ULTRASTRUCTURAL CHANGES IN CELLS OF THE RECTAL MUCOSA IN EXPERIMENTAL COLIBACILLOSIS

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The study of morphological changes in different parts of the intestine in colibacillosis is highly topical because of the widespread distribution of the disease and also of the direct dependence of development of the infectious process on the character of relations between microorganisms and cells in the different organs of the host. Research workers have paid great attention to the study of parts of the small intestine in acute intestinal infections, but have not undertaken a detailed analysis of changes taking place in the large intestine [2, 3, 7, 8]. The large intestine has a number of distinguishing features. First, an abundant bacterial flora exists in the large intestine. Second, the large intestine secretes enzymes which degrade various products of bacterial metabolism and substances which are components of dietary products. Third, different parts of the large intestine have their own particular features. This applies most of all to the rectum, which differs in certain anatomical and physiological respects from other parts of the large intestine [5, 9]: it is only from the lumen of the rectum that various substances are absorbed, and enter the blood stream directly; it has the richest innervation with sympathetic and parasympathetic nerves; the network of receptors involved in its stretching is most highly developed in the rectum; anaerobic, nonsporeforming bacteria live mainly in the rectum. Recent publications indicating that the epithelium of the rectal mucosa may be infected with human immunodeficiency virus must not be disregarded. These features of the rectum are bound to be reflected in the functioning of its mucosal cells under various conditions of infectious pathology.

The aim of the investigation was to study ultrastructural features of cells of the rectal mucosa and correlation between morphometric parameters of cell composition in colibacillosis, the most widespread acute intestinal infectious disease.

EXPERIMENTAL METHOD

Colibacillosis was produced by the method in [1]. To study the ultrastructure of the rectum material was fixed in a mixture of 1% glutaraldehyde solution and 4% formaldehyde solution in 0.05 M cacodylate buffer, postfixed in a 1% solution of OSO_4, and embedded in Vestopal. Material was taken 15 and 30 min, 1, 3, 6, 8, 14, and 18 h, and 1 and 2 days after infection. After staining with lead citrate, ultrathin sections were examined in the IEM-100B electron microscope. Semithin sections were stained with methylene blue — azure II and with basic fuchsine. In the morphometric investigation, conducted on paraffin sections through the rectum 4-5 μ thick, the overall density of infiltrating cells (ODIC) was determined, including plasma cells (PC), immunoglobulin-producing cells (IgPC), lymphocytes of the lamina propria ILL), mast cells (MC), granulocytes, and macrophages. The PAS reaction was carried out to detect goblet cells (GC), impregnation with silver by Grimelius' method for quantitative analysis of endocrinocytes (EC), IgPC were demonstrated by the direct immunoperoxidase method, and the relative percentage of intraepithelial lymphocytes (IL) was determined. EC, MC, and GC were counted in an area of 1 mm^2 of histological section of the rectum. The number of IL and GC also was counted per 100 epitheliocytes. The data were subjected to statistical analysis. Significance of differences between mean values compared was determined by Student's test.

