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REMODELING OF THE WALL OF THE ASCENDING COLON IN RATS UNDER THE INFLUENCE OF THE FOOD ADDITIVES COMPLEX

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Food additives are substances added to raw materials, finished products and medicines to extend the shelf life of the product, prevent the development of pathogenic microflora, and improve taste and organoleptic qualities. Monosodium glutamate, sodium nitrite and ponceau 4R are among the most widely used food additives. The experiment involved 84 mature male rats. The animals received 0.6 mg/kg sodium nitrite E250, 20 mg/kg monosodium glutamate E621 and 5 mg/kg ponceau 4R orally. Samples of the ascending colon of rats for histological examination were taken at 1, 4, 8, 12 and 16 weeks. It was found that oral administration of the food additives complex MSG, ponceau 4R and sodium nitrite for 16 weeks causes significant changes in the morphometric parameters of the wall of the ascending colon in rats. A pronounced reaction of the intestinal mucosa is characterized by the destruction of intestinal epithelial cells and disruption of the crypt structure with subsequent incomplete recovery at late follow-up.

Key words: food additives, monosodium glutamate, sodium nitrite, ponceau 4R, ascendenig colon.

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РЕМОДЕЛЮВАННЯ СТІНКИ ВИСХІДНОЇ ОБОДОВОЇ КИШКИ ЩУРІВ ЗА УМОВ ВПЛИВУ КОМПЛЕКСУ ХАРЧОВИХ ДОБАВОК

Харчові добавки – це речовини, які додаються до сировини, готових продуктів та лікарських препаратів з метою подовження терміну зберігання продукту, запобігання розвитку патогенної мікрофлори, поліпшення смакових та органолептичних якостей. Monosodium glutamate, sodium nitrite and ponceau 4R є одними з найбільш широко вживаних харчових добавок. The experiment involved 84 mature male rats. Тварини отримували 0.6 mg/kg sodium nitrite E250, 20 mg/kg monosodium glutamate E621 and 5 mg/kg ponceau 4R перорально. Відбір зразків висхідної ободової кишки щурів for histological examination was carried out at 1, 4, 8, 12 and 16 weeks. Встановлено, що пероральне застосування комплексу харчових добавок MSG, понсо 4R та нітриту натрію упродовж 16-ти тижнів зумовлює достовірні зміни морфометричних показників стінки висхідної ободової кишки у щурів. Характерною є виражена реакція слизової оболонки кишки, що характеризується деструкцією кишкових епітеліоцитів та порушенням структури крипт з подальшим неповним відновленням на пізніх термінах спостереження.

Ключові слова: харчові добавки, глутамат натрію, нітрит натрію, понсо 4R, висхідна ободова кишка.

The study is a fragment of the research project "Restructuring of the organs of the immune, respiratory and excretory systems under the effect of various exogenous factors (monosodium glutamate, sodium nitrite, ethanol, methacrylate)", state registration No. 0121U108234.

The main purpose of using food additives is to extend the shelf life of the product, prevent the development of pathogenic microflora, and improve taste and organoleptic qualities. Monosodium glutamate, sodium nitrite and ponceau 4R are food additives that have different purposes and are widely used in combination in food, medicines and animal feed. All three substances are sodium salts by their chemical nature. The use and acceptable daily intake of food additives in Europe is regulated and periodically reviewed by the European Food Safety Authority. It should also be noted that for children, the

daily intake of food additives is lower than for adults [1, 5, 14]. The effect of each food additive has been studied separately, as evidenced by recent publications. Only a few studies have directly focused on the effects of monosodium glutamate, sodium nitrite, and ponceau 4R on the intestine. It is reported that nitrites increase mucus secretion by intestinal goblet cells, which is considered a positive aspect [7]. In addition, nitrites cause vasodilation and lower blood pressure [6, 12]. The food supplement monosodium glutamate is used to model a variety of pathological conditions, including obesity, diabetes, and central nervous system disorders. The excitotoxicity of monosodium glutamate and its ability to penetrate the blood-brain barrier have been reported [2,4,9]. Food coloring, in particular, ponceau 4R, is used not only in food but also in medicines for children [11]. The FAO/WHO Expert Committee on Food Additives (JECFA) last revised the Acceptable Daily Intake of ponceau 4R in 2011. Consumption of ponceau 4R is associated with allergic reactions and negative effects on children's behavior. In some countries, consumers are demanding additional product labeling to avoid the use of dyes [10].

