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## MORPHOFUNCTIONAL CHARACTERISTIC OF HEMOMICROCIRCULATORY BED IN THE RED BONE MARROW WHEN MODELING AN ACUTE ASEPTIC INFLAMMATION IN RATS

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In modern medicine, the structural components in various organs and tissues have been studied in great detail, but at the same time there are no works which would study hemomicrocirculation in the red bone marrow in the modeling of acute aseptic inflammation in rats, where reactive changes of morphological and functional character take place. The purpose of the work was to study the morphological changes of the components of the hemomicrocirculatory bed in the red bone marrow of rats when modeling an acute aseptic inflammation. The work was performed on 50 mongrel white rats, divided into 2 groups: Group I–5 intact animals, Group II included 45 animals, which were simulated an acute aseptic peritonitis. In experimental groups of animal, an immediate, but reversible, increase in the penetration of venules and capillaries was observed, due to the active reduction of microfilaments in endothelial cells. In vascular lumens, during the experiment, a sludge syndrome was observed, which led to the difficulty of perfusion of blood corpuscles through the wall of the venule, which is a morphological confirmation of the inflammatory process.

**Key words:** red bone marrow, hemomicrocirculatory bed, inflammation.

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One of the problems of morphology is the study of the principles of structural components and the functioning of hemomicrocirculatory pathways on the level of the bloodstream, as a link that provides the whole complex of metabolic processes of the organism. In modern medicine, the structural components in various organs and tissues have been studied in great detail [1, 5, 8, 10, 12], but at the same time there are no works which would study hemomicrocirculation in the red bone marrow in the modeling of acute aseptic inflammation in rats, where reactive changes of morphological and functional character take place [3].

The study of the morphology and the functions of a vascular bed in red bone marrow, in normal and in different pathological conditions, allows us to determine which morphological mechanisms occur during blood regeneration, since a large number of blood corpuscles dies in an adult organism daily, while the number of blood cells in the peripheral bed remains quite stable. This is due to the fact that the dead cells are replaced by new ones that are formed in the organs of hematopoiesis [2, 3].

As it is known, the magistral vessels, which provide the trophism of the red bone marrow of the rat's femur, did not undergo a significant change in topographic anatomy in all experimental groups [2]. It is morphologically found that components of the hemomicrocirculatory bed in the red marrow of the rats make a multicomponent system, which consists of a certain set of typical units: arterioles, capillaries and venules. Blood capillaries of the red bone marrow in a rat have distinct differences and are both nonfenestrated and fenestrated. Nonfenestrated blood capillaries that provide the trophism of the red marrow are a continuation of the blood vessels that branch off the precapillary arterioles that adhere sufficiently close to the bone tissue. Sinusoidal (fenestrated) capillaries branch off the somatic capillaries and are located predominantly in cells, perforating the erythroblastic islets [2, 3]. Thus, the fact of the topographic differentiation of a single integral hemomicrocirculatory bed of the red marrow has been established, which results in a peculiar functional distribution.

**The purpose** of the work was to study the morphological changes of the components of the hemomicrocirculatory bed in the red bone marrow of rats when modeling an acute aseptic inflammation.

**Materials and methods.** The work was performed on 50 mongrel white rats, divided into 2 groups: Group I – 5 intact animals, Group II included 45 animals, which were simulated an acute aseptic peritonitis by intraperitoneal administration of 5 mg  $\lambda$ -carrageenan (Sigma, USA) in 1 ml isotonic solution of sodium chloride for one animal.

The animals were withdrawn from the experiment on the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup>, 10<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 30<sup>th</sup> day by an overdose of thiopental anesthesia. The study of red bone marrow was carried out in accordance with the established terms of the experiment.

The material for microscopic study was taken immediately after euthanasia of animals, the femur carefully was separated from soft tissues, followed by its fixation in 10% formalin solution for 24–48 hours. Subsequently, fragments of the femur were subjected to decalcification with ethylenediaminetetraacetic

acid. The material of the red bone marrow was thickened in paraffin according to the generally accepted technique and sections were produced on the sliding microtome snuff micro-tissue MC-2 in thickness (4-5) microns, which were stained with hematoxylin and eosin according to Van Gieson[4].

