ULTRASTRUCTURE OF CECAL ENDOCRINE CELLS IN NORMAL MICE AND MICE WITH EXPERIMENTAL ESCHERICHIOSIS

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Morphology in the field of gastrointestinal endocrinology has made considerable advances in its interpretations of function. The writers showed previously [2] that five types of endocrine cells can be identified in different parts of the large intestine of mice and six types in the rectum. The cecum and, in particular, its distal part, regarded as the possible morphologic and functional equivalent of the human appendix, plays the role of a polyfunctional organ, one of its most important functions being endocrine [7]. In this connection both the identification of endocrinocytes and their modification in experimental escherichiosis, the most widespread of the acute intestinal infections, in which involvement of gastrointestinal hormones is proven [4], has not yet been sufficiently well characterized morphologically.

EXPERIMENTAL METHOD

Experimental escherichiosis was produced by the method described previously [1]. Small fragments from the distal part of the mouse cecum were taken between 15 min and 2 weeks after exposure to a culture of *Escherichia coli.* For quantitative analysis of the endocrinocytes, material was fixed in 10% neutral formalin and embedded in paraffin wax. Histologic sections were stained with silver nitrate by Grimelius' method and the number of endocrinocytes was counted in 1 mm^2 section through the intestine. The results were subjected to statistical analysis by Student's test. Material for electron microscopy was fixed in a mixture of 0.5% glutaraldehyde and 4% formaldehyde in 0.05 M cacodylate buffer, and postfixed in 1% osmium tetroxide solution in the same buffer, after which it was washed, dehydrated, and embedded in Vestopal. Ultrathin sections after staining with lead citrate were examined in the JEM-100C electron microscope.

EXPERIMENTAL RESULTS

We identified five types of endocrinocytes in the mouse cecum: EC, D_1, L_1, X, and PP cells (Fig. 1). These types of cells, like other endocrinocytes, were described fully at the ultrastructural levels by the present writers previously [2]. However, a description of some ultrastructural features of these types in the cecum and their cytotopography must be given. A study of histological sections gave information on the histo- and cytotopography of the endocrine cells. These cells are found most frequently in the crypts and in the lamina propria of the mucous membrane (LPMM). In the crypts they are most numerous at the base, where the neighboring cells are brush-border epitheliocytes and, sometimes, goblet cells and intraepithelial lymphocytes. In the epithelium of the cuppola of the lymphoid nodules endocrinocytes most frequently adjoin brush-border epitheliocytes and M cells.

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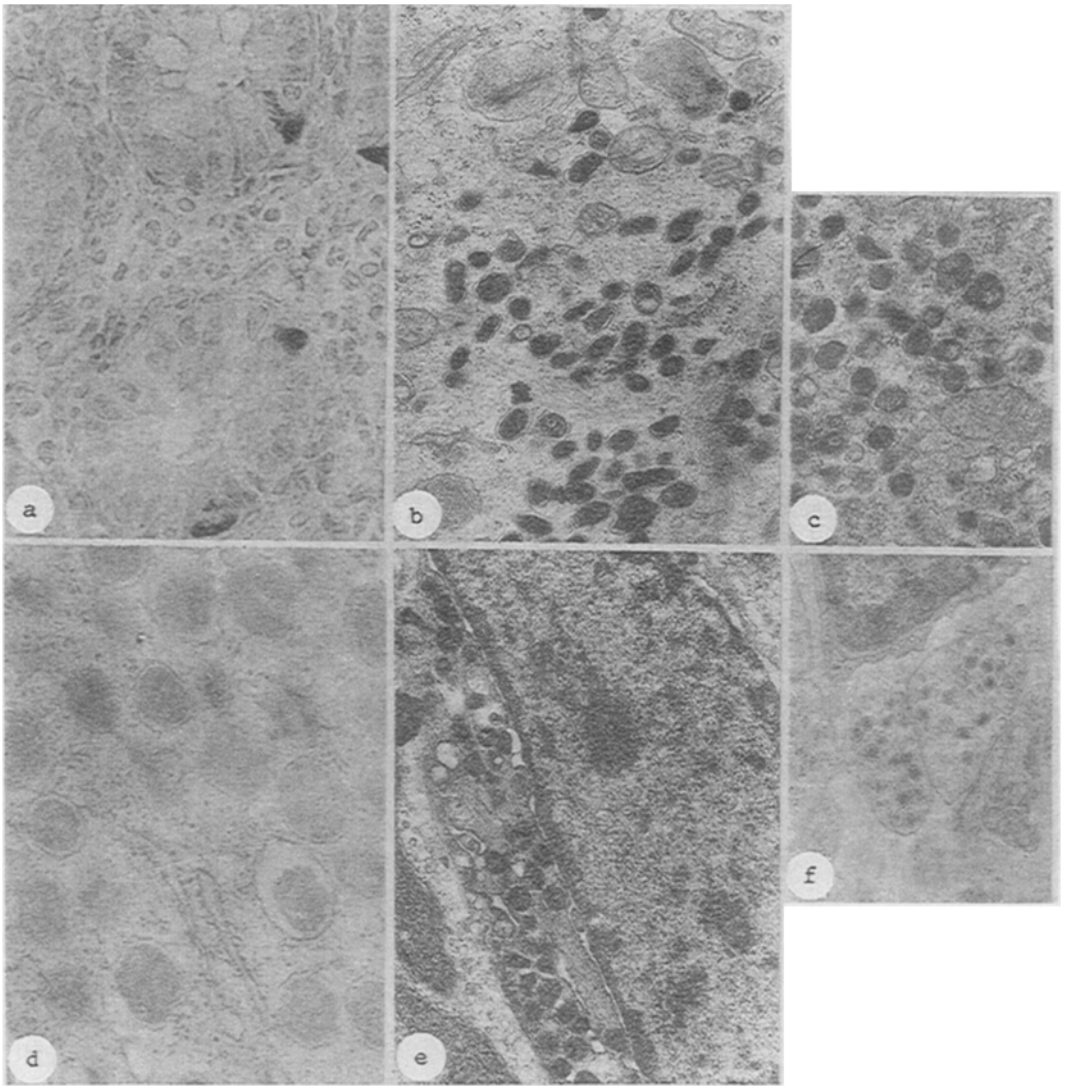

