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ULTRASTRUCTURAL CHARACTERISTICS OF THE RAT GASTRIC FUNDIC WALL AFTER THE IMPACT OF THE COMPLEX OF FOOD ADDITIVES

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The main submicroscopic changes in the gastric wall of rats after exposure to monosodium glutamate, sodium nitrite and Ponceau 4R were determined. It was found that the effect of a complex of food additives on the mucous membrane of the fundus of the stomach of rats led to a violation of secretion and secretion, which at the ultrastructural level was manifested by violations of architecture and electron density of secretory granules in major exocrinocytes, cervical mucocytes and superficial epithelium. The impact of the complex of food additives on the mucous membrane of the rat gastric fundus is manifested primarily by dystrophic changes in superficial scrobiculus exocrinocytes, which are in direct contact with exogenous factors, as well as in the exocrinocytes of gastric glands; disorders of microcirculation are also noted.

Key words: fundus of stomach, submicroscopic changes, food additives, mucous membrane, rats.

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УЛЬТРАСТРУКТУРНА ХАРАКТЕРИСТИКА ФУНДАЛЬНОГО ВІДДІЛУ СТІНКИ ШЛУНКУ ЩУРІВ ПІСЛЯ ДІЇ КОМПЛЕКСУ ХАРЧОВИХ ДОБАВОК

У роботі визначені основні субмікроскопічні зміни у стінці шлунку щурів після дії глутамату натрію, нітриту натрію та Понсо 4R. Встановлено, що дія комплексу харчових добавок на слизову оболонку фундального відділу шлунку щурів призводила до порушення секретоутворення і секретовиведення, що на ультраструктурному рівні проявлялось порушеннями архітектоніки і електронної щільності секреторних гранул у головних екзокриноцитах, шийкових мукоцитах та поверхнево-ямковому епітелії. Вплив комплексу харчових добавок на слизову оболонку фундальної частини шлунку щурів проявляється, насамперед, дистрофічними змінами екзокриноцитів як поверхнево-ямкових, які зазнаються безпосереднього контакту із екзогенними чинниками, так і у складі шлункових залоз. Також, спостерігаються розлади мікроциркуляції.

Ключові слова: фундальний відділ шлунка, субмікроскопічні зміни, харчові добавки, слизова оболонка, щури.

The work is a fragment of research work "Regularities of organs, tissues and vascular-nervous formations morphogenesis in the norm, at pathology and under the influence of external factors", state registration No. 0118U004457.

The stomach is one of the key organs of the digestive system with its own structural features. It performs a number of such important functions as excretory, endocrine, absorption, neutralizing, motor and formation of the antianemic factor of Castle [6]. Currently, a laboratory rat is one of the most popular experimental models for the study of anatomical, physiological and biochemical relationships in the digestive system.

The rat is most widely used in surgical studies of the abdominal cavity and the study of morphology, functions and diseases under the effect of complex of chemical agents. Albino rats are the most preferable ones for use by the researchers in the experimental modeling of the digestive system disorders [2, 12].

Nowadays, food additives of synthetic origin are considered the most dangerous ones [15]. The main reason is that they are xenobiotics, which the human body has not experienced during its evolutionary development and, therefore, it lacks enzymes that can convert them into non-toxic metabolites [3, 5, 8]. Some food colorants are also considered dangerous [9].

The purpose of the study was to determine the main submicroscopic changes in the gastric wall caused by the effect of monosodium glutamate, sodium nitrite and Ponceau 4R.

Materials and methods. The study involved 84 outbred mature male rats. The rats of the control group consumed drinking water and received saline per os. The rats of the experimental group were given access to water *ad libitum* and administered with 0.6 mg/kg of sodium nitrite, 20 mg/kg of monosodium glutamate and 5 mg/kg of Ponceau 4R in 0.5 ml of distilled water once a day orally. The doses of food additives twice lowered the allowable normal rate in food products. Prior to the animals' sacrifice, the evaluation of the rats' adaptive behavior with the use of the "open field" test was made [14].

The rats were sacrificed within 1, 4, 8, 12 and 16 weeks under thiopentone anesthesia overdose. Upon the rodents' euthanasia, the fragments of the gastric fundic wall were fixed in 2.5 % of glutaraldehyde solution. Subsequently, the pieces of the gastric wall were embedded into epont-812 [10]. Ultrathin slices were made on the LKB-3 (Sweden) ultramicrotome. The sections were contrasted first in the 1 % uranyl acetate solution in

methanol, followed up with the Reynold's lead citrate stain. The sections were studied in the PEM – 125 K (serial number 38-76, TU 25-07-871-70) electron microscope at accelerating voltage (50–75) kW.

The housing of the animals and experimental part of the study has been carried out in compliance with the "General Ethical Rules for Conducting Animal Experiments", adopted by the First National Congress on Bioethics and the requirements of the international principles of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [4].