Morphometry and microphotography were performed using Biorex-3 BM-500T microscope with digital DCM 900 photomicrographic attachment with programs adapted for such studies.

Statistical processing of morphometric data was performed using the program Exel [6].

During the experiment, the international principles of the European Convention on “the Protection of Vertebrate Animals used for Experiments and Other Scientific Purposes” (Strasbourg, 1986) were kept [11].

**The results of the research and discussion.** In the study of histologic preparations, it was found that the red bone marrow of the intact rats consisted of the stromal, vascular and cellular components. The stromal component is represented by the bone lamellae and reticular tissue. The vascular component consisted of arterioles, capillaries of somatic and sinusoidal type, and venules. The cell component is represented by cells: proerythroblasts, basophilic erythroblast, polychromatophilic, and orthochromic erythroblasts, which formed an erythroblastic islet with a centrally located macrophage.

As a result of the morphometric study, the average lumen diameters of the vessels in the hemomicrocirculatory bed of the red marrow in the rats of the intact group were established: for arterioles –  $18.47 \pm 1.06 \mu\text{m}$ ; for somatic capillaries –  $5.71 \pm 0.98 \mu\text{m}$ ; for sinusoidal capillaries –  $28.53 \pm 2.37 \mu\text{m}$ ; for venules –  $50.97 \pm 3.28 \mu\text{m}$ . In their lumens, erythrocytes in the form of double-concaved discs were determined predominantly.

The average diameter of the somatic capillaries was 4.9 times smaller than of the sinusoidal capillary, which indicates their different functional significance.

The conducted histological studies showed that the magistral vessels that provide the trophism of the red marrow in the femur of the rats did not undergo anatomically significant changes in all experimental groups.

Having done the morphologic and morphometric analysis of the average lumen diameter of the hemomicrocirculatory bed in the experimental group, it was found that during the whole experiment the mean value was uneven and a vascular reaction of the red marrow was revealed, which was manifested in the form of enlargement of its lumens and spasming at different terms of the experiment.

The analysis of the average diameters of arterioles showed that on the 1<sup>st</sup> day of the experiment there was a decrease in their lumen, compared with the intact group of animals, which continued to decrease to the 2<sup>nd</sup> day. From the 3<sup>rd</sup> and 5<sup>th</sup> days, an increase in the arterioles' diameter was observed, with  $p < 0,05$ , in comparison with the previous term. The maximum mean of the average diameters of the arterioles was reached on the 10<sup>th</sup> day, expanding by 1.17 times, at  $p < 0.05$ , in comparison with the previous experimental period, from the 14<sup>th</sup> to the 21<sup>st</sup> day their expansion was observed. From the 30<sup>th</sup> day, there was a reliable tendency, at  $p < 0,05$ , in comparison with the previous experimental period, to the restoration of the parameters of the average lumen diameter in arteriols (table).

Table

**Changes' dynamics in the components of the red marrow hemomicrocirculatory bed during experimental inflammation ( $\mu\text{m}$ )**

Day	Arterioles	Capillaries	Venules
Intact animals	$18,47 \pm 1,06$	$28,53 \pm 2,37$	$50,97 \pm 3,28$
1 day	$17,56 \pm 1,04^*$	$55,87 \pm 2,92^*$	$77,51 \pm 2,88^*$
2 day	$15,64 \pm 1,08^*$	$52,21 \pm 2,94^*, *$	$79,01 \pm 2,79^*, *$
3 day	$16,07 \pm 1,09^*$	$53,35 \pm 2,83^*$	$85,11 \pm 2,67^{*, **}$
5 day	$19,81 \pm 1,07^{*, *}$	$45,46 \pm 2,91^{*, *}$	$89,19 \pm 2,29^{*, *}$
7 day	$28,08 \pm 1,06^{*, *}$	$54,21 \pm 2,92^{*, *}$	$90,21 \pm 2,86^{*, *}$
10 day	$32,73 \pm 1,05^*$	$54,73 \pm 2,48^*$	$92,14 \pm 2,67^{*, *}$
14 day	$26,12 \pm 1,08^*$	$55,34 \pm 2,49^{*, *}$	$58,11 \pm 2,92^{*, *}$
21 day	$20,71 \pm 1,05^{*, *}$	$36,81 \pm 2,26^{*, *}$	$57,08 \pm 2,87^{*, *}$
30 day	$19,21 \pm 1,07$	$29,44 \pm 2,92$	$51,48 \pm 3,84$

Note 1. \* –  $p < 0,05$  in comparison with the contact group.

