Ultrastructural Changes in Smooth Muscle Cells of the Small Intestine in Suckling Rabbits with Experimental Cholera

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Ultrastructural study of the small intestine in suckling rabbits with experimental cholera revealed involvement of the inner and outer smooth muscle layers into the pathological process. Smooth muscle cells were characterized by vacuolar and fatty degeneration and focal colliquative necrosis. Apoptosis played little role in gastrointestinal motility disturbances. The presence of considerable amounts of fluid in intestinal loops reflects peristaltic dysfunction due to generalized damage to smooth muscle cells.

Key Words: *cholera; smooth muscle cells; small intestine; electron microscopy*

Secretion in the small intestine induced by cholera toxin (CT) [1,9] or virulent strains of cholera vibrios was studied in details [8]. Much attention was given to ultrastructural changes in epithelial cells of the small intestine [5,6,9], the role of intramural ganglia [2,3], and effects of lidocaine [4] and adrenoblockers during experimental cholera [7].

Administration of CT in the ileum induces myoelectric activity, so-called migrating complex of action potential presented by single contraction of circular muscles [13]. Rapid action potential bursts in combination with fluid secretion were observed in adult New Zealand albino rabbits after intraintestinal infusion of the *E. coli* lysate with enterotoxin cloned from *Salmonella typhimurium* [14].

Other types of intestinal motility, including retrograde giant and giant migrating contractions, remained unchanged during cholera [11,12]. CT markedly reduces the duration of migrating motor complexes via the direct effect on the intestinal wall, increases the percentage of phase II activity, and inhibits migrating contraction clusters due to an indirect effect of CT determined by fluid accumulation [10].

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Here we studied ultrastructural changes in intestinal smooth muscles 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 4-day food withdrawal. Soda (1 ml, 3%) was administered into the stomach through a polyethylene tube for neutralization the gastric content. The procedure was followed by infusion of 18-h El Tor vibrios (1 ml) and soda (0.5 ml). The infecting dose estimated by standard optical opacity was 10^5 microbial cells and ensured the development of cholera syndrome and accumulation of a transparent or turbid serous fluid containing vibrios in high concentration (10^8-10^9 microbial cells/ml) in the intestine on the next day. The lifespan of suckling rabbits was 24-48 h. The animals were killed with a lethal dose of nembutal after 1 day. The control group included 4 suckling rabbits intragastrically receiving 1.5 ml soda and 1 ml isotonic sodium chloride. Segments of the jejunum taken at a distance of 15 cm distally to the duodenum were examined under an electron microscope. The samples were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) at 4°C for

