

Original article

Reprint

Levels of CD45+ and CD3+ cells in the liver during physical exertion of varying intensity and after administration of meldonium

Alexander A. Yusov  , Olga V. Alpidovskaya 
 yusov1961@yandexl.ru

Chuvash State University, Cheboksary, Russia

Received 27 December, 2024, Accepted 29 September 2025



© This article is an open access publication. Russian Text. Published in *Saratov Journal of Medical Scientific Research* 2025; 21 (3): 340-345. <https://doi.org/10.15275/ssmj2103340> ISSN 1995-0039.

Abstract:

Objective: to assess the proportion of leukocytes (CD45+ cells) and T-lymphocytes (CD3+ cells) in the liver during physical exertion of varying intensity with and without the use of meldonium.

Materials and Methods. The experiments were conducted on male Wistar rats performing physical exercise of varying intensity. Meldonium was added to the feed of the experimental animals at a rate of 100–120 mg/kg of body weight during water-based exercise. After the experiment, liver samples were taken, sections were prepared and stained with hematoxylin and eosin, and immunohistochemical reactions were performed for CD45+ and CD3+ cell markers.

Results. Under heavy physical exertion, the proportions of CD45+ and CD3+ cells in the liver parenchyma increased 3.2- and 2.9-fold, while perivascularly they increased threefold and 6.3-fold ($p=0.001$), respectively, vs. the intact group. After the administration of meldonium during heavy physical exertion, the percentage of CD45+ cells decreased in the liver parenchyma and perivascularly by 1.8 times ($p=0.008$), the proportion of CD3+ cells declined by 1.7 times in the liver parenchyma ($p=0.001$) and by 2.3 times around the blood vessels ($p=0.004$) vs. intact animals. Histologically, inflammation and degenerative changes in the liver tissue decreased.

Conclusion. The use of meldonium during heavy physical exertion led to a decrease in the percentage of CD45+ and CD3+ cells due to a reduction in the degree of inflammatory infiltration of the liver tissue and the severity of the destructive process.

Keywords: physical exertion, liver, CD45+, CD3+, meldonium

Cite as: Yusov AA, Alpidovskaya OV. Levels of CD45+ and CD3+ cells in the liver during physical exertion of varying intensity and after administration of meldonium. *Saratov Medical Journal* 2025; 6 (3): e0304. <https://doi.org/10.15275/sarmj.2025.0304>

Introduction

The favorable impact of sports on health has never been questioned and has long been scientifically proven. However, there is a high probability of sudden death due to acute physical overexertion occurring during high-intensity training or competition [1–5]. The sudden death of a young and apparently healthy person becomes a tangible tragedy for society and the family, especially if it is related to athletes who have always been a symbol of health and strength. The development of an adverse outcome is associated with a violation of the regimen of physical exercise, especially in the presence of hidden pathology, for which intense overexertion becomes a triggering factor.

The liver is an immune organ containing cells of both innate and adaptive immunity [6–8]. Leukocyte populations in the liver provide a protective barrier by contributing to the influx of lymphocytes into it [9]. Lymphocytes interact with hepatocytes owing to the fenestrated endothelium and the absence of a basal membrane, which facilitates intercellular contacts between resident immune cells and nonhematopoietic liver cells [10]. Lymphocyte priming occurs with the immune response development to various factors. Dysregulatory mechanisms yield inflammatory reaction and disruption of the organ's architecture due to the

development of fibrosis. Leukocytes and various populations of T-lymphocytes participate in these processes [10, 11]. Studying the reaction of leukocytes and lymphocytes in degenerative processes and liver regeneration can serve as a basis for finding ways to correct these changes. One of the medicines with proven efficacy is meldonium, which possesses a membrane-protective effect contributing to the restoration of cytoplasmic protrusions (microvilli of hepatocytes). Meldonium improves cell metabolism, contributes to effective ATP transport, and optimizes oxygen consumption by cells [12].

The objective of our study was to assess the proportion of leukocytes (CD45+ cells) and T-lymphocytes (CD3+ cells) in the liver during varying levels of physical exertion with and without the use of meldonium.

