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## RESEARCH OF EFFECTS BY DONATORS NITROGEN OF OXYGEN IN COMPLEX THERAPY DURING EXPERIMENTAL PERITONITIS

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### Abstract

The research was conducted on 175 white rats of reproductive age (3 months), weight of animals - 180-220 g. Animals were divided into 4 groups:

Group 1 - 20 intact animals. Group 2 - 50 rats with simulated fecal peritonitis.

Group 3 - 50 rats with simulated fecal peritonitis with subsequent antibiotic correction and debridement by chlorhexidine solution. Group 4 - 50 rats with simulated fecal peritonitis with subsequent antibiotic correction, chlorhexidine debridement and endothelial dysfunction correction with the use of a nitric oxide donor. Fecal peritonitis was modeled using injection of 10% fecal suspension in a dose of 0.5 ml per 100 g of animal weight in the abdominal cavity of laboratory animals by puncture method (Lazarenko V.A., et al., 2016, patent No. 233826).

The following results were obtained.

Histologically confirmed the development of experimental peritonitis in all groups of the experiment. In the group №2 on the third day the deterioration of the studied structures was established. In the group in which the experimental peritonitis was corrected by antibiotic therapy and chlorhexidine remediation, the effectiveness of the correction was morphologically proven. In group 4, macroscopic and microscopic examinations of the intestine, liver, and abdomen did not reveal significant differences from the histological picture we observed in rats of the intact group.

**Keywords: peritonitis; experimental model; histological examination; correction; L-arginin**

**Introduction.** Among all postoperative intra-abdominal complications, widespread peritonitis ranks first, accounting for 23.2% of cases [1].

Foreign authors give mortality figures for diffuse forms of peritonitis in the range from 20 to 25% [2, 3].

According to some authors, during the first 5 years after peritonitis, 35% of patients develop complications of vascular dysmetabolism, of which 65% die within 10 years [4]. The main etiopathogenesis factor of endothelial dysfunction is the development of endotoxin aggression [5]. Endogenous intoxication, in turn, develops due to intestinal failure, changes in the quantity and quality of intestinal microflora, the concentration of toxins and microorganisms in the vascular bed and in the lumen of the abdominal cavity [6].

Until recently, the problem of long-term complications after peritonitis in the form of vascular accidents (heart attacks and strokes) has not been considered in the context of other surgical pathologies.

**Materials and methods of research:**

The research was conducted on 175 white rats of reproductive age (3 months), weight of animals - 180-220 g. Animals were divided into 4 groups:

Group 1 - 20 intact animals.

Group 2 - 50 rats with simulated fecal peritonitis.

Group 3 - 50 rats with simulated fecal peritonitis with subsequent antibiotic correction and debridement by chlorhexidine solution.

Group 4 - 50 rats with simulated fecal peritonitis with subsequent antibiotic correction, chlorhexidine debridement and endothelial dysfunction correction with the use of a nitric oxide donor.

Fecal peritonitis was modeled using injection of 10% fecal suspension in a dose of 0.5 ml per 100 g of animal weight in the abdominal cavity of laboratory animals by puncture method [7].

Upper median laparotomy and abdominal revision were performed on anesthetized animals of groups №3 and №4. Inflammatory exudate was removed using an electroaspirator. Carried out the rehabilitation of the abdominal cavity to obtain clean wash water. Then the wound was drained and sutured in layers. Animals were properly anesthetized with sodium thiopenta during the manipulations.

Antibiotic correction was performed as follows. Ceftriaxone dissolved in 0.2 ml of isotonic sodium chloride solution at a dose of 5 mg / 100 grams was injected intramuscularly into the right thigh.

Nitric oxide donor administration a solution of L-arginine (SIMESTA, made in China, quality standard USP32) was carried out by intragastric injection of L-arginine solution in 0.9% sodium chloride solution at a dose of 500 mg / kg [8] through a syringe with a feeding tube. The volume of the solution depended on the weight of the animal and did not exceed 1 ml. The drug was administered once a day before morning feeding, daily for 10 days [8].

Research was conducted in accordance with the "Rules for carrying out works using experimental animals", approved by the Order of the Ministry of Health of Ukraine No. 249 of 01.03.2012 and the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruel Treatment" (as amended on December 15, 2009, and 10/16/2012).

Celluloid slices 7-9  $\mu\text{m}$  thick were prepared from the liver and small intestine according to the generally accepted method, which were stained by hematoxylin and eosin (H&E) with further examination under a light microscope [9].

### **Research results**

*The results obtained on the first day after simulation of fecal peritonitis.*

Macroscopic examination of the abdominal cavity 1 day after simulation of fecal peritonitis shows swelling and turbidity of the abdominal surface, on the small loops and large intestine are determined deposits (moderate) of homogeneous whitish translucent masses. The vessels of the abdomen are full-blooded, dilated. At the bottom of the abdominal cavity is 1-2.0 ml of turbid fluid. Visually, the liver without significant changes. Its color is brownish-purple, the leading edge is sharp, the surface is smooth, slightly cloudy.

