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Ultrastructural Changes in Small Intestinal Lipofibroblasts of Suckling Rabbits with Experimental Cholera

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Ultrastructural analysis of the jejunum in suckling rabbits showed that lipofibroblasts localized in the submucosa and adjacent to crypts contain lipid inclusions (granules) with typical "melting" surface. Lipofibroblasts contained moderately widened cisternae of the granular endoplasmic reticulum and few mitochondria with dense matrix and poorly developed cristae. Experimental cholera was usually accompanied by a decrease in the number of lipid inclusions, and only in some cases by accumulation of lipid material. Our results suggest that the material accumulated in granules plays a role in the pathogenesis of cholera.

Key Words: *cholera; lipofibroblasts; small intestine; electron microscopy*

Lipid inclusions of prostaglandins (PG) or their precursors are present in the cytoplasm of interstitial cells in the renal medulla [8]. Ultrastructural changes in these cells were studied during generalized Schwarzman phenomenon in rabbits [7], endotoxin shock in rats and dogs [11], and experimental cholera in suckling rabbits [11]. Functional heterogeneity of lipofibroblasts (LFB) was previously reported [9]. LFB, or lipid interstitial cells, play a role in the synthesis of surfactant in the lungs [14], functional activity of the renal countercurrent system, and local homeostasis in the liver [9].

Endotheliocytes, tissue basophils (mast cells), fibroblasts, migrating capillary leukocytes, arteriolar smooth muscle cells, and myocytes of longitudinal and circular muscles are good candidates for intestinal PG-synthesizing cells during cholera intoxication [12]. There is little likelihood that smooth myocytes are involved in the synthesis of PG during cholera. Cell damage during cholera is accompanied by peristaltic disorders [5]. It is unlikely that the structures with well-known function can act as PG-synthesizing cells [1]. LFB as a possible source of PG were not identified in the small intestine of suckling rabbits. Published data show that during cholera the basal content of cholera PGE_2 in cells of the lamina propria 6-fold surpassed that in enterocytes [13].

Here we studied LFB in the small intestine of suckling rabbits and ultrastructural changes in these cells during experimental cholera.

MATERIALS AND METHODS

Experiments were performed on 10-12-day-old suckling rabbits (n=16). The animals were infected with cholera after 1-day starvation. Soda solution (1 ml, 3%) was administered into the stomach through a polyethylene tube to neutralize the gastric content. This treatment was followed by infusion of an 18-h culture of *Vibrio cholerae eltor P-5879* Inaba vibrios (1 ml) and soda (0.5 ml). The infecting dose estimated by optical opacity was 10^5 microbial cells. Treatment with this dose was accompanied by the development of cholera syndrome and accumulation of a transparent or semitransparent serous fluid with vibrios in high concentration (10^8-10^9 microbial cells/ml) in the intestine. The lifespan of suckling rabbits was 24-48 h. The ani-

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mals were killed with nembutal in a lethal dose on the next day. The control group included 4 suckling rabbits receiving 1.5 ml soda and 1 ml isotonic sodium chloride. For electron microscopy, segments of the jejunum taken at a distance of 15 cm distal to the duodenum were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) at 4°C for 1 h, postfixed with 1% OsO_4 in the same buffer at 4°C for 1 h, dehydrated with ascending alcohols, and embedded into Epon 812. Semithin sections were stained with toluidine blue. Sections of blocks were prepared on a LKB-8800 ultramicrotome, contrasted with uranyl acetate