Materials and Methods

The experiments were conducted on male Wistar rats ($n=60$) weighing 240 g. The animals were subjected to physical exercise of varying intensity. For instance, rats in Group 1 were subjected to light physical exertion, for which they were placed in a basin filled with water (29–32 °C), where they swam for 15 min. Rats in Group 2 spent 30 min doing water-based exercises (moderate physical exertion). To

model heavy physical exertion (Group 3), the animals swam in the basin until they were exhausted and began to drown. This usually occurred after 55-59 minutes spent in the water [2, 3]. A total of 10 swimming sessions were performed. The animals were taken out of the experiment immediately after the last session (10 animals per group) and 30 days after the end of the experiments (10 animals per group). Meldonium was added to the diet of experimental animals at a dose of 100–120 mg/kg body weight during the water-based exercise. Three control groups of rats (10 per group) were used for comparison. These rats also performed similar water exercises of varying intensity but were not given meldonium. Another group used for control included intact rats, which did not exercise.

All laboratory animal manipulations were performed in accordance with GOST 33216–2014, Guidelines for the Care and Maintenance of Laboratory Animals. Rules for the Care and Maintenance of Laboratory Rodents and Rabbits. The experimental design was approved by the Ethics Committee of the Mari State University (Protocol No. 1 of April 28, 2023).

Animals were euthanized by guillotine decapitation. After the animals were withdrawn from the experiment, their livers were atraumatically harvested for further examination. Parts of the right lobe of the liver were fixed in 10% formalin in phosphate buffer, embedded in paraffin, followed by the preparation of histological sections (5 μ m thick) using the MTP-120 tissue processor (SLEE Medical GmbH, Germany) and Tissue-Tek® TEC™ 5 (Sakura Seiki Co, Japan). The sections were stained with hematoxylin and eosin. An immunohistochemical (IHC) reaction for CD-45 markers was performed. Primary antibody: Purified Mouse Anti-Rat CD45 clone OX-1 (BD Pharmingen, Inc., USA), 1:30 dilution. Secondary antibody: Biotin Goat Anti-Mouse Ig, Multiple Adsorption, polyclonal (BD Biosciences, USA), 1:50 dilution; and CD-3 Purified Mouse Anti-Rat CD3 clone G 4.18 (BD Pharmingen, Inc., USA), 1:30 dilution; Biotin Goat Anti-Mouse Ig, Multiple Adsorption, polyclonal (BD Biosciences, USA), 1:50 dilution, according to a standard method. An indirect peroxidase method was used to identify epitopes. Cells were counted in 40 fields of view (1 mm²-section).

Statistical processing on collected data was performed using STATISTICA version 10.0.228.2 (StatSoft, USA). Normality of data distribution was tested using the Kolmogorov-Smirnov and Lilliefors tests. To determine the statistical significance of differences between groups, a one-way analysis of variance (ANOVA) was employed, followed by Tukey's honestly significant difference test ($p \leq 0.05$). For non-normal distributions, we used the Kruskal-Wallis ANOVA, followed by pairwise comparisons via the Mann-Whitney test.

Results

With light physical exertion, histological examination revealed plethora and minor degenerative changes in the liver. The proportion of CD45+ cells did not change either with or without meldonium (Tables 1 and 2). With moderate physical exertion, pronounced degeneration and necrobiotic changes were determined (Figure 1a and b). IHC reaction revealed changes in the CD45+ cell level, which was accompanied by a 1.8-fold increase in the percentage of cells in the liver parenchyma (Table 1) and twofold increase

perivascularly ($p=0.002$) vs. the intact group (Table 1). Histological examination of the liver in Group 3 revealed cellular swelling in the centrilobular zone and ballooning degeneration of periportal hepatocytes, along with necrobiotic changes confirmed by the fact that the nuclei were in a state of karyopyknosis and karyolysis (Figure 1c). Remnants of lightly eosinophilic cytoplasm were determined around the nuclei or along the cell membranes. Pronounced inflammatory infiltration in the liver was detected (Figure 1d). We noted the following changes in the IHC reaction: the proportion of CD45+ cells increased 3.2-fold in the liver parenchyma and threefold perivascularly ($p=0.001$) vs. the intact group (Table 1). After administration of meldonium during heavy physical exertion, the proportion of CD45+ cells in the liver parenchyma and perivascularly decreased 1.8-fold ($p=0.008$) vs. the intact group (Table 1).

