ULTRASTRUCTURAL AND MORPHOMETRIC ANALYSIS OF THE RESPONSE OF PANETH'S CELLS TO CHOLERA TOXIN

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This investigation is part of a study of the mechanisms of action of cholera toxin on the intestinal epithelium. Previous studies of this problem [1, 2] have been devoted to several stages of the mechanism of action of cholera toxin on different types of epithelial cells: prismatic, goblet, and marginal cells of the Peyer's patch. On the basis of these investigations a new secretion-filtration theory has been put forward [6] to explain the syndrome of rapid intestinal dehydration in cholera toxemia. However, the role of Paneth's cells in response to cholera toxin at different periods of their functional activity has not yet been explained. Yet it must be pointed out that the role of their secretion in small intestinal function likewise has not yet been fully explained [7, 8]. Accordingly, the concrete aim of this investigation was to establish, at the ultrastructural level, after how many hours cholera toxin reaches the surface of the Paneth's cells, how their functional activity is modified, and at what stage is the activity of the cell organelles altered.

METHODS

Experiments were carried out on 40 immature male guinea pigs weighing 250-300 g. Of the 40 animals 20 were given cholera toxin (Sigma) in a volume of 0.5 ml of physiological saline, in a dose of 0.4 mg/100 g body weight, by injection into the duodenum. Pieces of experimental material were taken from all parts of the small intestine under ether anesthesia 3, 6, 12, and 24 h after administration. Physiological saline alone was given under the same conditions to 20 control animals. Material for electron-microscopic investigation was fixed n 2.5% glutaraldehyde solution in cacodylate buffer, pH 7.3, postfixed in a 2% solution of osmium tetroxide, dehydrated in acetones of increasing strength,

TABLE 1. Values of $\bar{X}S$ of Secretion of Paneth's Cells in Experimental and Control Groups

Time of taking material, h	$\bar{X}S$ of secretion in one cell in experimental group (administration of cholera toxin)	$\bar{X}S$ of secretion in one cell in control group (administration of physiological saline)
3	16,19	10,78
6	4,65	11,2
12	7,29	14,5
24	11,32	14,28

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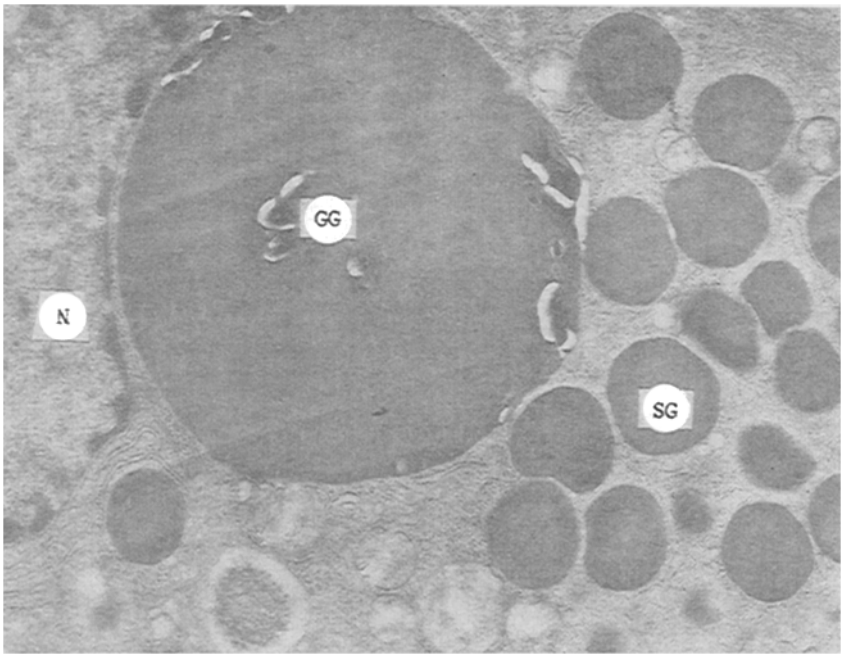

Fig. 1. Paneth cell in small intestine of guinea pig 3 h after injection of cholera toxin. N) Nucleus; SG) secretory granules; GG) giant granule. 6700 ×.

and embedded in Epon-Araldite. Semithin and ultrathin sections were cut on a "Reichert" ultramicrotome (Austria). Semithin sections were stained with methylene blue—azure II—fuchsine. The block was then trimmed to a point on Paneth's cells in different parts of the small intestine. Ultrathin section were stained with lead citrate and studied in the JEM-100B electron microscope (Japan). On morphometric analysis the area of secretion was determined only in hose Paneth's cells which, on electron micrographs, appeared whole and contained a nucleus. Altogether 160 Paneth's cells were investigated in the eight experimental groups of animals, 20 in each group. The functional capacity of the cells was compared in terms of the mean area of secretion of a single cell, calculated by the equation

$$\bar{X}S \text{ secretion of 1 cell} - \bar{X}n \cdot \bar{X}S \text{ secretion in 1 granule},$$

where $\bar{X}$ is the mean, n the number of granules in one cell, and S is the area. The "Mop-Videoplan" apparatus (Reichert, Austria) was used for counting.