On microscopic examination of the small intestine, the serous membrane is swollen and infiltrated with lymphoid elements. The intestinal mucosa also shows signs of edema and lymphoid infiltration, although visually the density of this infiltrate is lower than in the serous

membrane. Vessels are dilated, blood supply is increased, part of the vessels is spasmed. Myocytes with dark nuclei, their cytoplasm is pale. The same pattern (Fig. 1) is observed in the submucosal plate. As for the mucosa, it should be noted the small height of the villi, in some of them the tip is broken. The epithelial cells are swollen, some of them look like goblets. In the middle the vessel is spasmed, and infiltration by lymphoid elements is observed.

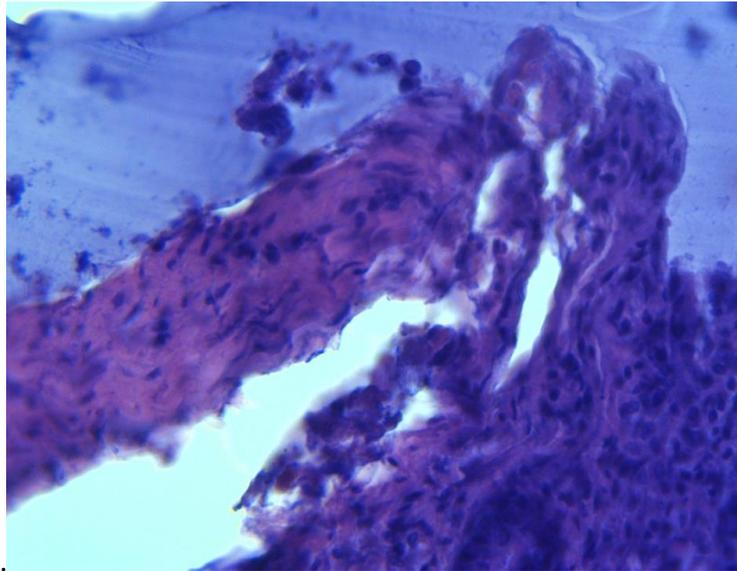


Figure 1 - Small intestine rat. The first day of modeling peritonitis. Infiltration of muscular and serous membranes. Edema of the serous membrane. Color: H&E, x100

At microscopic research of a liver the partial organization of a parenchyma is defined. Between the lobular layers is slightly thickened due to edema. Hepatocytes around the central vein are collected in beams, in another space they are arranged in a disorderly manner. Hepatocytes with enlarged pale-colored nuclei, in which the fibrous-granular pattern of chromatin is determined. Their cytoplasm is also pale basophil. In some hepatocytes in the cytoplasm are defined vacuoles of different sizes, in some there are eosinophilic inclusions. In general, we can state the inflammatory process in the small intestine and the manifestations of dystrophies in the liver parenchyma (Fig. 2). The vessels are dilated to the state of the gaps.

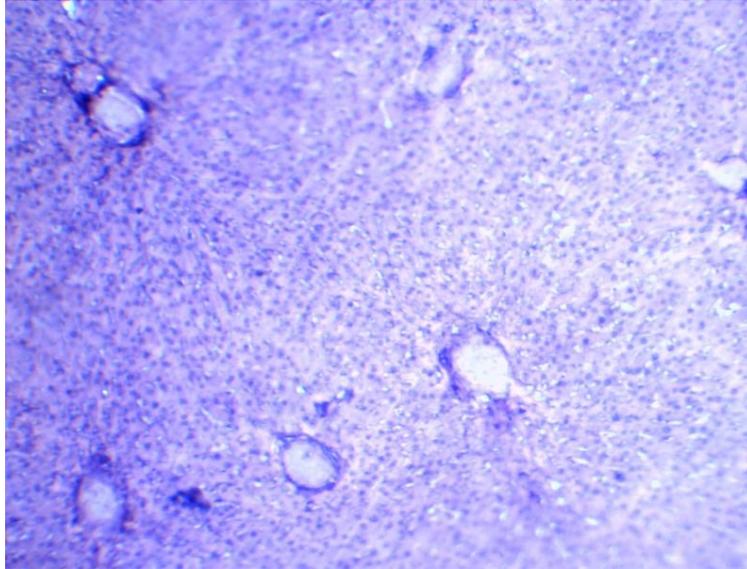


Figure 2 - Rat liver. The first day of the study. Hepatocyte disorder. Cytoplasmic vacuolization. Dilation of blood vessels. Stain: H&E, x100

*Histological examination research on the third day after simulation of fecal peritonitis*

Macroscopic examination of the abdominal organs on the 3rd day of peritonitis simulation determined the preservation of up to 1.5 ml of slightly turbid fluid. Abdominal membrane surface also retains turbidity, but its significant edema is not detected. The peculiarity of this term of the experiment is the adhesion of some intestine loops (they are separated during the revision with some effort) and the presence on the intestine surface accumulations of whitish homogeneous masses. The size of these accumulations ranged from 0.1 cm to 0.3 cm. On visual assessment, the surface of the liver is smooth, not shiny, brown liver tissue.

