According to our results, thioctacide is more effective than flavobione. After use of flavobione, unlike thioctacide, no increase was observed in the gain in weight of the regenerating liver in the irradiated rats, nor of the number of cells in the liver, but changes in the concentration and total content of DNA and histones were significantly less than in unprotected animals. These results suggest that flavobione has a greater effect on the structure of chromatin and on DNA and histone synthesis than on mitosis.

These results are evidence that hepatoprotective agents (thioctacide and flavobione) diminish latent damage to the genetic material of the liver caused by irradiation, and they thus stimulate regeneration.

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ULTRASTRUCTURAL CHANGES IN INTERNEURONAL JUNCTIONS IN INTRAMURAL GANGLIA OF THE SMALL INTESTINE OF SUCKLING RABBITS WITH EXPERIMENTAL CHOLERA

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KEY WORDS: small intestine; cholera; ganglia

The secretory and motor activity of the small intestine is regulated by intramural ganglia of the autonomic nervous system, which consists of assemblages of neurons involved in the mechanism of various lesions arising the gastrointestinal tract [5].

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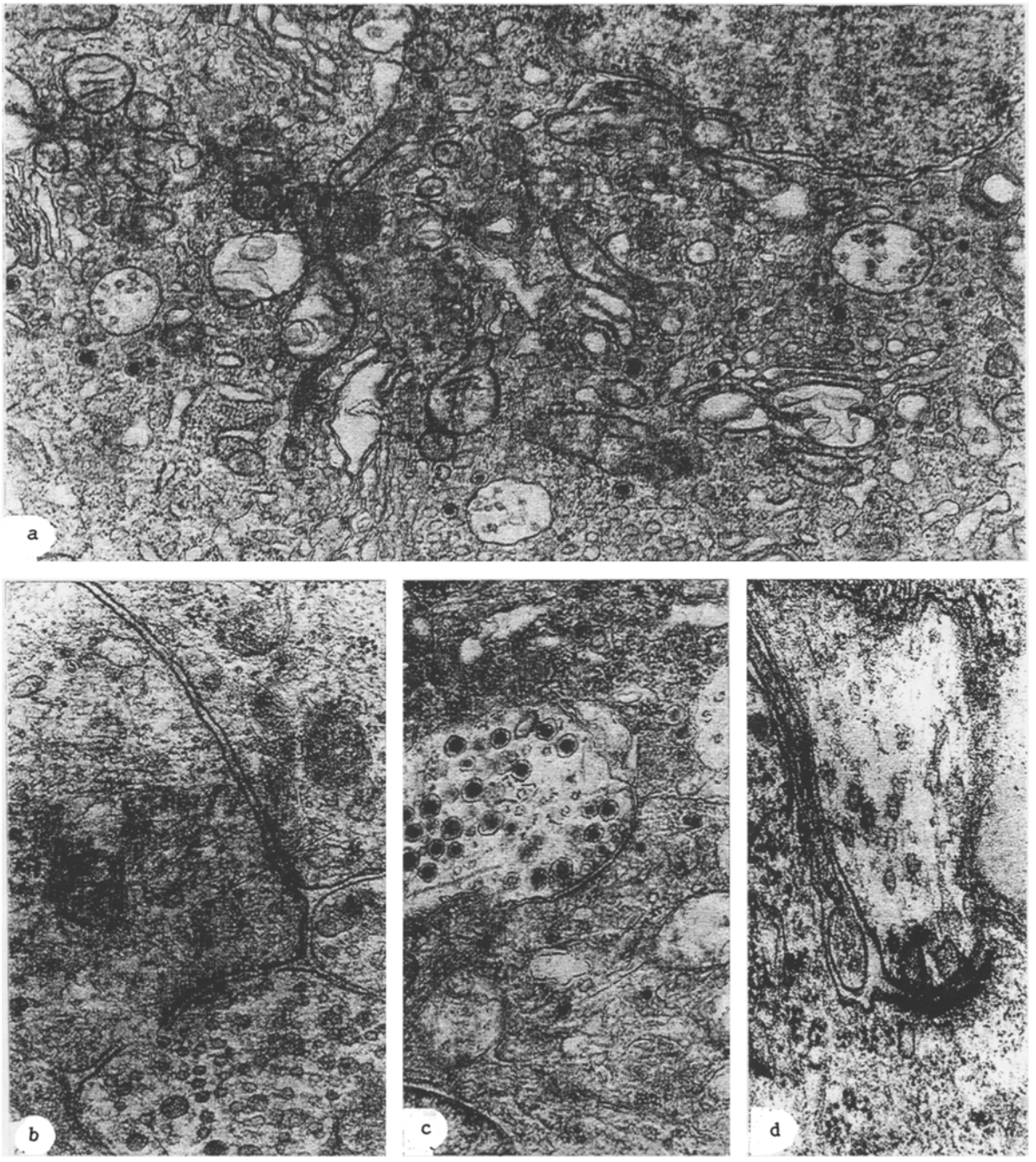