RESULTS

The values for $\bar{X}S$ of secretion in one cell in the experimental and control groups are given in Table 1.

Giant granules, for which S_{max} reached 4.09 mm^2 compared with S_{max} = 2.8 mm^2 in the control group, began to appear in the cytoplasm of the Paneth cells 3 h after injection of the cholera toxin. A sharp decrease in $\bar{X}S$ of the secretion in one cell took place 6 h after injection of the cholera toxin, in which the value of this parameter fell by 2.4 times compared with the control group, in agreement with data in the literature showing that the cytoplasm of the Paneth cells in the mucosal crypts of the small intestine of germ-free rats, carriers of *Vibrio cholerae*, contains far fewer secretory granules, evidently, in the opinion of the authors cited, due to the intensification of secretion in response to injection of vibrios [4].

Marked ultrastructual changes were found in the Paneth cells 6 h after exposure to cholera toxin. The number of microvilli in the apical part of the cells decreased, the villi were shortened, and they disappeared during active secretion (Fig. 2a).

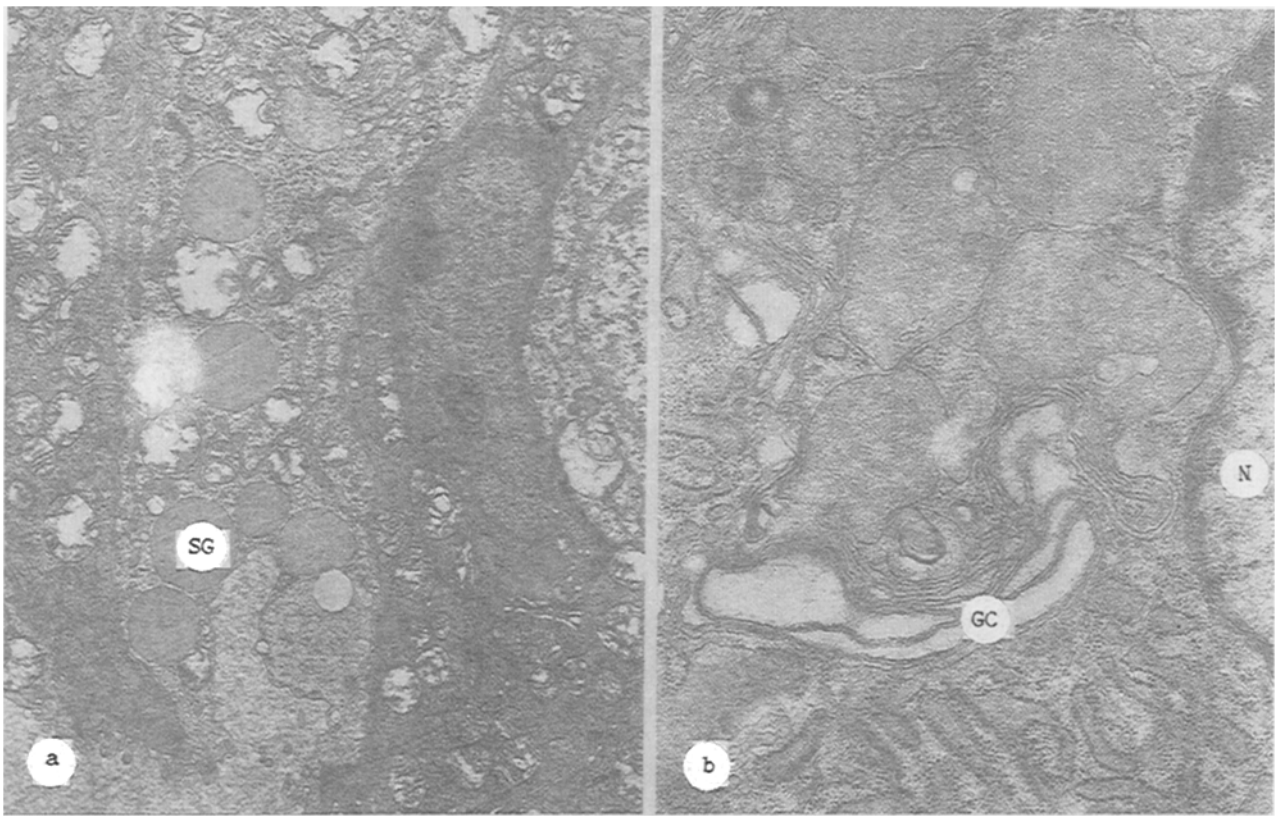

Fig. 2. Paneth cell in duodenum of guinea pig 6 h after injection of cholera toxin. N) Nucleus; SG) secretory granules; GC) Golgi complex. Magnification: a) 5000 ×; b) 20,000 ×.

In Paneth cells under normal conditions (Fig. 3) secretory granules with a narrow halo and with a core of low electron density are located mainly in the supranuclear region and lie in close contact with structures of the Golgi complex, whereas granules with high electron density are found chiefly in the apical part of the cell. This distribution of granules reflects the natural process of their maturation [3].