On microscopic examination, the serous membrane of the small intestine is swollen, but the visual degree of scattering of fibrous fibers is less than in the transverse period of the study (it is possible to visually separate fairly thick bundles of fibers, single fibers are not separated). Infiltration of lymphoid elements takes place, but their placement is quite sparse, in contrast to the previous stage.

In the muscular membrane, the edematous changes of the myositis bundles are not diffuse, as in the previous observation period, but are defined as separate fields. In this shell of the small intestine is a number of lymphoid elements. Muscular and serous vessels of

moderate blood supply, some spasms persist. The color of the cytoplasm of myocytes is moderate, the nuclei are elongated, dark. The submucosal plate is quite dense, violation of fibrous fibers occurs in separate fields. Lymphoid infiltration is represented by a disturbed placement of a number of lymphocytes.

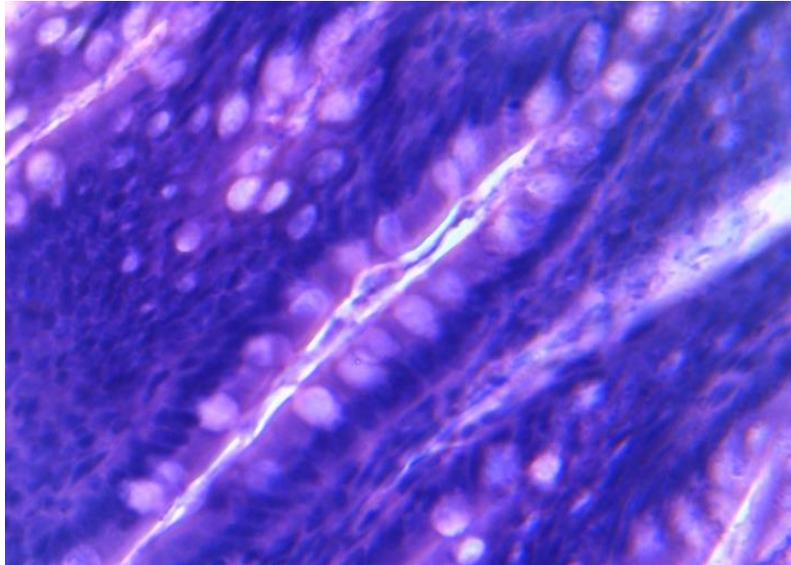


Figure 3 - The mucous membrane of the rats' small intestine. 3rd day of modeling peritonitis. Infiltration of the villi. Goblet-shaped expansion of part of the cells. H&E stain, x300

Villi of mucous membrane are different heights, in general, they are undamaged, although in isolated cases there is damage to the tips. Epitheliocytes are mostly close to normal, although the nuclei in most of them are rounded, but they are located in one layer. The cytoplasm is swollen, with transformation into goblet cells. Lymphoid elements that fill the villi are placed with moderate density (Fig. 3). Vessels in a part of villi of a usual kind, in a part their spasm is inserted.

Microscopic examination of the liver revealed the preservation of the lobular organization of the parenchyma, between the partial layers are thin, quite dense. Hepatocytes in the central part of the lobe are collected in beams, in the peripheral part of the lobe they form a single array. Central vein of moderate blood supply. The nuclei of part of hepatocytes of medium size of moderate staining. Part of the hepatocytes contains enlarged nuclei, well stained with a pronounced granular-fibrous pattern of chromatin. Cytoplasmic vacuolization of hepatocytes is preserved. Between some hepatocytes there are small inclusions of homogeneous eosinophilic mass (fig. 4).

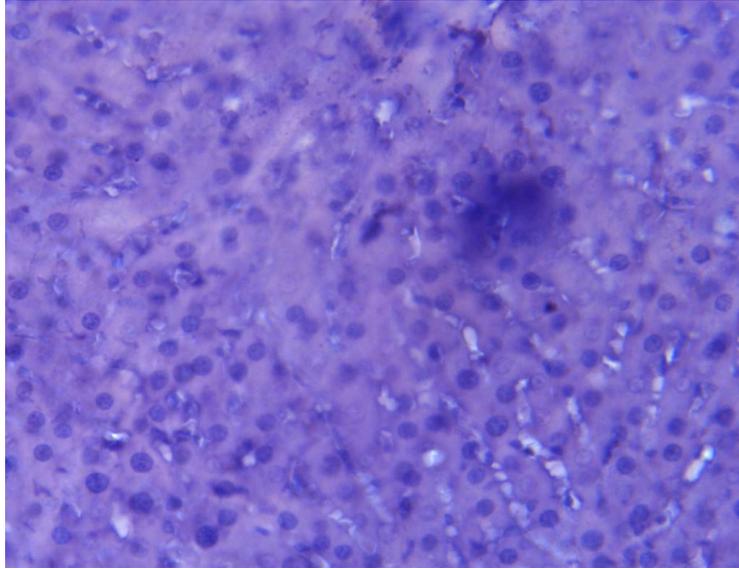


Figure 4 - Rat liver. 3rd day of modeling peritonitis. Hepatocytes and vacuoles in the cytoplasm are different in size. Stain: H&E, x300