Note 2. \*\* –  $p < 0,05$  in comparison with the previous observation term.

Histologically, at the light-optical and ultramicroscopic levels, the signs of the inflammatory process were determined even in the wall of the arterioles. It was found that though the wall of the arterioles retained the typical three-layer structure, but the nuclei of the endotheliocytes were intensively protruded into the vessels' lumen, the internal elastic membrane, when stained with a polychrome dye, had the appearance of a basophilic strip that formed numerous tall layers. Such changes in the internal elastic membrane were not observed in the intact group of animals. It was determined that the overwhelming

majority of the nuclei of smooth myocytes in the middle membrane of arterioles acquired various forms, and the arteriol's lumen itself was densely filled with blood corpuscles.

It was established that the metabolic link of the hemomicrocirculatory bed, on the introduction of  $\lambda$ -carrageenan, reacted with a dilation from the 1<sup>st</sup> to the 3<sup>rd</sup> day of the experiment, which was conditioned, first of all, by the development of tissue hypoxia in the red marrow due to spasm of resistive vessels, but the wall retained a layered structure. An electron microscopic study of the elements of the hemomicrocirculatory bed has established that from the 3<sup>rd</sup> day of observation, changes in the luminal circuit of endotheliocytes, which has a non-uniform run, due to numerous protrusions, the basement membrane maintains continuity. On the 5<sup>th</sup> day, the decrease in arteriol diameter was observed, in comparison with the previous term, but these parameters significantly differ from the intact group at  $p < 0.05$ . From the 7<sup>th</sup> to the 14<sup>th</sup> day, the expansion of the average diameter of the vessels appeared at  $p < 0.05$ , in comparison with the previous term, and starting from the 30<sup>th</sup> day of the experiment, their diameter approached to the parameters of the intact group of animals.

It was proved that the average diameter of venules, during the experiment, also significantly increased, the analysis of the parameters of the diameters indicates that the increase was irregular, thus from the 1<sup>st</sup> to the 7<sup>th</sup> day, the gradual expansion of the vessels was observed. The maximum value of the venules diameters was reached on the 10<sup>th</sup> day of observation, with  $p < 0.05$ , in comparison with the previous experiment term and the indicators of the intact group of animals. The 5<sup>th</sup>, 7<sup>th</sup> and 10<sup>th</sup> days were characterized by expanded venules diameters, whereas from the 21<sup>st</sup> day, there was a decrease in the diameter of vessels' lumens, with  $p < 0.05$  as compared to the previous observation period, and from the 30<sup>th</sup> day of the experiment their diameter approached the indicators of the intact group of animals.

Morphologically, the wall of the capillaries was thinned, the restoration of the morphofunctional state of metabolic hemomicrovessels in the early stages of the experiment was not observed. Histologically and morphometrically, it was found that capillaries and venules also tended to expand in response to acute aseptic inflammation and remained enlarged to the end of the experiment.

The wall of the venules preserved the typical structure, but it was thinned, hyperemia was observed, and the blood corpuscles densely filled the lumens.

The ultramicroscopic examination revealed changes that were manifested by decompensation processes in the selective penetration and barrier function of the microvascular wall. Endothelial cells became swollen, which led to the formation of folds, lacuna, protrusions, and as a result, the size and shape of the vascular lumen changed significantly, from the right round or oval to the wrong one. Alongside with this, endothelial cells showed a loss of the order and the uniformity of microfilaments and myofilaments in myocytes, as well as the separation of endothelial contacts with the formation of clefts through which an excess fluid penetrated into the parenchyma of the red marrow from the blood plasma. As a result of these pathomorphological changes at the ultramicroscopic level, it is noted that in the areas of folds and protrusions of the endotheliocytes cytoplasm, the merge of pinocytic vesicle and the formation of vacuoles took place, which led to their subsequent separation into vascular lumen and subsequent necrotic and apoptotic changes.

The reaction of the microvessels in the experimental group had a general tendency of development and manifested itself in the form of spasms with subsequent dilation of the arterioles in response to the expansion of the lumen of capillaries and venules and had a gradual nature.

