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Choleragen-Induced Changes in the Ultrastructure of Epitheliocytes from the Large Intestine of Conventional and Gnotobiotic Minipigs

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The dynamics of changes in the absorbing epitheliocytes and goblet cells is studied in minipigs 1, 3, 6, 18, and 24 h after intragastral administration of cholera toxin. Light and dark cells are identified after the treatment. The dark cells are probably protected against the toxin by paracrine E_c-cells persisting throughout the entire observation period. Changes in the organelles of absorbing epitheliocytes appear 3 h after the treatment and persist for 24 h. During this period, the goblet cells actively secrete mucus.

Key Words: *choleragen; large intestine epitheliocyte; minipig; gnotobiotic and conventional animals*

In recent decades, minipigs have been widely used in laboratory research, since their internal organs are very similar to those of humans [2,10,11]. It should be noted that the intestinal microflora of minipigs of the Svetlogorsk population is identical or close to the symbiotic microflora of human intestine [3]. Therefore, the data obtained on minipigs have been extrapolated to humans [6]. However, the ultrastructure of epitheliocytes from the large intestine of gnotobiotic minipigs in the norm and during choleragenic intoxication has not been studied.

MATERIALS AND METHODS

This paper presents experimental data obtained on 15 gnotobiotic and 2 conventional minipigs (500-1500 g) after intragastral administration of cholera toxin (500-800 μg) in physiological saline. The large intestine and epitheliocytes were studied 1, 3, 6, 18, and 24 h after administration of the toxin. After a 24-hour period of food deprivation, the minipigs were given 1% sodium bicarbonate solution (pH 7.2) to neutralize gastric juice, after which cholera toxin was administered. Two control animals were given the same volume of physiological saline.

Specimens for light and electron microscopy were fixed with glutaraldehyde and postfixed with osmium tetroxide in a cacodylate buffer (pH 7.3). The specimens were passed through increased ethanol concentrations and embedded in Epon-Araldite. Both semithin and ultrathin sections were examined.

RESULTS

It has been generally recognized that gnotobiotic conditions affect all vital systems of the organism. The most pronounced ultrastructural changes occur

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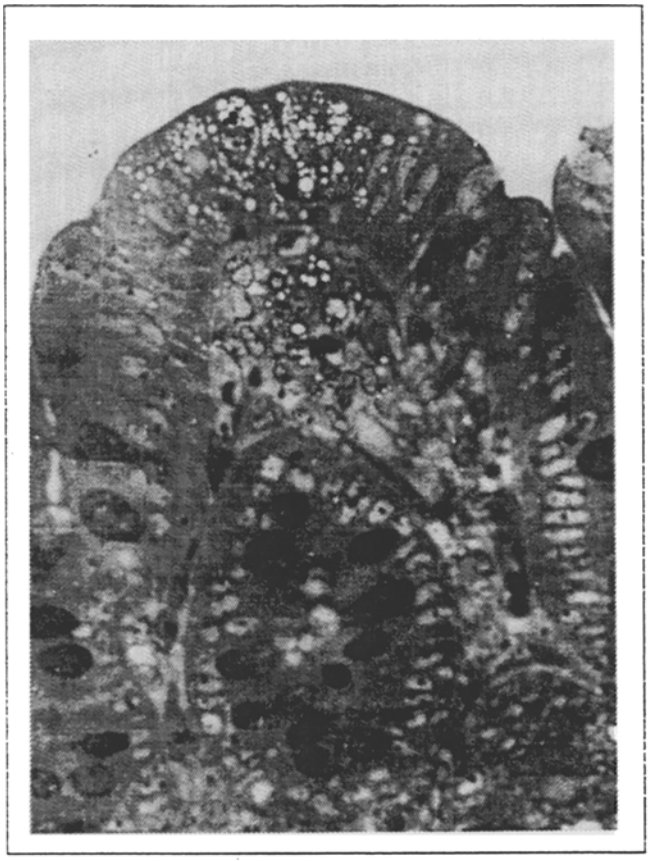

Fig. 1. Large vacuoles in epitheliocytes from the large intestine of a minipig 3 h after administration of cholera toxin. Semithin section, azure—methylene blue staining, ×400.