*Histological examination results on the first day after simulation of fecal peritonitis with correction with antibiotics (12 hours after the first injection) and remediation with chlorhexidine*

Up to 1.5 ml of fluid was found in the abdominal cavity, it is vicious and quite transparent. The leaf of the abdomen that covers the intestine is visually swollen, there are single deposits of whitish masses in the form of individual lumps. Abdominal vessels of moderate blood supply. The color of the liver is brown with a crimson tinge. At microscopic research of a small bowel the serous cover is swollen, bunches of fibers are moderately scattered. Fibrocytes with oval dark nuclei. There is a moderate, diffuse infiltration of lymphocytes (Fig. 5). Vessels are partially spasmed, partially moderately stagnant blood supply. The muscle membrane also shows signs of edema. It identifies small clusters of lymphocytes, mostly near blood vessels. Myocytes are pale in color with dark small oval nuclei. A similar pattern is observed in the submucosal plate. In the mucosa of the normal form, epitheliocytes in them with enlarged rounded nuclei, and swollen cytoplasm. The villi are low, but intact, the vessels in the center of the villi are of moderate blood supply. Infiltrated mucosa and villi with a moderate number of lymphocytes.

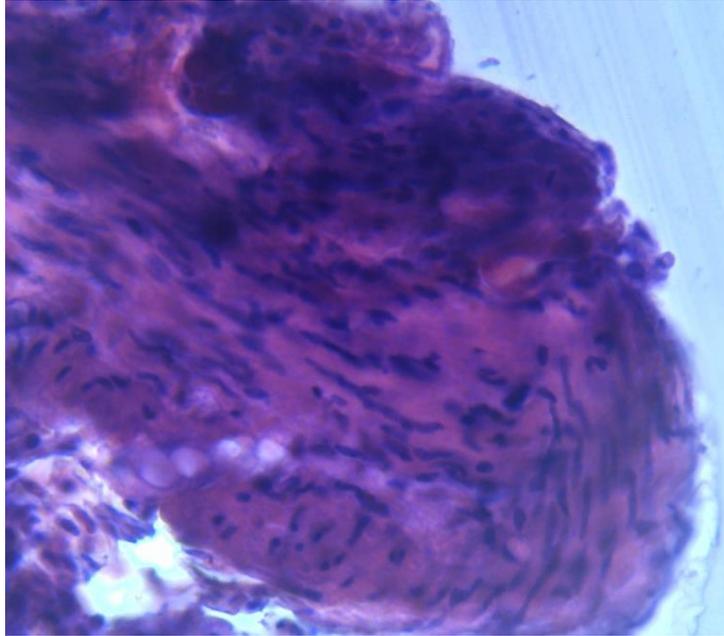


Figure 5 - Small intestine of the rat. 1 day of development of simulated peritonitis with correction by antibiotics and remediation with chlorhexidine. A fragment of the serous membrane. Edema, enlarged fibroblast nuclei, a few lymphocytes. Stain: H&E, x300

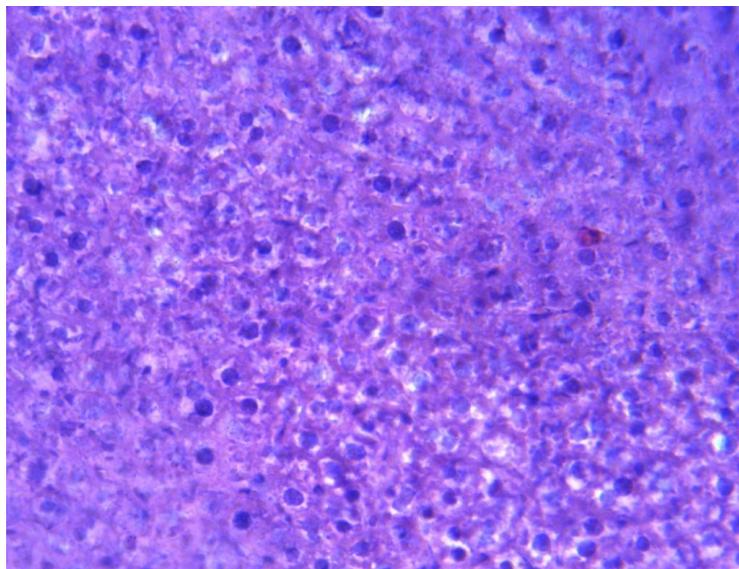


Figure 6 - Rat liver. 1st day of simulated peritonitis development with correction by antibiotics and rehabilitation with chlorhexidine. Poor ordering of hepatocytes. Distribution of inter-beam spaces. The nuclei of hepatocytes are enlarged, the vacuoles of the cytoplasm. Stain: H&E, x300.

