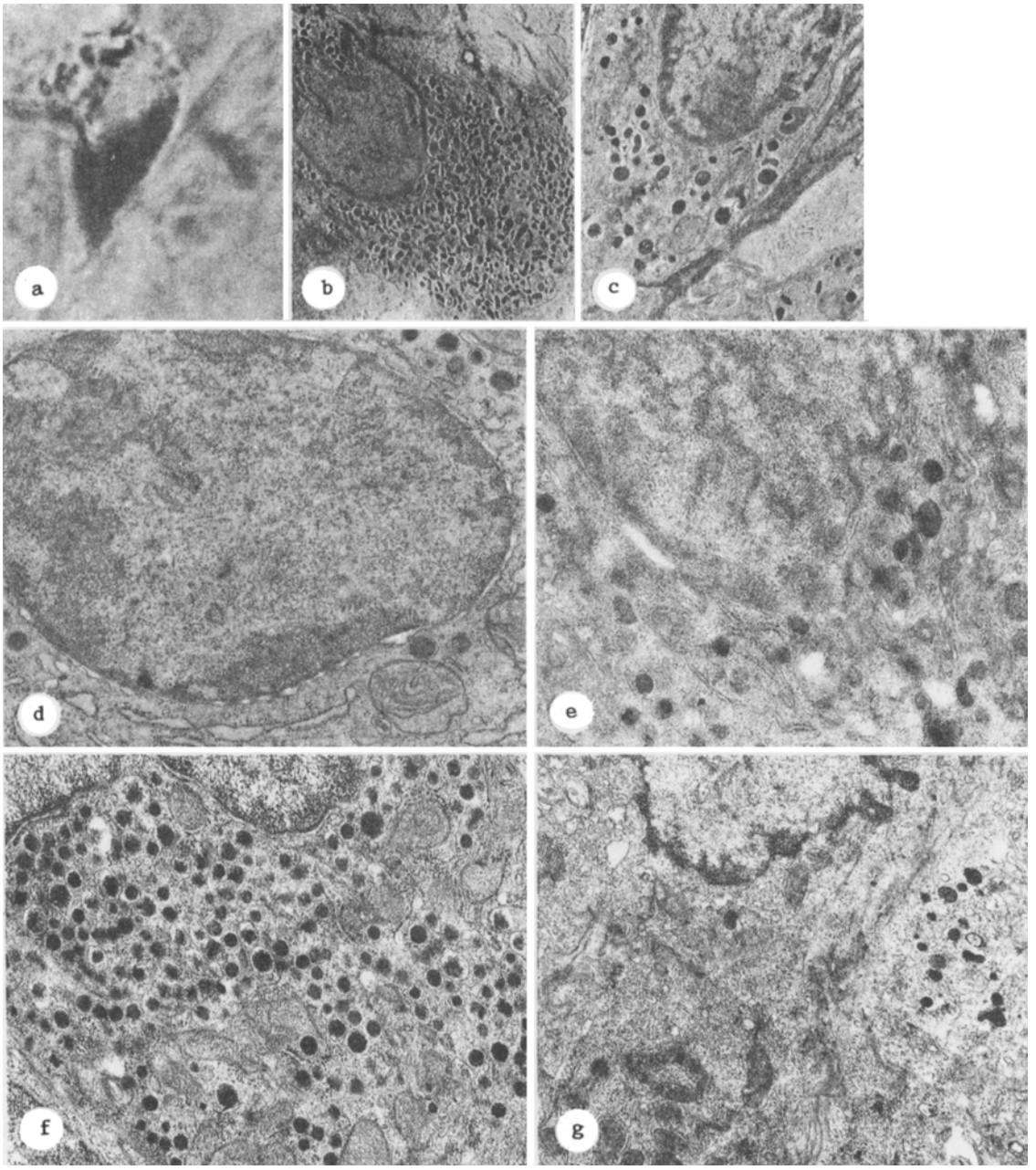

Fig. 1. Different types of endocrine cells in mouse intestine: a) endocrine cell in duodenal crypt, Grimelius' stain. 400×; b) EC cell in duodenal crypt of control mouse. 5000×; c) slight degranulation of EC cell in duodenal crypt 15 min after infection. 7000×; d) Degranulation of G-cell and dilatation of cisterns of RER with its partial degranulation. Duodenal crypt 15 min after infection. 11,600×; e) D-cell in duodenal crypt 15 min after infection. Moderate degranulation of cell with slight dilatation of cisterns of SER. 11,880×; f) S-cell in duodenal crypt of control mouse. 21,000×; 9) Degranulation of S cell in duodenal crypt 15 min after infection. 12,000×.

nuclear pores, and the appearance of mitochondria with a pale matrix and widened intracristal spaces. After 6 h and in the later stages, only repair processes and accumulation of secretory granules in the endocrine cells could be observed.

The investigation showed that considerable changes are observed in *Escherichia coli* infection in the endocrine cells of different parts of the intestine,and they differ in nature in different parts of the small and large intestine. Changes were most marked in the duodenal endocrine cells, which are the first in the intestinal tract to be exposed to pathogenic action. We know from the literature that in other diseases of the gastrointestinal tract of an inflammatory character considerable