Results of the study and their discussion. The main and parietal exocrinocytes, as well as mucous cells and cervical mucocytes and endocrinocytes, have been found in the gastric glands of rats of the control group. In the cervical mucocytes, mitosis figures have been quite often found, which is a morphological confirmation of their cambial role in the gastric epithelium. The cells had an orbicular nucleus, sometimes with an irregular contour in the basal part. Ultramicroscopic examination reveals granules of medium electron-optical density and a developed Golgi complex. On the bottom of the fundal glands, the main exocrinocytes have been mainly found. They have a pyramidal shape; the nucleus was visualized in the basal part of the cytoplasm. The granules, surrounded by the membrane, have been found above the nuclei. The surface contains microvilli. Few mitochondria, homogeneously located throughout the cytoplasm, have been found. Lysosomes have been noted in the supranuclear portion (Fig. 1a). Triangular parietal exocrinocytes with centric nuclei and a wide base, adjacent to the basement membrane were located mainly in the necks of the fundal glands. Ultrastructurally, numerous mitochondria and intracellular tubules, called tubulovesicles, have been found in the cytoplasm (Fig. 1b). Among endocrinocytes, in the gastric fundus, EC, ECL, P and D_1 cells have been found. EC-cells were orbicular with electron light cytoplasm. The granules showed pronounced polymorphism. The nuclei were mostly bean-shaped. Peripheral condensed chromatin formed a wide stripe along the caryolemma (Fig. 1c).

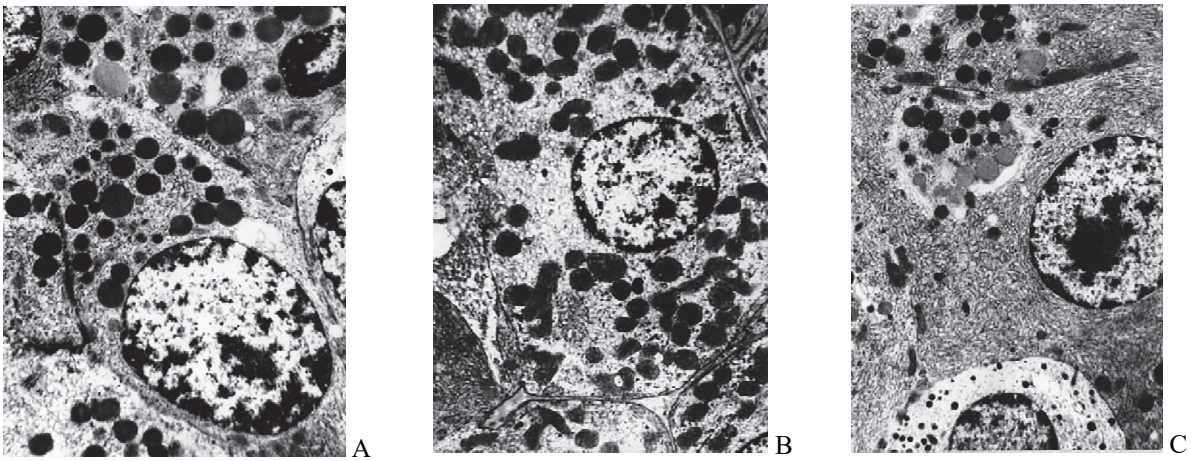

Fig. 1. The main exocrinocyte (a), the parietal exocrinocyte (b) and the EC-endocrinocyte (c) in the fundal gland of the stomach of the rat of control group. Electronogram. 8000×magnification.

Dystrophic changes of epitheliocytes of the superficial scrobiculus epithelium have been found in the gastric fundus on week 1-4 of the experiment. Vacuoles of different size were found in the cytoplasm of individual cells. The number of secretory granules was reduced (Fig. 2a).