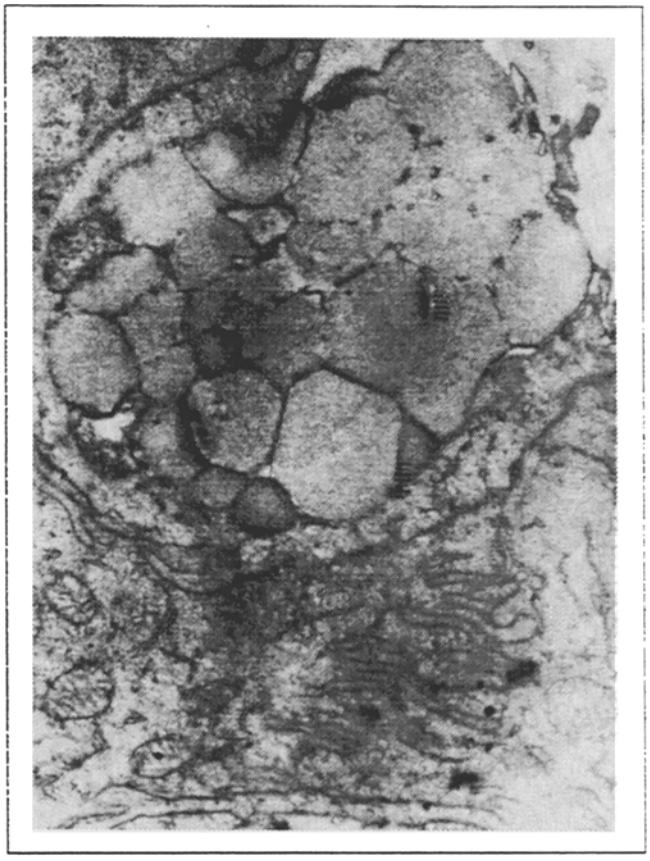

Fig. 2. Ultrastructure of the goblet cell from the large intestine of a minipig 24 h after administration of cholera toxin. Staining by the method of Reynolds, ×30,000.

in the organs that normally contact with microorganisms and microbial products [7].

Examination of semithin sections (azure and methylene blue staining) of the large intestine of conventional animals revealed epitheliocytes with small vacuoles in the apical part (1 and 3 h after treatment, Fig. 1). Numerous small and large vesicles were observed under an electron microscope. They fused and formed larger vesicles. Six hours after administration of cholera toxin, a slight diffuse edema of the epitheliocyte cytoplasm and destruction of mitochondria occurred in some cells; the nuclei with disperse chromatin were located predominantly in the basal part of the cell. These changes were observed in all examined epitheliocytes. The goblet cells actively secreted mucus. The intensity of these changes increased up to the 18th hour. However, large vacuoles did not appear. The majority of mitochondria were deenergized. Numerous mucus-producing goblet cells were seen. Twenty-four hours later, these changes persisted only in single cells, the bulk of epitheliocytes remaining practically unaltered. However, a considerable number of active mucus-secreting goblet cells were still present (Fig. 2). Throughout the observation period, smoothed and pin-shaped microvilli were less abundant than in the control animals (Fig. 3, *a*, *b*). No animals died in this experimental series.

Uniform changes were revealed by electron microscopy in the large intestine of gnotobiotic animals 1-3 h after administration of cholera toxin. The microvilli and glycocalyx were intact, the terminal web was loosened, and abundant mono- and polysomes were present in the epitheliocyte cytoplasm. The components of the smooth endoplasmic reticulum were dilated, and the signs of hyperplasia appeared in the rough endoplasmic reticulum; some membranes were degranulated. Hyperchromatous nuclei were localized in the cell center. Most goblet cells were empty. Twenty-four hours later, the ultrastructure of epitheliocytes did not differ from that of the control animals. However, empty goblet cells were numerous.

Thus, our data obtained on conventional minipigs agree with those obtained on other animals [1,4,8,9]. Our findings suggest that the ultrastructure of epitheliocytes from the large intestine reflects the disturbed reabsorption capacity and enhanced secretory function of goblet cells. This effect lasted 24 hours.