In experimental groups of animal, an immediate, but reversible, increase in the penetration of venules and capillaries was observed, due to the active reduction of microfilaments in endothelial cells (Fig. 1).

In vascular lumens, during the experiment, a sludge syndrome was observed, which led to the difficulty of perfusion of blood corpuscles through the wall of the venule, which is a morphological confirmation of the inflammatory process (Fig. 2).

In the previously conducted experimental studies, on the state of the vessels of the hemomicrocirculatory bed, under the influence of an acute aseptic inflammation, the differentiated response of the vascular system is traced. Thus, the comparative analysis of morphometric parameters of the diameter of the elements in the hemomicrocirculatory bed of the adrenal medulla revealed the following changes: for the 1 day the diameter of the arterioles increased reliably compared to the indicator of the intact group ( $p < 0.05$ ) and decreased unreliably by 7-10 days ( $p > 0.05$ ). The exchange link of the hemomicrocirculatory bed in the adrenal cortex of the II<sup>nd</sup> group also varied in different directions.

Comparing capillary diameter changes with intact animal data, a significant decrease in capillary diameter was revealed on the 5<sup>th</sup> and 21<sup>st</sup> days ( $p < 0.05$ ), with a maximum increase on the 3<sup>rd</sup> and 14<sup>th</sup> days of the study, the difference in significance was ( $p < 0.05$ ). In the statistical analysis of the average diameter

of the capacitive link (venules) in the hemomicrocirculatory bed of the adrenal cortex, the following changes were established: a significant decrease of the indicator compared to the intact group ( $p < 0.05$ ) was observed on the 1<sup>st</sup> day. The same trend preserved for 7 days, within 2-3 and 10-14 days a significant increase in venule diameter was detected ( $p < 0.05$ ).

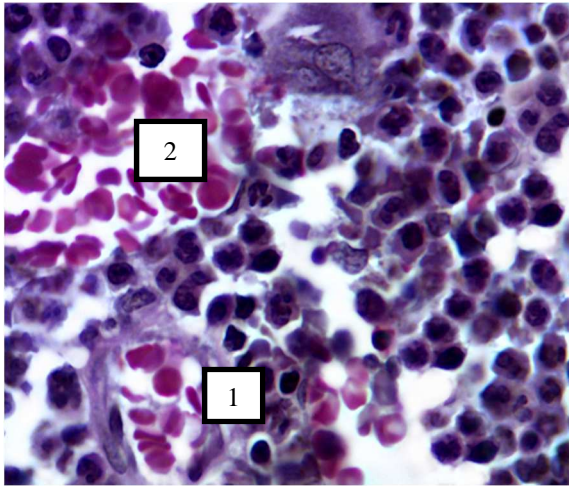


Figure 1. Red bone marrow of rats in the early stages of acute aseptic inflammation. 1 – arteriole; 2 – venula. Coloring: polychrome dye. Increase: ocular: 10; object.100 (oil immersion).

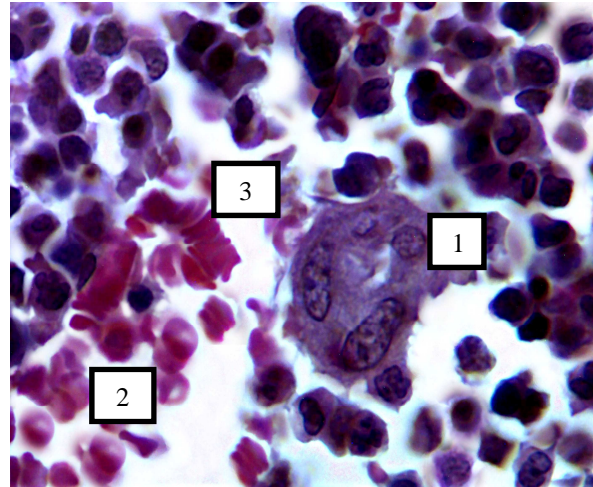


Figure 2 Red bone marrow of rats in the late stages of acute aseptic inflammation. 1 - megakaryocyte; 2 - venules; 3 - sludge-syndrome. Coloring polychrome dye. Increase: ocular: 10; object100 (oil immersion).

