The pathological process in the lungs of diabetic rats also is aggravated by a deficiency in the phagocyte system – polymorphs and alveolar macrophages. Diabetic patients are well known to be prone to serious infectious diseases. Investigations on diabetics have demonstrated the functional insufficiency of the polymorphs and, in particular, a decrease in chemotaxis [9] and phagocytic activity [12, 14].

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# ULTRASTRUCTURE OF THE DUODENAL EPITHELIAL CELLS IN THE EARLY STAGES OF EXPERIMENTAL COLIBACILLOSIS


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The leading role in the diarrheal syndrome caused by enterotoxigenic strains of Escherichia coli is ascribed to the thermolabile enterotoxin of these bacteria. The enterotoxin, binding firmly with the specific receptor of the apical plasmalemma of the epithelial cell, activates adenylate cyclase [7-9], as a result of which the cAMP concentration is increased and secretion of water and electrolytes stimulated. Activation of the adenylate cyclase of the epithelial cells also takes place as a result of the action of certain gastrointestinal hormones of the endocrine cells of the gastrointestinal tract [9]. The endocrine cells are known to secrete their contents either into the vascular system or into the interstitital space, through which they exert their action on neighboring target cells [6]. Endocrine cells of the APUD system of the gastrointenstinal tract have been shown to be most numerous in the proximal part of the small intestine [5], which is the most sensitive part to the action of the enterotoxins of E. coli [10]. Although the ultrastructure of the epithelial cells of the small intestine has been studied in some intestinal infections, problems concerned with changes in the duodenum in colibacillosis, especially in the earliest stages of the disease, remain virtually unstudied.

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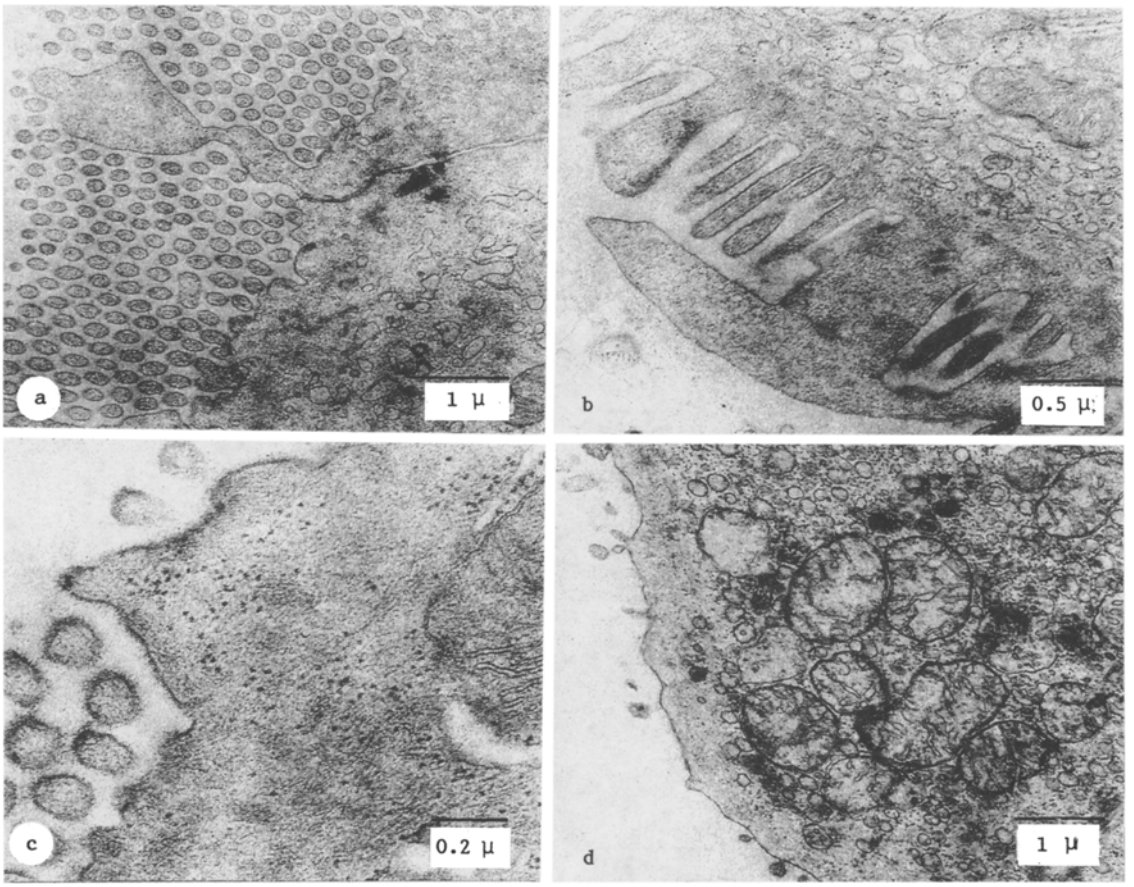


