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REMODELING OF THE RAT GASTRIC WALL COMPONENTS UNDER THE EFFECT OF COMPLEX FOOD ADDITIVES

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The purpose of the study was to establish the dynamics of changes in the metric components of the structural components of the rat gastric wall with long-term use of a complex of food additives: sodium nitrite, sodium glutamate and Ponceau 4R. The use of a complex of food additives (monosodium glutamate, sodium nitrite and Ponceau 4R leads to structural and metric changes in the fundus of the fundus of the stomach. At 4 weeks is determined by severe hyperhydration and microcirculation disorders in all membranes. Other components do not recover to the values in the control group, destructive phenomena develop in the mucous membrane, and pronounced leukocyte infiltration in the submucosal.

Key words: gastric wall, sodium nitrite, sodium glutamate, Ponceau 4R, rats.

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СТРУКТУРНА ПЕРЕБУДОВА КОМПОНЕНТІВ СТІНКИ ШЛУНКУ ЩУРІВ ЗА УМОВ ВПЛИВУ КОМПЛЕКСУ ХАРЧОВИХ ДОБАВОК

Метою роботи було встановити динаміку змін метричних показників структурних компонентів стінки фундальної частини шлунку щурів при тривалому застосуванні комплексу харчових добавок: нітриту натрію, глутамату натрію та Понсо 4R. Вживання комплексу харчових добавок (глутамату натрію, нітриту натрію та Понсо 4R призводить до структурних і метричних змін у стінці фундального відділу шлунку. На 4 тижні визначається виражена гіпергідратація і розлади мікроциркуляції у всіх оболонках. На пізніх термінах спостереження спостерігається відновлення метричних показників у м'язовій і серозній оболонках. Інші компоненти не відновлюються до значень у контрольній групі, у слизовій оболонці розвиваються деструктивні явища, у підслизовій – виражена лейкоцитарна інфільтрація.

Ключові слова: стінка шлунку, нітрит натрію, глутамат натрію, Понсо 4R, щури.

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Currently, a laboratory rat is one of the most popular experimental models for studying the anatomical, physiological and biochemical correlations in the digestive system. The digestive system of humans and rats is a homologous functional system that has much in common in terms of structure and function of the organs [8, 12, 14].

Due to the increasing demand for food in modern society and to increase demand in food production, manufacturers currently use different types of food additives. Food additives of synthetic origin are considered the most harmful, because they are xenobiotics [2].

Sodium nitrite is widely used as a food additive in production of canned meat, delicacies, as well as canned fish to improve the consumer properties of the product. Some products can contain up to 700 mg of nitrates per 1 kg. Under the action of nitrites, which are used in sausage production as a food additive, the functional activity of mitochondria decreases, which leads to a deficiency in the tissues of macroenergetic compounds [4, 6, 10].

Monosodium glutamate (E621) is widely used in the world of marketing as a flavor enhancer and is added to many processed foods. Monosodium glutamate added to food products (≤10 g/kg) enhances its natural taste properties, weakened in the course of processing and storage, disguises certain negative components of the flavor and smell. Currently, about 50 % of on-the-shelf products contain the above additive, with the average daily human consumption of about 0.3–1.0 g in European highly developed industrial countries [2, 7].

The dramatic increase and uncontrolled use of synthetic food colorants added to various types of goods to enhance their appearance or compensate for natural color variations is of great concerned. [11].

The study of the effect of complex food additives (sodium nitrite, monosodium glutamate and Ponceau 4R) on the adaptive responses of rats has established behavioral disorders of experimental animals. From the first week of observation, the rats showed anxiety, fear, dull adaptive reactions, decreased activity and emotional disturbances, which were exacerbated by week 16 of the experiment [13].

Therefore, recently, scientists of various profiles are devoting to the study of the mechanisms of its toxic effects, as well as the study of compensatory-adaptive reactions in response to entry into the body.

The purpose of the study was to establish the dynamics of changes in the metric parameters of the structural components of the rats' gastric fundus wall in long-term use of complex food additives: sodium nitrite, monosodium glutamate and Ponceau 4R.