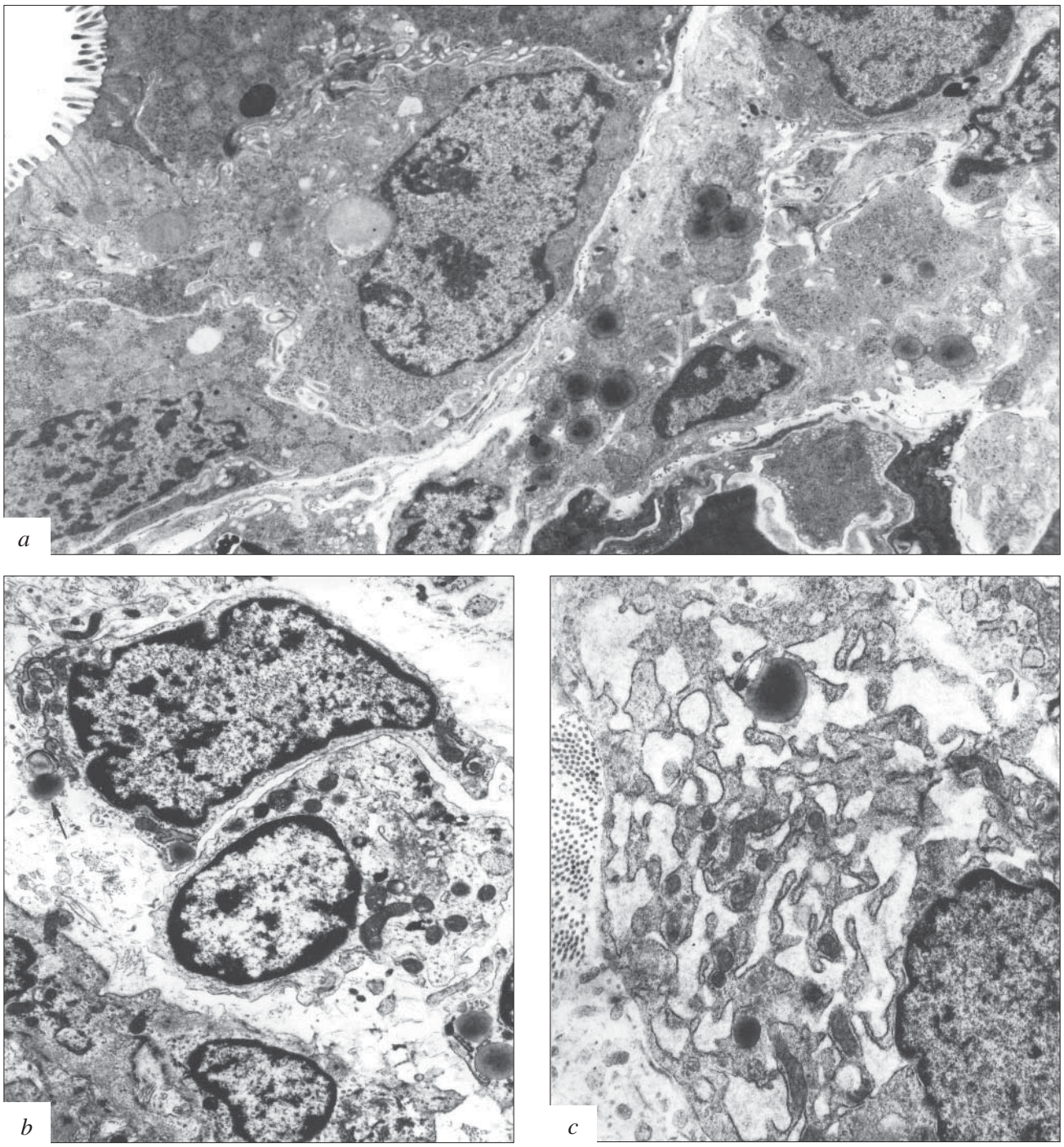

Fig. 1. Ultrastructural characteristics of lipofibroblasts in the small intestine of suckling rabbits under control conditions (*a*) and during experimental cholera (*b*, *c*). Conglomerates of lipid inclusions in lipofibroblast adjacent to the crypt (*a*, ×3000). Destruction of the lipofibroblast plasmalemma and sharp decrease in the amount of lipid inclusions (arrow, release of the lipid granule, *b*, ×4000). Sharply widened tubules of the granular endoplasmic reticulum with lipid inclusions (*c*, ×5000).

