SUBMICROSCOPIC FEATURES OF CECAL CELLS IN EXPERIMENTAL ESCHERICHIOSIS

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The intestinal lymphoid tissue is regarded as an important component of the entire immune system of the body. Its distribution along the course of the gastrointestinal tract is variable. One site of greatest concentration of intestinal lymphoid tissue is the cecum and its vermiform appendix [4, 6]. We have shown [3] that the mouse cecum contains a considerable concentration of lymphoid tissue, especially in its distal part, which can be regarded as the morphological and physiological equivalent of the appendix.

The aim of this investigation was to study the ultrastructural features of the various cells found in the cecum (chiefly in its distal part), in the most widely prevalent acute intestinal infection, namely Escherichiosis.

EXPERIMENTAL METHOD

Escherichiosis was produced by the method in [1]. To study the ultrastructure of the cecum, material was fixed in a mixture of 1% glutaraldehyde solution and 4% formaldehyde solution in 0.05 M cacodylate buffer, post-fixed in 5% OSO_4 solution, and embedded in Vestopal. The material was taken 15 and 30 min, 1, 2, 6, 8, 14, and 24 h, and 1 and 2 weeks after infection. Ultrathin sections were stained with lead citrate and examined in the JEM-100B electron microscope (Japan).

EXPERIMENTAL RESULTS

The cell composition of the mucous membrane of the mouse cecum is very heterogeneous (Fig. 1a-d). Epithelial cells are represented by the following populations: brush-border epitheliocytes, goblet cells, Paneth's cells, M cells, and endocrinocytes (of 5 types). Intraepithelial lymphocytes also are found quite frequently. A characteristic feature of the cell composition of the cecum is the presence of the above-mentioned M cells in the cupola of the lymphoid nodules, and the presence of both diffuse and grouped foci of lymphoid tissue, consisting of lymphocytes, plasma cells, reticulocytes, etc. Incidentally, both epithelial and lymphoid cells react to a stimulus as cellular assemblies, but when absorption is disturbed, as we observed during diarrhea, changes in the brush border epitheliocytes naturally assume the greatest prominence. Ultrastructural changes in the epithelial cells of the distal part of the cecum were observed in the earliest stages after infection (15 min to 1 h). These changes mainly affected the apical

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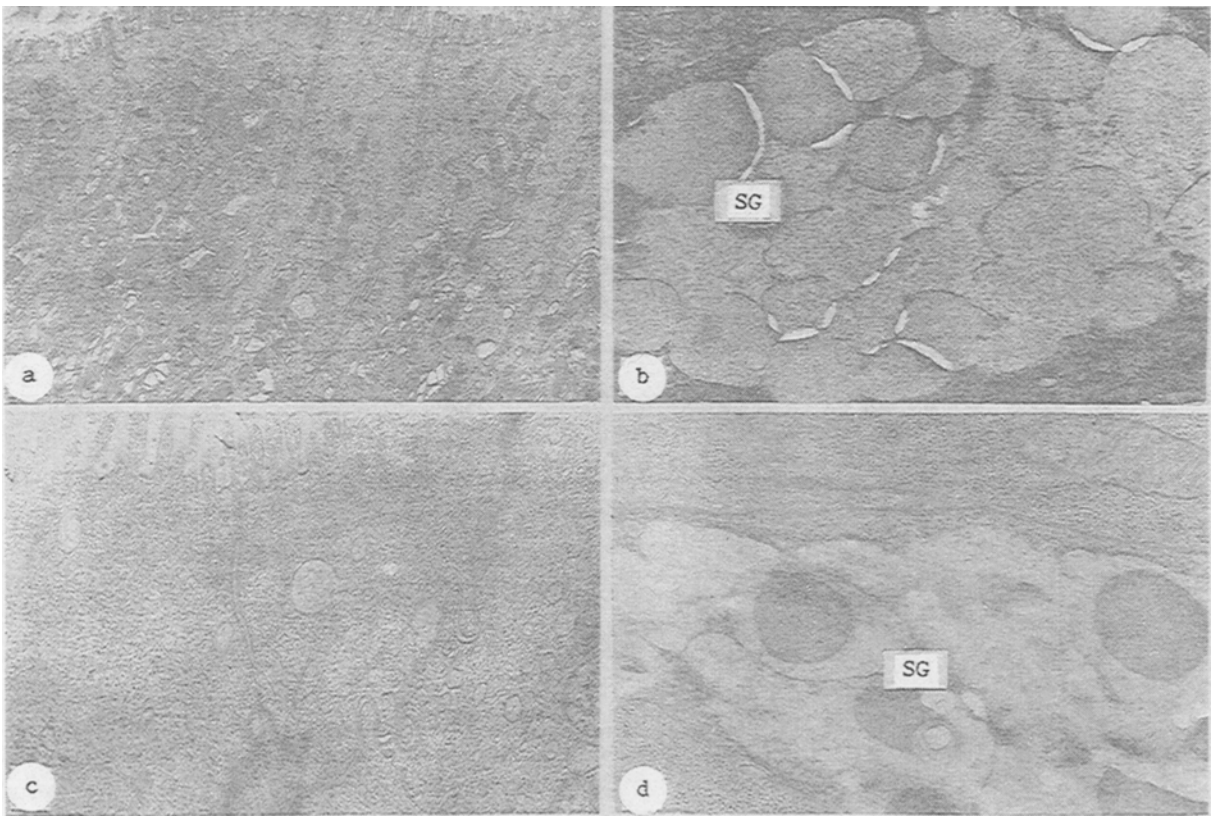