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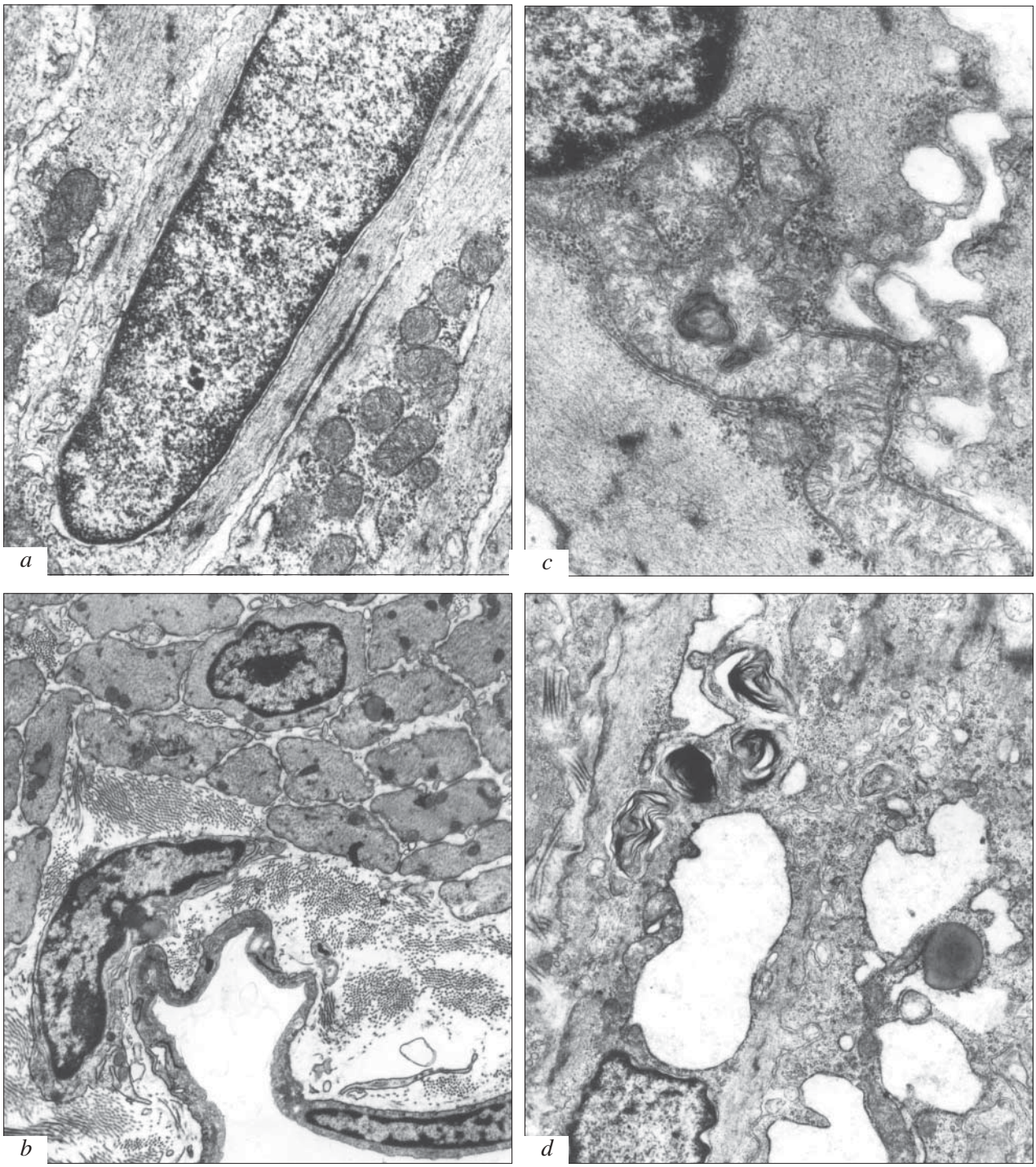

Fig. 1. Ultrastructural characteristics of smooth muscle cells in the small intestine of suckling rabbits under control conditions (*a*, *b*) and during experimental cholera (*c*, *d*). Adjacent cells in the zone of contact forming nexuses; intact oval nucleus, mitochondria, and sarcoplasmic reticulum (*a*, longitudinal section, ×10,000; *b*, transverse section, ×3000). Changes in the shape of mitochondria and appearance of 2 myelin figures in the matrix (*c*, ×8000). Pronounced widening of cisternae of the granular reticulum, their transformation into large cavities, and appearance of myelin figures in the sarcoplasm (*d*, ×6000).

1 h, postfixed with 1% OsO_4 in the same buffer at 4°C for 1 h, dehydrated in assenting alcohols, and embedded into Epon 812. Semithin sections were stained with toluidine blue, blocks containing smooth muscle fibers were selected, the orientation of muscle fibers and changes in the inner (circular) and outer (longitudinal) layers were analyzed. Sections obtained from these blocks were prepared on a LKB-8800 ultramicrotome, contrasted with uranyl acetate and lead citrate, and examined under a JEM-100 B electron microscope.