Thirty days after the experiment, we observed normalization of the liver histology with light and moderate physical exertion, while no degenerative or necrobiotic changes were observed. The percentage of CD45+ cells was similar to the intact group (Table 1). During heavy physical exertion, small areas of connective tissue were observed in the peripheral parts of hepatic lobules. As for CD45+ cells, their number decreased 2.1-fold in the liver and 2.3-fold vs. the animals that did not receive meldonium (heavy physical exertion; Table 1).

The IHC reaction for CD3+ cells revealed no significant changes under light physical exertion, either with or without administration of meldonium. With moderate physical exertion without meldonium, the share of CD3+ cells increased 3.8-fold ($p=0.04$) perivascularly vs. the intact group and 2.9-fold ($p=0.02$) vs. Group 2 (with meldonium). Under severe physical exertion, the fraction of cells increased 2.9-fold in the liver parenchyma and 6.3-fold perivascularly ($p=0.001$) vs. the intact group (Table 1). We observed clusters of CD3+ cells: in different fields of view, CD3+ cells were present in the form of chains surrounding the vessels (Figure 2). With meldonium, the percentage of CD3+ cells was lower than without meldonium (Table 1).

Thirty days after the experiment, with light physical exertion (without and with meldonium), the proportion of CD3+ cells remained unchanged, compared with the intact group. With moderate physical exertion (with meldonium), a twofold reduction in the percentage of CD3+ cells was observed in the liver parenchyma and a threefold decrease in the perivascular area ($p=0.006$) vs. the animals without meldonium administration. With severe physical exertion (with meldonium), the share of CD3+ cells decreased 2.5-fold in the liver parenchyma and 3.2-fold perivascularly ($p=0.004$) vs. the group untreated with meldonium.

Table 1. Proportion of CD45+ cells (%) under experimental conditions and with meldonium administration

Group	CD45+ cells in the liver parenchyma n/mm ² (%)	Min/max	Perivascular CD45+ cells in the liver n/mm ² (%)	Min/max	Total number of CD3+ cells in the liver section, n/mm ² (%)
Intact	12.1±2.1	7.4–19.9	16.4±2.9	9.3–26.4	28.5±2.1
Study groups					
1	12.9±1.9/12.3±1.7	9.1–17.8/9.2–17.5	16.9±2.2/16.6±1.8	9.5–22.4/9.8–22.9	29.8±1.9/28.9±1.9
2	21.4±3.2/17.9±1.4	16.5–26.9/13.4–24.2	32.3±3.7*/21.9±1.6 p=0.002	26.2–41.3/17.4–32.1	53.7±3.2/39.8±1.4
3	38.3±4.2/ 21.2±2.9	32.6–41.5/15.8–26.5	49.9±4.8** p=0.001 29.7±1.9*** p=0.008	38.9–55.6/21.3–36.7	88.2±4.2/50.9±2.9
30 days after experiment					
Physical exertion					
Light	12.3±2.2/12.2±1.2	8.9–17.5/9.0–17.2	16.5±2.5/16.6±2.1	10.2–21.8/10.4–21.6	28.8±2.2/28.8±1.2
Moderate	18.1±2.1/13.1±1.9	14.7–23.5/9.2–26.4	21.3±3.9/17.1±1.5**** p=0.004	17.3–28.9/14.1–23.2	38.4±2.1/30.2±1.9
Heavy	37.8±3.3/17.8±3.1	29.4–43.1/ 14.5–23.8	48.4±5.1/20.6±4.3**** p=0.002	39.2–52.4/ 17.3–26.1	86.2±3.3/38.4±3.1

Statistical significance of differences is denoted as follows: *Group 2 vs. intact group; **Group 3 (without meldonium) vs. intact group; ***Group 3 without vs. with meldonium administration; 30 days after the experiment: ****Group 2 (with meldonium) vs. intact group; *****Group 3 without vs. with meldonium administration.