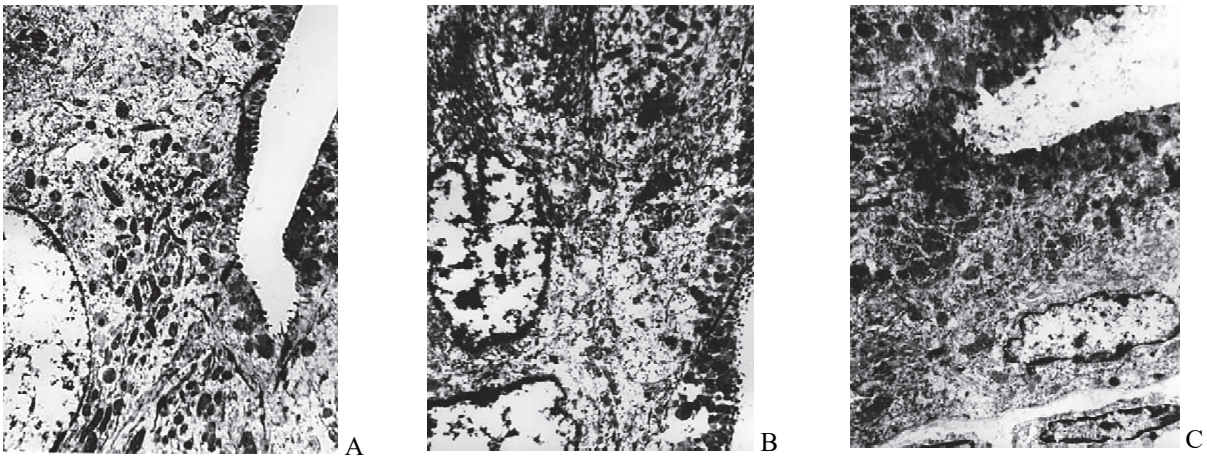

Fig. 2. The superficial scrobiculus epithelial cells of the rat gastric fundus on week 4 (a), 12 (b) and 16 (c) of the experiment. Electronogram. 8000×magnification.

Intensification of dystrophic changes and occurrence of destructive changes was found in the superficial scrobiculus epithelium of the gastric fundus on week 4 to 12. The number of secretory granules progressively

decreased, and the "desolation" areas were found in the apical cytoplasm. Karyopyknosis was detected in the epitheliocytes. The centric nuclei of irregular shape with numerous invaginations were found in the cells, containing mainly decondensed chromatin (Fig. 2b). Apoptotic bodies were noted in the cytoplasm.

Electron microscopic examination revealed that by week 16, the integrity of the superficial scrobiculus epithelium of the gastric fundus was partially restored. The nuclei were found in the basal parts of the cytoplasm, had an oval shape and a long axis oriented parallel to the basement membrane. Peripheral condensed chromatin was detected in the form of a thin stripe. Decondensed chromatin was noted in the center of the nuclei. A significant number of mitochondria and secretory granules were visualized in the apical cytoplasm of the superficial scrobiculus epithelial cells, which had an enhanced electron-optical density, and elements of the Golgi complex were visualized above the nuclei (Fig. 2c).

On weeks 1-4 of the experiment, in the bodies of the fundic glands, the lumens, in the cross sections, had an irregular shape, and the content was of low electron-optical density. Thickening and compaction of the apical plasmalemma were noted at the ultramicroscopic level. Secretory granules of the main exocrinocytes showed polymorphism in both size and content. Along with the medium-sized electron-dense granules, the large ones of medium-electron-optical densities were found (Fig. 3a).

On weeks 8-12, we found dystrophic and destructive changes in the exocrinocytes of the gastric fundic glands. Secretory disorders resulted in the increased number of secretory granules in the main exocrinocytes with enhanced electron density. They were localized not only in the apical cytoplasm, but also in its middle portions, surrounding the nucleus with pronounced chromatin condensation. Cisterns of the granular EPR, located in the basal parts of the cells, were dilated.

Subsequently, during the observation up to week 16, a decrease in the number of secretory granules, the size of the nucleus and chromatin condensation was noted in the main exocrinocytes. The electron-optical density of the cistern of the granular EPR was significantly decreased. Large vacuoles were detected in the cytoplasm (Fig. 3b).

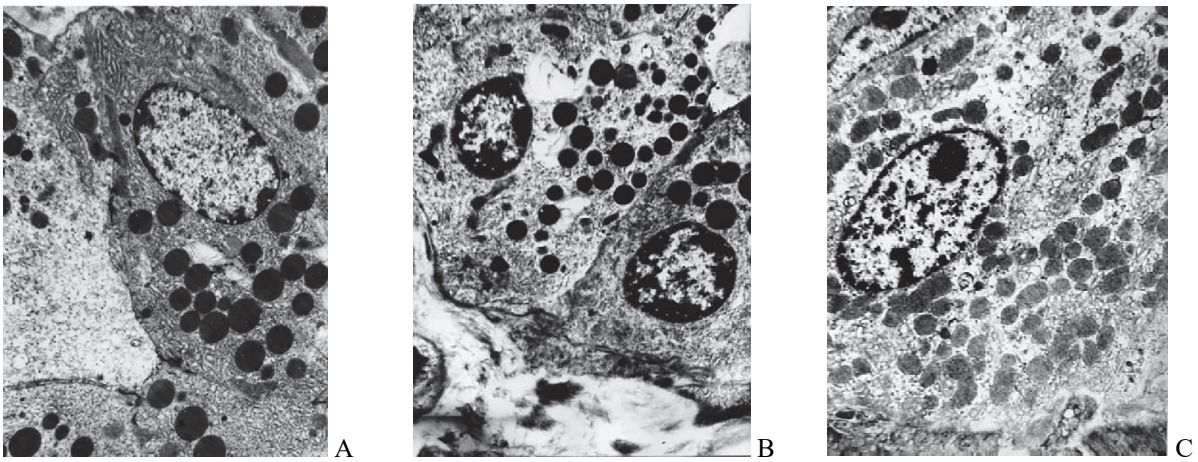

Fig. 3. The main exocrinocyte on week 4 of experiment (a). The main (b) and parietal (c) exocrinocytes on week 16 of the observation. Electronogram. 8000×magnification.