As can be seen from the brief review of sources, these food additives have different mechanisms of action on organs and systems. However, the effect of monosodium glutamate, sodium nitrite and ponceau 4R in combination is poorly understood. The synergistic, or vice versa, antagonistic effect of the above substances in combination on the human and animal body is questionable.

The purpose of the study was to establish the remodeling dynamics of the ascending colon wall of rats under the influence of a food additives complex.

Materials and methods. The experiment involved 84 mature male rats (Rattus norvegicus) weighing 204.5±0.67 g, which were obtained from the experimental-biological clinic of the University. All procedures followed the standard rules established by the commission of Poltava State Medical University on ethical issues and bioethics (order of the rector No. 330 of May 30, 2020) in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). The rats of all groups had access to food and water ad libitum. The animals were sacrificed under thiopentone anesthesia overdose. The rats of control group (n=14) consumed drinking water and were administered with saline orally. The rats of the experimental group, with access to water ad libitum, were administered with 0.6 mg/kg sodium nitrite E250 (Uralchem, China), 20 mg/kg monosodium glutamate E621 (Multichem, China) and 5 mg/kg ponceau 4R E124 (Multichem, China) in 0.5 ml of distilled water once daily orally. Collection of samples for histological examination was carried out at 1, 4, 8, 12 and 16 weeks. After euthanasia the rats were dissected following the method of complete evisceration. The ascending colon was removed and fixed with a 10 % neutral formalin solution. The material was washed and prepared for paraffin embedding according to standard techniques [13]. Sections of 5-10 μm thick were obtained using the manual rotary microtome HistoLine. Histological sections were stained with hematoxylin and eosin (H&E). Series of histological slide's photomicrographs from objectives 4x and 10x were captured by a microscope Levenhuk D740T attached to a digital 5.1 Mpx kit camera. Photo fixation and morphometry were performed in Levenhuk Lite software. The data were expressed as the means ± standard error of the mean. Statistical processing of morphometric data was performed using the Excel software, that analyzed using parametric Student's t-test. The data was expressed as the means ± standard error of the mean. Besides, $p<0.05$ was considered to be statistically significant.

Results of the study and their discussion. According to the results of morphometric studies of the components of the wall of the ascending colon of rats, it was found that its total thickness in the control group of animals was 530.21±14.98 μm, the thickness of the mucosa was 360.14±10.44 μm, the thickness of the submucosa was 86.04±3.12 μm, the thickness of the muscularis was 143.02±6.49 μm and the thickness of the serosa was 6.94±0.16 μm (Table 1).

Table 1

Dynamics of changes in the thickness of the wall structures of the ascending colon in rats of control and experimental groups

Observation period, weeks	Overall wall thickness	Mucous membrane thickness	Submucosal base thickness	Muscle sheath thickness	Serous membrane thickness
Control (n=14)	530.21±14.98	360.14±10.44	86.04±3.12	143.02±6.49	6.94±0.16
1 (n=14)	734,89±6.85 *	500,12±4.04 *	92.47±1.54 *	146.19±4.91	6.55±0.09 *
4 (n=14)	672.48±19.52 *,**	432.44±3.61 *,**	68.62±3.71 *,**	133.18±2.01 *,**	5.17±0.15 *,**
8 (n=14)	598.6±9.72 *,**	386.43±3.69 *.**	59.71±4.99 *,**	144.61±2.38 **	5.35±0.1 *,**
12 (n=14)	607.53±10.28 *	417.3±3.21 *,**	76.84±1.61*,**	123.55±3.76 *,**	4.53±0.12 *,**
16 (n=14)	611.54±10.47 *	418.5±8.73 *	50.92±1.48 *,**	130.53±4.19 *	4.2±0.08 *,**

Notes: data are mean and standard deviation; *$p<0.05$ compared with the control group, **$p<0.05$ compared with the previous observation period.