Fig. 1. Different types of epithelial cells in cecum of control BALB/c mouse: a) brush-border epitheliocytes (6000×), b) goblet cell (21,000×), c) M-cell in cupola of lymphoid nodule (36,000×), d) Paneth's cell (34,000×). SG) secretory granules.

part of the brush-border epitheliocytes: microvilli, terminal zone, and upper part of the cytoplasm. Degranulation of the endocrine cells also was observed quite frequently, and this is the subject of a separate investigation. Very small degrees of widening of the intercellular spaces also were observed, more especially at the level of the basal part of the epithelial cells.

Gradually, as the duration of exposure to the pathogenic agent increased, hydration both of the cells themselves and of the intercellular spaces increased in intensity. In some parts of the epithelial layer widening of the intercellular spaces between neighboring brush-border epitheliocytes increased in both volume and extent. Meanwhile in some areas active brush-border epitheliocytes were observed, as shown by hypertrophy and hyperplasia of the lamellar complex, an increase in the number of mitochondria and cisterns of the rough endoplasmic reticulum (RER), and increased density and osmiophilia of the cytoplasm.

Changes in the microvilli 3 h after infection were less frequently observed but signs of vesiculation of the brush-border epitheliocytes increased. The M-cells showed very slight changes due to their number of organelles. Considerable widening of the perinuclear spaces was observed in individual cells. The goblet cells were in stages of both accumulation and extrusion of secretion, evidence of commencing processes of defense against damaging factors and manifested as intensive mucus formation. In the lamina propria of the mucous membrane (LPMM) the number of cellular associations was increased (Fig. 2a); they consisted of lymphocytes, plasma cells, eosinophils, mast cells, reticulocytes, and other connective-tissue cells.

