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The influence of dietary supplements on the adaptive processes in animals after physical stress

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Abstract

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The aim of our research is to study the morphological changes in the well-studied laboratory animals during the period of their physical stress and use of dietary supplements aimed to hasten animals' recovery, and to correct their organism functional capability. The data got from the research will be applied for powerlifting practices. Complex research was made for the first time to reveal the morphological changes in mice which were under physical stress and used vegetable and animal adaptogenes in their meals at the same time (maral root tincture, pantocrine tincture, and magnolia vine seeds tincture and adsorbed male bee brood and pantocrine for hamsters). It was found that the use of adaptogenic agents before the physical stress contributes to positive changes in the morphology and physiology of the blood and increases the physical endurance of the experimental animals. However, when the experimental and control groups are exposed to the maximal physiological activity during 28 days it can result in their overtraining.

Keywords: mice; hamsters; morphology; maral root; magnolia; vine seeds; adsorbed

Introduction

One of the most important problems nowadays in the activation of biological processes of athletes' organisms is to develop a system for the recovery of the morphological and physiological functions of the organism after physical stress (Blagonravov et al., 2016). When an organism is under high physical stress, there are functional disorders, which in some cases become chronic. The influence of the physical activity on the morphology of individual organs, muscle fibers of animals and of a human being, on the structure of veins and the activity of the blood-making organs, on the morphology of cartilage and joints is reflected in the results of numerous studies of the scientists (Safonov, 2018; Khabibullin, 2016; 2017; 2017b; Khabibullin et al., 2016; 2017).

To improve the physical efficiency and to reduce the negative effect of the physical stress on the body, many scientists such us Yakimoskii et al. (2017), Kuznetsov et al. (2017) and Khabibullin et al. (2018) propose to use biological active substances which are vegetable and animal adaptogenes.

The efficiency of the use of adaptogenes to improve physical endurance and working capacity in animals exposed to intensive physical activity is proved by the research results of such scientists as Kreider et al. (2009), Khabibullin

and Fazlayeva (2014), Anikina et al. (2017), Novoselov et al. (2016) and Sokolova et al. (2016). Hoene et al. (2018), Panossian et al. (1999), Ristow et al. (2009) confirm that these substances accelerate the body's recovery process, correct its functional abilities, affect the structure of muscle fibers, kidneys, spleen, cardiac muscle, blood morphology, and histological structure of individual organs. Thus, we can say that many authors share the opinion that the acceleration of the body recovery after physical activity is possible by taking certain adaptogenes. However, under experimental conditions, the influence of plant and animal adaptogenes in the comparative aspect on the morphology of the complex of organs and muscle tissue, on the morphological and biochemical blood parameters, as well as on the dynamics of the formation of the working capacity of the experimental animals after physical activity has not been studied, so the problem is still relevant.

The purpose of our research is to study the morphological changes in mice and hamsters exposed to physical stress and using biologically active substances to accelerate their recovery and to the correction of the organism functional ability. Our task was to take a comparative assessment of anatomic and physical characteristics of mice and hamsters during their physical activity and the use of adaptogenes such as maral root tincture, magnolia vine seeds tincture and of the adsorbed male bee brood. We also wanted to reveal morphological changes in their organs, and the dynamics of glycogen content in the liver of experimental mice after their physical stress and after the use of maral root tincture, pantocrine tincture, to study the morphology of blood cells of mice and hamsters after their physical activity and application of adaptogenes. One more aim was to establish the dynamics of the formation of physical endurance in experimental mice during the period of their physical activity and application of plant and animal adaptogenes.

Material and Methods

The methodological basis of this work is a set of general biological and special methods, including empirical and theoretical studies. In the course of the study, we used methods arising from the tasks solved during the research. Among the methods we used are observations, experimental method, method of modelling, physiological, histological, histochemical and morphological methods of blood tests, statistical analysis and generalization. The essence of these approaches is in a systematic approach aimed to study morphological changes in mice, when using adaptogenes during physical stress and in general with the coordinated functioning of all constituent structures of the organism, which is the basis for determining the reliability of the study results. To determine the physical activity effect on the organism of the experimental mice, we used the technique of "forced swimming" by Porsalt held during 28 days in two experimental batches of the studies.

Four experimental groups of mice were formed to study the effects of adaptogenes (maral root tincture, pantocrine tincture and the combination of pantocrine tincture and Ovesol) on their physical endurance, the anatomy of their internal organs, live weight gain, histology of some organs, histochemical changes in liver, blood morphology and biochemistry.

The weight of internal organs and the live weight gain were determined by the weighing method described in the articles of Balakhonov et al. (2017), Mikashinovich et al. (2017), Khabibullin and Fazlayeva (2013), Avdeeva et al. (2016). Standard methods were used to make histological research followed by the staining of paraffin sections with haematoxylin-eosin according to the Van Gieson method. Histochemical studies of the liver for the glycogen presence by Mac Manus were made. Morphological blood tests were performed on blood smears stained by the Romanovsky-Gimza method with the counting test of 200 cells. The number of erythrocytes and leukocytes was determined by the cell counting test in Goryaev's chamber. Statistical processing of the research results was performed using Microsoft Excel for Windows – XP program. The difference accuracy (P) was determined taking into account the indicators of the Student's test. Statistical and mathematical data processing was also used during the research like in the studies of Khabibullin et al. (2018), Islamgulov et al. (2018), Dementyev et al. (2018), Lubova et al. (2018), Bagautdinov et al. (2018). Experimental studies were carried out on 60 white mice and 75 hamsters at the Department of Small Animal Science and Animal Breeding, Federal State Budgetary Educational Institution of Higher Education Bashkir State Agrarian University. Mice and hamsters were selected for the groups on the principle of analogues: live weight, sex, age.