On the lumen side, the surface of the mucosa is represented by high columnar epithelium and goblet cells. The lumen of the shallow crypts was formed by goblet cells and low-differentiated colonocytes in the area of the crypt bottom. A dense layer of mucus was clearly visualized on the surface of the mucosa. The mucous membrane was separated from the submucosa by its own plate and a thin muscular plate. The intestinal mucosa had folds (Morgagni's columns), which were formed due to the thickening of the submucosa. In the thickness of the loose connective tissue, at the base of the Morgagni column, there were numerous vessels of different caliber and large ganglion cells belonging to the structures of Meissner's plexus. The muscle sheath was visualized in the form of two layers - a more developed inner circular layer and an outer longitudinal layer. Between the two layers of the muscle membrane, the ganglion cells of the myenteric plexus (myoplexus myentericus), also known as Auerbach's complex, were clearly visualized. Externally, the intestine was covered with a thin serous membrane (Fig. 1a).

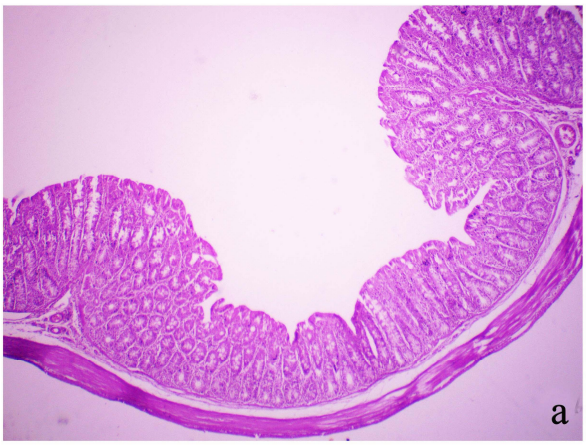

Fig. 1a. Structure of the ascending colon of the control group in rats Hematoxylin and eosin staining. 100×magnification.

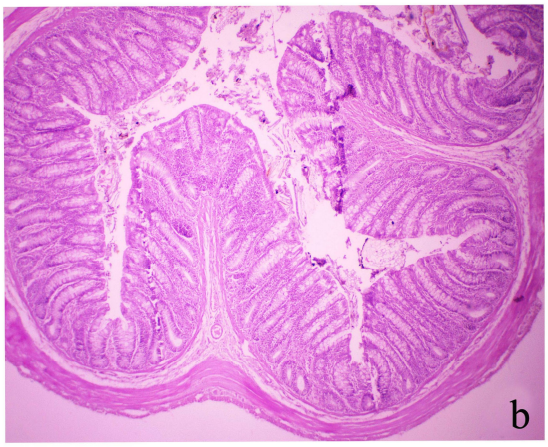

Fig. 1b. Structure of the ascending colon of rats at the 1st week of observation. Hematoxylin and eosin staining. 100×magnification.

At 1 week of consumption of the complex of food additives in the rats of the experimental group, a significant increase in the total wall thickness of the ascending colon was observed - 734.89±6.85 μm, which was 26.83 % more than in the control ($p<0.05$). There was an increase in the thickness of the intestinal mucosa - 500.12±4.04 μm, which was 38.87 % more than the control ($p<0.05$). There was a significant increase in the thickness of the submucosal base to 92.47±1.54 μm - 7.47 % more than in the control group ($p<0.05$). The thickness of the muscularis was 146.19±4.91 μm, which did not differ significantly from the control values. The thickness of the serous membrane was 6.55±0.09, which is 5.62 % less than in the control group ($p<0.05$).

At 1 week of observation in the colon ascendens of rats, pronounced changes in the mucous membrane were detected. On the lumen side, the mucosa was covered with columnar colonocytes. In the lumen of the crypts, secretion accumulation was recorded. Moderate cellular infiltration of the lamina propria and loose connective tissue between the crypts was observed. There was a thickening of the submucosal base due to tissue edema (Fig. 1b).

On the 4th week of the experiment, the total wall thickness of the ascending colon was 672.48±19.52 μm. This indicator was higher than the control by 26.83 % ($p<0.05$), and also decreased compared to the previous observation period by 8.49 % ($p<0.05$). The thickness of the mucous membrane of the ascending colon was 432.44±3.61 μm. This indicator was significantly higher than the control results by 20.08 % ($p<0.05$), and at the same time was 13.53 % less than the results of the 1st week of observation. The thickness of the submucosal base was 68.62±3.71 μm, which was 20.25 % less than in the control group and 25.79 % less than in the previous observation period ($p<0.05$). The thickness of the muscularis sheath was significantly less than that of the control by 6.88 %, as well as by 8.9 % ($p<0.05$) compared to the results of the 1st week of observation and amounted to 133.18±2.01 μm. The thickness of the serous membrane was 5.17±0.15 μm. This indicator was significantly lower compared to the control and the results of the previous observation period by 6.88 % and 8.9 %, respectively ($p<0.05$). The thickness of the serous membrane was 5.17±0.15 μm, this result was significantly less than that of the control group and the results of the 1st week of observation by 25.5 % and 21.07 %, respectively ($p<0.05$).