Table 2. Proportion of CD3+ cells (%) with and without meldonium administration

Group	CD45+ cells in the liver parenchyma n/mm ² (%)	Min/max	Perivascular CD45+ cells in the liver n/mm ² (%)	Min/max	Total number of CD3+ cells in the liver section, n/mm ² (%)
Intact group	2.4±1.3	1.8–4.4	3.8±1.7	2.1–8.0	6.2±1.3
Group 1	2.8±1.7/2.7±1.9	1.6–4.6/1.5–4.1	4.1±1.5/3.9±1.5	2.2–8.8/2.1–8.3	6.9±1.7/6.6±1.7
Group 2	5.6±2.6/3.8±2.3	2.9–9.9/1.9–5.9	14.3±1.8*/4.9±1.4 p=0.04	7.9–23.3/2.3–8.9	19.9±1.3/8.7±2.1
Group 3	6.9±1.1/4.1±1.7	3.8–9.8/2.2–7.6	23.8±2.1** p=0.001 8.8±1.8*** p=0.004	17.2–31.8/ 5.2–12.4	30.7±3.1/12.9±2.3
30 days after experiment					
Physical exertion					
Light	2.7±0.9/2.6±1.9	1.2–4.4/1.1–4.2	3.9±0.8/3.8±1.3	1.8–7.1/1.7–7.0	6.6±1.3/6.4±1.1
Moderate	7.5±0.8/3.5±1.7	4.2–8.9/2.2–6.9	13.8±0.6****/4.6±1.6 p=0.006	6.2–19.7/2.2–8.8	21.3±1.3/8.1±1.8
Heavy	9.7±0.8/3.9±1.2	5.6–16.3/2.1–6.8	21.9±2.7*****/6.8±2.1 p=0.004	15.9–32.4/3.2–11.4	28.6±1.3/10.7±1.9

Statistical significance of differences is denoted as follows: *Group 2 vs. intact group; **Group 3 (without meldonium) vs. intact group; ***Group 3 without vs. with meldonium administration; 30 days after the experiment: ****Group 2 (with meldonium) vs. intact group; *****Group 3 without vs. with meldonium administration.

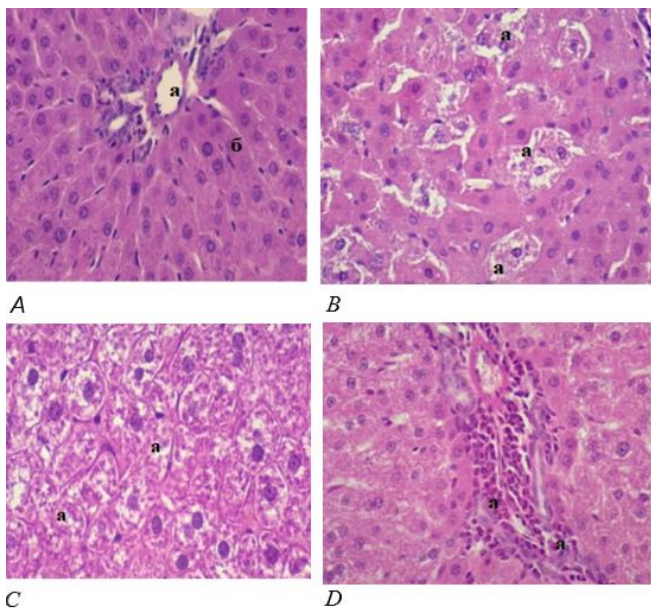


Figure 1. Microscopic changes in the liver: A – in intact animals: a, central vein; b, hepatic lobules; B – Group 2: a, necrobiosis of individual cells; C – Group 3: a, cells with karyolysis; D – a, cellular infiltration. Staining with hematoxylin and eosin, magnification 400×.

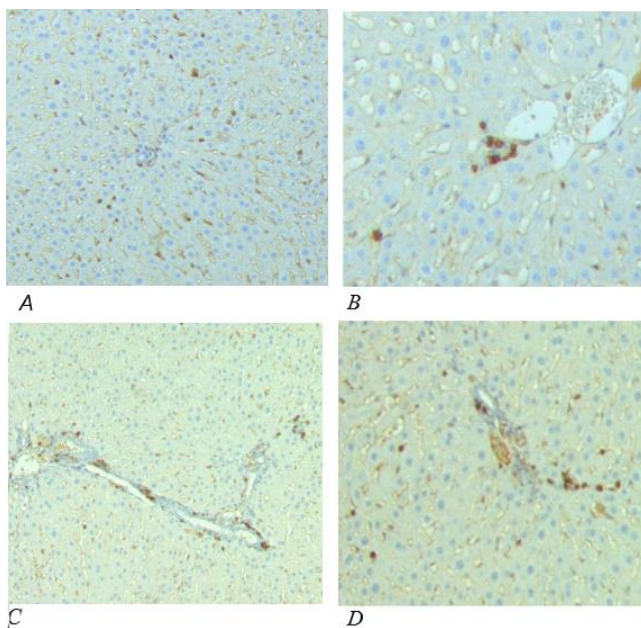


Figure 2. Immunohistochemical reaction for CD3+ cells in the liver: a, in intact animals; b, groups of CD3+ cells against the background of vacuolar dystrophy; c and d, clusters of CD3+ cells perivascularly and in the liver parenchyma (brown arrow-shaped cells); a, c – magnification 100×; b, d – magnification 400×.