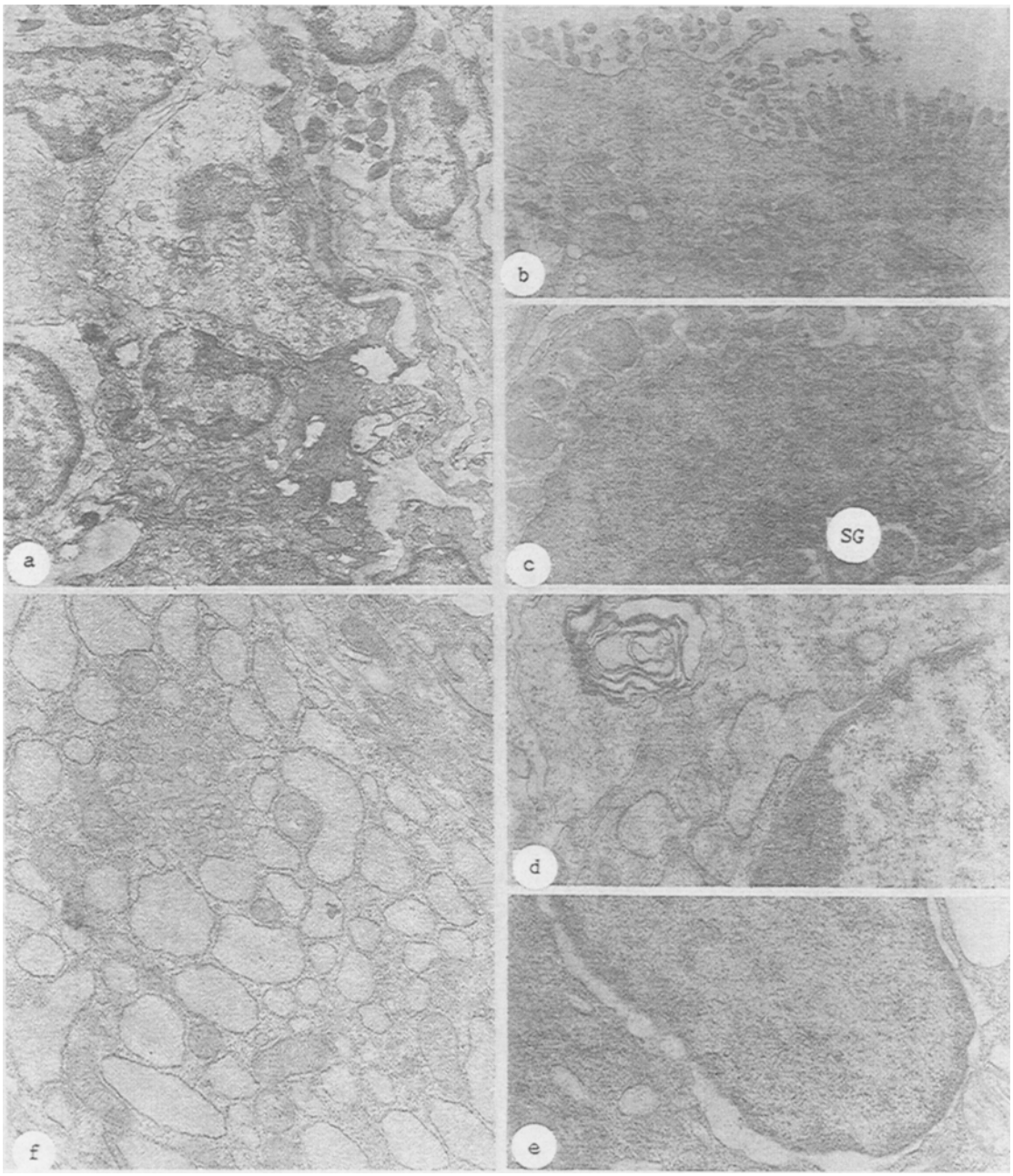

Fig. 2. Ultrastructural changes of different cells of cecum in experimental escherichiosis: a) cellular associations in lamina propria of mucous membrane: lymphocytes, plasma cells, eosinophils, nerve endings (3 h after infection, 10,000×); b) disorganization, and widening of microvilli and disorientation of microfilaments of terminal zone of a brush-border epitheliocyte (6 h after infection, 17,000×); c) mast cell in stage of granule accumulation (6 h after infection, 29,000×); d) local widening of perinuclear space and of cisterns of RER, myelinlike structure present (12 h after infection, 36,000×); e) marked widening of perinuclear space and cisterns of RER, with their partial degranulation (12 h after infection, 36,000×); f) widening of cisterns of RER and vesiculation of cytoplasm of a plasma cell (12 h after infection, 20,000×).