Fig. 1. Different types of endocrine cells from distal part of cecum of control mouse: a) endocrine cells in crypts and in lamina propria of mucous membrane Grimelius' stain, 140×; b) EC1 cell at base of crypt, 33,000×; c) D_1 cell in crypt, 45,000×; d) L cell in crypt, 36,000×; e) X cell in crypt, 18,000×; f) PP cells in lamina propria of mucous membrane, 18,000×.

Cells of EC type have subtypes (EC_1, EC_2, ED_n) that differ from one another only in the size of their granules: in EC_1 cells the diameter of the granules is 290 ± 49 nm. In EC_2 cells they are largest (diameter 330 ± 63 nm); in EC_n they are smallest (diameter 190 ± 38 nm). Granules of secretion in these cells are distributed mainly in the basal part from the nucleus and have a characteristic beanlike shape. This is the most numerous subpopulation of endocrinocytes in the mouse cecum and it produces serotonin and substance P.

Second place for wideness of spread is occupied by cells of the D_1 type. These cells are oval or round in shape, small (diameter 150 ± 36 nm), and possess homogeneous, round secretory granules of average osmiophilia, with a small pale interval near the paragranular membrane. Compared with other types of endocrinocytes, their organelles are well developed. This type is found at the base of the crypts and it produces vaso-intestinal polypeptide (VIP).

Cells of the L, X, and PP types are found less frequently in the mouse cecum than those of the EC and D_1 types, and are located in the basal part of the crypts. In escherichiosis, these cells are identified more frequently, for they are activated by the conditions of the infectious process, as we demonstrated in relation to other secretory cells, namely goblet cells and Paneth's cells.

TABLE 1. Number of Endocrinocytes in 1 mm^2 of Histologic Section

Number of cells Period of infection						
Control	1 h	6 h	14 h	24 h	1week	2weeks
28±5	17±3	15±3	14±2	11±2	19±4	22±2

