aluronic acid content in the pulp of the teeth and in the ganglia was unchanged. In all ganglia the difference in the number of changed cells from the control was not statistically significant ($P > 0.5$).

Analysis of the results of this investigation enabled the various local anesthetics studied to be arranged in the following order of increasing effectiveness for preventing histomorphological changes in the teeth, periodontal tissues, and ganglia when used in preparing teeth for metal crowning: procaine, celnovocain, trimecaine, and lidocaine. By contrast with procaine and celnovocain, local anesthetics of the xylidine series (trimecaine and lidocaine) block the conduction of nociceptive impulses along perivascular nerve fibers of the teeth and periodontal tissues. The facts described above indicate that trimecaine and lidocaine can be recommended for extensive use in the practice of orthopedic stomatology during preparation of teeth for crowning.

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SCANNING ELECTRON MICROSCOPY AND X-RAY MICROANALYSIS OF THE EFFECT OF CHOLERA TOXIN ON THE SMALL INTESTINE OF SUCKLING RABBITS

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KEY WORDS: scanning electron microscopy; x-ray microanalysis; small intestine; cholera toxin.

Ultrastructural changes in the epitheliocytes of the small intestine during the development of a rapid intestinal dehydration syndrome have now been well studied by transmission electron microscopy [1, 2, 6]. However, information on changes in the surface of the small intestine caused by the action of cholera toxin is still fragmentary [9]. Furthermore, in cholera, liquid and HCO_3^-, Na^+, and to a lesser degree, K^+ ions accumulate in the intestinal lumen [11]. So far, however, the question of changes in ion transport in different parts of the villi and their differential involvement in this pathological process remains in doubt.

It was accordingly decided to study the normal structure of the surface of the epithelium of the small intestine in suckling rabbits and its changes under the influence of cholera toxin, and also to investigate the distribution of elements in sections by the aid of x-ray microanalysis.

EXPERIMENTAL METHOD

The action of cholera toxin was studied in experiments according to the method in [8]. Cholera toxin, obtained from *Vibrio cholerae* strain 569 B, Pakistan line, Inaba serotype

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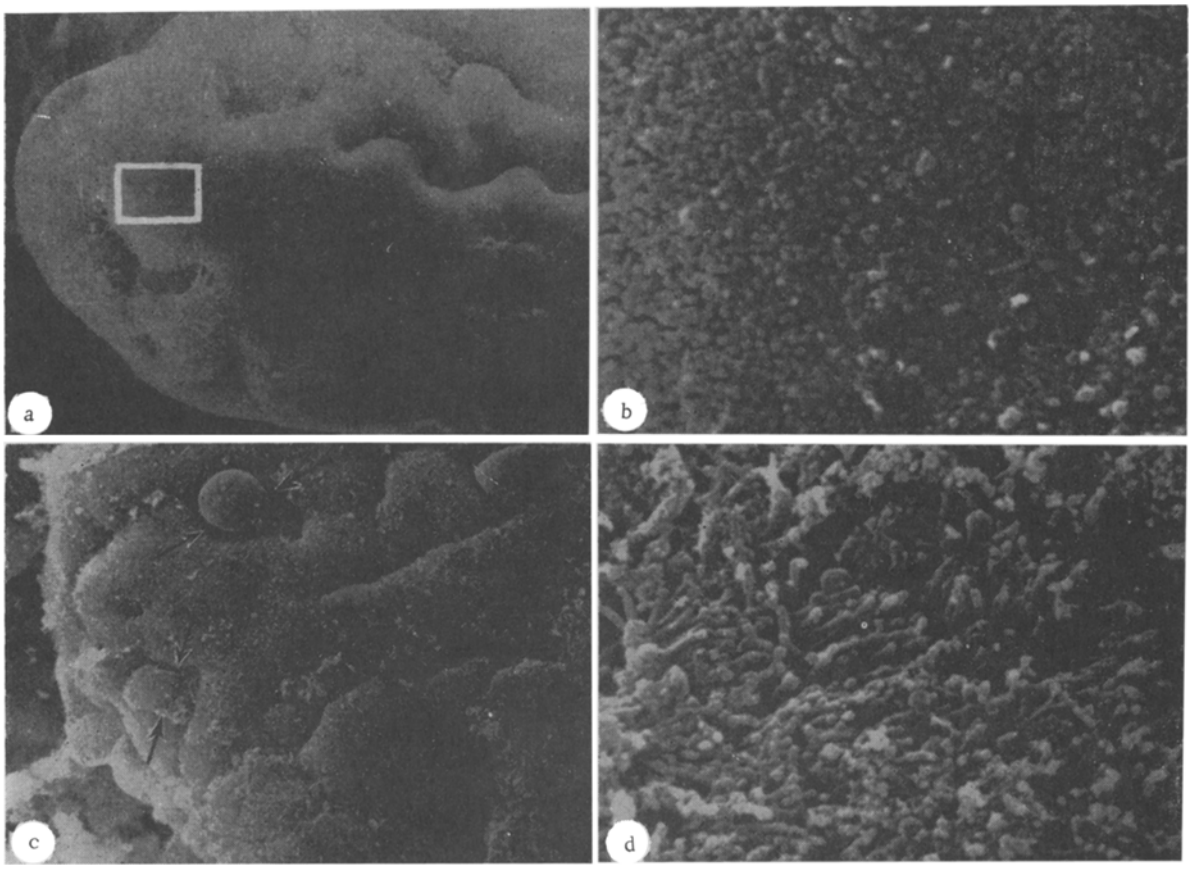