The scheme of experimental studies is presented in Table 1.

White mice and hamsters used for the experiment were kept under the conditions prescribed by the Rules of Laboratory Animal Care in vivarium according to the Directive 2010/63/EU. Determination of the effect of physical activity on the animals on experiment was performed by using the technique of "forced floating" by Porsalt within 28 days. Three groups of mice of 20 heads in each and three groups of hamsters of 25 heads were formed to study the effect of adaptogenes on the physical endurance, morphological macro- and microscopic changes in internal organs, histochemical parameters of the liver, blood morphology and live weight gain.

Table 1. Scheme of experimental studies of the effect of physical activity and the use of adaptogenes on animals

Group	Medication	Posology and administration
Mice		
Experimental group 1	Maral root tincture	2 mql from the 1st to the 7th experiment day, 4 mql from the 8th to the 14th day and 6 mql from the 15th to the 21st day
Experimental group 2	Pantocrine tincture	2 mql from the 1st to the 7th experiment day, 4 mql from the 8th to the 14th day and 6 mql from the 15th to the 21st day
Control group	Distilled water	2 mql from the 1st to the 7th experiment day, 4 mql from the 8th to the 14th day and 6 mql from the 15th to the 21st day
Hamsters		
Experimental group 1	Magnolia vine seeds tincture	4 mql from the 1st to the 7th day, 6 mql from the 8th to the 14th day and 8 mql from the 15th to the 21st day
Experimental group 2	Adsorbed male bee brood	4 mql from the 1st to the 7th day, 6 mql from the 8th to the 14th day and 8 mql from the 15th to the 21st day
Control group	Distilled water	4 mql from the 1st to the 7th day, 6 mql from the 8th to the 14th day and 8 mql from the 15th to the 21st day

Live weight gain was determined by weighing. Histological studies were carried out with the use of general histology methods, followed by staining of paraffin sections with haematoxylin-eosin and according to the method of Van Gieson. For the histochemical studies of the liver for the presence of glycogen the Mac Manus method was used. Blood smears stained by Romanovsky-Gimza with a count of 200 cells were used for the morphological blood tests. Blood tests were performed with the use of Haematology test panel Haema 8-01 – "Astra". Statistical processing of the research results was performed using Microsoft Excel for Windows-XP. Statistical significance (p) was determined taking into account the indicators of the Student's test.

Results and Discussion

The results of the study are presented in Figure 1, which shows the dynamics of changes in the live weight of experimental mice during the experiment period. Weighing, conducted at the end of the experiment, showed that the increase in live weight gain of mice of the first group for the experimental period made 2.37 g, which is 3.17 g less than the live weight gain in mice of the control group. In mice of the second group it was 5.66 g and exceeded the indicator of the control group by 0.46 g.

The study showed that the swimming activity dynamics in experimental mice at the beginning of the experiment did not changed much and ranged from 49.60 s to 51.60 s. Seven days later after the beginning of the experiment, the duration of swimming in all groups was lower than before the experiment ranging from 47.90 s to 49.00 s. Fourteen days later after the beginning of the experiment, the duration of swimming increased sharply in all groups, and this indicator was especially significant in the second group where it increased by 128.40 s.

The swimming duration indicators of the second and the third groups in comparison with those of the control group increased by 97.0 s and by 93.60 s respectively. The swimming activity of the mice of experimental and control groups by the end of the experiment is presented in Figure 2.

Morphological changes in organs of experimental mice after the use of maral root and pantocrine on the background of physical stress.

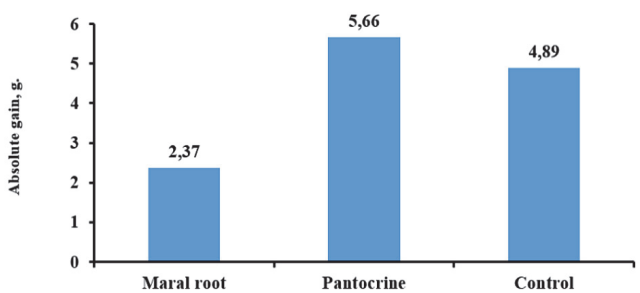

Fig. 1. Absolute live weight gain of mice during the experiment

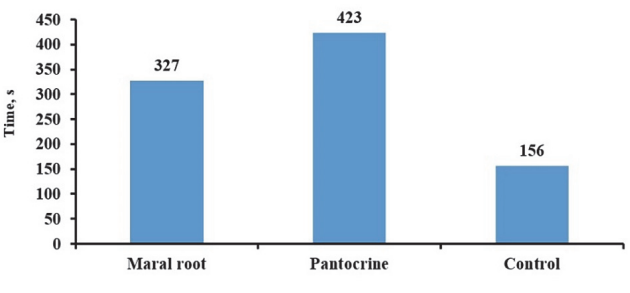

Fig. 2. Swimming activity of mice after the experiment

Morphological changes in the skeletal muscles of the control group that were under physical stress without the influence of adaptogenes, skeletal muscle tissue formed by bundles of muscle fibers, which were mostly located in parallel rows, had a number of pathomorphological changes. Cross-striation of mascularskeletal fibers caused by the interchange of dark and light discs was not revealed everywhere. There were determined the symptoms of vacuolar degeneration and of swelling of muscle fibers. In some places there were areas where the space between the individual muscle cells was expanded due to the hydropic fluid.

