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Histological Examinations of the Influence of 6-Hydroxydopamine (6-OH-DA) upon the Ileum of the Guinea Pig

Badania histologiczne nad wpływem 6-hydroksydopaminy (6-OH-DA)
na jelito kręte świnki morskiej

Examinations of the influence of 6-OH-DA (6-hydroxydopamine) upon the structure of nerve cells and their axons have been conducted by many authors and in various aspects (1, 2, 8, 10). They have concerned histochemistry, fluorescence and ultrastructure of neurons and have shown that the substance evokes various changes in the perikaryons and axons of adrenergic neurons.

The wall of the small intestine is innervated by external autonomyous nerves and fibres of the intramural autonomyous plexuses. External autonomyous fibres make numerous anastomoses with the neurons of the two intramural plexuses and ganglia: the myenteric one (Auerbach's) and the submucosal one (Meissner's) (6). Both ganglia run along the length of the intestine and their neurons apart from making anastomoses, send axons to the nonstrained muscles cells and glandular cells in the mucosa. As a consequence, the active phase is easily carried over along the alimentary system stimulating action of nonstrained muscles and glandular cells. 6-OH-DA exerts strong action upon the nerve cells and ends, that is why we have decided to examine the changes taking place in the ileum when the substance starts to operate.

MATERIAL AND METHODS

The examinations were conducted on 24 guinea pigs, which were divided into two groups: the experimental one and the control one.

The animals from the experimental group were administered 6-OH-DA with 1% solution of ascorbic acid in 0.9% NaCl as the solvent in intraperitoneal injections. Each guinea pig was administered 6-OH-DA in 2 doses. The first dose was 100 mg/kg of body mass, and the second dose (administered after 24 hrs) — 250 mg/kg of body mass. After the next 24 hrs the animals were decapitated and the ileum was collected for histological examination.

The animals from the control group were administered only the solvent (1% of the ascorbic acid in 0.9% NaCl) which was also divided into two doses, the following procedure being the same as in the case of the animals from the experimental group.

The collected material was preserved in glutane aldehyde buffored with phosphatic buffer and 1% water solution of osmium tetroxide. Fragments of ileum after being dehydrated were the

preserved in "Spurr" resin and cut into half-thin sections by means of Reichert's ultramicrotome. They were stained with solution methylen blue and azur II. Light microscope was used for the evaluation of preparations and photographic records.

RESULTS

The control group

The structure of the ileum wall was regular. Intestinal glands and numerous lymphocytes were observed in the mucosa. Few neurons of the Meisner's plexus were observed in the submucosa. Between circular and longitudinal tunica muscularis Auerbach's nerve ganglia were noticeable (Fig. 1 and 2).

The experimental group

In some intestinal glands mucous cells filled with large amount of secreta were observed (Fig. 3). In the submucosa, in Meissner's ganglia, several types of nerve cells arranged closely side by side could be distinguished (Fig. 4).

In the circular tunica muscularis between miocytes, interstitial cells (Cajal's) with numerous granulations in the neuroplasm became visible (Fig. 5). Between circular and longitudinal tunica muscularis, in Auerbach's plexuses, neurons belonging to three basic types of cells appearing there were noticeable. Cell nuclei of some neurons were situated near the cell membrane. Also the cells perikaryons differed in intensity of satining and appearance of granulation (Fig. 6). Ganglia of nerve fibres surrounded the plexuses and connected them one to another (Fig. 7). Fragments of intestine with circular tunica muscularis as if remaining in the constriction phase, were also observed (Fig. 8). In several experimental animals mucosa and submucosa were almost completely destroyed (Fig. 9).

DISCUSSION

6-OH-DA is the substance having fast and strong action. When used to induce degeneration of the axons of adrenergic neurons it first removes noradrenaline from the nerve ends and then takes its place. According to literature (5, 9, 11) nerve ends degenerate only when sufficiently high doses of 6-OH-DA are administered. Below the minimum threshold level 6-OH-DA does not induce destruction, although amins are present in the vesiculars situated in the nerve ends' area. The studies conducted in recent years (1, 3—5, 12) indicate that 6-OH-DA can be used for chemical sympathectomy. In the course of examination of the influence of 6-OH-DA upon the peripheral nervous system, Cobb and Bennet (1) noticed that the substance was absorbed by neurons,

which was reflected in the appearance of vesiculas in the neuroplasm. However they achieved such results only after administering small doses of the examined substance. After administering big doses of substances, apart from big, electronically dense vesiculas, also necrotic mitochondria occurred. In the Auerbach's myenteric plexuses after administration of 6-OH-DA the authors noticed vesiculas only in a few cells.