Fig. 1. Disturbances of configuration of microvilli of brush-border epitheliocytes of mouse duodenum in experimental colibacillosis. a, b) 15 min after infection (magnification 17,000 and 22,100 respectively); c, d) 30 min after infection (magnification 48,600 and 14,400 respectively).

The aim of this investigation was study ultrastructural changes in the brush-border epitheliocytes and endocrine cells of the duodenum, and also to compare these changes with the dynamics of adenylate cyclase activity in the epitheliocytes in the early stages of experimental colibacillosis.

## EXPERIMENTAL METHOD

Experimental colibacillosis was produced by the method described previously [1]. Altogether 35 mice were used in the experiments. The comparison group consisted of 5 uninfected mice, which were given physiological saline instead of the culture of E. coli perorally. Pieces of tissue from the duodenum were taken 15, 30 and 60 min and 3, 6, 12, and 24 hr after infection, and fixed in a mixture of 1% glutaraldehyde and 4% paraformaldehyde in 0.05 M cacodylate buffer, pH 7.2. After postfixation in a 1% solution of osmium tetroxide in the same buffer, the tissue was dehydrated and embedded in a mixture of Epon and Araldite or in Vestopal W. Ultrathin sections were cut on an LKB ultrotome, stained with lead citrate, and studied in the JEM-100B electron microscope. Adenylate cyclase activity on the epitheliocyte membranes was demonstrated by the method described previously [2].

## EXPERIMENTAL RESULTS

In the earliest stages of development of experimental colibacillosis 15 min after infection, changes in the ultrastructure of the brush-border epitheliocytes related mainly to their microvilli and took the form of thickening and disturbance of their configuration, with the formation of fungiform and clavate forms (Fig. 1a, b). Some degree of heterogeneity of the mitochondria of individual epitheliocytes was noted. Investigation of the endocrine cells of the g and EC type revealed a decrease in the number of secretory granules, exocytosis of the granules in the intercellular spaces, and sometimes their fusion. The mitochondria of the endocrine cells had a translucent matrix and reduction of their