Microscopic examination of the liver determined the preservation of the lobular organization of its parenchyma. The interparticle layers are thin and dense. The vessels of the triads are full-blooded. Hepatocytes in the lobe in the central zone are collected in beams, on the periphery form a solid array. Inter-beam spaces are common, Kupffer cells are swollen. Hepatocytes of medium size with enlarged nuclei, cytoplasm of the lump part. In some there are small and medium-sized vacuoles. Homogeneous inclusions in hepatocytes and intercellular space do not occur (Fig. 6). We can say about a moderate inflammatory reaction in the intestine and a moderate dystrophic reaction of the liver.

*Histological examination results on the third day after simulation of fecal peritonitis during antibiotic correction and remediation with chlorhexidine*

At macroscopic research of an abdominal cavity the following is established. The surface of the abdomen is visually swollen, but clean, transparent. Deposits on the leaf of the abdomen covering the intestine are not defined. Adhesion of intestinal loops was also not determined. The fluid in the abdominal cavity is almost undetectable.

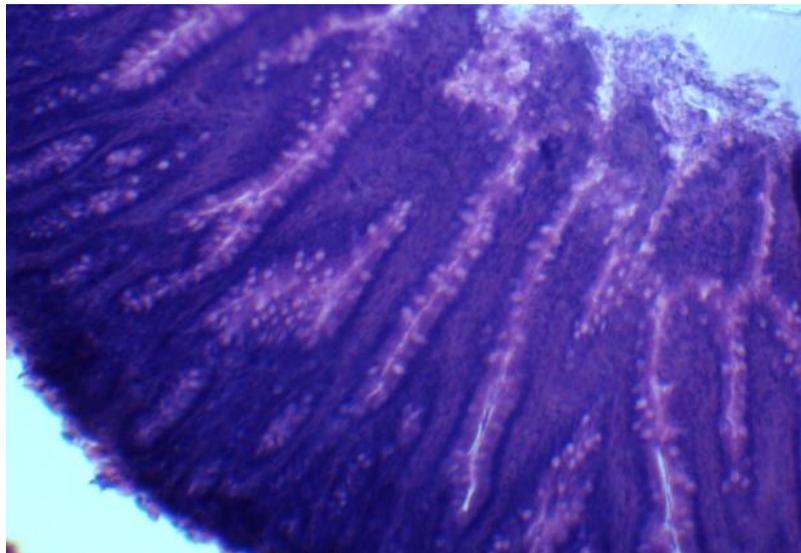


Figure 7 - Small intestine of the rat. 3rd day simulated peritonitis development with correction by antibiotics and remediation with chlorhexidine. Villi with a moderate content of histo-lymphoid elements, an increase in the goblet epitheliocytes of the villi. Stain: H&E, x100.

The liver is covered with a smooth leaf of the abdomen, its edge is sharp, the color of the tissue is brown.

Microscopic examination of the small intestine at this stage of the experiment showed the presence of edematous violation of the bundles of fibrous fibers, although there are areas where these bundles are tightly packed. Fibrocytes in moderate amounts of normal form. Epitheliocytes that cover the serous membrane - with enlarged fuzzy nuclei. Lymphocytes are diffusely scattered in moderation. The muscular membrane consists of myocytes of normal appearance, although the color of their cytoplasm is somewhat pale. Inter-bundle layers are widespread due to edema. There are lymphocytes, diffusely, in small quantities. Vessels of moderate blood supply. In the mucous membrane of the gland of normal appearance. Epitheliocytes with juicy colored medium-sized nuclei. Hairs of different heights, epithelium in the most usual kind but on a lateral back there are the increased cells which cytoplasm contains a large amount of mucus. In the middle of the villi is a moderate amount of histio-lymphoid elements and a vessel of moderate blood supply (Fig. 7).

At microscopic research of liver special changes of structure in comparison with control are not defined, only there is a presence of small vacuoles in some hepatocytes, and lumpiness of cytoplasm (fig. 8).

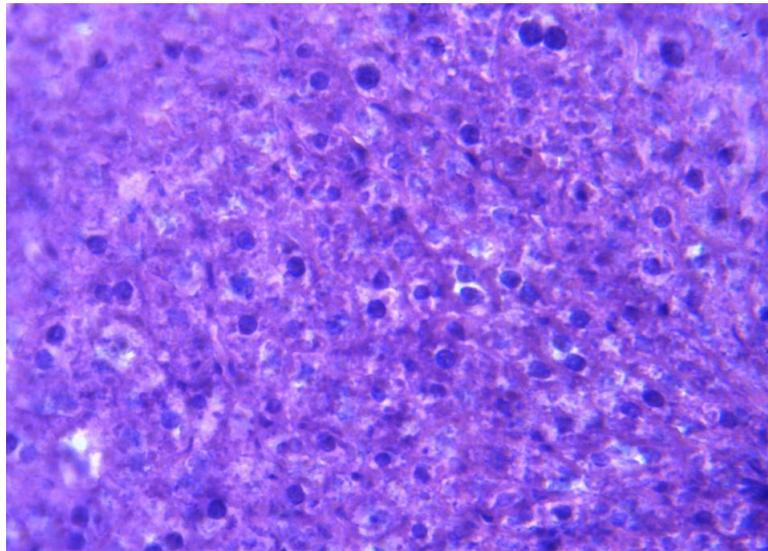


Figure 8 - Periphery of the hepatic lobe. 3rd day of simulated peritonitis development with correction by antibiotics and remediation with chlorhexidine. Hepatocytes with a lumpy cytoplasm, some with small vacuoles. Stain: H&E, x300.