Myenteric nerve plexuses in the small intestine of guinea pig were carefully examined by means of electron microscope by Zhou and Komuro (12). They distinguished three types of the cells appearing in this area: gap junction-rich cells, glycogen-rich cells and fibroblast-like cells. These make up something like an omentum that is connected with myocytes and connective tissue. These cells are surrounded by nerve fibres of two kinds: one with oval, light vesiculars, and the other with flattened vesiculars. Lewellyn-Smith (7) claim that nerve fibres running between the plexuses and winding around them are situated mainly in the circular tunica muscularis. Despite that, neurotransmission concerns the layers of longitudinal muscles as well. Hadzijahic et al. (3) decided to myenteric plexuses surgically and chemically. It turned out that the number of neurons in the plexuses was significantly reduced. Morphological changes in the intestinal villi, in the depth of the crypts and in the thickness of the tunica muscularis were especially vivid. The changes concerned only the further fragments of the small intestine, those beneath the damage. In the remaining fragments the changes were minimal.

In our examinations 6-OH-DA affected all layers of the ileum of the experimental animals. The action of mucous cells of many intestinal glands in the mucose was stimulated. In the submucosa more cells in the Meissner's plexuses were observed. In the circular tunica muscularis of the experimental ileum intersticial cells (Cajal's) acting as "starters" of intestine peristaltis became apparent. Neuroplasm of these cells was rich in granulations. Between circular and longitudinal tunica muscularis, in the Auerbach's plexuses several types of nerve cells were observed. Some of them had their nuclei moved towards the periphery, which is an untypical phenomenon. Perikaryons of these cells were stained with different intensity.

In several experimental animals destruction of the mucosa and submucosa took place after administering of 6-OH-DA.

According to the authors, the described morphological changes, that could be observed even by means of a light microscope, were caused by the relatively high dose of 6-OH-DA.

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EXPLANATIONS TO FIGURES

Fig. 1. The ileum of the guinea pig, control group. Methylene blue and azur II. Magn. 200×.

Fig. 2. The ileum of the guinea pig (tunica mucosa), control group. Methylene blue and azur II. Magn. 200×.

Fig. 3. The ileum of the guinea pig (tunica mucosa), experimental group. Methylene blue and azur II. Magn. 200×.

Fig. 4. The plexus submucosus (Meissner) of the ileum, experimental group. Methylene blue and azur II. Magn. 200×.

Fig. 5. The interstitial cells (Cajal's) of the stratum circulare of tunica muscularis, experimental group. Methylene blue and azur II. Magn. 200×.

Fig. 6. The plexus myentericus (Auerbach) of the ileum, experimental group. Methylene blue and azur II. Magn. 200×.

Fig. 7. The plexus myentericus (Auerbach) of the ileum, experimental group. Methylene blue and azur II. Magn. 200×.

Fig. 8. The plexus myentericus (Auerbach) of the ileum, experimental group. Methylene blue and azur II. Magn. 200×.

Fig. 9. The ileum of the guinea pig, experimental group. Methylene blue and azur II. Magn. 200×.