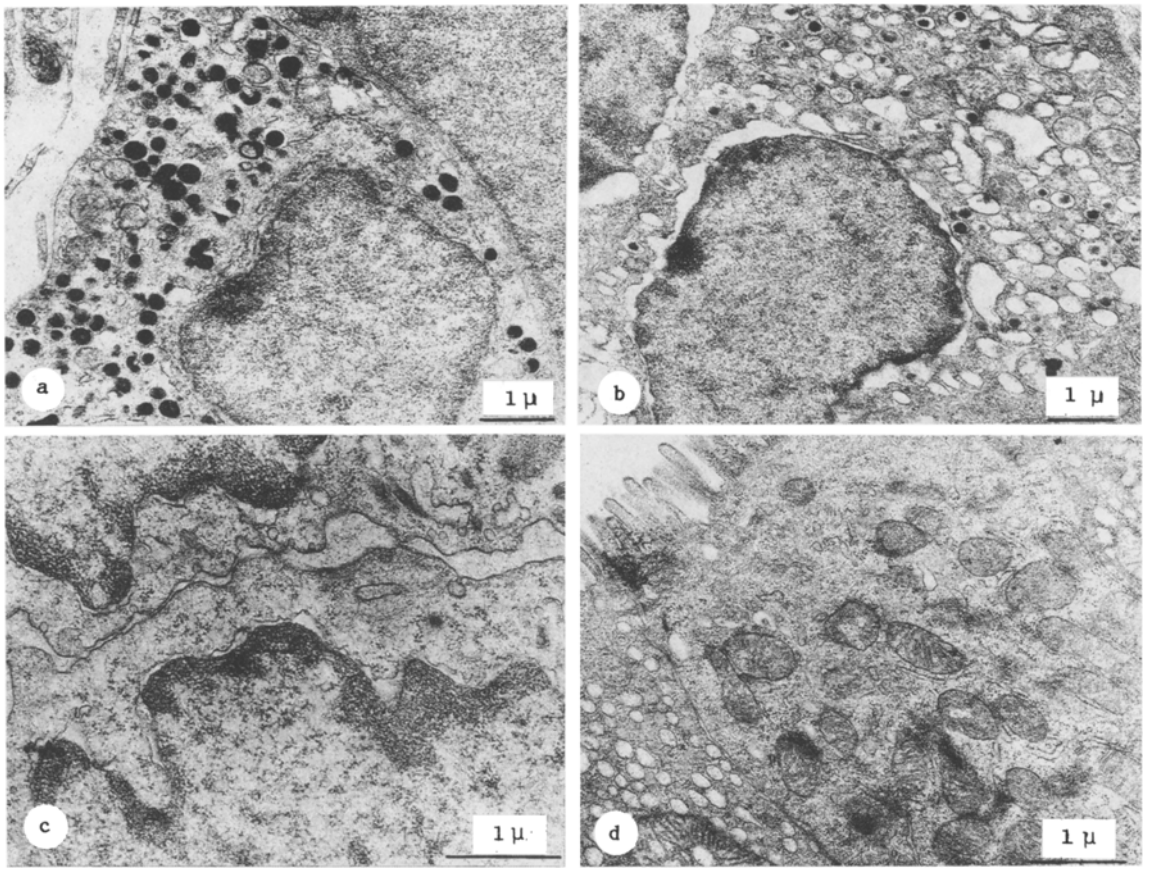


Fig. 2. Ultrastructure of epithelial cells of mouse duodenum in experimental colibacillosis. a, b) Different degree of maturation of granules in g-cells 30 min after infection (9800 ×), c) widening of intercellular and perinuclear space 1 h after infection (18,200 ×), d) brush-border epitheliocytes with different degrees of changes 14 h after infection (12,800 ×).

cristae, whereas myelin-like structures and degranulation of the rough endoplasmic reticulum (RER) were found in the cytoplasm.

The microvilli of certain epitheliocytes 30 min after infection became even wider and flatter, and sometimes disappeared virtually completely, while their apical plasmalemma remained intact. The matrix of the altered microvilli had a marked similarity with the ultrastructure of the terminal zone and contained many microfilaments, free ribosomes, and microvesicles (Fig. 1c, d). Many vesicles of various sizes were found in the cytoplasm, where they were located mainly in the apical part of the epithelial cells, and had finely granular contents of average and low electron density. Widening of RER with its partial degranulation were observed in some cells. The mitochondria of the brush-border epitheliocytes were round, they were located mainly in the supranuclear zone, and they had a translucent matrix, while some of them showed separation of the outer and inner membranes with the formation of small cavities, sometimes with rupture of the membrane. Structural changes also were intensified in the endocrine cells, reflecting differences in their functional state. Endocrine cells, both completely empty or containing solitary granules, as well as other filled with many granules of different sizes and different electron density, were found (Fig. 2a, b). Secretory granules were distributed mainly around the periphery of the cytoplasm, and some were even in close proximity with the lateral plasmalemma. An active lamellar complex and disorientation and fragmentation of the mitochondrial cristae were found in some of them.