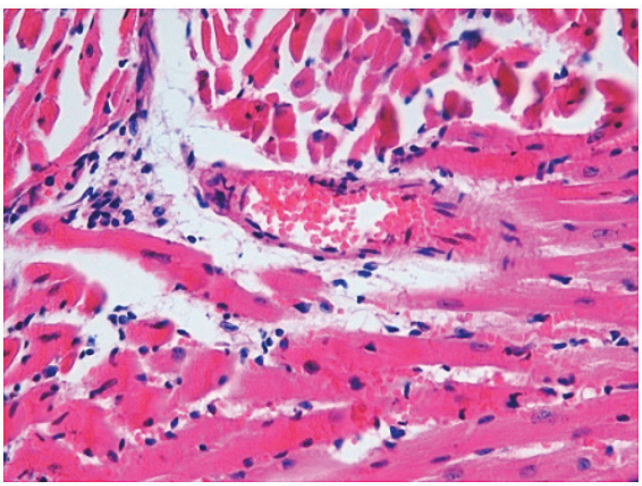

Fig. 3. Perivascular oedema of skeletal muscle tissue of an animal of the control group; staining with haematoxylin-eosin; micro-photo ocular 10, lens 20

In some areas there were the signs of intracellular edema, but also partial fragmentation and focal lumpy myocytes breakdown. In such areas, the collapsing myocytes sometimes merged, disrupting the correct architecture of the muscle bundle. Various cellular elements such as single neutrophils, macrophages, fibroblasts were determined. There was a pretty severe reaction on the part of the vascular bed: the interstices of the vessels were dilated and filled with erythrocytes. There were revealed the signs of moderately expressed perivascular edema (Figure 3). The vascular wall was swollen and tumid. There was a significant amount of free-lying red blood cells in the form of blood strokes between muscle fibers. In the lumen of the vessels the presence of sludge was noticed. Muscle tissue of the animals of the first group differed from that of the control group. There were fewer signs of dystrophic changes in muscle cells. Swelling of tissues decreased, muscle fibers were located more compact and the tissue was denser (Figure 4).

The number of red blood cells between muscle fibers decreased. The blood vessels located in the endomysium were quieter and firmly to the muscle fibers. All blood vessels are of moderate congestion. The vascular wall edema was dissipating. The nuclei of muscle fibers lay in the sarcoplasm under the sarcolemma and were clearly visible. The sarcoplasm and the myofibrils were stained with oxyphilic pink. The complete recovery of the skeletal muscles of the animals which used pantocrine during physical stress didn't also occur (Figure 5). Structural and functional unit of the skeletal muscle tissue in the experimental mice were made of bundles of cross-striated muscle fibers, which didn't always have transverse striations. Individual fibers remained swol-

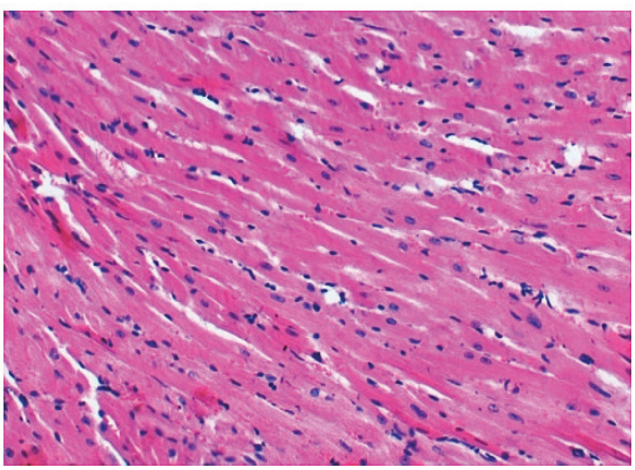

Fig. 4. Skeletal muscle tissue of an animal receiving maral root tincture; staining with haematoxylin-eosin; micro-photo ocular 10, lens 20

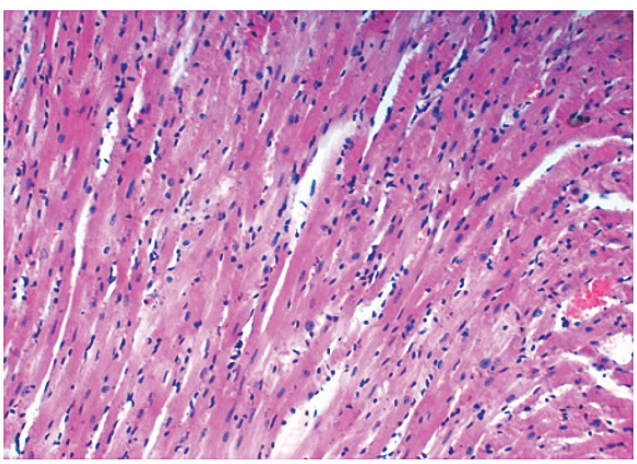

Fig. 5. Skeletal muscle tissue of an animal receiving pantocrine; staining with haematoxylin-eosin; micro-photo ocular 10, lens 10

len and sometimes destroyed with the infiltration of cellular elements such as macrophages and fibroblasts.

Morphological changes in cardiac muscle in animals of the control group who did not receive adaptogenes during physical stress, on histological preparations of myocardium, first of all, there were signs of blood vessels' expansion and their filling with blood (Figure 6). In some areas around the vessels there was hemorrhagic suffusion of the heart muscle tissue. Cardiomyocytes had signs of significant dystrophic changes. In some areas, both in the deep layers of the heart and in the pericardium, there were inflammatory infiltrating cells. There was a big amount of lymphocytes, macrophages, fibroblasts in the infiltrate. In the myocardium of the control group of animals there were also seen edematous zones with the dilution of cardiomyocytes. Perivascular cell infiltrates were also detected here.