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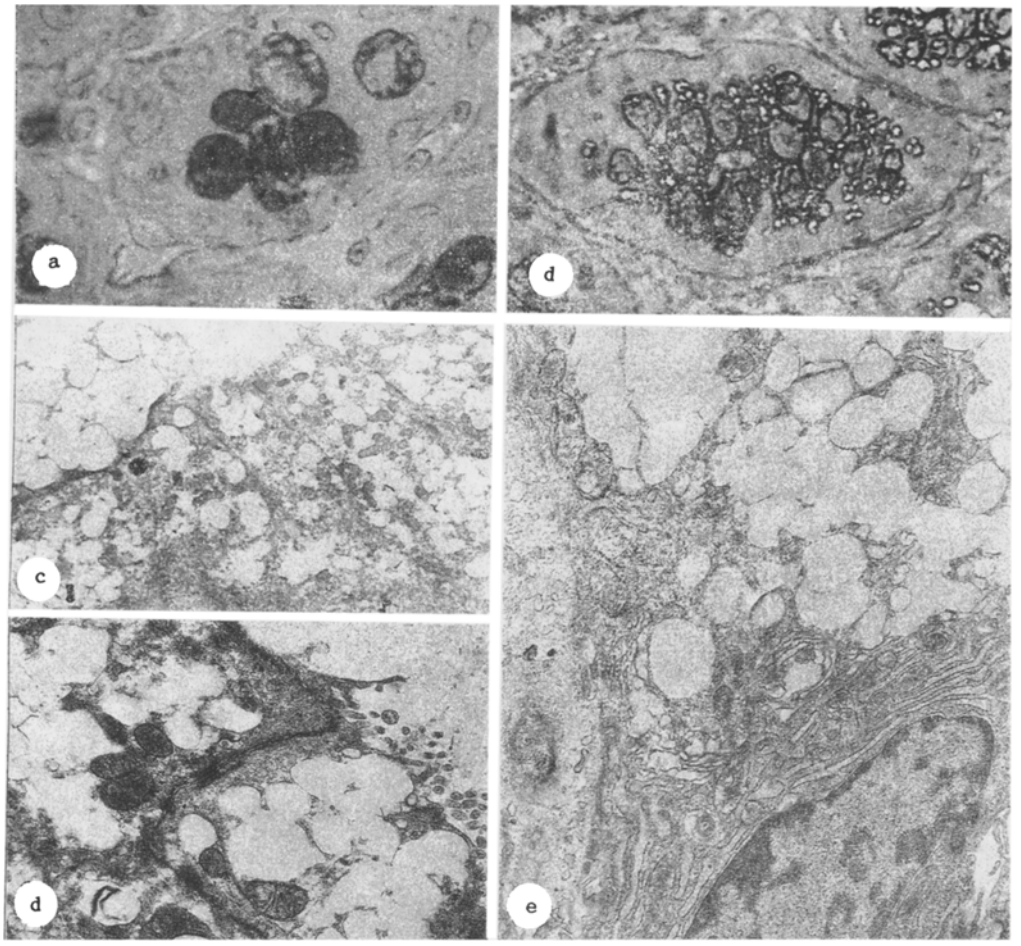

Fig. 1. GC of mouse rectal mucosa: a) control animal, 120×; b) 14 h after infection with culture of Escherichia coli. 100×; c) at different stages of maturation and extrusion of secretory granules 15 min after infection. 4000×; d) extrusion and accumulation of secretory granules after 30 min. 10,000×; e) activation of intracellular organelles after 1 h. 20,000×.

EXPERIMENTAL RESULTS

Electron-microscopic investigation of the rectal epithelium revealed the following types of cells: brush border epitheliocytes, GC, and occasionally Paneth's cells and six types of endocrinocytes (EC, D_1, X, L, P, PP). As early as 15 min after infection, many active GC were observed, and after 14 h their number had increased considerably (Fig. 1a-d). After 30 min to 1 h, GC had an active Golgi complex, a rough endoplasmic reticulum (RER), and a smooth endoplasmic reticulum (Fig. 1e). From 3 to 6 h after infection, a focus of inflammatory infiltration was found in the stroma of the lamina propria, consisting mainly of lymphocytes, plasma cells, eosinophils, and mast cells (Fig. 2a-d). These cells were extremely closely bound with nerve terminals, which are most marked in this part of the large intestine (Fig. 2c). After 6-8 h distinct changes were found in the brush-border epitheliocytes. Many monosomes and polysomes appeared in the cytoplasm of these cells and signs of vesiculation and microplasmatosis were often observed (Fig. 3a, b). Foci of local necrosis were seen in individual brush-border epitheliocytes (Fig. 3c). Accumulation of secretory granules was observed in the endocrinocytes until 14 h after infection. Signs of degranulation of these cells were found mainly from 1 to 8 h after infection. The number of granules varied in most cells. Characteristically their number in the rectal cells was considerably less than that of the corresponding cells in the small intestine, but greater than in other parts of the large intestine [8, 11]. Considerable changes in stromal cells of the lamina propria, where many PC are concentrated, were observed after 12-24 h. Dilatation of the cisterns of the RER was observed in many of them (Fig. 3d). From the 1st to the 2nd day after infection numerous cell associations, active nerve terminals, and blood capillaries with large numbers of micropinocytotic vesicles in the endothelial cells were found in the lamina propria of the mucosa.