The severity of the ultrastructural changes in the epithelial cells 1 h after infection of the animals continued to increase. The vesicular cytoplasm of the brush-border epitheliocytes extended as far as the basement membrane. Coated vesicles of different shapes and sizes were found in the paranuclear zone and apical part of these cells. The RER was greatly widened and partly degranulated, and many free monosomes and polysomes were found in the cytoplasm. The mitochondria were grossly hypertrophied, with a pale matrix.

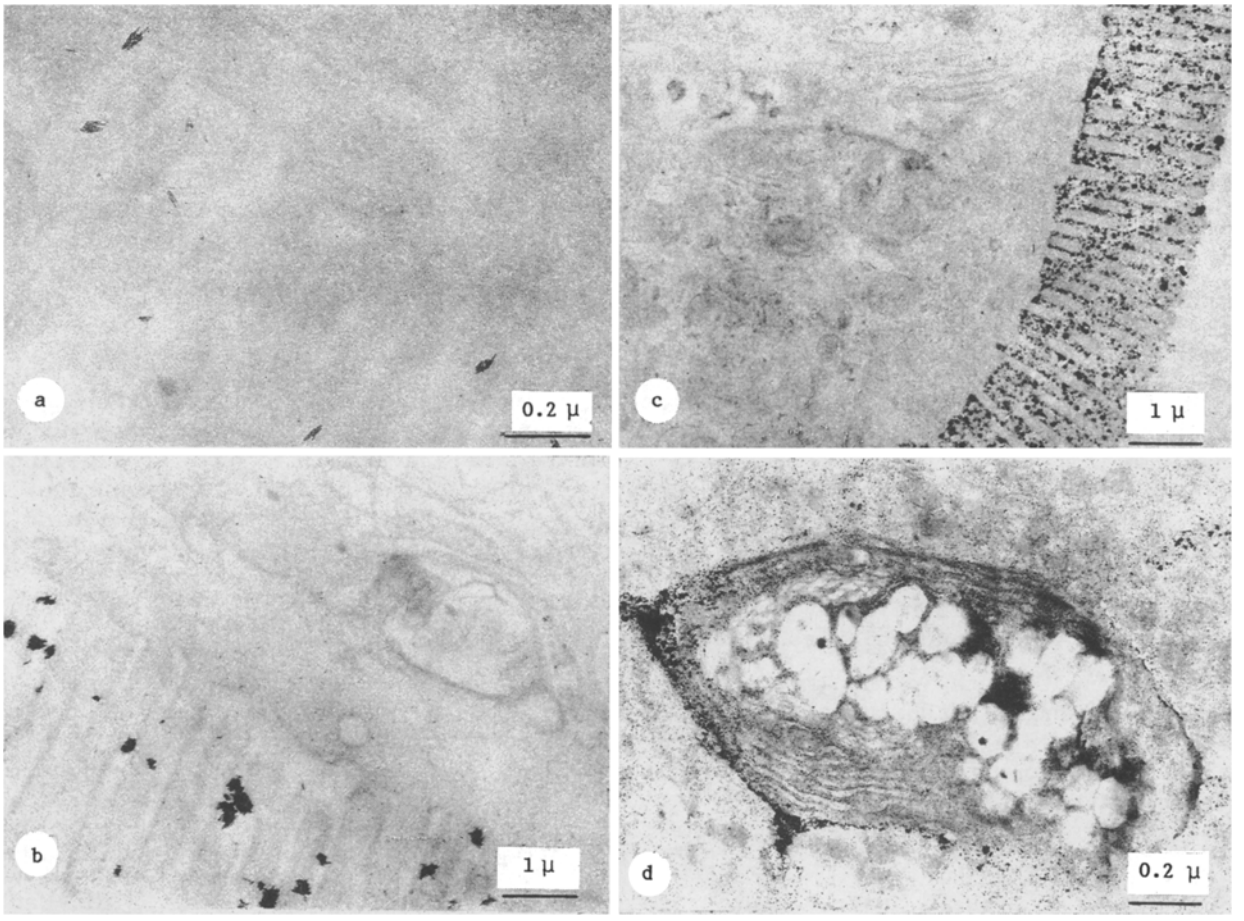


Fig. 3. Adenylate cyclase activity on apical (a, b, c) and lateral plasmalemma of mouse duodenal epitheliocyte. Unstained preparations. a) Control mice (52,800 ×), b) 15 min after infection (37,800 ×), c) 30 min (9000 ×), and d) 60 min after infection (8500 ×).