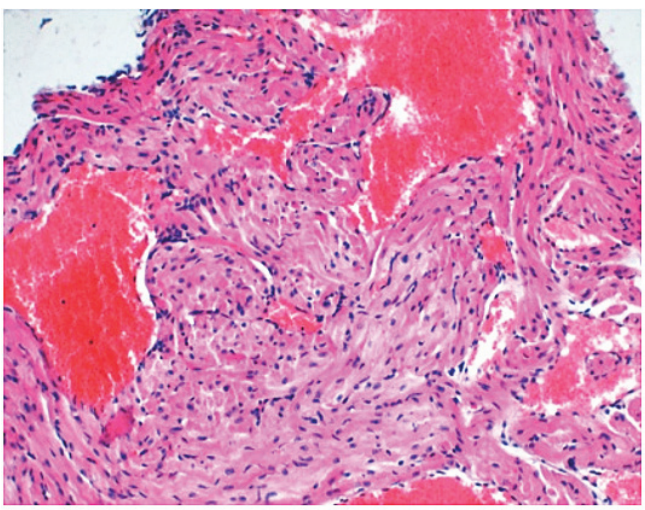

Fig. 6. Myocardium cardiomyocytes of an animal from the control group; staining with haematoxylin-eosin; micro-photo ocular 10, lens 10

The heart muscle of the animals receiving maral root tincture during physical stress differed from that of the control group animals. Though dystrophic changes in muscle cells were decreasing, they didn't disappear completely. Vascular walls of some vessels didn't have any pathological changes. But most of the vessels were full-blooded, there were cardiomyocytes around them with mild dystrophic changes (Figure 7).

On the part of the pericardial area, pathological changes also did not disappear completely, small inflammatory cell infiltrates continued to be detected, and the vessels were expanded and blood-filled. In the group of the animals receiving pantocrine during physical stress, there was no complete restoration of myocardial tissue. Single cardiomyocytes had

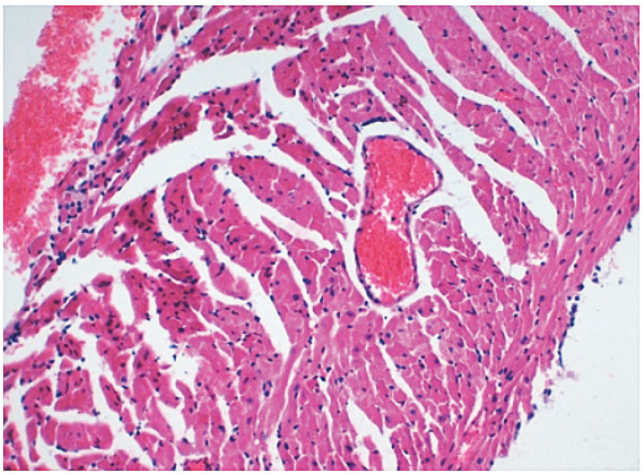

Fig. 7. Cardiomyocytes of an animal from group 1; staining with haematoxylin-eosin; micro-photo ocular 10, lens 40

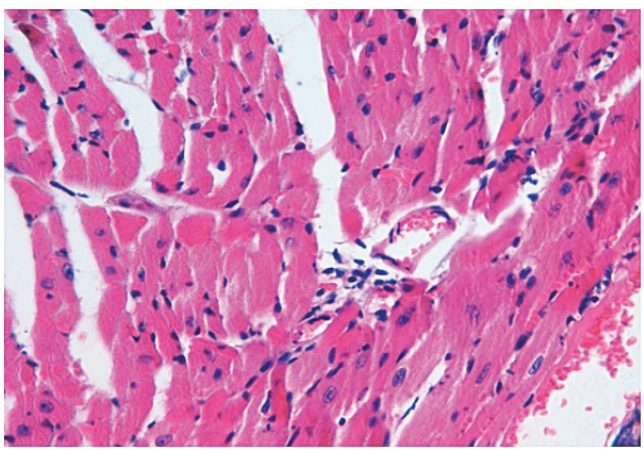

Fig. 8. Heart muscle tissue of the animals receiving pantocrine; staining with haematoxylin-eosin; micro-photo ocular 10, lens 20

the signs of dystrophic changes. In some places there was assembly of the red blood cells as far as single red blood cells. Some larger blood vessels were blood-filled and a little edematous. Some vessels had the usual structure, although there were signs of mild edema around them (Figure 8).

During the experimental studies conducted on experimental hamsters which used dietary supplements in their food after physical stress certain changes in the heart muscle were also observed. As an example, there can be blood vessels of the heart with severe hyperemia, followed by exudation of the liquid part of the blood (non-cystic exudate), and then migration of leukocytes through the walls of the micro-

circulatory bed (cystic exudate) into the pericapillary zone. In some cases, it is noted the destruction of endothelial cells with the release of red blood cells outside the blood vessel. There are areas of the myocardium with significant blood stagnation in the venous vessels, especially closer to the epicardium, when the blood vessel is significantly expanded. Such "swelling or expansion" of blood vessels can lead to their rupture, followed by destruction of the muscle tissue of the heart, i.e. to a heart attack. The use of adaptogenes leads to a certain stabilization of the blood supply system of the organs to be studied. Thus, animals which are under significant physical activity are also in a stressful situation which results in changes in their vital organs. First of all, it affects the cardiovascular system of animals.

Morphological changes in the lungs of the mice from the control group were characterized by the fact that both the intrapulmonary small bronchus, lined with two-three-row ciliated epithelium on the basal membrane, and terminal bronchioles, lined with a single layer cylindrical epithelium with ciliated cells, changed not much. But there were signs of the lungs circulatory system disorder which attracted the attention. Probably, the functions of the inflow and the outflow of the blood from an organ were disturbed as both arterial and venous vessels were expanded and blood-filled. Microscopically, a strong expansion of individual alveoli and their rupture were also found. In the alveolar and interalveolar septa, there were punctate hemorrhages and red blood cells going out into the lumen of the alveoli (Figure 9). In some areas, small clusters of lymphoid and other cells were detected along the blood vessels and airways of the animal lung. But around the individual vessels, cellular inflammatory infiltrates were quite large and diffusely spread from the vessels to a considerable distance.