On the 5<sup>th</sup> -7<sup>th</sup> days and 21-30 days there was a significant decrease in diameters ( $p < 0.05$ ). Examining the changes in the diameter of the sinusoidal capillaries and venous sinuses during aseptic inflammation, certain common features were revealed - on the 1<sup>st</sup> day of inflammation, there was a significant increase in the diameter of sinusoids and venous sinuses ( $p < 0.05$ ). But on the 2<sup>nd</sup> -3<sup>rd</sup> days, a sharp decrease in the diameter of both units ( $p < 0.05$ ) was detected, from the 5<sup>th</sup> day of inflammation, the diameter of sinusoids significantly increased ( $p < 0.05$ ), whereas, from the 7<sup>th</sup> day, an unreliable increase in the size of venous sinuses was observed ( $p > 0.05$ ) [7].

In the studies of the reaction of the hemomicrocirculatory bed in the mucous membrane of the empty intestine during transplantation of cryopreserved placenta at the acute aseptic inflammation of the peritoneum in rats, it was found that the arterioles and capillaries initially decrease in diameter substantially on the 2<sup>nd</sup> day, and then increase substantially in diameter on the 3<sup>rd</sup>-10<sup>th</sup> day. Thus, with the introduction of cryopreserved placenta on the background of acute aseptic inflammation of the peritoneum, the development of inflammation and its correction is reduced by 4-5 days [9], and at the same time the reaction to the restoration of the blood vessels of the hemomicrocirculatory bed can be traced at the later stages of the experiment.

As a result of complex morphological researches it is found out that in the modeling of an acute aseptic inflammation significant morphological changes of the hemomicrocirculatory bed in the red marrow occur. The capillaries of the sinusoidal type are the main link of the vascular bed, as it is necessary for the organism's vital activity to have daily blood renovation, and this process is provided by the capillary bed of the red bone marrow.

### Conclusions

1. At the early stages of the experiment during acute aseptic inflammation (1st - 14th day) there were significant changes of vessels of the hemomicrocirculatory bed in the red marrow. Arterioles spasmed on the 2<sup>nd</sup> day, their average lumen diameter significantly decreased by 1.18 times. From the 7<sup>th</sup> to the 14<sup>th</sup> day, they tended to expand with a reliable maximum value on the 10<sup>th</sup> day in 1.77 times. The capillaries of the sinusoidal type reached maximal expansion on the 1<sup>st</sup> day, having increased in 1.96 times, and the venules' lumen on the 3<sup>rd</sup> day increased in 1.67 times ( $p < 0.05$ ), which corresponded to the general principles of vascular reactions in response to the inflammatory process.

2. At the late terms of the experimental inflammation (21<sup>st</sup> and 30<sup>th</sup> days) it was found morphometrically that the arterioles were significantly expanded in 1.12 times on the 21<sup>st</sup> day and till the 30<sup>th</sup> day they did not acquire the values of the intact group of animals. The capillaries had increased lumens – in 1.29 times on the 21<sup>st</sup> day, and on the 30<sup>th</sup> day this figure almost reached the norm. At the late terms of the study venules remained expanded, which confirmed the continuation of the vascular reaction in response to the acute aseptic inflammation.