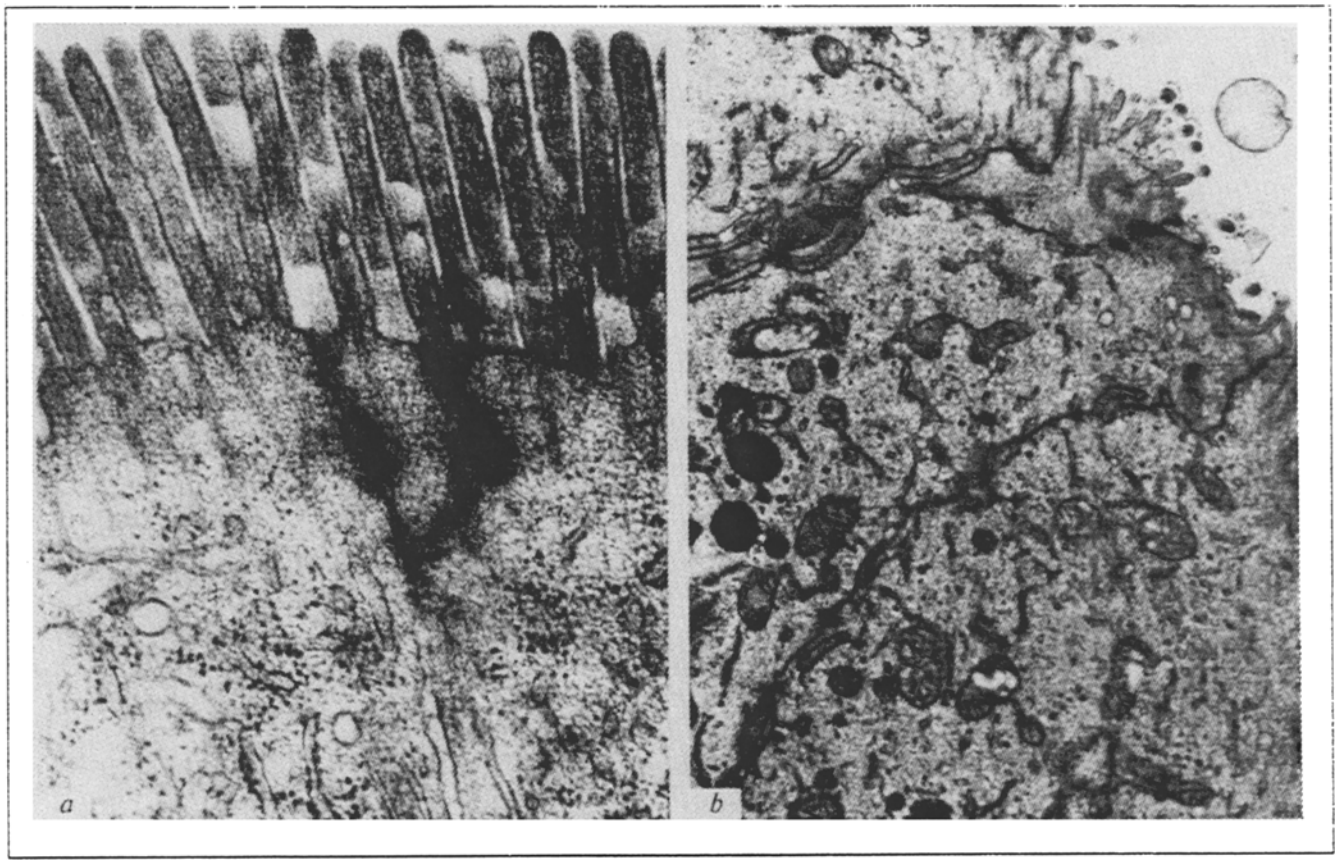

Fig. 3. Ultrastructure of the apical part of epitheliocytes from the large intestine of a minipig before (*a*, ×75,000) and 3 h after (*b*, ×33,000) administration of cholera toxin. Staining by the method of Reynolds.

Cholera toxin induced no pronounced changes in the dark epitheliocytes of the large intestine from gnotobiotic minipigs. In these cells, the enhanced protein synthesis ensured cell functioning. The secretion of mucus by goblet cells was increased and persisted for 24 h.

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