In the lungs of the mice of the test group receiving the maral root tincture during physical stress, only in certain areas continued to be identified pathologically altered areas containing blood-filled vessels and diffuse cellular infiltrates. In some areas, only small blood vessels were filled with blood (Figure 10). A characteristic feature of the histological structure of the lung of this experimental group of mice is a slight increase in the number of lymphoid cells along the airways and accompanying blood vessels. In the pulmonary tissue of mice of the experimental group, which was given the pantocrine during physical stress, in comparison with the control group, pathological changes were less pronounced. In the respiratory section of the lung alveolar sacs were lined with typical alveolar epithelium. Inside the septa, there were many tightly packed blood capillaries with red blood cells inside them. Around separate blood vessels and terminal bronchioles lymphoid accumulations were revealed (Figure 11).

Morphological changes in the spleen in mice of the control group are covered with a connective tissue capsule and numerous connective tissue crossbeams – trabeculae go from the capsule into the body. Reticular tissue is the basis of the parenchyma of the spleen. The white pulp which is of a smaller volume is well seen, and the red pulp has a significant volume. The red pulp, which makes up most of the volume of the spleen, is detected between venous sinuses and consists of reticular tissue containing a large number of

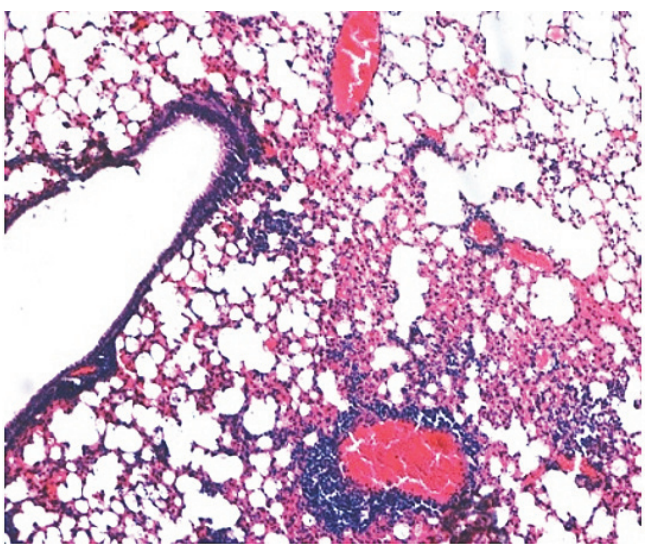

Fig. 9. Blood-filled vessels of the lung of the animals from the control group; staining with haematoxylin-eosin; micro-photo ocular 10, lens 40

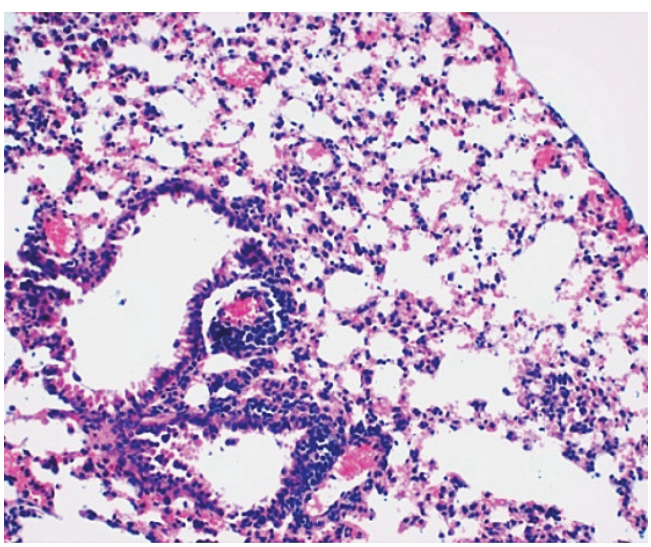

Fig. 10. Lung structure of a mouse receiving the maral root tincture; staining with haematoxylin-eosin; micro-photo ocular 10, lens 10

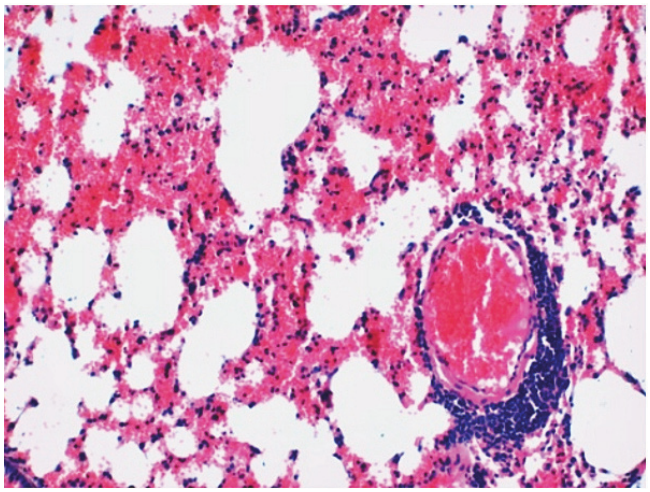

Fig. 11. Blood-filled vessel of the tissue of an animal receiving pantocrine; staining with haematoxylin-eosin; micro-photo ocular 10, lens 10

red blood cells, lymphocytes, macrophages (Figure 12).