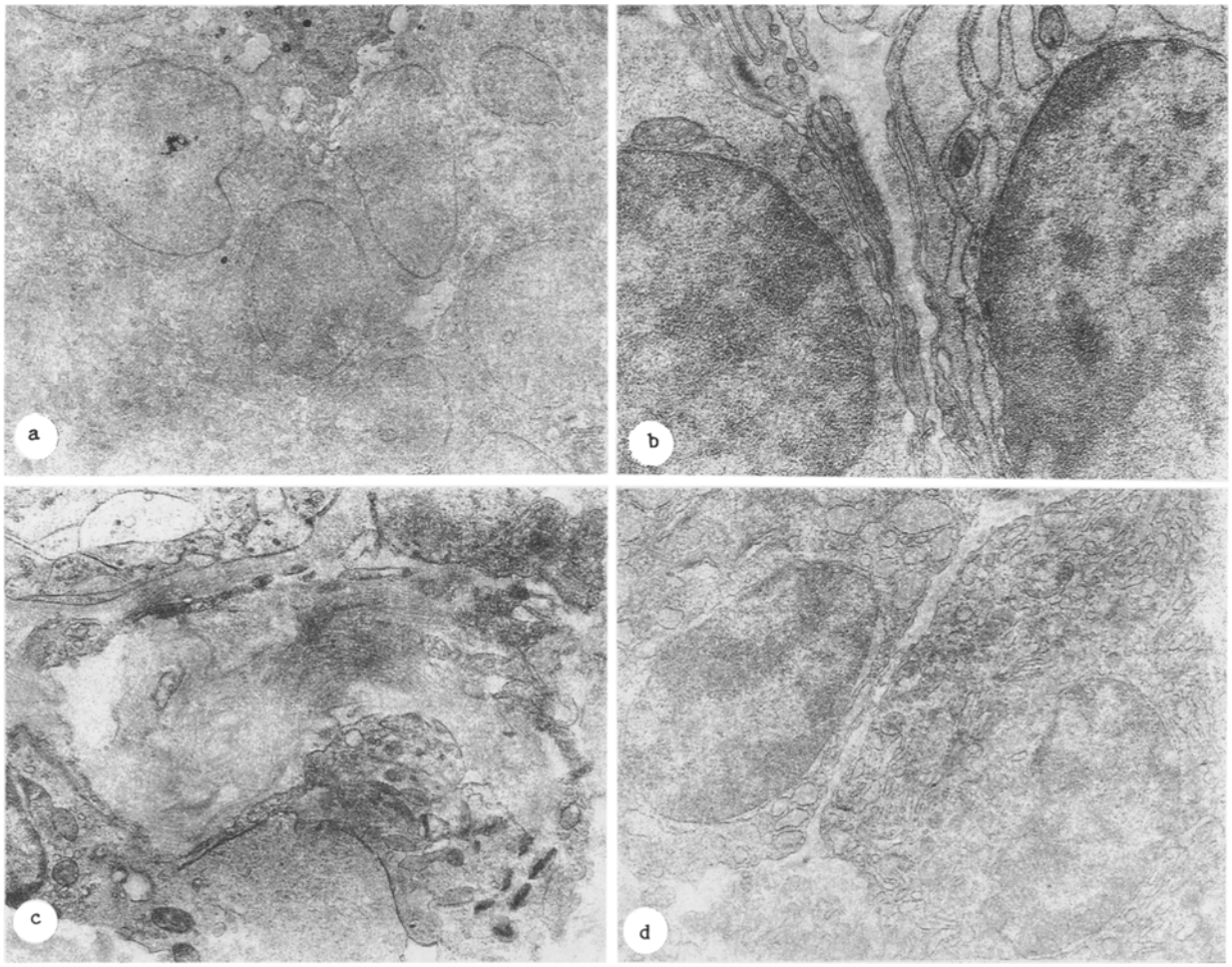

Fig. 2. Stromal cells of lamina propria of mouse rectal mucosa. a) Concentrations of lymphocytes with active intracellular organelles in their cytoplasm, 3 h after infection. 5400×; b) activated B lymphocyte and plasma cell with dilated cisterns of RER 6 h after infection. 20,000×; c) junction between eosinophil, collagen fibrils, and active nerve terminals after 6 h. 10,000×; d) MC with widened perinuclear spaces and active Golgi complex after 6 h. 10,000×.

Morphometric analysis revealed significant quantitative changes in the structural elements (Table 1). At the height of the disease, for instance, an increase was found in the number of GC, which are most active in this part of the intestine. GC were found in stages of both accumulation and extrusion of the secretory granules, and are secreted with the aid of cells with merocrine, apocrine, and holocrine types of secretion. This alternation of functions of GC, taking place in the rectum, is determined both by the character of their cytotopography and by the chemical composition of mucin [4, 10, 12], which differs considerably in its physicochemical characteristics not only in the large and small intestines, but also in different parts of the large intestine itself. Mucus formation in the rectum is observed in a considerable volume in other intestinal infections also [3, 7, 13].