Discussion

Our study revealed that light physical exertion (with or without meldonium) did not significantly alter the share of CD45+ cells. Moderate physical exertion yielded an increase in the percentage of leukocytes (CD45+ cells). Heavy physical exertion triggered an increase in the proportion of CD45+ cells in the liver parenchyma and perivascularly, which differed from the intact group. Histological examination revealed necrotic and exudative reactions accompanied by necrobiosis, cell necrosis, and inflammatory infiltration. Meldonium administration during moderate to heavy water-based exercise altered the IHC pattern of liver sections, decreasing the percentage of CD45+ cells both perivascularly and in the liver parenchyma. However, the proportion of CD45+ cells remained higher during the studied water-based exercise of varying exertion intensity than in the intact group. Thirty days after the experiment, the proportion of CD45+ cells reached the level seen in intact animals under moderate physical exertion, but remained slightly higher than in the intact group under heavy exercise.

The percentage of T-lymphocytes in the light physical exertion group (with and without meldonium) did not change vs. the intact animals. With moderate and heavy intensity exercise, the share of CD3+ cells increased vs. the intact group. The increase in the percentage of CD3+ cells was associated with an inflammatory response in the liver associated with T-lymphocytes. With meldonium, the share of CD3+ cells decreased with moderate and heavy physical exertion, but remained higher than in the intact group. Thirty days after the end of the experiment, the percentage of CD3+ cells approached the level seen in the intact group under moderate exercise. This can be explained by high regenerative capacity of the liver, restoration of its histoarchitecture, and the absence of destructive changes. With heavy physical exertion and meldonium administration, we also observed a statistically significant reduction in the studied parameter both in the liver parenchyma and perivascularly.

According to some authors [13], during periods of heavy exercise, the total level of T-cells (CD3+) and helper lymphocytes in the blood decreased, while the proportion of suppressors remained unchanged and T-lymphocyte activity (DNA synthesis and response to T-mitogen) remained elevated. The authors found that a decrease in the blood population of CD3+ and CD4+ cells indicated compromised level of cellular immune defense.

Another study discovered that during physical exercise in a subchronic experiment, the levels of neutrophils and eosinophils increased. This was consistent with data indicating that physical exercise can be associated with inflammation and promote the production of interleukins, along with an increase in circulating neutrophils and eosinophils [14]. According to E.N. Ermolaeva et al. [15], rats that performed moderate exercise of subjected to physical exertion near maximum intensity, leukocyte levels increased.

Researchers examining an effect of the CCL4 toxicant on the liver observed an increase in CD45+ and CD3+ cells, along with the development of lymphocytic and leukocyte infiltration in the liver. The use of aminophthalhydrazide resulted in a diminution in leukocyte infiltration and a sharp reduction in CD45+ and CD3+ cell numbers to levels seen in intact animals [16].

Conclusion

We observed that during strenuous physical activity without the use of meldonium, lymphocytic and leukocytic infiltration developed in the liver, indicating a reaction of the cellular component of immunogenesis. After taking meldonium, a reduction in the proportions of CD45+ and CD3+ cells was observed due to a less pronounced inflammatory infiltration in the liver tissue and destructive changes.

Author contributions. The authors contributed equally to the preparation of the manuscript.

Conflict of interest. The authors declare no conflicts of interest.