In the spleen of mice of the experimental group, who received the maral root tincture after physical stress, as well as in the control group, the white and red pulp is well defined. But the area of the white pulp in the spleen of this group of animals in comparison of the control group, significantly increased by the growth of the number of lymph nodes. Some lymph nodes are even fused. Light centers of reproduction are revealed almost in all lymph nodes. They contain a significant amount of lymphoblast with mitotic figures. Due to

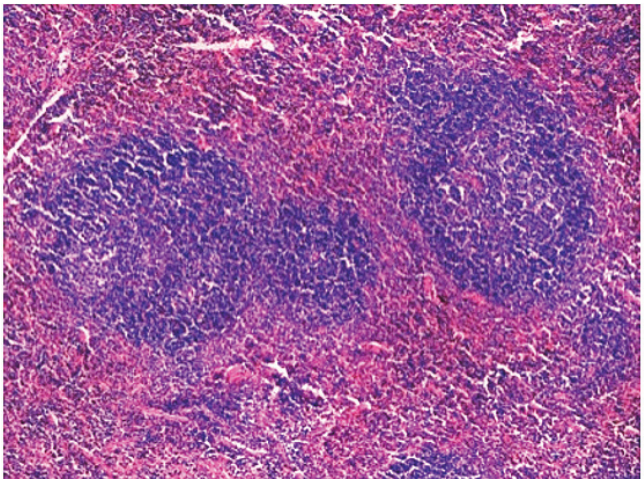

Fig. 12. Red and white pulp of the spleen of an animal from the control group; staining with haematoxylin-eosin; micro-photo ocular 10, lens 10

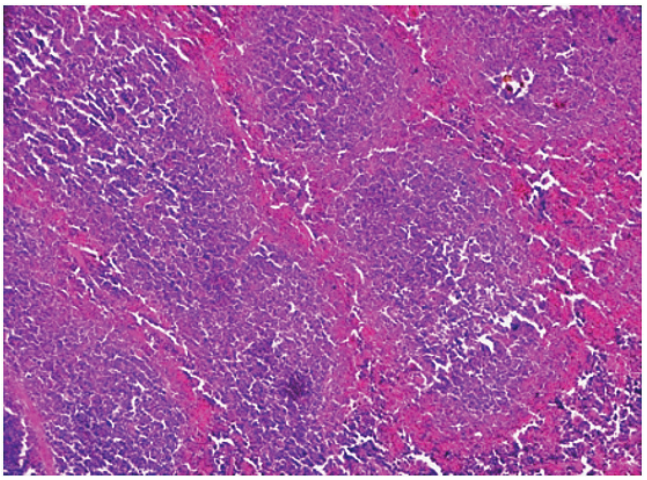

Fig. 13. Increase in the number of the lymph nodes in the spleen of an animal receiving the maral root tincture; staining with haematoxylin-eosin; micro-photo ocular 10, lens 20

the increase in the white pulp area, the width of the red pulp decreases accordingly. Lymphoid tissue grows not only due to the increase in the size of the follicles, but also due to the increase of the number of lymphoid cells of the splenic bands (Figure 13). In the spleen of mice of the experimental group who received pantocrine after exercise, the zones of the white pulp with the central artery and the red pulp consisting of venous sinuses and splenic strands are also clearly identified (Figure 14).

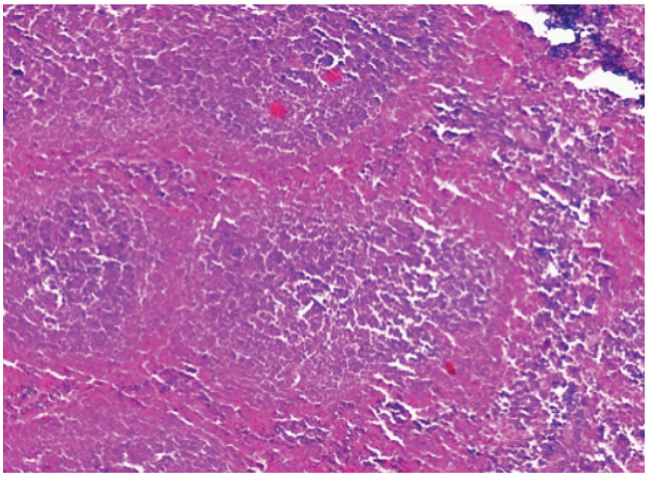

Fig. 14. White and red pulp of the spleen of an animal receiving pantocrine; staining with haematoxylin-eosin; micro-photo ocular 10, lens 10

Morphological changes in the kidneys in the control group of mice revealed pathomorphological changes, indicating to congestion. Most blood vessels contained blood elements. In the cortical substance, where renal corpuscles were determined, a large number of free-lying red blood cells were also determined. Between the brain rays in the renal labyrinth there were revealed the signs of severe congestion, accompanied by the destruction of the cells adjacent to these areas of the proximal and distal tubules. In some parts the cells were subjected to dystrophic changes in the proximal and distal tubules. Between the convoluted proximal and distal tubules, blood cell clusters were determined (Figure 15).

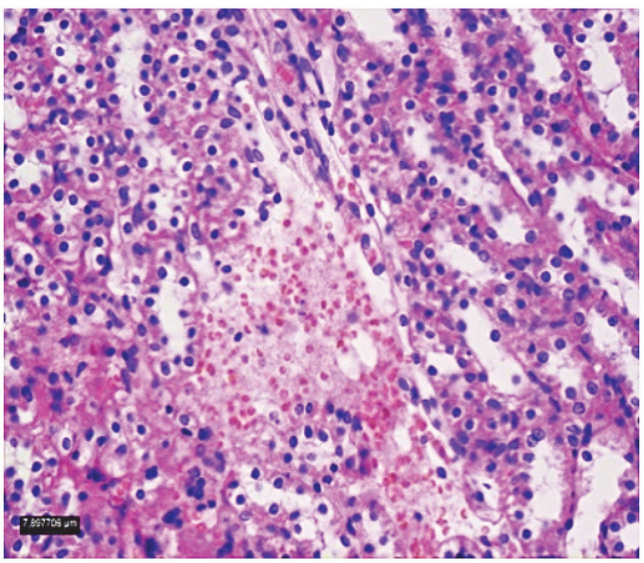

Fig. 15. Venous hyperaemia of the renal cortex of a mouse from the control group; staining with haematoxylin-eosin; micro-photo ocular 10, lens 40