Materials and methods. 84 outbred mature male rats were involved into the study. Control rats consumed drinking water and received saline per os. The rats of the experimental group were given access to water ad libitum and, supplementary, consumed 10 % sodium nitrite solution. Monosodium glutamate was administered at a dose of 20 mg/kg in 0.5 ml of distilled water, Ponceau 4R at a dose of 5 mg/kg in 0.5 ml of distilled water once daily per os. The doses of food additives were twice lower the allowable normal rate in foods. To evaluate the adaptive behavior, the rats were exposed to the "open field" test. [13].

The animals were sacrificed within 1, 4, 8 and 16 weeks under thiopentone anesthesia overdose. For histological study, after euthanasia of the animals, fragments of the gastric fundus wall were fixed in 10 % neutral formalin solution for three days. Subsequently, the fragments, fixed in formalin, were embedded into paraffin [1]. After staining with hematoxylin and eosin, sections of the 5–10 μm thick were studied in the Biorex 3 light microscopefter equipped with DCM 900 digital microphoto attachment. To obtain semi-thin sections, the material was fixed in glutaraldehyde and embedded in Epon-812. Sections of 1–2 μm thick were stained with toluidine blue with pH 8.4. The thickness of mucous, submucous, muscle, serous membranes and the total thickness of the gastric wall were determined using the morphometric method. Statistical processing of morphometric data was performed using the Excel program [9].

Results of the study and their discussion. The findings of the morphometric study have established that in animals of the control group, the mean values of the total gastric fundus wall thickness were 1364.26±27.86 μm (fig. 1, table 1).

Table 1

Morphometric characteristics of the rats' gastric fundus wall (μm)

Parameters / Time period	Total wall thickness	Mucous membrane thickness	Submucous membrane thickness	Muscle membrane thickness	Serous membrane thickness
Control group	1364.26±27.86	676.72±16.06	122.72±9.12	462.94±12.6	10.01±0.91
Week 1	1118.66±20.34 *	589.42±16.01 *	103.08±8.24 *	544.92±14.29 *	7.42±0.69 *
Week 4	1740.63±25.86 *,**	722.51±19.98 *,**	167.09±11.36 *,**	569.18±10.33 *	12.08±0.71 *,**
Week 8	922.53±20.03 *,**	551.21±15.74 *,**	140.02±12.51 *,**	447.18±9.24 *,**	11.09±0.59 *,**
Week 12	1177.15±22.14 *,**	564.67±15.65 *	147.13±14.62 *	463.90±8.03 *	12.77±0.64 *,**
Week 16	1214.87±22.79 *	589.22±15.02 *	141.41±9.75 *	468.41±7.94 *	10.35±0.74 **

Note * – $p<0.05$ compared to the control group;

** – $p<0.05$ compared to the previous time period of the observation.

After 1 week of consumption of the complex food additives, the value significantly decreased by 10.97 % and accounted for 1118.66±20.34 μm. On week 4 of the observation, a dramatic thickening of the gastric wall by 55.58 % was established and the values reached 1740.63±25.86 μm, induced by prominent hyperhydration of the connective tissue (fig. 2). By week 8, the rate decreased by 47 % compared to the previous period of observation ($p<0.05$), accounting for 922.53±20.03 μm (table 1). From week 12 of the experiment, a gradual recovery of the values of the total thickness of the gastric fundus wall by 27.67 % (1177.15±22.14 μm, $p<0.05$), after 16 weeks – by 11.69 % to 1214.87±22.79 μm, though they were significantly lower compared to the control group (table 1).

The analysis of the dynamics of metric parameters of mucosal thickness has found that after a week of the observation of experimental animals, the average values of mucosal thickness significantly decreased by 12.97 %, and by week 4 the value increased by 22.58 % ($p<0.05$), compared to the previous time period and exceeded the value in the control group. The defined phenomenon is caused by the connective tissue edema and vascular plethora (fig. 2). On week 8 of the experiment, a decrease in the average thickness of the mucous membrane by 23.71 % was noted, the values were by 18.55 % lower compared to the control group ($p<0.05$). Changes in the metric parameters were caused by desquamation of the surface layers of the mucous membrane, established by histological study (fig. 3). On week 12 and 16, the values did not recover to the reference ones, and did not differ significantly, although there was a

tendency to increase (table 1). On week 16, toluidine blue stain of the semi-thin sections with pH 8.4 revealed an increase in mucin-containing cells in the fundal glands (fig. 4).