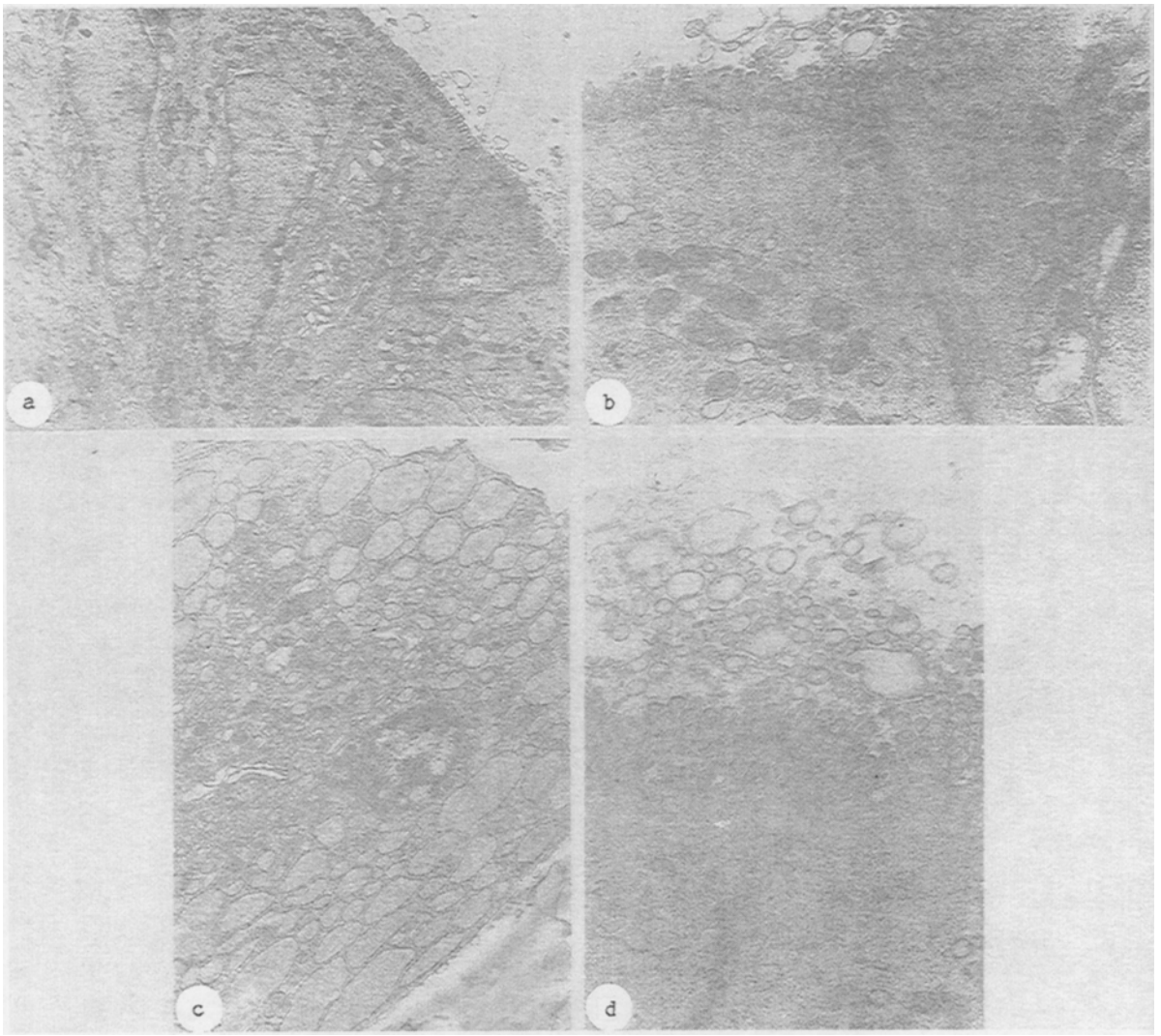

Fig. 3. Ultrastructural changes in different cecal cells in experimental escherichiosis 1-2 weeks after infection: a) moderate widening of RER in individual brush-border epitheliocytes, irregular orientation of microvilli (6000×); b) regular orientation of microvilli and microfilaments in brush-border epitheliocytes (19,000×); c) intraepithelial plasma cell with widened cisterns of RER, hyperplasia of Golgi complex and microvesiculation of cytoplasm (14,000×); d) release of many microvesicles into intestinal lumen (21,000×).

After 6 h the microvilli of the brush-border epitheliocytes became more regularly oriented, but their disorganization and fragmentation and the disorientation of the microfilaments in the terminal zones of the brush-border epitheliocytes, widening and degranulation of the RER, and vesiculation of the cytoplasm in individual cells still remained (Fig. 2b, c).

The severity of the ultrastructural changes described previously in the brush-border epitheliocytes diminished 8-14 h after infection, but in some cells considerable widening of the perinuclear space and of the cisterns of the RER and their partial degranulation were still observed (Fig. 2d, e). Well defined repair processes were in evidence

at these times, and were reflected by synthesis of numerous protein molecules due to new formation of the RER from the outer nuclear membrane, and the appearance of free monosomes and polysomes in the cell cytoplasm. These processes compensate for the considerable loss of proteins observed in the early period of the diarrhea syndrome. Just as at the previous time, intraepithelial lymphocytes and plasma cells with dilated cisterns of their RER and with an active lamellar complex were observed (Fig. 2f). Besides repair processes, activation of cells of the lymphoid series, located both inside the epithelial layer (intraepithelial lymphocytes and various cellular associations of the lamina propria of the mucous membrane) and in the grouped lymphoid tissue (grouped lymphoid nodules) also was observed.

Ultrastructural changes 24 h after infection mainly were reminiscent of the general pattern of the previous time. However, vesiculation of the cytoplasm of the brush-border epitheliocytes and changes affecting RER were a little less marked.