Microscopic changes in the colon ascendens of rats at 4 weeks of observation were characterized by a gradual decrease in the total thickness of the intestinal wall compared to 1 week of the experiment. Such changes were probably due to pathological processes in the mucosal tissues. Intensive desquamation

of the surface epithelium was observed. There was a total disruption of the crypt structure, which was well visualized in the longitudinal section of the intestine. Cellular infiltration of the mucosa and submucosa increased in the areas of localization of intestinal-associated lymphoid tissue (Fig. 2a).

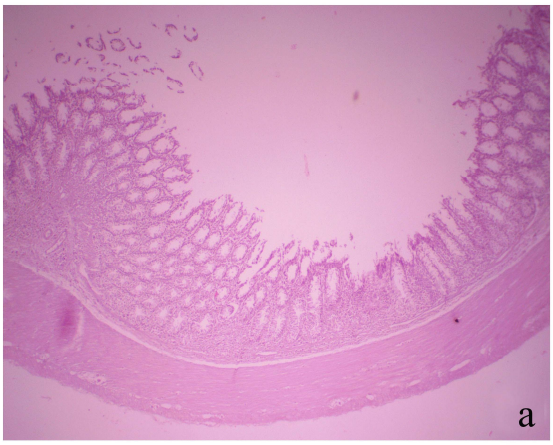

Fig. 2a. Structure of the ascending colon of rats on the 4th week of observation. Hematoxylin and eosin staining. 100× magnification.

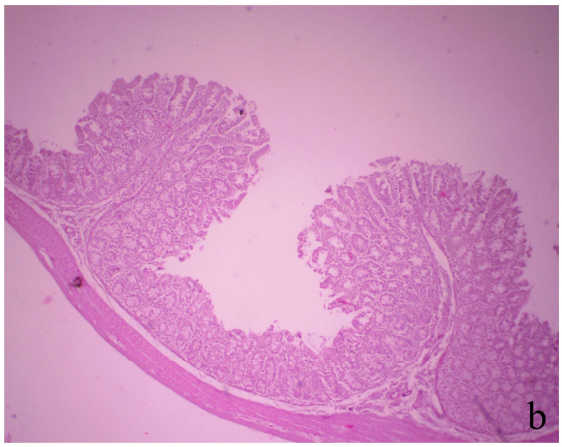

Fig. 2b. Structure of the ascending colon of rats at week 8 of observation. Hematoxylin and eosin staining. 100×magnification.

At the 8th week of observation, the total wall thickness of the ascending part of the rat colon was 598.6±9.72 μm. This indicator was significantly higher than the control results by 12.9 %, and continued to decrease relative to the results of the 4th week of observation - by a total of 10.99 % ($p<0.05$). The thickness of the intestinal mucosa was in the range of 386.43±3.69 μm and was significantly lower than in the control group and compared to the previous observation period by 7.7 % and 10.64 %, respectively ($p<0.05$). The thickness of the submucosal base was 59.71±4.99 μm, this indicator was significantly reduced compared to the control and the previous observation period by 30.6 % and 12.98 %, respectively ($p<0.05$). The thickness of the muscularis was within 144.61±2.38 μm, this result was significantly higher compared to the 8th week of observation by 3.48 % ($p<0.05$). The thickness of the serous membrane was 5.35±0.1 μm, this indicator was significantly lower than the control by 22.91 %, and at the same time exceeded the results of the 4th week of observation by 3.48 % ($p<0.05$).

The histological picture of the wall of the colon ascendency of rats on the 8th week of observation was similar to the picture of the 4th week of the experiment. Characteristic for this period were changes in the intestinal mucosa in the form of intense desquamation of epithelial cells, as well as pronounced cellular infiltration (Fig. 2b).