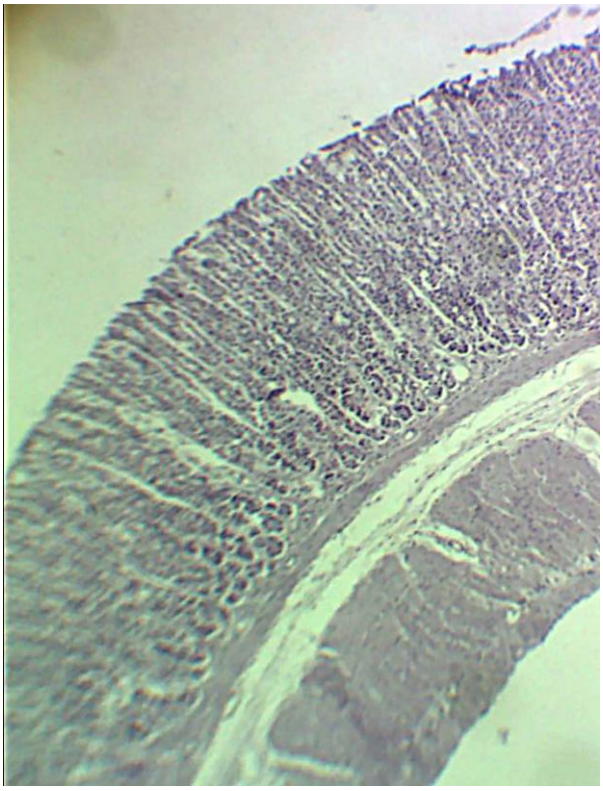

Fig. 1. The wall of the rats of control group. Microimage. H&E stain; ×100 magnification.

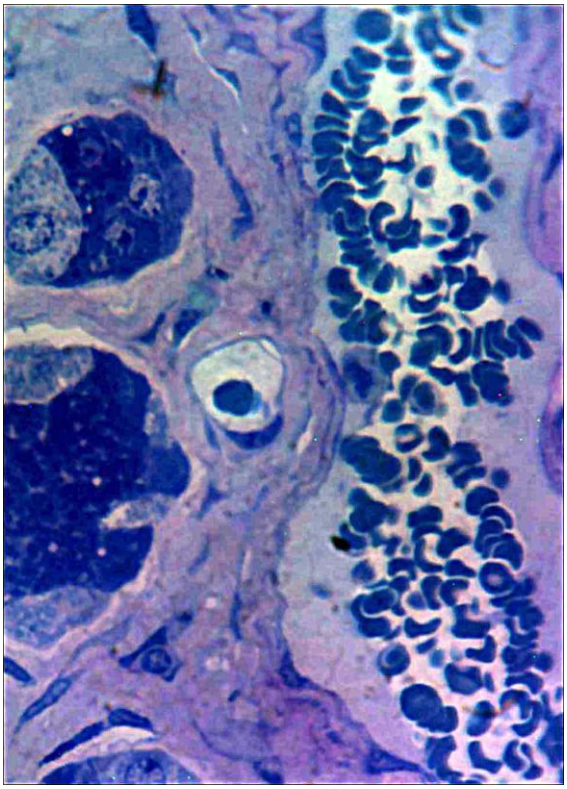

Fig. 2. Hyperhydration of the rats' gastric wall mucosa on week 4 of the experiment. Semi-thin section. Toluidine blue stain; ×1000 magnification.