Fig. 1. Surface of apical part of villus of small intestine of suckling rabbit under normal conditions (a, b) and 3 h after injection of cholera toxin (c, d). Drying by critical point method, spraying with gold. a) 1000×; b) regular arrangement of microvilli, 10,000×; c) secreted mucus is present (arrow), 900×; d) haphazard arrangement of microvilli, 900×.

(from the Mikrob Institute, Saratov), was injected intramuscularly into 28 suckling rabbits in a dose of 0.3 ml. The same volume of physiological saline was injected into 12 animals of the control group.

A segment of small intestine was removed 3 h after injection of the toxin and washed with sucrose buffer consisting of 0.25 M sucrose, 10 mM Tris-HCl, pH 7.4. Fixation was carried out in a 2.5% solution of glutaraldehyde in 0.1 M phosphate buffer for 24 h at 4-8°C. After washing, the tissue was postfixed in 1% osmium tetroxide solution in the same buffer. The tissues were dehydrated in acetones of increasing strength, then dried in air or by the method of passing through the critical point, and were sprayed with gold (20 nm). The preparations were examined and photographed in the S-500 Hitachi scanning electron microscope under an accelerating voltage of 25 kV.

Pieces of small intestine for x-ray microanalysis (2-3 mm^2) were washed with sucrose buffer and immediately frozen in isopentane cooled with liquid nitrogen. Frozen sections 10 μ thick, used for analysis, were mounted on polished carbon disks. The sections were subjected to sublimation drying at between —40 and —60°C under a pressure of 10^{-4}-10^{-5} Torr for 3 h, after which the temperature was slowly raised to room temperature. The spectra were recorded from an area of 25 μ^2 in four different regions of the epithelial layer of the villi: apex, middle, base, and crypt. The sections were examined in secondary electrons in the same microscope (Fig. 3), on a Kevex 5100 x-ray spectrometer (USA) [4]. Student's t-test was used for the statistical analysis of the result.

EXPERIMENTAL RESULTS

Under low power, folds in which the orifices of goblet cells were frequently found, could be seen on the surface of the villi of the small intestine of the control animals (Fig. 1a).