References

1. Bilash S.M. Structural reconstruction of the hemomicrocirculatory bed of the gastric wall during experimental gastritis and the introduction of the preparation "Platex-placental". Clinical Anatomy and Operative Surgery. 2013; 12 (3): 44-6.
2. Bilash S.M., Boruta N.V. Morphology of the red bone marrow at the introduction of cryopreserved placenta in the background of acute aseptic inflammation in the early stages of the experiment. The Scientific Method 2017; 1.6 (6): 21–24.
3. Boruta N.V. Morphological changes of structural elements of red bone marrow of rats in acute aseptic inflammation of the peritoneum / Bulletin on problems of biology and medicine. 2017;1(135):273-277.
4. Goralsky L.P., Khomych V.T., Kononsky O.I. Fundamentals of histological technique and morphofunctional methods of research in norm and at pathology. Zhytomyr: Polissya; 2011;288 p.
5. Kramarenko D.R., Yeroshenko H.A., Nebesna Z.M. Structural adjustment of the capacitive unit of the hemomicrocirculatory bed after the action of 1% ester of methacrylic acid. The world of medicine and biology. 2019; 3 (69): 194-97.
6. Lapach S.N., Chubenko A.B., Babich P.N. Statistical Methods in Biomedical Researches Using Excel. Kiev: Morion; 2000: 320.
7. Shotarenko T.A., Shepitko V.I. Reaction of hemomicrocirculatory bed of adrenal cortex and adrenal medulla during acute aseptic peritonitis. The world of medicine and biology. 2015; 4 (54): 139-41.
8. Shepitko V.I., Yeroshenko G.A. Morphofunctional characteristic of the mandibular salivary gland of rats in experimental acute aseptic sialadenitis. Bulletin on problems of biology and medicine. 2012; 1(91): 238-41.
9. Shepitko K.V. The reaction of the hemomicrocirculatory bed of the mucous membrane of the empty intestine at the transplantation of cryopreserved placenta during acute aseptic inflammation of the peritoneum in rats. Bulletin on problems of biology and medicine. 2015; 4 (2): 255-60.
10. Shepitko V.I., Skotarenko T.A., Yeroshenko G.A., Lysachenko O.D. Ultrastructural characterization of adrenal cortex in acute aseptic peritonitis and its correction by introduction of cryopreserved placenta. The world of medicine and biology. 2016; 3 (57): 161-64.
11. European convention for the protection of vertebrate animals used for experimental and others scientific purposes. Strasbourg: Council of Europ. 1986;123.52.
12. Yeroshenko G.A., Shevchenko K.V., Yakushko O.S. Hsee of morphometric characteristics of rat salivary glands hemomicrovasculature capacity component under normal conditions and in ethanol chronic intoxication. Bulletin on problems of biology and medicine. 2018;3(65):149-52.

Рефераты

**МОРФОФУНКЦИОНАЛЬНА ХАРАКТЕРИСТИКА ГЕМОМІКРОЦИРКУЛЯТОРНОГО РУСЛА ЧЕРВОНОГО КІСТКОВОГО МОЗКУ ПРИ МОДЕЛЮВАННІ ГОСТРОГО АСЕПТИЧНОГО ЗАПАЛЕННЯ У ЩУРІВ**

**Борута Н.В.**

В сучасній медицині досить докладно досліджені структурні компоненти в різних органах і тканинах, і поряд з тим відсутні роботи в яких розглядаються дослідження гемомікроциркуляції в червоному кістковому мозку при моделюванні гострого асептичного запалення у щурів, де і відбуваються реактивні зміни морфологічного та функціонального характеру

В експериментальній групі тварин спостерігалось негайне, але реверсивне, збільшення проникності венул і капілярів, завдяки активному скороченню мікрофіламентів в ендотеліальних клітинах.

В просвітах судин, під час експерименту, спостерігався сладж-синдром, який призвів до утруднення перфузії формених елементів крові через стінку венул, що є морфологічним підтвердженням запального процесу.

**Ключові слова:** червоний кістковий мозок, гемомікроциркуляторне русло, запалення.

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**МОРФОФУНКЦИОНАЛЬНАЯ ХАРАКТЕРИСТИКА ГЕМОМІКРОЦИРКУЛЯТОРНОГО РУСЛА КРАСНОГО КОСТНОГО МОЗГА ПРИ МОДЕЛИРОВАНИИ ОСТРОГО АСЕПТИЧЕСКОГО ВОСПАЛЕНИЯ У КРЫС**

**Борута Н.В.**

В современной медицине достаточно подробно исследованы структурные компоненты в разных органах и тканях, вместе с тем отсутствует работы в которых рассматриваются исследования гемомікроциркуляції в красном костном мозге при моделировании острого асептического воспаления у крыс, где и происходят реактивные изменения морфологического и функционального характера В экспериментальной группе животных наблюдалось реверсивное увеличение проницаемости венул и капилляров, благодаря активному сокращению микрофиламентов в эндотелиальных клетках.

В просветах сосудов, во время эксперимента наблюдался сладж-синдром, который привел к затруднению перфузии форменных элементов крови через стенку венул, что является морфологическим подтверждением воспалительного процесса.

**Ключевые слова:** красный костный мозг, гемомікроциркуляторное русло, воспаления.

Рецензент Шепітько В.І.