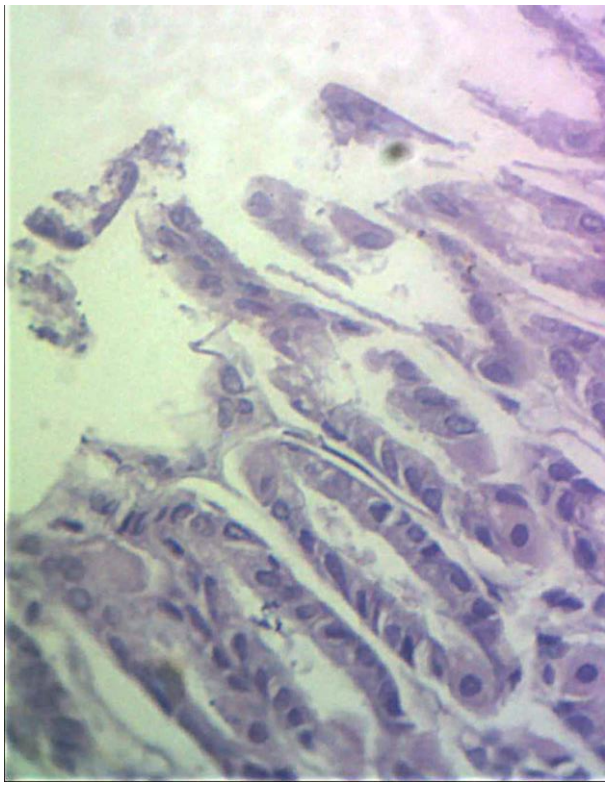

Fig. 3. Desquamation of the rats' gastric superficial epithelium on week 8 of the experiment. Microimage. H&E stain; × 400 magnification.

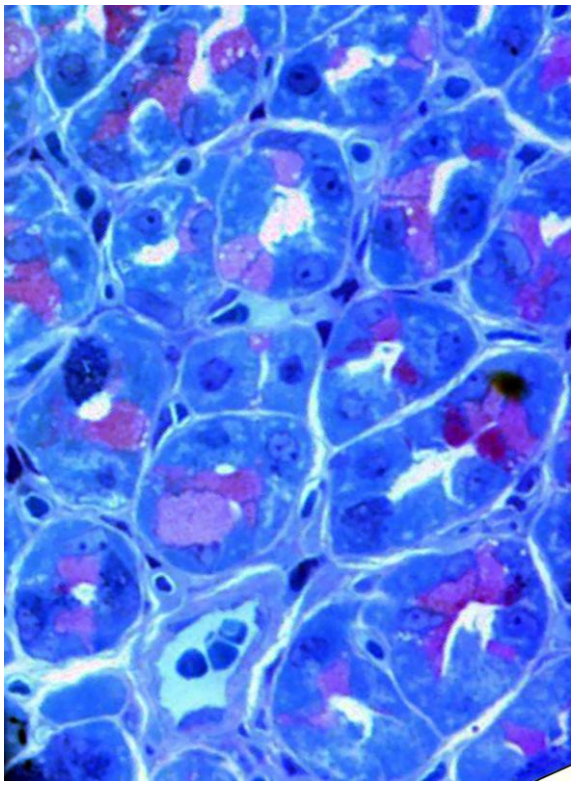

Fig. 4. Emergence of mucocytes in the rats' gastric fundal glands on week 16 of the experiment. Semi-thin section. Toluidine blue stain; × 400 magnification.

Changes in the average thickness of the submucous layer at the early stages of observation were similar to the thickness of the mucous membrane (table 1). However, the tendency to decrease and restore

indicators did not lead to normalization – the values were by 15.23 % significantly higher compared to the control group ($p<0.05$) and histological study revealed diffuse infiltration by leukocytic cells.

The least pronounced changes in the average thickness were found in the muscle plate. On week 1 and 4, its thickening by 22.95 % ($p<0.05$) was detected most likely due to general hyperhydration and disrupted blood perfusion in the gastric wall. From week 8 of observation, the values did not differ significantly from the control group (table 1).

Changes in the serous membrane were manifested by a decrease in thickness by 25.9 % on week 1 of the experiment, an increase by 62.8 % to week 4 ($p<0.05$) and a gradual decrease in values on week 8 and 12. By week 16, the parameter did not differ significantly from the control group (table 1).

The revealed changes in the metric parameters of the structural components of the rats' gastric fundus wall of rats after the consumption of glutamate, sodium nitrite and Ponceau 4R are primarily caused by their direct effect on the surface of the gastric mucosa, which leads to alteration and exudation and is stereotyped for many aggressive factors. [3, 5]. However, the findings of our study showed that from week 8 of the effect of the complex food additives, dystrophic and destructive changes developed in the mucous membrane, which persist until the end of observation. Entering of the stimuli into the thickness of the gastric wall leads to disorders of hemomicrocirculation and hyperhydration of connective tissue.

Conclusion

Consumption of the complex food additives (monosodium glutamate, sodium nitrite and Ponceau 4R) leads to structural and metric changes in the gastric fundus wall. On week 4, a prominent hyperhydration and microcirculation disorders in all membranes is observed. At the later stages of observation, the recovery of the metric parameters in the muscle and serous membranes were noted. Other components do not recover to the values in the control group, destructive phenomena develop in the mucous membrane, and pronounced leukocyte infiltration in the submucous membrane is observed.

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