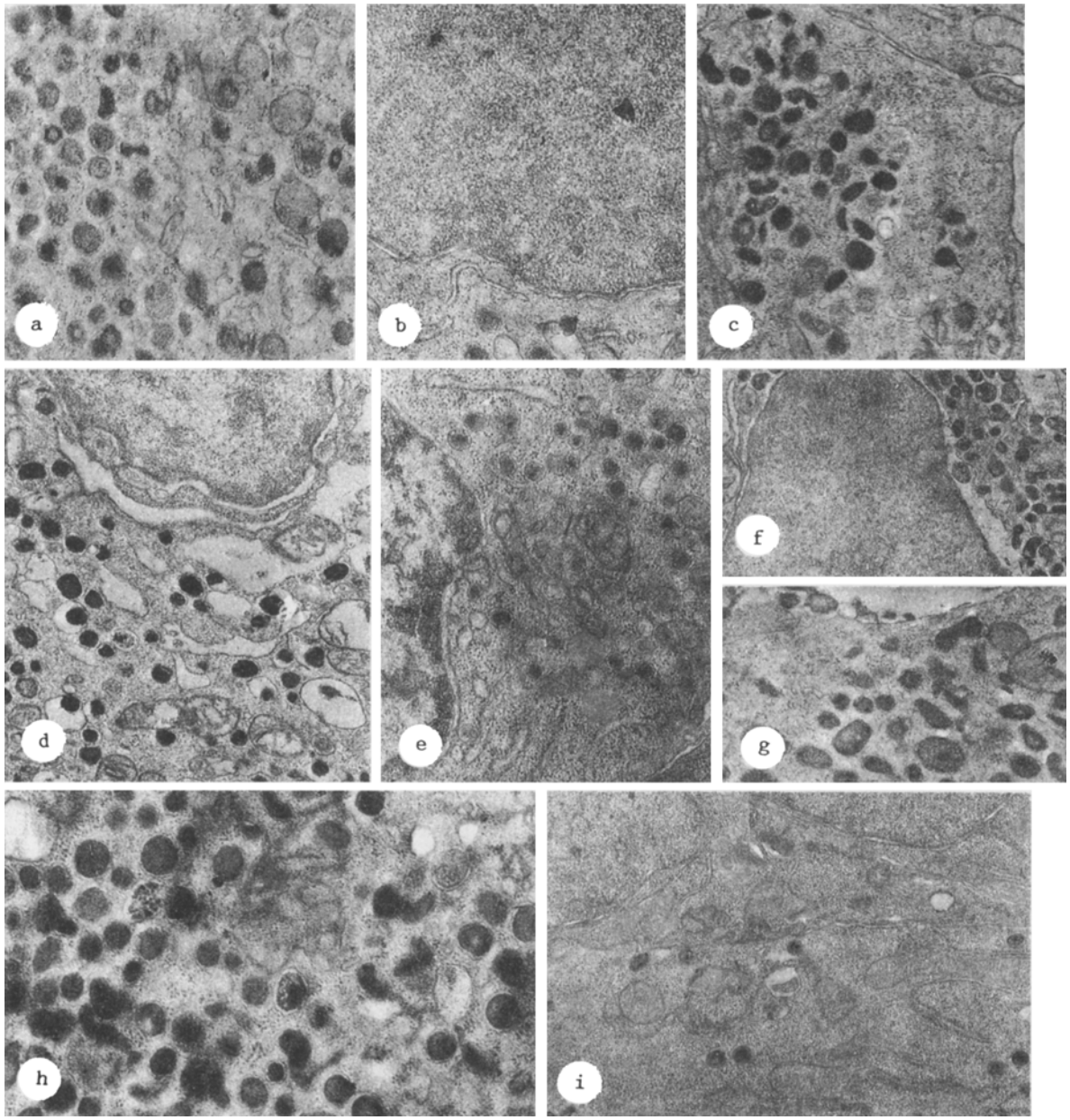

Fig. 2. Different types of endocrine cells of mouse intestine: a) D-cell of duodenal villus of control mouse. 14,000×; b) Degranulation of D-cell of duodenal villus 30 min after infection. 16,000×; c) Slight degranulation of EC-cell of duodenal villus 1 h after infection. 15,000×; d) Different stages of maturation of secretory granules, widening of SER and RER of perinuclear space in G-cell of duodenal villus 1 h after infection. 11,800×; e) Few secretory granules, widening of intercellular spaces and moderate widening of RER and SER in D_1-cell of duodenal crypt 1 h after infection. 22,650×; f) Widening of perinuclear space which contains immature secretory granules of duodenal EC-cell 1 h after infection. 10,000×; 9) Exocytosis into intercellular space of secretory granules of duodenal EC-cell 6 h after infection. 15,000×; h) Different stages of maturation of secretory granules in duodenal L-cell 6 h after infection. 16,000×; i) Considerable degranulation, many monosomes and polysomes in duodenal P-cell 14 h after infection. 17,000×.

changes are observed similarly in the duodenal endocrine cells, involving their degranulation and destruction of individual organelles [2, 4, 7, 9, 12]. On the other hand, in the modern view the intestinal endocrine apparatus can be regarded as a polyfunctional endocrine gland [3], involved to some degree or other in the infectious process. This process is initiated by gastrin, produced by G-cells, which we found only in the duodenum. Next to be involved in the process are EC-cells,