In the course of the tubules small clusters of lymphoid cells are revealed. The mice of the test group receiving after exercise the maral root tincture, the cortex is well defined, the brain matter is close to the middle of the kidney. Pathological changes are less pronounced than in the control group of animals. The structural and functional units of the kidney-nephrons, consisting of the renal corpuscle, located in the cortical substance, the tubules of the nephron of the proximal zone, the loop of the distal zone and then the next collecting tube are clearly visible. The proximal zone, narrow and distal nephrons are surrounded by blood capillaries with moderate blood filling, but there are small areas with venous hyperemia (Figure 16). In some places there are diffuse foci of lymphoid cells.

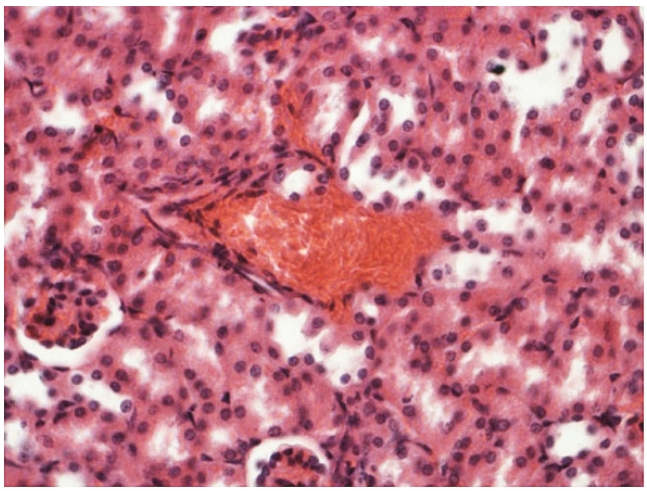

Fig. 16. Venous hyperaemia of the renal cortex when an animal is given the maral root tincture; staining with haematoxylin-eosin; micro-photo ocular 10, lens 40

We couldn't find most evident changes in the histostructure of the kidneys of animals of the experimental group, receiving pantocrine. It can only be noted that some venous vessels show signs of congestion. The accumulation of red blood cells is determined in the renal corpuscle. Dystrophic changes in the cytoplasm in some cells of the tubules are found. Small clusters of lymphoid cells are revealed around the tubules of the nephron and blood vessels (Figure 17). Thus, histological changes in the kidneys of animals of the

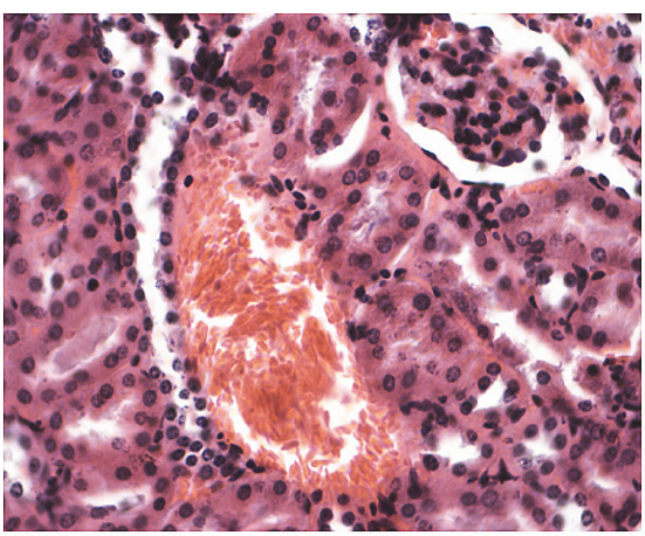

Fig. 17. Vascular congestion of some kidney venous vessels of an animal receiving pantocrine; staining with haematoxylin-eosin; micro-photo ocular 10, lens 10

control group after increased physical activity without the use of adaptogenes revealed signs of severe congestion, accompanied by the destruction of the cells adjacent to the proximal and distal zones of the tubules. After using plant and animal adaptogenes such as the maral root tincture and pantocrine the congestion signs are weakened.

Morphological changes in the liver of mice of the control group are distention and blood-filling of interlobular vessels. It is evident that both the inflow and the outflow of the blood from the liver were disturbed, as central veins were also dilated and blood-filled. Red blood cells were determined in sinusoidal capillaries. Small assembly of cellular elements around the dilated blood vessels and a bit further was found. Most hepatocytes were characterized by dystrophic changes in the cytoplasm, expressed in varying degrees. The signs of granular dystrophy, reflecting the increasing swelling of mitochondria, of hydropic vacuolar dystrophy with the enlightenment of the cytoplasm of cells due to the expansion of the tanks of the endoplasmic network and of balloon dystrophy provoked by the accumulation of a large amount of fluid in the hyaloplasm, were determined (Figure 18). Detected in hepatocytes nuclei were round, different in size, of basophilic tinction. Nucleoli in the nuclei were not visible. In many areas parenchyma trabecular structure was damaged and hepatocytes were distributed randomly.