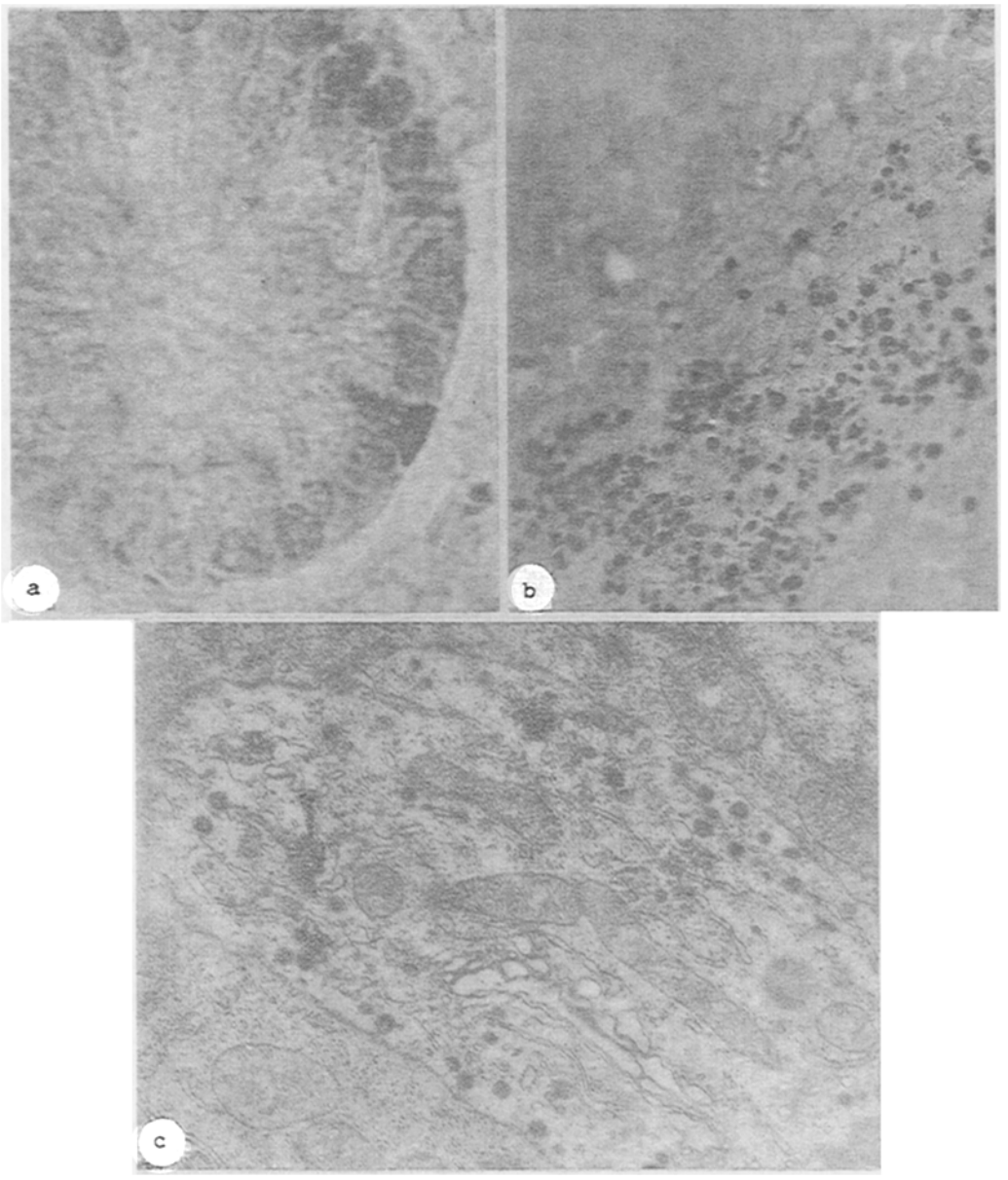

Fig. 2. Different types of endocrine cells in distal part of cecum in experimental escherichiosis 1 h after infection: a) endocrinocytes in crypt and in lamina propria of mucous membrane. Grimelius' stain, 220×; b) in EC_1 cell and neighboring brush-border epitheliocytes – widening of intercellular spaces, cisterns of rough endoplasmic reticulum, and local translucencies of hyaloplasm, 14,000×; c) few secretory granules, local translucencies of hyaloplasm, myelinlike structures, and hypertrophy and hyperplasia of Golgi complex in a D_1 cell, 20,000×.