The writers showed previously [6] that in experimental colibacillosis marked degranulation of endocrinocytes takes place. These data apply mainly to endocrine cells of the small intestine. In the rectum this process is observed from 1 to 8 h after infection, and it is followed by accumulation of secretory granules, and mainly in cells of the EC type, producing serotonin and substance P. At the same time an increase was observed in the number of plasma cells and IgPC in the lamina propria, in association with eosinophils and MC. The greatest peak of migration of lymphoid cells into the epithelium occurs after 6 h, when the number of LL was sharply reduced and the number of IL increased. This process is undulating in character, and its amplitude is repeated approximately every 6 h for 2-3 days. The trend of the change in the number of granulocytes and macrophages in experimental colibacillosis was not statistically significant, and accordingly the data are not shown separately in Table 1. It is an interesting fact also that after 1-2 h the principal morphological changes were observed in cells of the lamina propria

TABLE 1. Morphometric Parameters of Cell Populations in the Rectum in Experimental Colibacillosis (M ± m)

	Control	Time after infection, h						
		1	3	6	8	14	18	24
ODIC	11 914±327	13 151±713	12 877±469	10 807±328	13 177±603	16 627±416	18 541±102	12 955±221
PC	4 724±398 (40±2 %)	3 255±210 (25±3 %)	5 636±900 (44±2 %)	9 273±141 (86±4 %)	8 759±771 (67±3 %)	5 204±273 (31±6 %)	6 431±92 (35±5 %)	1 934±163 (15±5 %)
IgPC	3 168±84 (29±3 %)	2 175±115 (67±5 %)	3 293±193 (58±3 %)	6 731±101 (73±7 %)	7 003±73 (80±9 %)	2 081±93 (40±3 %)	4 395±35 (68±4 %)	1 134±46 (59±5 %)
LL	1 728±151 (15±0,5 %)	2 447±281 (19±0,4 %)	2 153±174 (17±0,4 %)	1 272±93 (12±0,3 %)	2 570±115 (20±0,2 %)	1 093±94 (7±0,2 %)	1 875±124 (15±0,6 %)	1 354±101 (10±0,5 %)
IL	6,8±0,4	6,0±0,4	8,3±0,5	9,7±0,7	6,4±0,6	2,0±0,2	3,9±0,4	5,2±0,3
GC	35±2	35±2	23±2	29±4	33±5	55±4	45±2	49±2
Endocrinocytes	53±1	42±1	39±2	35±3	47±5	68±1	63±3	68±4

Legend. $p < 0.05$.