Histological examination results on the twenty-first day after simulation of fecal peritonitis in the correction of antibiotics and remediation with chlorhexidine

Microscopic examination of the cat found that the serous membrane was almost normal in appearance. There are single areas of edematous violation of fibrinous fibers bundles. The epitheliocytes of the cubic epithelium partially retain the enlarged rounded nuclei. Vessels of moderate blood supply, some with thickened fibrous walls.

The muscular membrane does not differ in structure and appearance from that of control animals. The submucosal plate is dense, lymphocyte infiltration is not defined, part of the vessels with a thickened wall. Single fibrocytes of normal appearance.

In the mucous gland tubular, epitheliocytes with small juicy nuclei and homogeneous cytoplasm. The villi are also not visually different from the control. The epithelium that covers them has oval nuclei, which are located "palisade".

Microscopic examination of the liver did not reveal a significant difference in comparison with the data of intact animals, except for the expansion of the peripheral zone, where hepatocytes are not ordered.

*Histological examination results on the first day after simulation of fecal peritonitis in the correction of antibiotics, rehabilitation with chlorhexidine and the use of L-arginine solution*

Microscopic examination of the abdominal organs 1 day after modeling peritonitis, and 12 hours after the first administration of the corrective complex showed the following. No fluid was detected in the abdominal cavity. The surface of the abdomen is cloudy. Deposits of protein masses on the surface of the abdomen are not defined. The vessels of the abdomen are full-blooded, well visualized over the entire surface of the abdomen.

Microscopic examination of the intestine revealed that adventitia of the small intestine with signs of moderate edema (bundles of fibrous fibers are somewhat disturbed), a moderate number of fibrocytes, their nuclei are juicy, oval. Small vessels of adventitia of moderate blood supply. Lymphocytes are diffusely scattered in adventitia, their number is visually smaller than in rats with uncorrected peritonitis. Muscle plate with swollen common intercostal spaces. Normal-looking myocytes. The submucosal plate also has a normal appearance. Vessels of its moderate blood supply. Lymphoid elements are scattered throughout the plate in moderation. In the mucous membrane of the gland of normal tubular shape, epitheliocytes, which line them with enlarged nuclei, the cytoplasm is slightly swollen. The villi of the mucosa are quite high, the epithelium that covers them with oval nuclei, dark, the cytoplasm is slightly swollen. Some epitheliocytes are enlarged and their cytoplasm contains a lot of mucus (Fig. 9).

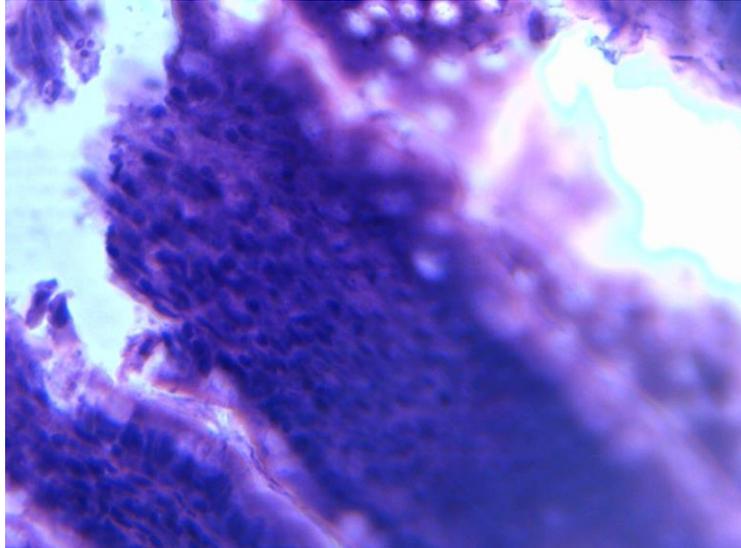


Figure 9 - The villi of the small intestine of the rat. 1 day of development of simulated peritonitis with correction by antibiotics, remediation with chlorhexidine and when using a solution of L-arginine. Enlargement of epitheliocytes with mucus filling of cytoplasm. Edema of epitheliocytes. Stain: H&E, \*300