RESULTS

Light microscopy showed that administration of sodium chloride produced no changes in smooth muscle cells (SMC) of control animals. In suckling rabbits with experimental cholera fragmented nuclei, lipid inclusions, small and large vacuoles were seen in the sarcoplasm of SMC on semithin sections. Nerve cells of the Meissner's and Auerbach's plexus were found in the submucosal and intermuscular layer, respectively. Ultrastructural changes in these cells during experimental cholera were described previously [2,3,8].

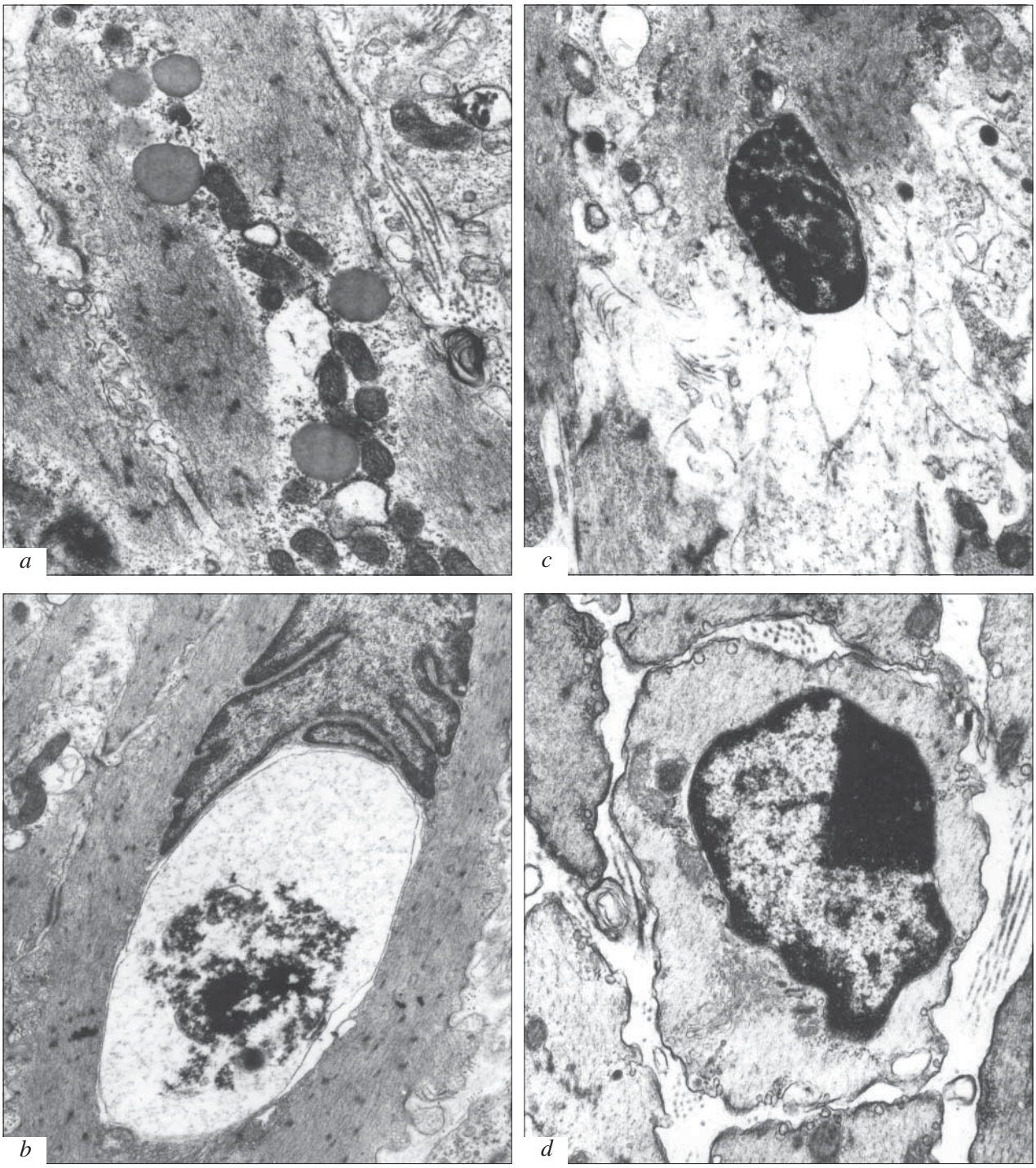

Fig. 2. Ultrastructural characteristics of smooth muscle cells in the small intestine of suckling rabbits with experimental cholera. Numerous lipid inclusions in the sarcoplasm (*a*, ×8000). Large cavity bounded by the single membrane; intact laminar complex and mitochondria with partial lysis of the matrix in the lower area (*b*, ×5000). Karyopyknosis and focal colliquation necrosis of the smooth muscle cell (*c*, ×6000). Initial stage of apoptosis in the smooth muscle cell (*d*, ×5000).

Electron microscopy revealed no changes in SMC of the longitudinal and circular muscles in control animals. The sarcoplasm of cells included thin and thick filaments, intermediate filaments contacted with dense bodies. Cell nuclei were unchanged; Golgi complex, small clusters of round or oval mitochondria with the electron dense matrix, and membranes of the granular reticulum (single short cisternae) were sometimes seen near the pole (Fig. 1, *a*, *b*). Caveolae were aligned in a row under the plasma membrane. Nexuses of varying length were found between SMC in the area where they came within shortest distance of each other. These intercellular contacts are typical of excitable tissues and provide electrotonic conduction of slow waves and action potentials in cell bundle or between bundles.

The development of experimental cholera was accompanied by considerable changes in most SMC of the outer layer. Myelin-like structures (myelin figures) were often found.

Of particular interest was the sequence of formation of myelin figures in mitochondria and sarcoplasm.