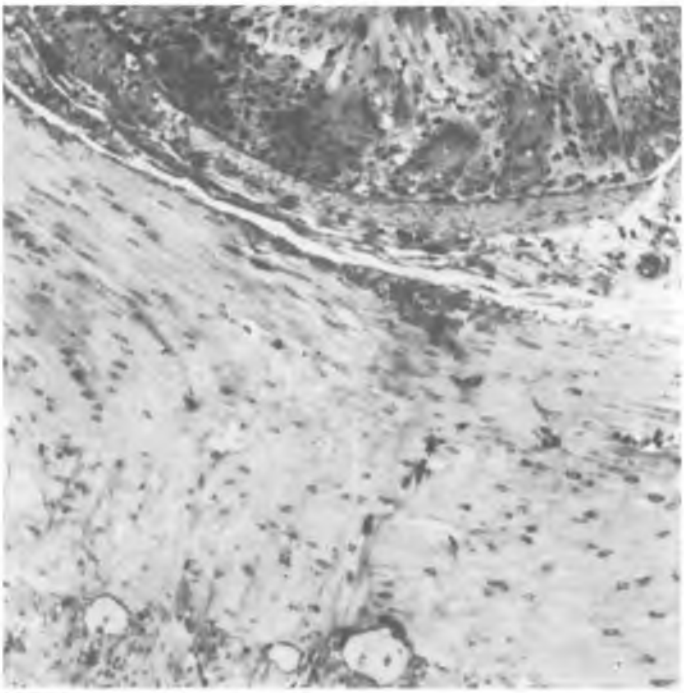

Fig. 1

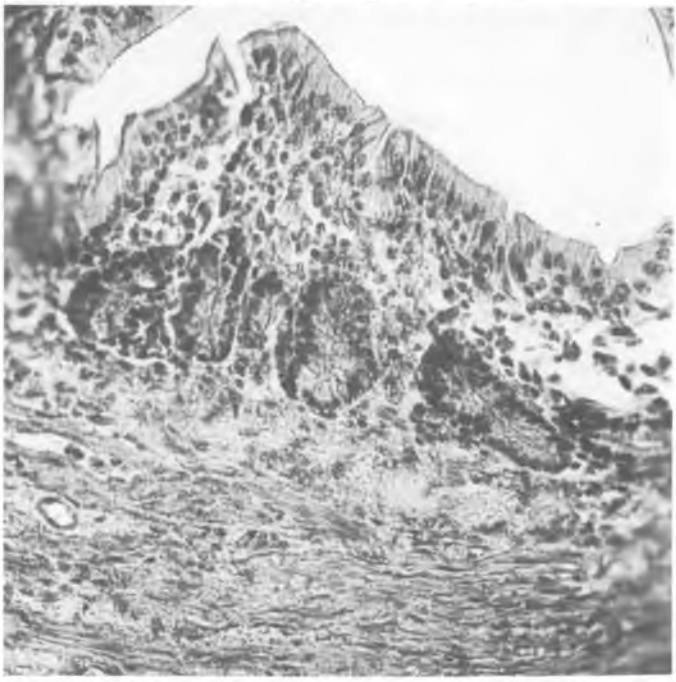

Fig. 2

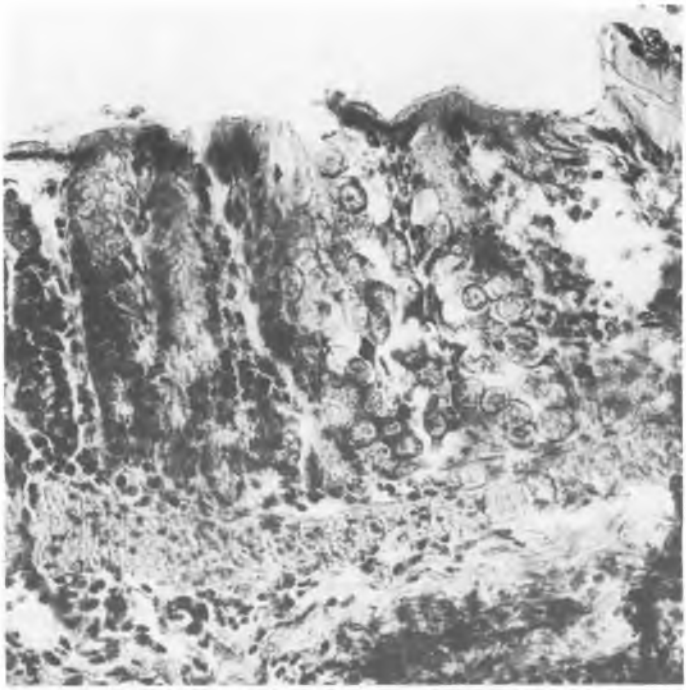

Fig. 3

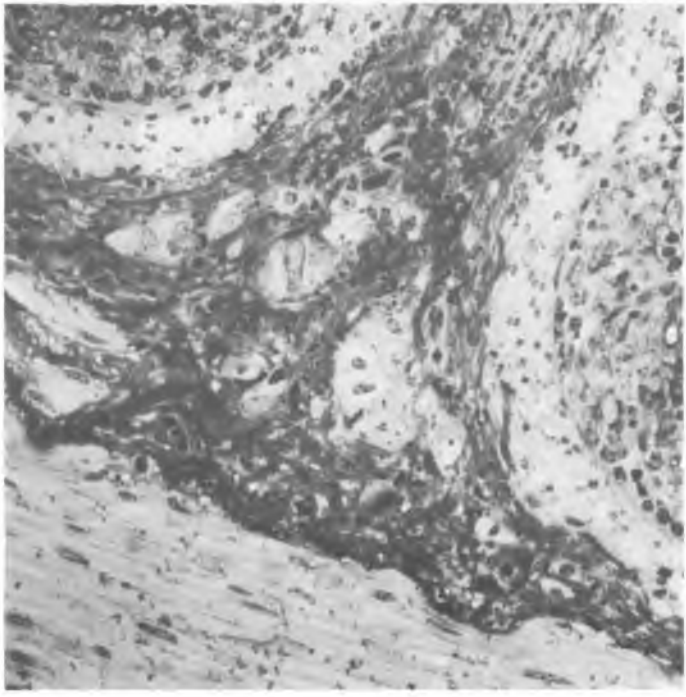

Fig. 4

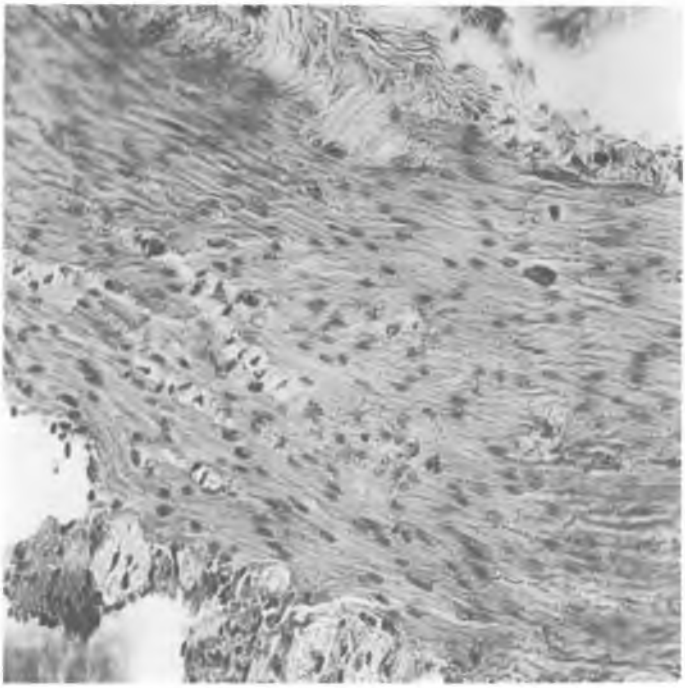

Fig. 5

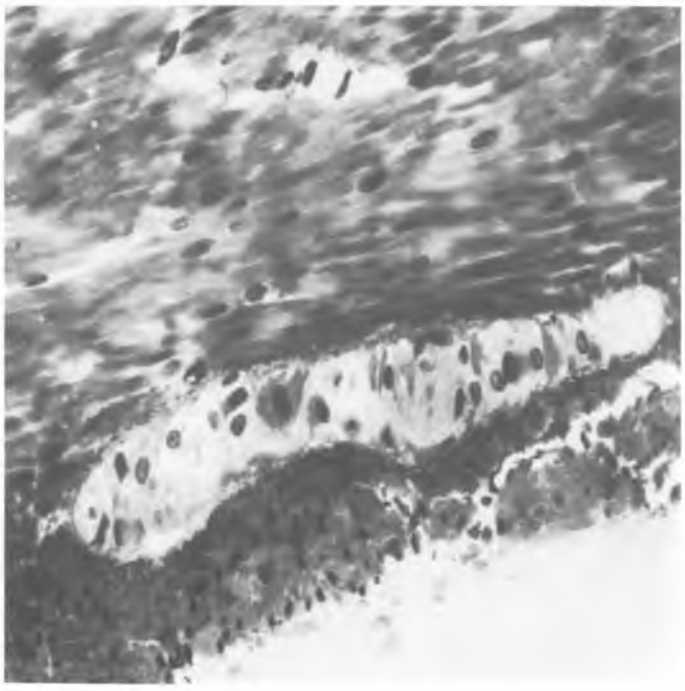

Fig. 6

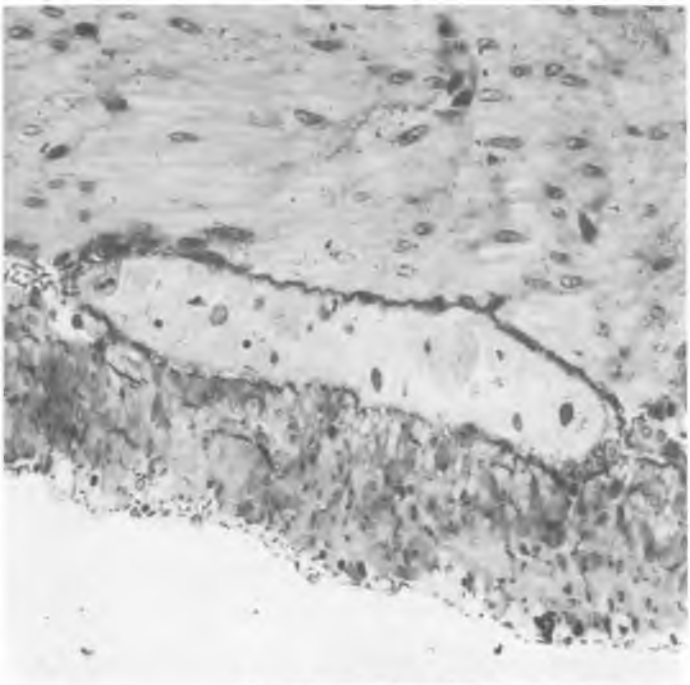

Fig. 7