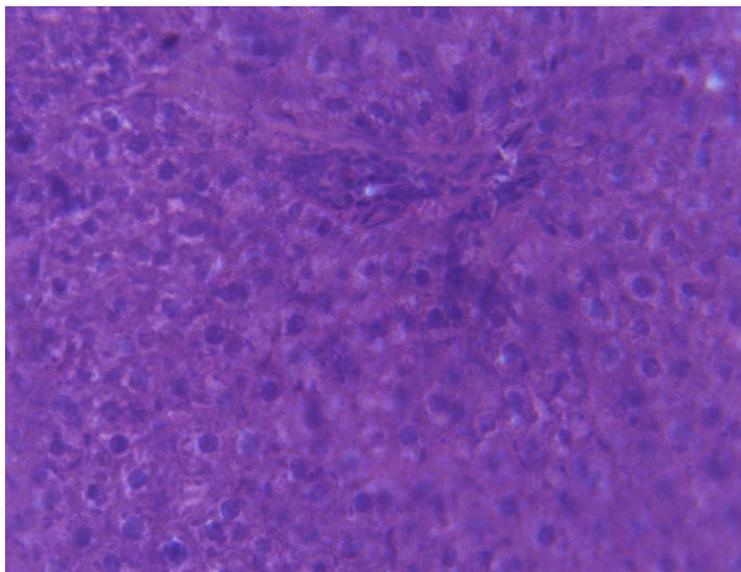


Figure 10 - Periphery of the rat liver lobe. 1st day of simulated peritonitis development with correction by antibiotics, remediation with chlorhexidine and when using a solution of L-arginine. Hepatocytes with dark nuclei of different sizes, lumpy cytoplasm. Stain: H&E, x300.

The liver on microscopic examination has a lobular organization. Interparticle layers are somewhat common, due to edema. Triad vessels, central vein dilated, moderate blood supply. Hepatocytes of medium size, dinuclear hepatocytes are not defined. The nuclei are

medium to moderate. The cytoplasm of hepatocytes is lumpy. The organization of a parenchyma in lobes corresponds to that at intact rats (fig. 10).

*Histological examination results on the third day after simulation of fecal peritonitis in the correction of antibiotics, rehabilitation with chlorhexidine and the use of L-arginine solution*

At macroscopic research of abdominal organs condition on the 3rd day of experiment the following is established. The surface of the abdomen is moist, muddy in places, but generally shiny. The vessels are moderately full-blooded. Deposits on the surface of the abdomen are not defined.

At audit of intestines the phenomenon intestines loops sticking is not established, liquid in an abdominal cavity is not defined. Macroscopic the liver does not differ from that at healthy rats.

On microscopic examination of the intestine, the adventitial membrane is formed by densely packed thin bundles of fibrinous fibers and scattered fibrocytes of normal appearance. Small vessels are full-blooded. The muscle membrane differs from the control group by a slightly pale color of the myocytes cytoplasm and scattered single lymphocytes. The vessels are also full of blood. In myocytes, the nuclei are oval, juicy. The submucosal plate is visually dense, there are single lymphocytes. The intermediate is also dense, dark eosinophilic color.

The glands of the mucous membrane are tubular, their cytoplasm is weakly basophil, the nuclei are partially enlarged. Moderate color, partly small dark.

Villi of various heights, vessels in most of them of moderate blood supply, in a part of villi they are spasmed. Lymphoid cells are not densely arranged in the middle of the villi. The villi are covered with a single layer of epithelium. Most epitheliocytes have oval, medium-sized nuclei.

Although there are cells with an enlarged round light nucleus. Some epitheliocytes are sharply enlarged, with the cytoplasm full of mucus. The villi are all intact (Fig. 11).

In the liver, microscopic examination of the lobular structure is preserved. Interparticle layers are thin, dense. The vessels of the triads and the central vein are full-blooded. Hepatocytes in most of the lobe are packed in beams. They are medium, single core. The nuclei are rounded of moderate homogeneous color. The cytoplasm is lumpy. Inter-beam spaces are common. Kupffer cells are swollen. There are hepatocytes with small vacuoles in the cytoplasm. Intercellular deposits are not defined (fig. 12).

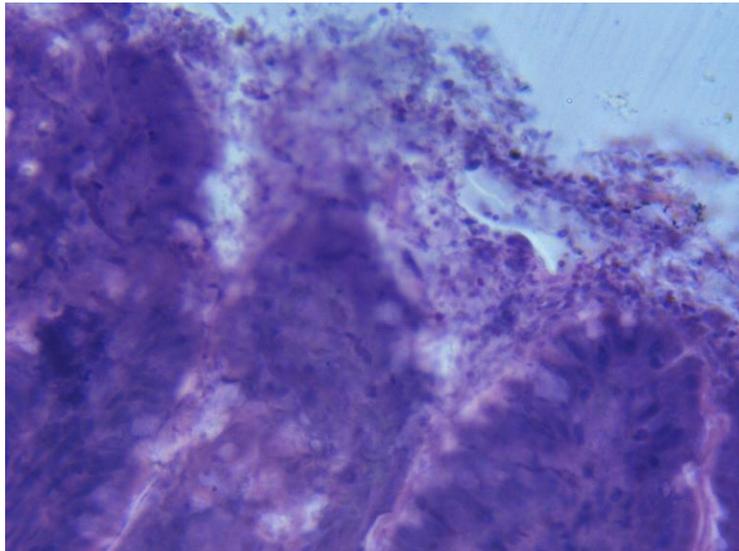


Figure 11 - The small intestine villi of the rat. 3rd day of simulated peritonitis development with correction by antibiotics, remediation with chlorhexidine and when using a solution of L-arginine. Epitheliocytes of the preserved villi with middle nuclei, some epitheliocytes with increased mucus content. Stain: H&E, x300