First, zones of lysis surrounded by the membrane appeared in the mitochondrial matrix or sarcoplasm of SMC, and then spiral structures appeared in these zones. Mitochondria contained 1-2 myelin figures (Fig. 1, *c*), and the sarcoplasm included 2-3-fold more figures than mitochondria (Fig. 1, *d*). Myelin figures were displaced to the cell boundary and were released into the stroma via exocytosis.

Membranes of the granular sarcoplasmic reticulum were vacuolized, dilated, and sometimes contained electron dense substances (Fig. 1, *d*). Cisternae of the reticulum sometimes opened into cavities that were formed after segregation of nuclear membrane leaflets. Fragments of organelles or cell membranes were revealed in some cavities.

A constant and typical sign in SMC was increasing number of lipid inclusions forming small aggregates (Fig. 2, *a*). The perinuclear space was regularly enlarged along the nuclear perimeter. The uneven surface had deep invaginations of the nuclear membrane. Giant cavities were surrounded by single membrane and contained digested residuals of organelles (Fig. 2, *b*). Dystrophic and necrotic changes during experimental cholera and pronounced diarrhea led to condensation of chromatin, constriction of the nucleus, partial bulging of the outer nuclear membrane, destruction of ultrastructures, and focal colliquation necrosis with fusion of the plasmalemma and part of the sarcoplasm (Fig. 2, *c*). Probably, SMC could undergo apoptosis. The initial stage of this process is characterized by chromatin condensation in the peripheral region of the nucleus. It should be emphasized that in this stage the membrane of intracellular organelles and sarcolemma were intact (Fig. 2, *d*). We believe that apoptosis in SMC is a rare process that plays little role in the development of abnormalities in gastrointestinal motility during cholera.

Ultrastructural study showed that experimental cholera is accompanied by changes in SMC of both layers in smooth muscles. Most pronounced changes are observed in the outer (longitudinal) layer of the jejunum. The development of diarrhea and overfilling of intestinal loops with the liquid content reflect peristaltic dysfunction and paralysis of smooth muscles in the gastrointestinal tract.

Ultrastructural changes in SMC probably underlie a variety of myoelectric phenomena reflecting motor dysfunction in the outer and inner smooth muscle layers of the intestine during cholera.

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