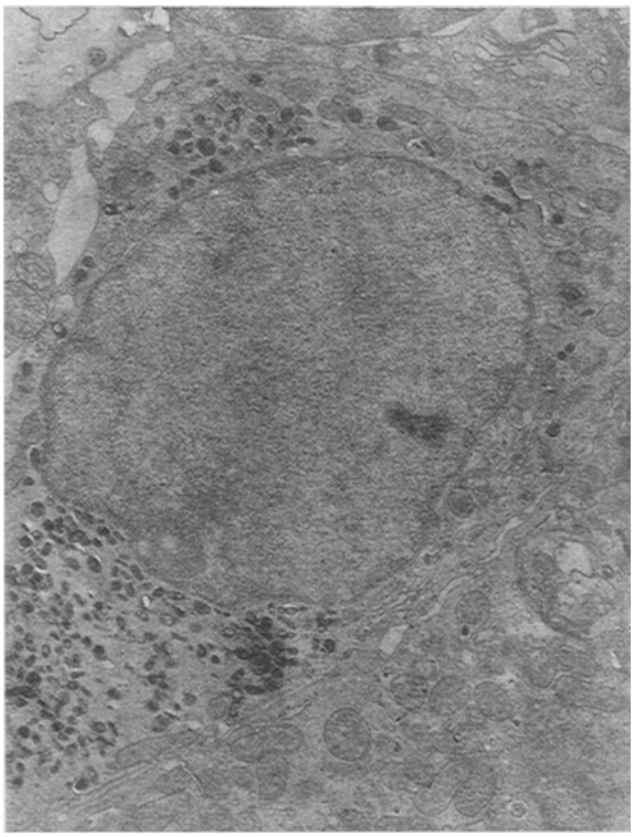

Fig. 3. EC_1 cell from distal part of mouse cecum in experimental escherichiosis, 12 h after infection. EC_1 cell in stage of accumulation of secretory granules, widening of intercellular spaces, 18,000×.

The largest endocrinocyte are L cells, which contain the largest granules (diameter 390 ± 89 nm); the granules are dark, round, homogenous, and distributed throughout the cytoplasm of the endocrinocytes; the pale interval near the paragranular membrane is narrow, but is more clearly defined in immature granules. These cells are located in the middle and basal parts of the crypts and they produce glicentin (enteroglucagon).

Cells of the X type have pale cytoplasm, their granules are heterogeneous but more often round or oval in shape, osmiophilic, and of average size (diameter 300 ± 25 nm). They are distributed in both middle and basal parts of the crypts, and what they secrete has not yet been established.

Cells of PP type are small, and so also are their granules (diameter 168 ± 23 nm). The granules of PP cells produce pancreatic polypeptides, and exhibit great diversity: they may be both homogeneous and osmiophilic (mature forms) and pale with granular contents, and oval or semilunar in shape (immature). The cells themselves are of average electron density and are located in the basal part of the crypts of the cecum and in LPMM.

The data in Table 1 show that the number of endocrinocytes in the cecum decreases until 24 h after infection. After the 1st week the number of endocrinocytes increases a little, but has not yet reached the control level by the 2nd week. This tendency is clearly visible also in sections stained by Grimelius' method (Figs. 1a and 2a).

Analysis of the ultrastructural changes shows that phenomena such as degranulation of a large proportion of endocrinocytes, are observed relatively early, beginning only 15 min after infection. The number of endocrine cells 1 h after infection was smaller than in the control, and besides cells filled with granules, many degranulated forms of cells appeared (Fig. 2a). Endocrinocytes of this kind could be observed during ultrastructural investigation, and not all types of endocrine cells found and described in the cecum underwent degranulation. These processes were more typical of cells of the EC and D_1 types (Fig. 2b, c). Together with a decrease in number of granules of these cells were very slight widening of the rough endoplasmic reticulum together with its partial degranulation could be

detected, but in some cases the perinuclear space also was widened. Degranulation in endocrinocytes was observed on average until 8 h after infection. Later, processes indicating accumulation of secretory granules were observed in these cells (Fig. 3), but the widening of the intercellular spaces which could be observed in other cell populations in experimental escherichiosis, still remained for quite a long time. Accumulation of secretory granules could be seen most demonstratively by studying the dynamics of the secretory reactions in a type EC cell.

In experimental escherichiosis we therefore observed considerable changes in the endocrine cells in different parts of the intestine, and these changes are not of the same significance in parts of the small and large intestine [5].

Only EC cells have been identified previously in the cecum [6, 7]. We found, however, five types of cells in this part of the intestine, and gave a detailed description of their ultrastructure. The important fact is that the endocrine apparatus of the intestine can be regarded as a polyfunctional endocrine gland [3]; the endocrinocytes of the cecum are a component of this gland. Products secreted by the endocrine cells have a marked influence on the course of the infectious process. Intensive secretion of serotonin, substance P, and vaso-intestinal polypeptide from cells of EC and D_1 types is one component of the pathogenesis of escherichiosis, in which secretion of glycentin and of pancreatic polypeptides is more stable.

Thus the ultrastructural characteristics of endocrinocytes of the mouse cecum indicate that the five types of cells identified in these parts reflect different degrees of functional load of the polypeptides and biogenic amines secreted under normal conditions and also during escherichiosis.

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