At the 12th week of the experiment, the total wall thickness of the ascending colon of rats was 607.53±10.28 μm, which was significantly higher than the control by 14.58 % ($p<0.05$). The thickness of the mucous membrane was 417.3±3.21 μm, which was significantly higher than the control and the previous observation period by 15.87 % and 7.8 %, respectively ($p<0.05$). The thickness of the submucosal base at this follow-up period was within 76.84±1.61 μm. This indicator was significantly lower than the control results by 10.69 % and at the same time increased by 28.69 % compared to the 8th week of observation ($p<0.05$). The thickness of the muscle sheath was 123.55±3.76 μm. This result was significantly lower compared to the control and previous observation periods by 13.61 % and 14.56 %, respectively ($p<0.05$). The thickness of the serous membrane was in the range of 4.53±0.12 μm, this indicator also decreased both relative to the control and the results of the 8th week of observation by 34.73 % and 15.33 %, respectively ($p<0.05$).

The histological picture of the rat colon ascendency at the 12th week of observation was characterized by an increase in the thickness of the mucosa due to the restoration of the structure of crypts and surface epithelium. The submucosa showed signs of moderate cellular infiltration (Fig. 3a).

On the 16th week of observation, the total wall thickness of the ascending colon of rats was 611.54±10.47 μm, this figure was significantly higher than the control results by 15.34 % ($p<0.05$). The thickness of the mucous membrane was in the range of 418.5±8.73 μm. This indicator was significantly higher than the control results by 16.2 % ($p<0.05$). The thickness of the submucosal base was 50.92±1.48 μm, which was significantly lower than the control and 12th week of observation by 40.82 % and 33.73 %, respectively ($p<0.05$). The thickness of the muscle membrane was 130.53±4.19 μm, which was significantly less than the control by 8.73 % ($p<0.05$). The thickness of the serous membrane was 4.2±0.08 μm, which was significantly less than the control and the results of the previous observation period by 39.48 % and 7.28 %, respectively.

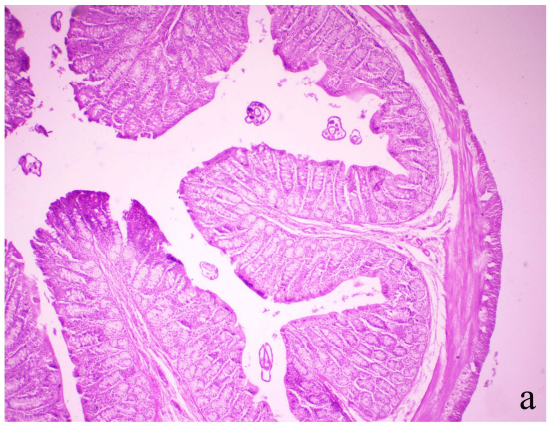

Fig. 3a. Structure of the ascending colon of rats at the 12th week of observation. Hematoxylin and eosin staining. 100×magnification.

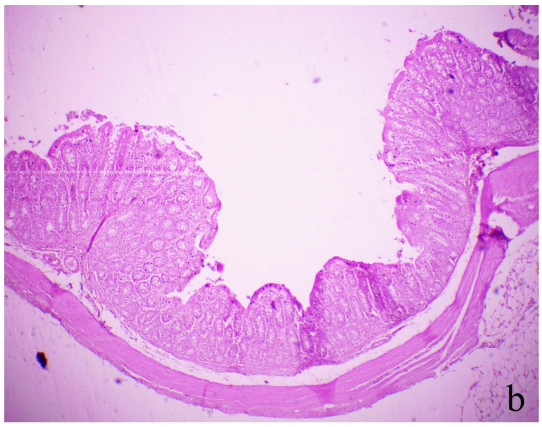

Fig. 3b. Structure of the ascending colon of rats at week 16 of observation. Hematoxylin and eosin staining. 100× magnification.

The histological picture of the wall of the colon ascendency of rats showed a partial compensatory restoration of its structures against the background of prolonged exposure to a complex of food additives (Fig. 3b). The restoration of the structure of the surface epithelium and crypts was observed against the background of preserved cellular infiltration.