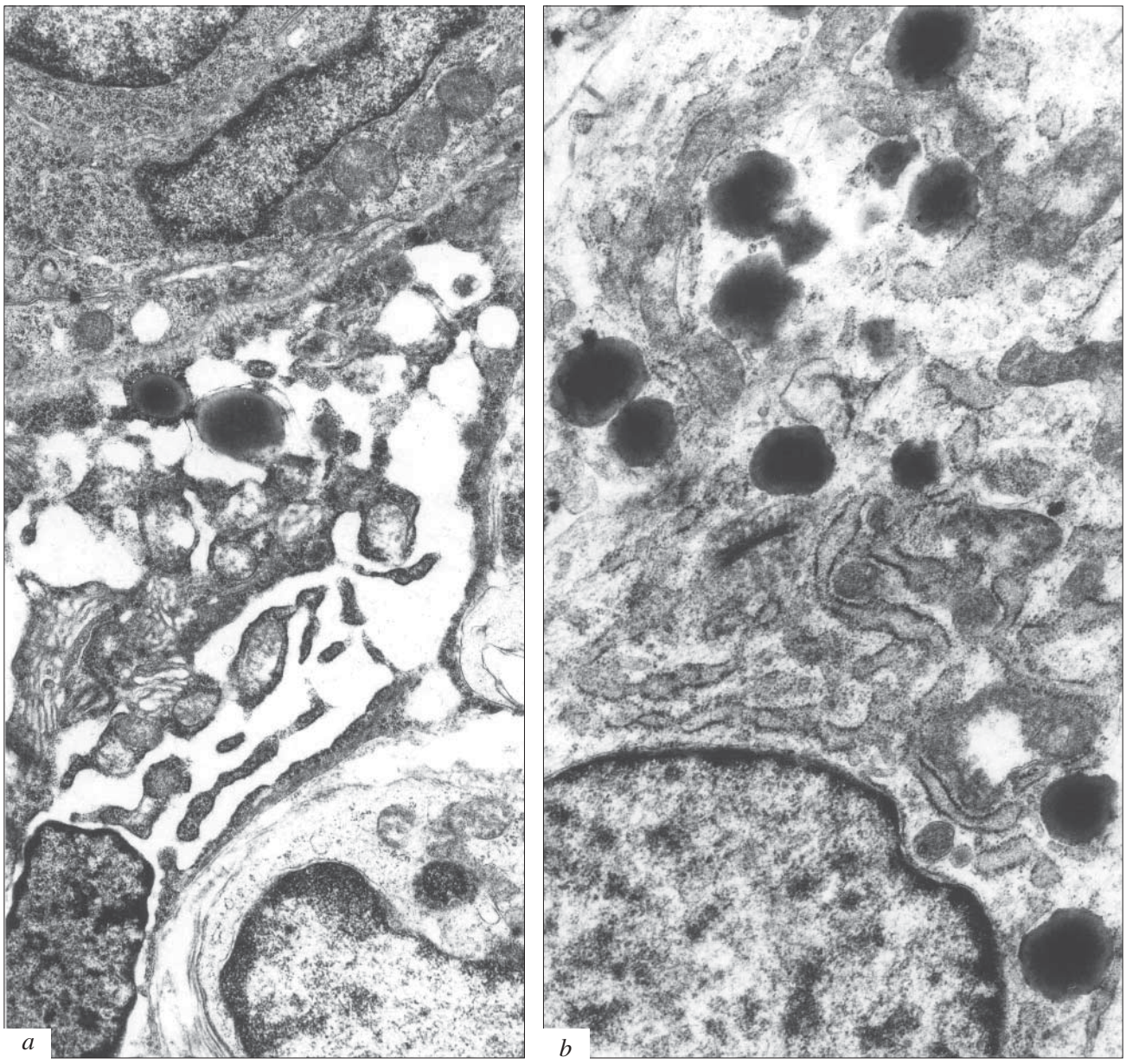

Fig. 2. Ultrastructural characteristics of lipofibroblasts in the small intestine of suckling rabbits with experimental cholera. Reduction of the lipid material, connection of the perinuclear space with sharply widened cisternae of the granular endoplasmic reticulum, swelling of mitochondria (*a*, ×5000). Increase in the amount of lipid inclusions in lipofibroblast, change in the shape of mitochondria, and appearance of osmiophilic material in sharply dilated tubules of the granular endoplasmic reticulum (*b*, ×8000).

and lead citrate, and examined under a JEM-100 B electron microscope.