Fig. 1. Ultrastructure of intramural ganglia after injection of cholera vibrios: a) reactive changes in a neuron (Meissner's plexus, after 1.5 h). 20,000×; b) formation of direct communication between axon and dendrite (arrows indicate nonsynaptic contact between neuron and dendrite). 35,000×; c) increase in area of contact zone in axosomatic synapse. 19,000×; d) pale type of degeneration of axosomatic synapse. 50,000× (b-d: Auerbach's plexus, after 4 h).

The most pathognomic sign of cholera is progressive diarrhea, associated with hypersecretion of enterocytes and leading to marked dehydration and disturbance of the electrolyte balance [6]. Physiological investigations have shown that an important role in the mechanism of choleragen-induced intestinal secretion is played by nervous influences [10, 11].

In this connection the study of ultrastructural disturbances in synaptic and nonsynaptic contacts of the submucous and intermuscular plexuses of the small intestine in the dynamics of development of experimental cholera.

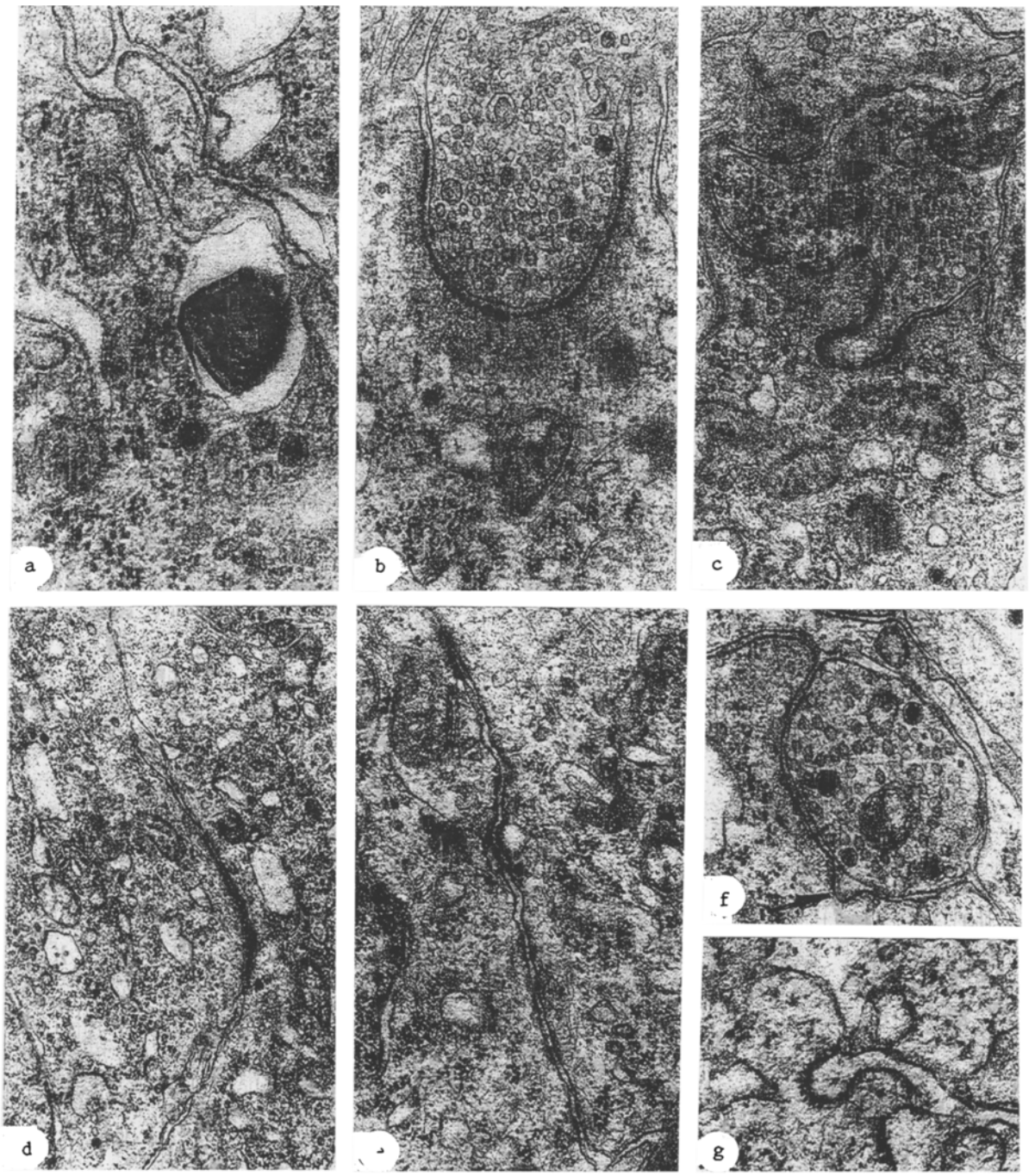

Fig. 2. Ultrastructure of intramural ganglia after injection of cholera vibrios (after 2 days): a) fragment of cytoplasm of neuron containing myelin figure (Meissner's plexus). 62,000×; b) axosomatic synapse with numerous synaptic vesicles, 34,000×; c) axosomatic synapse with complex configuration. 21,000×; d) increase in area of nonsynaptic contact zone. 14,000×; e) increase in number of nonsynaptic contact zone between two neurons. 32,000×; f) appearance of coated depression (arrow) on postsynaptic membrane. 39,000×; g) formation of coated depression on neuronal plasmalemma. 60,000× (b-g, Auerbach's plexus).