Dystrophic changes in the parietal exocrinocytes gradually progressed throughout observation and were manifested by a decrease in quantity, electron-optic density and destructuring of the cristae and dilatation of intracellular tubules (Fig. 3c).

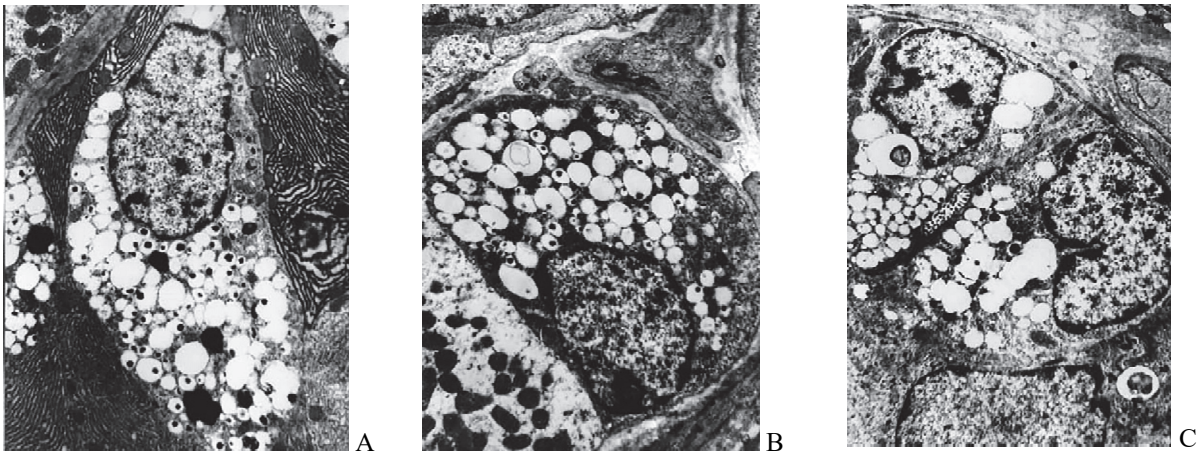

Fig. 4. Cervical mucocytes in the glands of the rat gastric fundus on week 4 (a), 12 (b) and 16 (c) of the observation. Electronogram. 8000×magnification.

Following weeks 1 to 4 of the experiment, dystrophic changes were detected in the cervical mucocytes, which were manifested by disrupted secretion and excretion. In the nuclei, oval in shape with irregular contours, localized in the basal parts of the cells, focal chromatin condensation was detected. Secretory granules in the apical cytoplasm showed polymorphism and merged to form large electron-transparent vacuoles. Electron-dense "nuclei" were visualized in the middle of some vacuoles (Fig. 4a).

Following weeks 4 to 12, in the cervical exocrinocytes, destructive changes caused by microcirculation disorders and swelling of the lamina propria were also noted. Their cytoplasm was compacted. The amount of heterochromatin in the nuclei increased. The number of electron-dense "nuclei" in the secretory granules decreased (Fig. 4b).

By week 16 of consumption of the complex of food additives, the areas of destruction of organelles and the formation of large vacuoles were noted in the cytoplasm of individual cells (Fig. 4c).

Thus, the impact of the complex of food additives on the mucous membrane of the rat gastric fundus is manifested primarily by dystrophic changes in superficial scrobiculus exocrinocytes, which are in direct contact with exogenous factors, as well as in the exocrinocytes of gastric glands; disorders of microcirculation are also noted.

The findings of our study are consistent with the findings of other investigators who studied the impact of monosodium glutamate on the liver [7] and vascular wall [11], and similar changes were found in the adrenal glands under the effect of the complex of food additives (monosodium glutamate, sodium nitrite and Ponceau 4R) [1].

The changes at the cellular level, established by the authors, were stereotypical for the impact of exogenous factors and manifested by dystrophic phenomena, namely, chromatin condensation, disruption of secretion and excretion processes in exocrinocytes, which was found in the study of the effect of chronic ethanol intoxication [13].

Conclusions

The impact of the complex of food additives on the mucous membrane of the rat gastric fundus led to disruption of secretion and excretion, which at the ultrastructural level, was manifested by disorders of architectonics and electronic density of secretory granules in the main exocrinocytes, cervical mucocytes and superficial scrobiculus epithelium. Dystrophic changes concomitant with microcirculation disorders were visible in the cells.

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