References

- Alpidovskaya OV, Malyshev II, Romanova LP. Changes in TGFB1 gene expression and TGF- β 1 level in the liver during physical exercise of varying intensity. *Bulletin of Experimental Biology and Medicine* 2025; 179(2): 203-7. (In Russ.) <https://www.doi.org/10.47056/0365-9615-2025-179-2-203-207>
- Alpidovskaya OV, Malyshev II, Romanova LP. Morphological changes in neurons of rats under physical exercise of varying intensity. *Medical Bulletin of the North Caucasus* 2024;19(1):49-52. (In Russ.) <https://www.doi.org/10.14300/mnnc.2024.19011>
- Malyshev II, Alpidovskaya OV, Romanova LP. The effect of physical exercise of varying intensity on cardiomyocyte hypertrophy and myocardial polyploidy in rats. *Siberian Journal of Clinical and Experimental Medicine* 2024; 39(1): 178-83. (In Russ.) <https://www.doi.org/10.29001/2073-8552-2024-39-1-178-183>
- Smirnova AD, Novitsky AV, Shmoilova AS, Schwartz YuG. The risk of sudden cardiac death in individuals engaged in strength training. *Russian Journal of Cardiology* 2021; 26(4S): 4394. (In Russ.) <https://www.doi.org/10.15829/1560-4071-2021-4394>
- Asif IM, Harmon KG. Incidence and etiology of sudden cardiac death: New updates for athletic departments. *Sports Health* 2017; 9(3): 268-79. <https://www.doi.org/10.1177/1941738117694153>
- Dusseau M, Martin E, Serriari N, Péguillet I. Human MAIT cells are xenobiotic-resistant, tissue-targeted, CD161hi IL-17-secreting T cells. *Blood* 2011; 117(4): 1250-9. <https://www.doi.org/10.1182/blood-2010-08-303339>
- Kurioka A, Walker LJ, Klenerman P, Willberg CB. MAIT cells: New guardians of the liver. *Clin Transl Immunology* 2016; 5(8): e98. <https://www.doi.org/10.1038/cti.2016.51>
- Hunter S, Willcox CR, Davey MS, Kasatskaya SA. Human liver infiltrating $\gamma\delta$ -T-cells are composed of clonally expanded circulating and tissue-resident populations. *J Hepatol.* 2018; 69(3): 654-65. <https://www.doi.org/10.1016/j.jhep.2018.05.007>
- Wang J, Holme TH, de Guevara LL. Phenotypic and functional status of intrahepatic T cells in chronic hepatitis C. *J Infect Dis.* 2006; 194(8): 1068-77. <https://www.doi.org/10.1086/507681>
- Mendes-Braz M, Martins JO. Diabetes mellitus and liver surgery: The effect of diabetes on oxidative stress and inflammation. *Mediators Inflamm.* 2018; 2018: 2456579. <https://www.doi.org/10.1155/2018/2456579>
- Chereshnev VA, Yushkov BG, Abidov MT. Morphogenetic function of immunocompetent cells in regenerative processes in the liver. *Immunology.* 2004; 4: 204-6. (In Russ.)
- Đurašević S, Stojković M, Sopta J. The effects of meldonium on the acute ischemia/reperfusion liver injury in rats. *Sci Rep.* 2021; 11(1): 1305. <https://www.doi.org/10.1038/s41598-020-80011-y>
- Afanasyeva IA. Indicators of the T-cell immune system in athletes during intensive training. *Scientific Notes of Lesgaft National State University of Physical Education, Sport and Health* 2007; 1. URL: <https://cyberleninka.ru/article/n/pokazateli-t-sistemy-immuniteta-u-sportsmenov-pri-intensivnyh-trenirovках> (28 Feb 2025). (In Russ.)
- Blinova TV, Strakhova LA, Kolesov SA. The influence of intense physical exertion on biochemical parameters of antioxidant defense systems and nitric oxide in competitive swimmers. *Occupational Medicine and Industrial Ecology* 2019; 59(10): 860-5. (In Russ.) <https://www.doi.org/10.31089/1026-9428-2019-59-10-860-865>
- Ermolaeva EN, Sashenkov SL, Surina-Marysheva EF, Petukhova VI. The immune profile of rats subjected to chronic physical exertion in the experiment. *Contemporary Issues of Science and Education* 2023; 4. (In Russ.) <https://www.doi.org/doi.org/10.17513/spno.32845>
- Shafigullina ZA, Gette IF, Danilova IG. Regenerative response of hepatocytes in diffuse toxic hepatitis. *Bulletin of the Ural Medical Academy of Science* 2020; 17(4): 313-22. (In Russ.) <https://www.doi.org/10.22138/2500-0918-2020-17-4-313-322>

Authors:

Alexander A. Yusov – PhD, Assistant Professor, Department of Normal and Pathological Physiology, <https://orcid.org/0000-0001-6079-1535>;

Olga V. Alpidovskaya – PhD, Assistant Professor, Department of General and Clinical Morphology and Forensic Medicine, <https://orcid.org/0009-0004-0232-3193>.