Toward the end of the first and during the second week after infection, an appreciable reduction in the intensity of the ultrastructural changes in different cells continued (Fig. 3a, b). Nevertheless, an increase was observed in the number of intraepithelial lymphocytes and plasma cells. It must be particularly mentioned that these cells have a quite powerful secretory apparatus: cisterns of the RER were dilated, elements of the Golgi lamellar complex underwent hypertrophy and hyperplasia, and vesiculation of the cytoplasm was increased (Fig. 3c). Widening of the perinuclear space and of the nuclear pores, which made direct contact with the dilated cisterns of the RER, also was observed in these cells. A powerful discharge of vesicles was observed from individual brush-border epitheliocytes (Fig. 3d). The cytoplasm of these epitheliocytes remained packed with an enormous number of the above-mentioned vesicles, of different sizes, evidence of both accumulation and secretion of vesicles by the brush-border epitheliocytes.

Thus the dynamics of experimental escherichiosis indicates a cytopathic action of the strain of *Escherichia coli* on the epithelial cells, and changes in the qualitative composition of the cells in the lamina propria of the mucous membrane of the cecum. An important fact is that in the earliest period after infection ultrastructural changes are taking place in the epithelial cells, affecting the microvilli and the terminal zone and cytoplasm of the brush-border epitheliocytes, accompanied by widening of the intercellular spaces. All these facts characterize disturbances primarily of water, electrolytes, and protein metabolism. Processes of absorption are disturbed in the brush-border epitheliocytes, and secretory processes are intensified in the goblet cells. The diversity and complexity of the structure and ultrastructure of the cell composition of the mouse cecum are the reasons for the unusual response reactions to introduction of a strain of *E. coli.* In our view the sequence of development of the pathological process in the cecum in experimental escherichiosis is as follows. Brush-border epitheliocytes are the first cells to react to the stimulus, with disturbance of absorption and metabolism and degranulation of the endocrine and mast cells. This is joined by the reaction of the goblet cells, as cells of the mucociliary barrier of the intestine, with intensification of mucus formation. Because of the large quantity of diffuse and grouped lymphoid tissue in the cecum, compared with other parts of the large intestine [2, 5], the earlier activation of local immune reactions, manifested by an increase in the number of cellular associations in the lamina propria of the mucous membrane, and also an increase in the number of intraepithelial lymphocytes and plasma cells, find an explanation. As a result of the considerable specialization of the cecum as an immunocompetent and endocrine organ, repair processes in brush-border epitheliocytes in experimental escherichiosis are more marked as regards both quality and time, so that it becomes possible to judge the flexibility of the defensive reactions to different kinds of abnormal stimulus, including bacterial aggression.

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LIPID METABOLISM IN EXPERIMENTAL VIRUS INFECTIONS

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Despite much progress in the study of the nature of virus infections in recent years their pathogenetic aspects have by no means been fully investigated. This is truest of all for metabolic disturbances, which may accompany virus diseases. Yet a comprehensive study of the role of viruses in disturbances of normal homeostasis at the whole body level is important not only from the standpoint of a fuller understanding of the pathogenesis of virus infections themselves, but also in order to establish the importance of particular viruses in what have been called metabolic somatic diseases, whose etiology and pathogenesis remain incompletely explained. One such disease is atherosclerosis, in respect of which an association with viruses has been established [4, 11, 12, 13].

The aim of this investigation was an experimental study of the effect of herpes simplex and influenza viruses on lipid metabolism. The blood serum lipid spectrum of the animals and the content of free lipids in cells of the aorta were studied in model experiments in vivo and in vitro. The choice of virus models was determined by the following factors: the wide distribution and massive prevalence of herpetic and influenzal infections caused by viruses, and the depth and severity of the damage caused to individual organs and systems in these diseases [3, 8].

EXPERIMENTAL METHOD

A model of herpetic infection, namely keratoconjunctivitis (HKC) was created in chinchilla rabbits weighing 2-2.5 kg, with the aid of type 1 herpes simplex virus (HSV1, Koptev strain), with an infecting dose (ID) of 100 LD_{50} [5]. A model of influenzal infection (II) was created in noninbred albino mice (weighing 15-20 g), using influenza virus (IV, strain A/Aichi/2/68 [H3N2]), with IV of 0.35 LD_{50} [2]. As inhibitor of KC we used Furavir, a guanine derivative (synthesized at the Institute of Organic Synthesis, Latvian Academy of Sciences), with combined administration in the form of intravenous injections (20 mg/kg daily for 8 days) together five repeated instillations of a 3% solution. As inhibitor of II we used remantadine (produced by the "Latbiofarm" Combine) in a dose of 12.5 mg/kg, given by the intragastric route 1 h before infection, and then three times, 24, 48, and 72 h respectively after infection. Each experimental group consisted of 6 animals. Concentrations of triglycerides (TG) were determined by the method in [15], total cholesterol (Chs) by Ilka's method [15], α-Chs by the same method after precipitation of β-lipo-

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