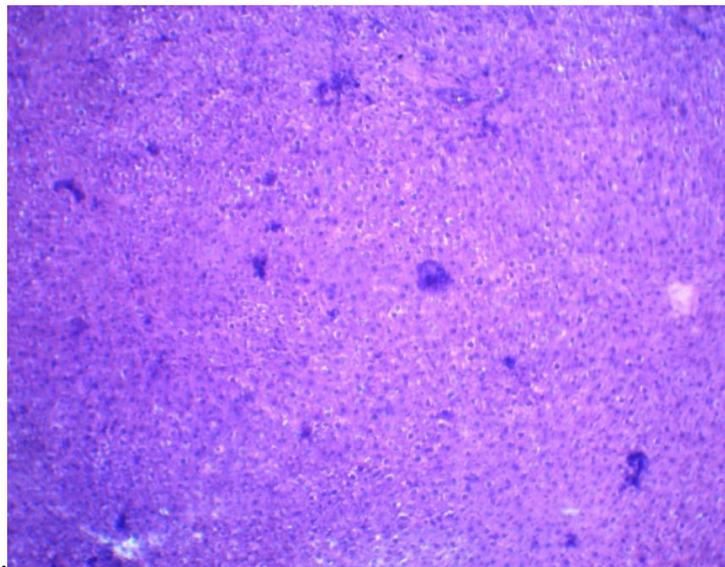


Figure 12. Rat liver. 3rd day of simulated peritonitis development with correction by antibiotics, remediation with chlorhexidine and when using a solution of L-arginine. Hepatocytes of moderate size. The cores are well painted. Different sizes. Elsewhere, vacuoles in hepatocytes. Stain: H&E, \*100.

*Histological examination results on the twenty-first day after simulation of fecal peritonitis in the correction of antibiotics, rehabilitation with chlorhexidine and the use of L-arginine solution*

Macroscopic and microscopic examinations of the intestine, liver and abdomen did not reveal significant differences from the picture we observed in control rats (Fig. 13).

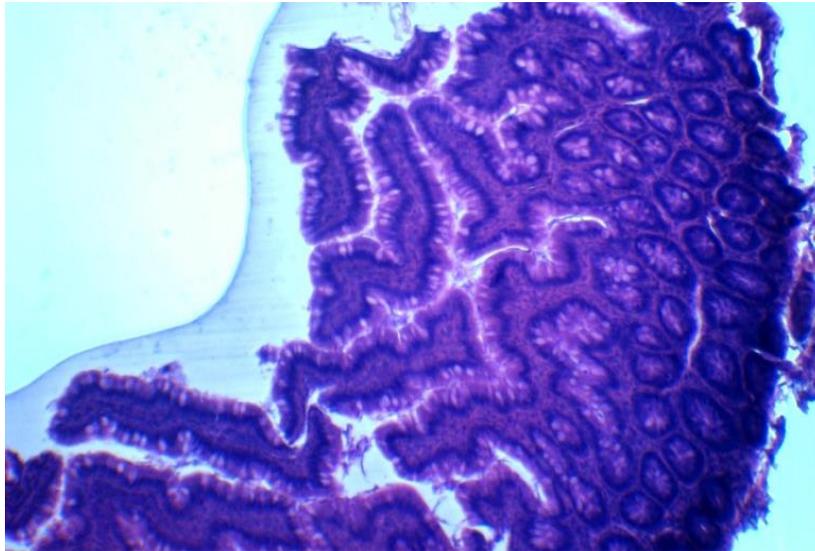


Figure 13 - The mucous membrane of the small intestine of rats. 21st day after simulation of peritonitis with antibiotics correction, remediation with chlorhexidine and when using a solution of L-arginine. Stain: H&E, x100.

### **Conclusions:**

1. Histologically confirmed the experimental peritonitis development in all groups of the experiment.
2. In group №2 on the third day the deterioration of the studied structures was established.
3. In the group in which the experimental peritonitis was corrected with antibiotic therapy and remediation with chlorhexidine morphologically proven effectiveness of the correction.
4. In the group of animals that received antibiotic therapy, remediation with chlorhexidine and correction by nitric oxide donor for the correction of simulated peritonitis, a more pronounced normalization of the state of the organism was histologically established.
5. At the last stage of the study in group 4, macroscopic and microscopic examinations of the intestine, liver and abdomen did not reveal significant differences from the histological picture we observed in rats of the intact group.

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