At this time widening of the intercellular spaces between adjacent brush-border epitheliocytes was clearly distinguishable over a considerable area, accompanied by local widening of the perinuclear space (Fig. 2c). Changes in the endocrine cells were intensified, and among them cells with numerous granules in different stages of maturation could be found; their RER was widened by the degranulated, and their cytoplasm contained many free monosomes and polysomes, and mitochondria with widened intracristal spaces.

Destructive processes were intensified 3-6 h after infection. Activation of the lamellar complex of the epithelial cells was noteworthy, with many free monosomes and polysomes appearing in their cytoplasm. In some brush-border epitheliocytes the normal orientation of the microfilaments in the terminal zone was disturbed and myelin-structures and cytophagosomes of enormous size were found.

No appreciable increase in the ultrastructural changes affecting the epithelial cells were found 12-24 h after infection. The general pattern was basically reminiscent of changes which had already taken place in the cells in the initial period (from 15 min to 6 h). However, in this period the transitional boundary between the terminal zone and cytoplasm had disappeared in individual cells, the cytoplasm was condensed, the RER invaginated, microplasmatosis was present, and the number of free monosomes and polysomes was reduced. Characteristically the changes described were local in character and affected individual epithelial cells; these changes, moreover, could also affect only certain structural elements of the same or a different cell, for example, only the microvilli, or, conversely, they could affect the cytoplasm, leading to its vesiculation (Fig. 2d).

The ultrastructural changes mentioned above were accompanied, with effect from 15 min after infection, by a definite increase in adenylate cyclase activity both on the apical plasmlemma and on the lateral plasmalemma of the brush-border epitheliocytes, as well as on the limiting membrane between the brush-border epitheliocytes and goblet-shaped epitheliocytes (Fig. 3a, b, c, d).

Thus changes taking place in the brush-border epitheliocytes and endocrine cells during the first 60 min after infection, as well as disturbances of configuration of the microvilli, vesiculation of the cytoplasm, widening of the intercellular spaces, degranulation of the endocrine cells, and activation of adenylate cyclase, are evidence of earlier disturbance of water and electrolyte exchange in the epitheliocytes in response to the action of metabolic products of enterotoxigenic E. coli. During subsequent periods after infection changes reflecting disturbance of protein metabolism were found: widening and degranulation of RER, an increase followed by a decrease in the number of free monosomes, polysomes, and coated vesicles in the cytoplasm. Activation of the lamellar compelx reflects potentiation of the detoxicating function of the epithelial cells.

The results of this investigation demonstrate the sequence of ultrastructural changes and the time course of adenylate cyclase activity in the epithelial cells of the duodenum. Even in the earliest stages of development of experimental colibacillosis growth changes take place in the microvilli of the brush-border epitheliocytes on account of swelling of the terminal zone in the region of the microvilli, with disturbance of their configuration and flattening or their almost complete disappearance. These changes are accompanied by increased adenylate cyclase activity on the apical plasmalemma of the brush-border epitheliocytes. Endocrine cells are involved at the same time in the pathological process, changes in their ultrastructure being evidence of a disturbance of their protein metabolism and revealing marked degranulation as early as 30 min after infection.

According to data in the literature, in various bacterial infections similar nonspecific structural changes in the intestinal epitheliocytes are observed [3, 4]. However, a combination of certain definite morphological and cytochemical reactions and their time course justify conclusions to be drawn regarding the characteristic ultrastructural changes in small intestinal epitheliocytes in the early stage of experimental colibacillosis.

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