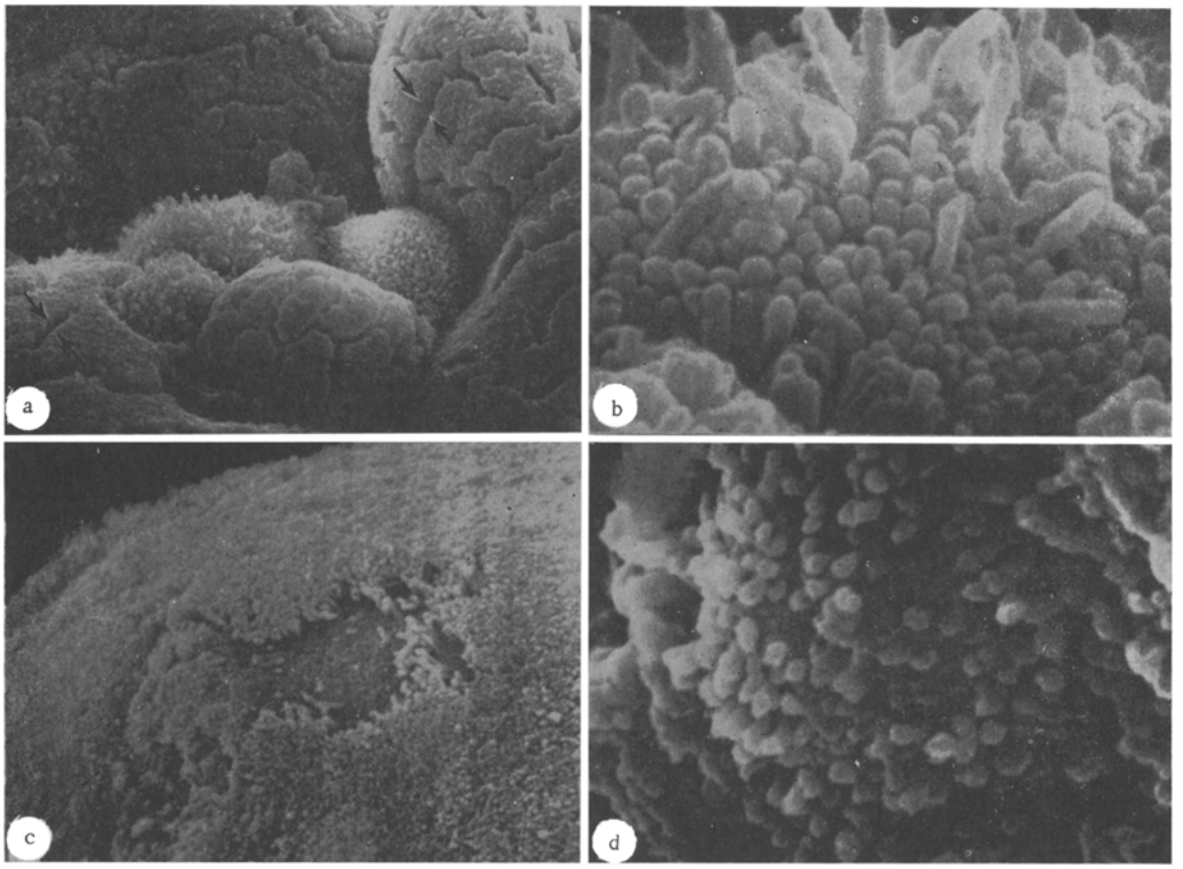
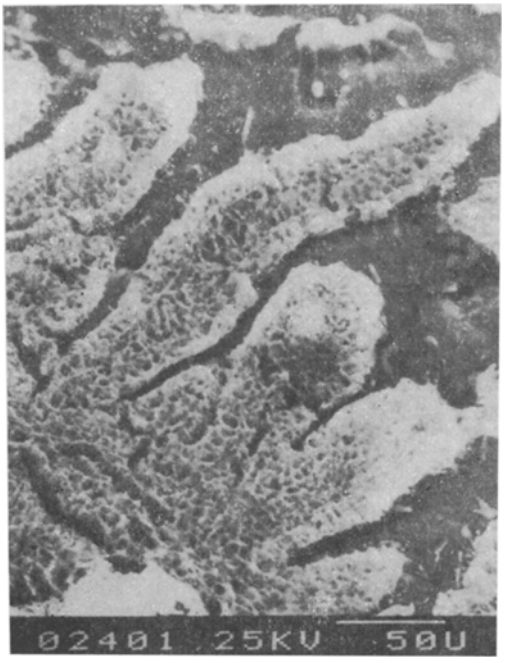