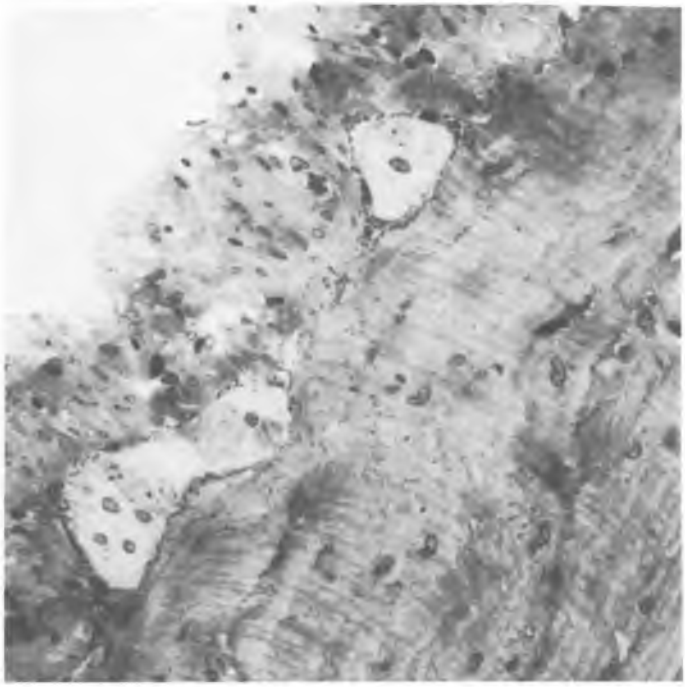

Fig. 8

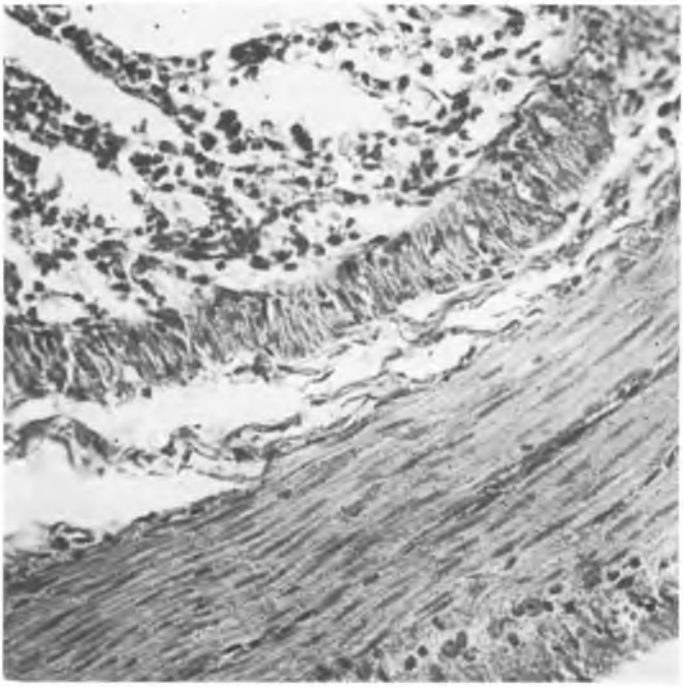

Fig. 9

STRESZCZENIE

Badano jelito kręte świnek morskich, którym podano dootrzewnowo 6-hydroksydopaminę w dwóch dawkach: 100 i 250 mg/kg masy ciała w odstępie 1 doby. Po 24 godz. zwierzęta dekapitowano. Materiał utrwalano w aldehydzie glutarowym. Półcienkie skrawki barwiono roztworem błękitu metylenowego i azuru II.

U zwierząt doświadczalnych uaktywnieniu uległy komórki śluzowe w nabłonku jelita krętego oraz komórki śródmiąższowe (Cajala) w warstwie mięśni okrężnych. W zwojach międzymięśniówkowych (Auerbacha) w komórkach nerwowych uwidoczniły się zmiany w zabarwieniu cytoplazmy bądź ułożeniu jądra.