EXPERIMENTAL METHOD

Experiments were carried out on 34 suckling rabbits aged 10-12 days, which were infected after preliminary starvation for 24 h. A preliminary dose of 1 ml of a 3% solution of soda was introduced through a polyethylene gas-

tric tube in order to neutralize the gastric contents, and this was followed by 1 ml of an 18-h culture of a virulent strain of Vibrio El-Tor 5879, and a further 0.5 ml of soda solution. The infecting dose, against an optical standard of turbidity, was 10^5 microbrial cells. A lethal dose of pentobarbital was injected into the animals 1.5 (5) and 4 (10) h and 1-2 days (13) later. In control experiments (2 rats in each series) 1.5 ml of soda solution and 1 ml of physiological saline were given to suckling rabbits. Pieces of small intestine, after glutaraldehyde and osmium fixation and dehydration, were embedded in Epon or Spurr medium. The ganglia were identified in semithin sections 1-2 μm thick, stained with toluidine blue. The ultrathin sections were cut on an "LKB 8800" microtome, stained on the grids with uranyl acetate and lead citrate, and examined in the JEM-100B electron microscope.

EXPERIMENTAL RESULTS

The electron-microscopic study of the epithelium and intramural ganglia of the small intestine of the suckling rabbits revealed no particular features and the results were similar to those obtained with other mammals [12, 13]. Besides highly specialized, typical junctions, nonsynaptic junctions characterized by the presence of electron-dense material on the adjacent membranes and the absence of synaptic vesicles in these areas, also were found.