Under the influence of cholera toxin the regular formation of granules is disturbed and their structure is unusual: the granules assume an irregular shape and are filled with contents of low electron density. Dislocation of the Golgi complex is observed and its cisterns are dilated (Fig. 2b).

By the 12th hour after injection of cholera toxin, $\bar{X}S$ of the secretion in one cell was reduced by half compared with the control group. Reduction of the functional activity of the cells during this period was accompanied by ultrastructural changes, such as the appearance of apoptosis and partial necrosis in some cells. The degree of the ultrastructural damage, moreover, varied, and some cells were structurally relatively undamaged.

After 24 h elements of recovery were found: the appearance of a large number of free monosomes and polysomes. The intracellular granules acquired high electron density, the membranes of the rough endoplasmic reticulum fused together, and juvenile forms of mitochondria (according to Shakhlamov, 1971) appeared [5].

The parameter of functional activity of the cell reached the lower limit of normal and morphometric data at this time indicate that the average area of secretion of one cell was 11.7 compared with an average area of secretion of one cell of 10.78 in the control group.

Thus, during exposure to cholera toxin, which reaches the crypts between 3 and 6 h after injection, there is a gradual decline of functional activity of the cell, accompanied by disturbance of granule synthesis in the region

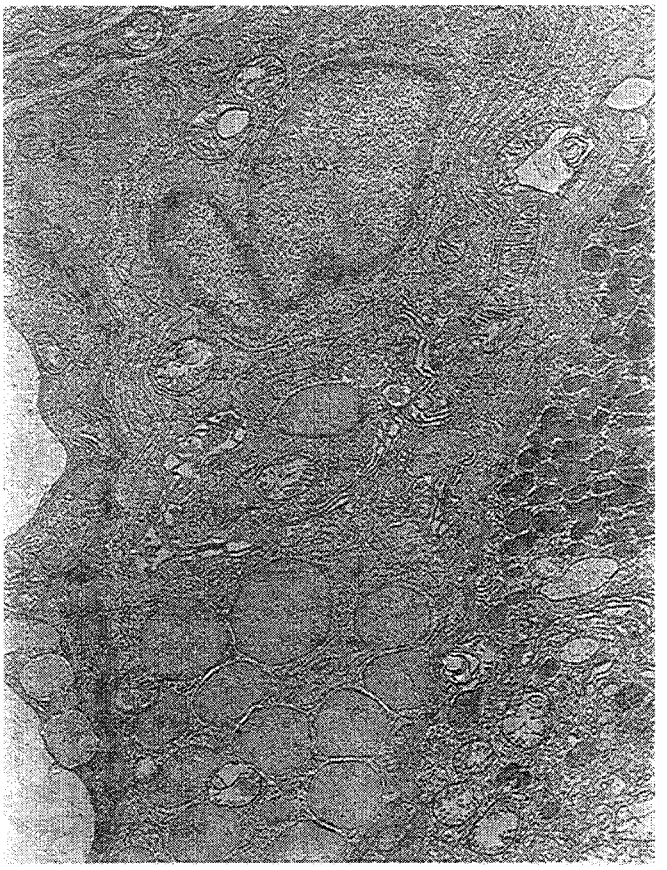

Fig. 3. Paneth cell in small intestine of guinea pig. Control. 5000 ×.

of the Golgi complex. The energy supply to the Paneth cells (changes in the mitochondria) and the protein-synthesizing apparatus are impaired.

Damage to the Paneth cells is focal in character and depends on the physiological activity of the cells at the time of exposure to cholera toxin.

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