Fig. 2. Changes in surface of villi of small intestine under the influence of cholera toxin. Air-drying, spraying with gold. a) Separation of groups of microvilli (arrow), 360×; b) haphazard arrangement of microvilli and changes in their length, 20,000 ×; c) disappearance of some microvilli, 5200×; d) bulluous expansions on ends of microvilli, 16.000×.

Under the influence of cholera toxin an increase in the number of goblet cells in a state of secretion was observed on the surface of the villi (Fig. 1c). Under high power microvilli covering the surface of the enterocytes became distinguishable. In the control, under high power the regular arrangement of the microvilli of the enterocytes could be seen (Fig. 1b). In the experimental animals, on some villi (up to 20-35% of their total number) regions with modified microvilli on the enterocytes were observed. These changes consisted essentially of an irregular arrangement of the microvilli (Fig. 1d), together with their lengthening or considerable shortening, or even total disappearance (Fig. 2c). The apices of these microvilli very often terminated in bullous expansions (Fig. 2d).

Observations showed that the method of drying used, by passing through the critical point (Fig. 1c), gave better preservation of the surface structures than did air-drying (Fig. 2a, b).

Changes in the surface of the rat intestine 3 h after introduction of cholera toxin in a dose of 1 μg/ml into the lumen were found by other investigators [9], but their observations were concerned mainly with the macrostructure of the villi, and the structure of the microvilli received little attention. These workers showed that the action of the toxin was manifested as separation of some groups of microvilli from others, mainly on the apices. An endoscopic study of the surface of the mucous membrane of the human jejunum in the period of cholera associated with the severest diarrhea revealed the presence of erosions and injuries with the appearance of red points [7, 10]. As other workers have pointed out, the action of some detergents and of bile acids and fatty acids may also lead to separation of groups of some microvilli from others, i.e., to changes similar to those which developed under the influence of cholera toxin [9]. This fact suggests that this process may be due to activation of some membrane-bound enzymes of the microvilli. These enzymes could be, for example, of the lipolytic kind, causing changes in viscosity of the lipid



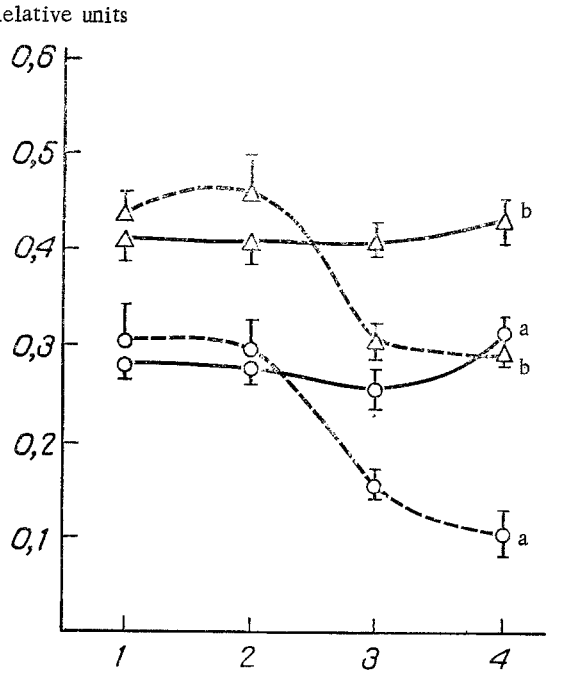


Fig. 3 Fig. 4

Fig. 3. Image of sublimation-dried frozen section (10 μ) in secondary electrons, 580×.