RESULTS

Light microscopy of semithin sections of the jejunum in control animals revealed the presence of LFB with irregular or elongated shape in the lamina propria of the mucosa in crypts. These cells had long polar and short lateral cytoplasmic processes.

LFB were adjacent to crypts and arranged in aggregates consisting of several cells (Fig. 1, *a*). The ultrastructural characteristic was the presence of lipid granules (inclusions) similar to those revealed in interstitial cells of the renal medulla in rats, dogs [10], and suckling rabbits [11]. Round or oval lipid inclusions formed conglomerates and had osmiophilic central area and light annular cytoplasm surrounded by "single-contour" membrane. Sometimes they looked like a half-moon structure. Another characteristic of LFB was the presence of short cisternae of the granular endoplasmic reticulum and individual mitochondria with poorly developed cristae. The cytoplasm had high electron density due to the presence of numerous free ribosomes and polysom.

LFB underwent two types of changes during experimental cholera. The first type included partial or complete disappearance of lipid inclusions, which was accompanied by ultrastructural injury to cells. Lipid inclusions were displaced to the plasma membrane, accumulated in cell processes, or present in the stroma (during plasmalemmal damage, Fig. 1, *b*). Light annular zone ("melting" surface) was widened. Osmiophilia of the central part in lipid granules considerably decreased.

Apart from transformation of the lipid material, we revealed deep invaginations of the plasmalemma in some LFB. These changes resulted in separation of the cytoplasm from cells. Microclasmatosis was followed by the appearance of fragments containing only lipids or organelles. We revealed lipid depletion of the cytoplasm and presence of sharply dilated tubules of the endoplasmic reticulum in some LFB (Fig. 1, *c*). The laminar complex was poorly developed and intact. Most mitochondria and nuclei remained unchanged. Some nuclei were characterized by partial widening of the perinuclear space. Individual mitochondria appeared as "rackets". The elongated handle was intact, while the wide frame included reduced cristae and lightened matrix.

Experimental cholera was accompanied by pronounced microcirculatory disturbances in intraganglionic capillaries of the submucosal Meissner's and intermuscular Auerbach's plexuses [2] and vessels of the right atrium [6], lungs [3], and renal cortex and medulla [4,11]. Progressive disorder of regional blood flow produced hypoxia and was followed by transformation of reactive signs typical of functional strain into degenerative changes. Hydropic degeneration of LFB during cholera was manifested in intracellular edema and widening of the perinuclear space connected with dilated structures of the granular endoplasmic reticulum (Fig. 2, *a*). Swollen mitochondria had no cristae. The Golgi complex was intact and exhibited very high resistance to hypoxia and damaging effect of choleric exo- and endotoxin.

The second type of changes in LFB was rarely observed. It consisted in accumulation of lipids in the cytoplasm (Fig. 2, *b*). These cells had widened cisternae of the granular endoplasmic reticulum with electron dense material. Mitochondria of various shapes were characterized by destruction of membranes and partial lysis of the mitochondrial matrix.

Quantitative and qualitative changes in lipid granules serve as a reliable criterion for comparative study of small intestinal LFB with renomedullary cells during cholera. Progression of cholera is followed by a decrease in the number of these granules, changes in topographic characteristics, appearance of granules with "melting" surface, and reduction of the lipid material [11]. Ultrastructural changes in the renal medulla were accompanied by a significant increase in PG concentration in the stroma [13] and capillaries of the small intestine [12]. Similar changes were observed in small intestinal LFB. The reduction of lipid inclusions was accompanied by an increase in PGE_2 concentration in the lamina propria and vessels of the small intestine. The appearance of cholera PGE_2 in considerable amounts leads to diarrhea.

Our results show that LFB of the lamina propria in the jejunal mucosa in suckling rabbits contain lipid granules (inclusions) similar to those identified in cells of the renal medulla. The development of experimental cholera was accompanied by opposite changes in the amount of lipids. The most common changes included reduction of the lipid material. LFB are reactive stromal structures of the jejunum. The total functional microsystem of these cells and epithelium is responsible for rapid intestinal dehydration during cholera.

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