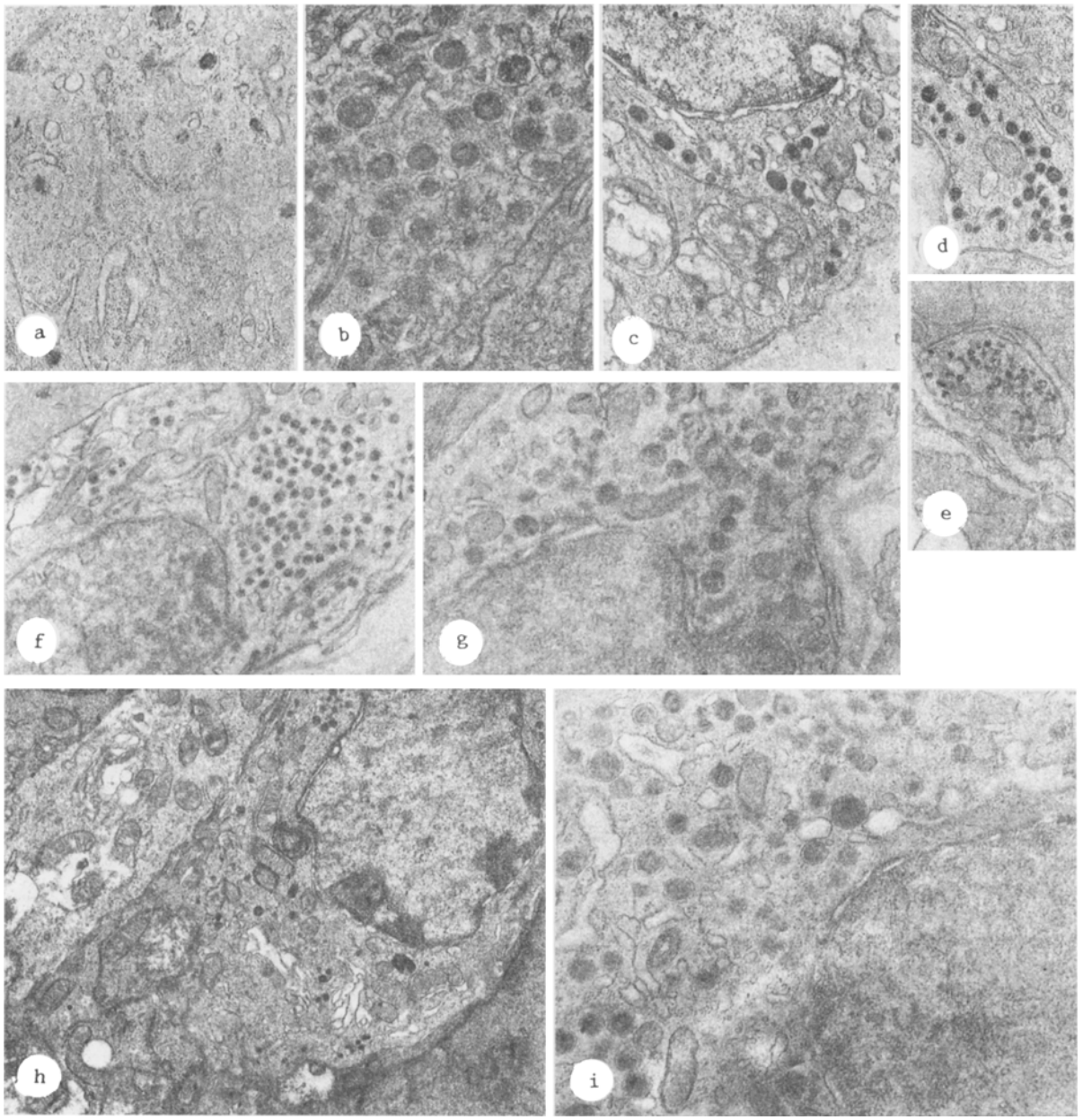

Fig. 3. Different types of endocrine cells in mouse intestine: a) many monosomes and polysomes in duodenal G-cell 24 h after infection. 15,000×; b) Accumulation of secretory granules in jejunal D-cell 24 h after infection. 15,000×; c) dilatation of cisterns of SER and RER in jejunal EC-cell 24 h after infection. 15,000×; d) PP-cell in rectum of control mouse. 15,000×; e) PP-cell in rectum 15 min after infection. 10,000×; f) Slight widening of RER and SER in D_1-cell in ascending colon 1 h after infection. 12,000×; g) Slight widening of RER and SER of X-cell in descending colon 1 h after infection. 18,000×; h) Exoendocrine cell in rectum 3 h after infection. 14,000×; i) Widening of RER and SER in L-cell in rectum 6 h after infection. 17,000×.

producing serotonin and substance P, and at almost the same time a secretion (somatostatin, VIP, glycentin, and secretin) is produced by cells such as those of the D, D_1, L, and S types, and finally the P-cells, producing bombesin, and X-cells whose secretion is still unknown, are involved in the process. The reaction of cells of the large intestine is varied, and mainly the endocrine cells of the rectum react to pathogenic influences. The discovery of mixed exo-endocrine cells is an interesting fact, for their presence can be regarded as the morphological manifestation of one way of restoration of the endocrine apparatus [2].

Morphological changes in the endocrine cells thus reflect differences in the degree of functional loading of different parts of the intestine in experimental *E. coli* infection.

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