Fig. 4. Changes in concentration of sodium (a) and chlorine (b) in epithelium of villus of rabbit small intestine in control (continuous line) and following administration of cholera toxin (broken line). Abscissa: 1) region of crypt of villus, 2) region of base, 3) central region, 4) apical region; ordinate, ratio of "pure" integral of peak for a given element to integral of background beneath that peak.

phase of the membranes [3], and that could evidently lead to changes in the structure of the microvilli. It is worth noting that the changes now observed in the surface of the epitheliocytes of the small intestine are in good agreement with results obtained in the writer's laboratory in a study of enterocyte ultrastructure by transmission electron microscopy after administration of cholera toxin [1, 2, 6].

The results of x-ray analysis showed that the potassium concentration in the enterocytes of the epithelial layer in animals of the control group had a tendency to increase in the direction from the crypts to the apices of the villi, whereas the concentration of chlorine, sulfur, phosphorus, and sodium did not change in that direction.

The difference in the distribution of potassium disappeared 3 h after injection of the cholera toxin and its level fell to the lower limits of the control values, but a significant difference appeared in the distribution of chlorine and sodium, which was not present in the control ($P < 0.001$). This difference was that the concentrations of both sodium and chlorine decreased parallel to one another toward the apex, and the greatest changes in the distribution of these elements occurred at the apices of the villi, whereas in the crypts and at the base of the epithelial layer of the villi their concentrations were equal in the animals of the experimental and control groups (Fig. 4). The concentrations of phosphorus and sulfur remained constant.

The results are evidence that under the influence of cholera toxin the transport at least of K^+, Na^+, and Cl^-, is disturbed; however, according to data in the literature, transport of HCO_3^- also is disturbed [11]. Unfortunately it was impossible to study its redistribution by the method used.

One manifestation of the action of cholera toxin is activation of adenylate cyclase [2]. It has recently been shown that cyclic adenosine monophosphate, accumulating as a result of this activation, evidently inhibits the electroneutral entry of NaCl through the apical membrane of the enterocytes, and in addition to this, it may perhaps also stimulate

either the combined secretion of NaCl or secretion of Cl^- only [11]. As a result of these processes NaCl accumulates in the lumen of the intestine in cholera intoxication. Inhibition of K,Na-activated adenosine triphosphatase also evidently takes place, with the consequent loss of intracellular potassium [5].

The action of cholera toxin in the stage of the developed syndrome of rapid intestinal dehydration thus causes marked changes in electrolyte transport and also in the ultrastructure of the microvilli of the enterocytes. All these changes are focal in character, with their predominant localization on the apical part of the villi.

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ACCUMULATION OF METHYLATED PURINES IN DNA OF RAT LIVER AND LARGE INTESTINE FOLLOWING REPEATED INJECTIONS OF 1,2-DIMETHYLHYDRAZINE

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KEY WORDS: methylation of DNA; accumulation of O^6-methylguanine; intestinal tumors.

The compound 1,2-dimethylhydrazine (DMH) is widely used in experimental cancer research to induce carcinoma of the large intestine [3]. DMH exerts its carcinogenic effect evidently through interaction with DNA, by methylating its bases. After administration of a single dose of DMH to rats it has been shown that purine bases of DNA of various organs are methylated [1, 11, 12]. Meanwhile O^6-methylguanine, which is ascribed a leading role in malignant transformation of cells [7], is formed in fairly large quantities and preserved for a long time in the DNA of the large intestine, and also of the liver [12].

It was accordingly decided a matter of fundamental importance to study the changes taking place in the structure of DNA in the liver and large intestine of rats during long-term weekly administration of DMH. With this dosage, as is usually used in experimental research, malignant intestinal tumors arise comparatively quickly and in practically all animals. At the same time, tumors of the liver do not develop in such animals [3].

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