The first signs of damage were observed 1.5 h after infection in the crypts, and consisted of solitary cases of adhesion of the cholera vibrios, mild degenerative changes in the epithelium, and increased permeability of the blood-enterocyte barrier. During this period changes were observed only in the neurons, and consisted of folding of the karyolemma, to form shallow invaginations in the nucleus, disturbance of the shape and swelling of the mitochondria, the appearance of secondary lysosomes, some degree of dilatation of the cisterns of the cytoplasmic reticulum and perinuclear space, hyperplasia and vacuolation of the lamellar complex, and an increase in the number of multivesicular bodies, and 80 on (Fig. 1a). Ultrastructural lesions in synaptic-junctions were not found.

This spectrum of morphological disturbances was sufficiently universal and corresponds to reactive changes whose features are similar for different types of cells, are reversible in character, and are aimed at preserving the functional features of the corresponding cells [1].

During subsequent periods of observation, i.e., during the period of adhesion of the cholera vibrios (4 h) and the development of a marked diarrhea syndrome (1-2 days), the lesions spread to the epithelium of the villi and ultrastructural changes in the intramural ganglia of the small intestine and the interneuronal junctions progressed.

Dystrophic changes, expressed as vacuole formation and lesions affecting the energy-producing and protein-synthesizing apparatuses, were recorded in the ganglion cells 4 h after infection. Meanwhile, in adrenergic neurons the number of synaptic vesicles decreased. At this stage noteworthy features included so-called perforated synapses, in which direct communications were present between cell processes (Fig. 1b). Here also, nonsynaptic avesicular interneuronal junctions of desmosomelike type could be seen (Fig. 1b). Usually granular vesicles were abundant in the adrenergic synapses, with, characteristically, an increase in area of the contacting surfaces between the pre- and postsynaptic parts (Fig. 1c). The number of synaptic vesicles in cholinergic synapses was sharply reduced, and they were detected a long way from the presynaptic membrane; the axoplasm of the presynaptic endings was translucent and appeared edematous (Fig. 1b). This state of the synapses corresponds to the pale type of degeneration and can be classed as damage of reversible type [3]. It must be emphasized that the ultrastructural changes in the synaptoarchitectonics in the intramural ganglia in cholera are nonspecific in character and have been described also in the CNS as a result of exposure to various extremal factors [2, 4, 7-9].

Manifestations of vacuolar degeneration were intensified after 1-2 days in the nerve cells. Large myelin figures could be seen in the cytoplasm of some neurons and in individual nerve fibers (Fig. 2a). As before, there were few granular vesicles in the neuron bodies. Two types of reaction could be identified in the cholinergic synapses. Most frequently these were contact with numerous synaptic vesicles, located by the presynaptic membrane, or uniformly distributed throughout the axoplasm, indicating an active state of the synaptic apparatus (Fig. 2b). Here also, beyond the postsynaptic membrane there was a dense accumulation of osmiophilic material. Sometimes the configuration of the synaptic plaque was modified (Fig. 2c). The number of synaptic vesicles decreased sharply, single mitochondria appeared swollen and the exoplasm was electron-translucent. It was noted that in all cases the extent of the pre- and postsynaptic membrane was increased by several times. This regular feature also extended to nonsynaptic contacts (Fig. 2d). It is interesting to note that 24 h after infection the number of these contacts between neurons or between neurons and dendrites was increased by several times (Fig. 2e). We also noted that at the height of the

diarrhea syndrome of cholera, coated depressions appeared in the zone of axosomatic synapses on the postsynaptic membrane (the neuronal plasmalemma) (Fig. 2f). It is interesting to note that coatings of this type may appear on adjacent surfaces between neighboring neurons (Fig. 2g). Meanwhile, the number of coated vesicles in the cytoplasm of the neurons increased.

During progression of the manifestations of cholera, reactive changes in the neurons thus were replaced by degenerative changes. The appearance of a large number of synaptic vesicles reflect an active state of the synapses. The combination of synaptic, nonsynaptic, and direct communications between neurons and processes facilitates both direct transport and transmembrane diffusion of ions and of certain mediators. Predominance of cholinergic influences over adrenergic in the intramural ganglia of the small intestine promotes hypersecretion of enterocytes and stimulation of intestinal motor activity.

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