Thus, at the 1st week of observation, the total wall thickness of the rat colon ascendens increases due to an increase in the thickness of the mucosa and submucosa, which is due to edema and progressive inflammation. At the same time, a decrease in the thickness of the serous membrane was recorded, which is likely due to its stretching due to the rapid increase in the thickness of the intestinal wall. From the 4th to the 8th week of the experiment, the total thickness of the intestinal wall decreases due to a decrease in the thickness of the mucosa and submucosa, which is explained by the destruction of mucosal structures and a simultaneous decrease in the edema of the submucosal tissues. Edema remains in the muscularis, as evidenced by an increase in its thickness. From the 12th to the 16th week, there is a gradual increase in the total thickness of the colon ascendes due to the restoration of mucosal structures. A sharp increase in the thickness of the submucosal base at week 12 is followed by a sharp decrease in its value at week 16. The thickness of the serous membrane did not approach the control values and was significantly reduced up to the 16th week of the experiment (Fig. 4).

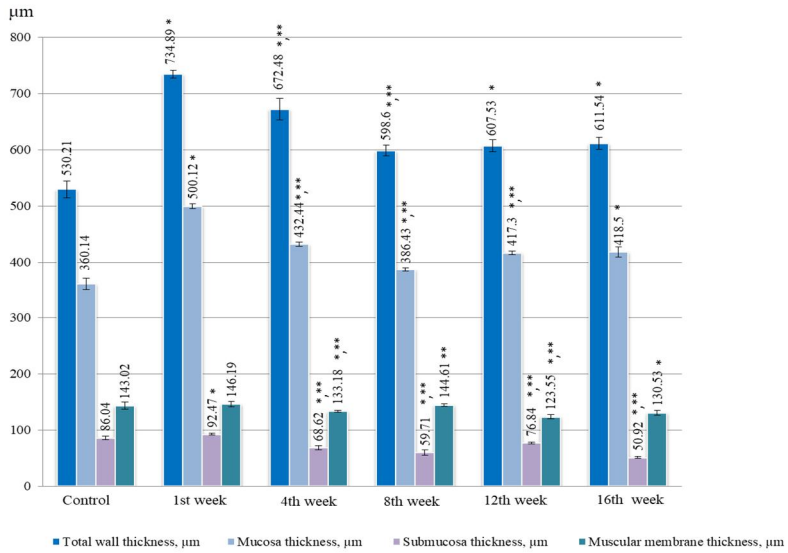

Fig. 4. Dynamics of changes in the thickness of the wall structures of the ascending colon of rats of the control and experimental groups. Data are mean and standard deviation. *p<0.05 compared to the control group, **p<0.05 compared to the previous observation period.

These results are consistent with the results of previous studies of the effect of a complex of food additives monosodium glutamate, sodium nitrite and ponceau 4r on the morphometric parameters of the duodenal wall of rats. It was found that in the case of consumption of a complex of food additives in the small intestine, a complex tissue reaction develops, which leads to changes in the morphometric parameters of the intestinal wall structures. Leukocyte infiltration, inflammatory reactions, and destruction of intestinal wall tissue with subsequent incomplete recovery are characteristic consequences of the use of the complex of these food additives [3].

The results of our studies indicate that the intestinal mucosa underwent major changes under the influence of a complex of food additives. In particular, the surface epithelium was damaged and the structure of the crypts was disturbed. It is known that normally the apical surface of intestinal epithelial cells is densely covered with so-called transmembrane mucins, which form an inner, tightly attached

layer of mucus. Goblet cells produce gel-forming mucins, these substances form the outer layer of mucus that is not attached to the epithelium. The outer layer of mucus in the large intestine is a substrate for bacteria, while the inner layer is impermeable to microorganisms [8]. Therefore, disruption of the mucous membrane structure due to the intake of a complex of dietary supplements can have consequences in the form of dysbiosis or the penetration of pathogenic bacteria through the intestinal barrier. The effect of the complex of monosodium glutamate, sodium nitrite and ponceau 4r on gastrointestinal exocrinocytes is confirmed by the research of Yachmin et al (2022). Thus, the use of a complex of food additives causes dystrophy of superficial exocrinocytes and exocrinocytes of the glands of the gastric mucosa [15].

Conclusions

The effect of the complex of food additives MSG, ponceau 4R and sodium nitrite causes significant changes in the morphometric parameters of the wall of the ascending colon in rats. A pronounced reaction of the intestinal mucosa is characterized by the destruction of intestinal epithelial cells and disruption of the crypt structure with subsequent incomplete recovery at late stages of observation.

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