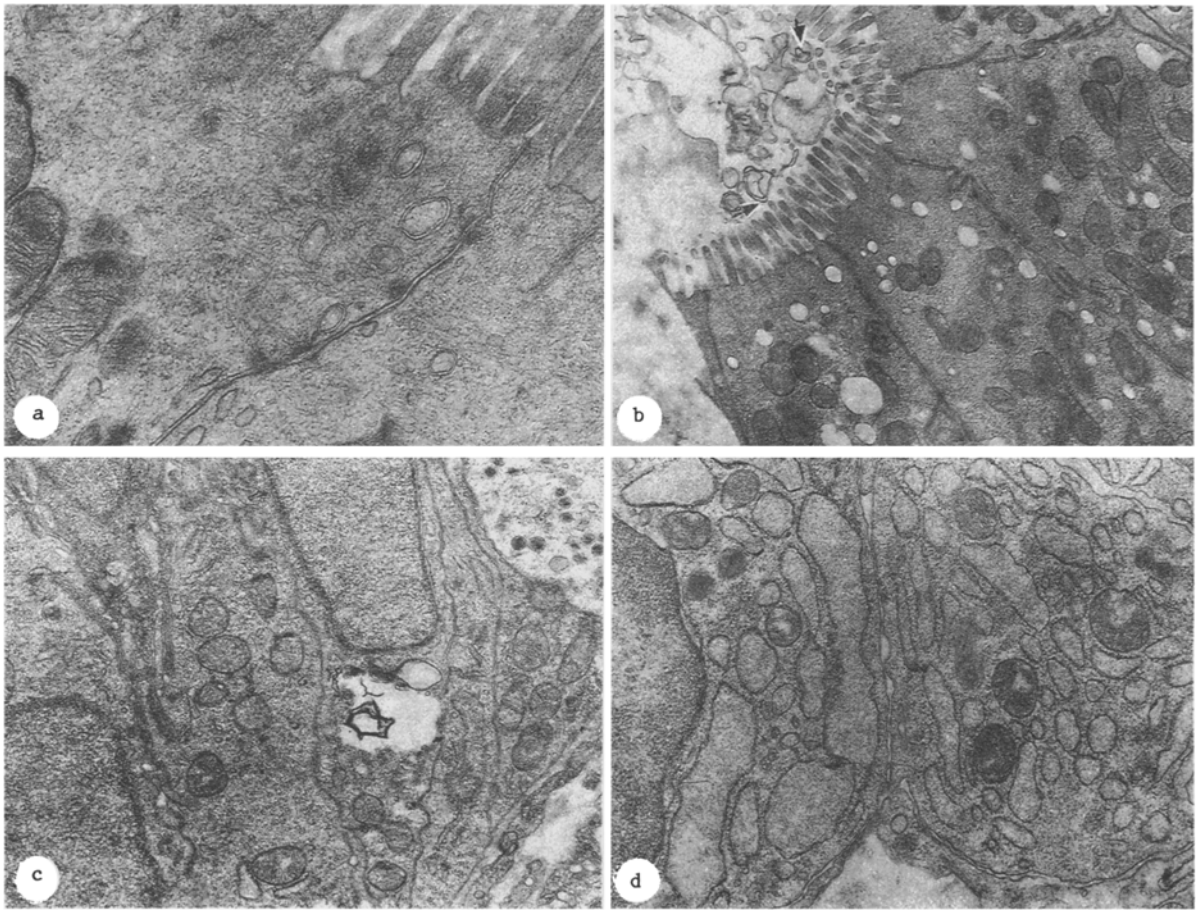

Fig. 3. Changes in epitheliocytes of control mouse. 30,000×; b) vesiculation and evidence of microplasmatosis (arrow) in brush-border epitheliocytes after 6 h. 14,000×; c) area of local necrosis visible in brush-border epitheliocyte; many monosomes and polysomes present in the neighboring epitheliocyte, and a few granules in a D_1-endocrinocyte after 8 h. 14,000×; d) considerable widening of RER in plasma cells with partial degranulation, after 24 h. 20,000×.

of the rectum, as is clearly seen from the high degree of vesiculation of the endothelial cells of the capllllaries and nerve terminals and activity of the lymphoid cells.

Thus in the early stages after infection the trend of the infectious process was characterized by definite morphological changes in the cell populations of the rectum, expressed as vesiculation of the brush-border epitheliocytes, considerable activation of goblet cells, degranulation of the endocrinocytes, and an increase in the number of cell associations in the lamina

propria, involving the participation of many lymphocytes and plasma cells, evidence of the development of inflammation with manifestations of local immune reactions in this part of the intestine.

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ACCUMULATION OF LIPOFUSCIN GRANULES IN ACUTE MYOCARDIAL INFARCTION AS A MODEL OF CELL AGING

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KEY WORDS: lipofuscin; myocardial infarction; electron microscopy

The aging process in man and animals is characterized by the accumulation of specific structures in cardiomyocytes [1, 7, 8, 10, 12], known as lipofuscin granules (LG). They are generally considered to be a kind of marker of the kinetics of aging [11, 13, 15, 16]. However, natural aging lasts quite a long time, and investigators have therefore attempted to produce a model of aging which would narrow this time interval. For this purpose, various cell cultures, in particular, have been widely used [2-4, 6, 9, 14]. The main parameter used to monitor the aging process in this case is a change in the number of LG. The results we obtained could be realistically interpreted only recently, when the membrane hypothesis of aging has become more widely adopted [11, 13, 15, 16]. Establishment of the fact that a key role in the formation of LG is played by the endoplasmic reticulum [3, 10], and also data indicating that the drug centrophenoxine (meclofenoxate) reduces the number of LG in cells, through its action at the receptor level on the plasma membrane [2, 4, 9], has made possible the development of a membrane-receptor hypothesis of aging, which is based on regular cellular mechanisms.

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