In the mice receiving the maral root tincture after exercise, hepatic lobules, which are of a polygonal shape at a cross-section, are constructed of hepatic fan-shaped plates radiating from the central vein to the periphery of the lobules. Signs of pronounced congestion in the liver were absent and there were signs of recovery of the trabecular structure of the liver parenchyma. Passing interlobular sinusoidal capillaries between the hepatic plates carrying blood from the periphery of the lobule to the central vein were visible. When put under microscope, properly located hepatic plates radiating from the central veins were also clearly visible (Figure 19). Some vessels were slightly dilated, and in their lumen there were seen cellular elements of blood, and after van Gieson's staining stagnant islands of yellow liquid probably those of blood plasma were also seen. Some parts of the parenchyma consisted of hepatocytes with mild dystrophic changes in the cytoplasm. Sinusoidal capillaries located between the hepatic plates are characterized by moderate vascular congestion.

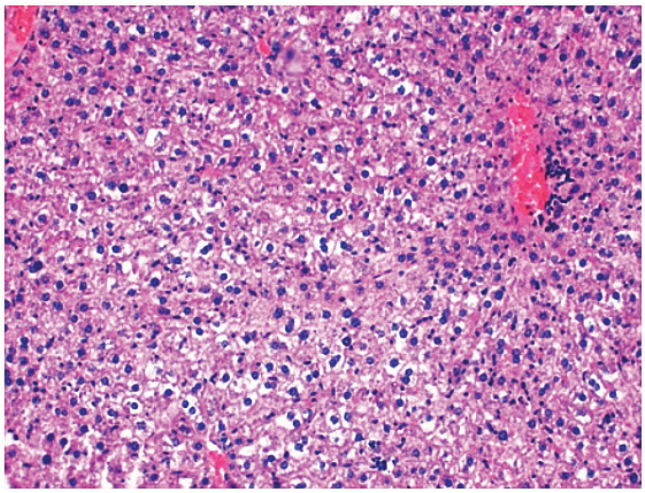

Fig. 18. Dystrophic changes in liver cells of an animal from control group; staining with haematoxylin-eosin; micro-photo ocular 10, lens 10

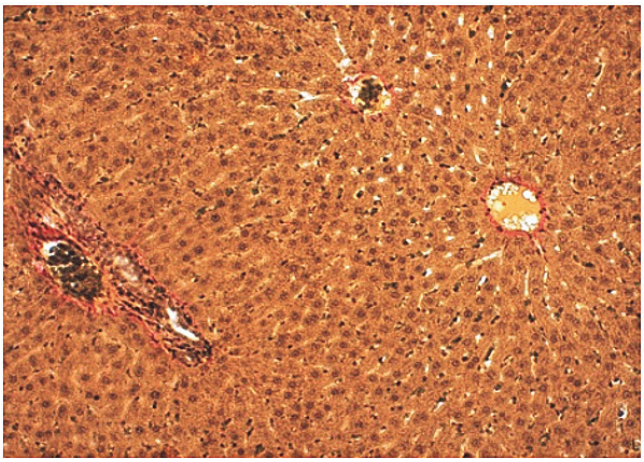

Fig. 19. Liver lobule with central vein and portal area in experimental mice of group 1; Van Gieson's stain; micro-photo ocular 10, lens 10

In mice of the second group, small lymphoid nodules in a small amount were determined in the liver of those animals which are from the experimental group receiving the pantocrine after exercise. There were signs of moderate dystrophic processes in the cytoplasm of hepatocytes. Each lobule consists of anastomosing hepatic plates radiating from the central vein to the periphery of the lobule (Figure 20). The nucleus is stained basophilic. There are hepatocytes with enlarged, or polyploid nuclei. Sometimes there are binuclear hepatocytes.

Histochemical studies of mouse liver for the presence of glycogen in cells showed that in the control group (Figure 21) in the cytoplasm of liver hepatocytes glycogen is determined in moderate amounts. Hepatocytes staining intensity varied from mild to moderate (from + to + +). Glycogen granules

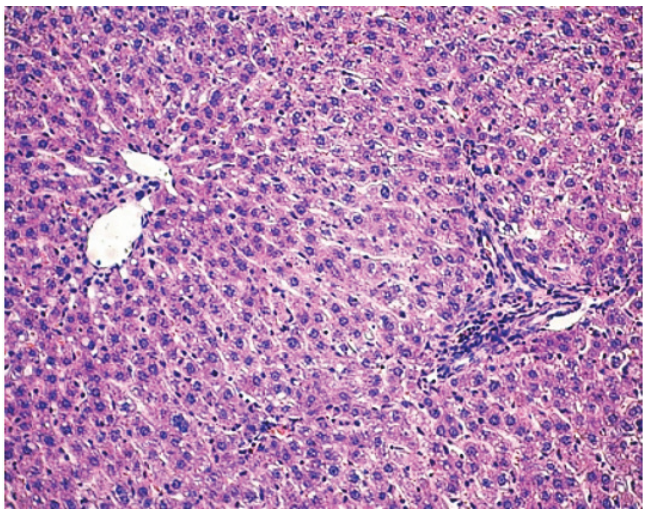

Fig. 20. Liver structure of an animal receiving pantocrine; staining with haematoxylin-eosin; micro-photo ocular 10, lens 10

were small and distributed evenly throughout the cytoplasm. During the histochemical reaction glycogen granules are detected evenly in the form of intense lilac staining in all areas of the liver lobule. Hepatocytes showed a moderate reaction to glycogen as far as forming intercellular sinusoid capillaries and hepatic cells surrounding larger blood vessels

In the hepatocytes of the liver of mice of the first group (Figure 22) there are some differences in histochemical reaction in comparison with the control group. Individual hepatocytes, especially located in the immediate vicinity and around the liver triad, show a fairly high response to glycogen (from + + + to + + + +), while in other parts of the liver lobule histochemical reaction in hepatocytes remains moderate (+ and + +). Glycogen granules are distributed evenly in hepatocytes around most blood vessels of different caliber. Moderate reaction to glycogen is determined here (+ and + +). In the experimental group of mice receiving pantocrine after exercise (Figure 23) reaction to glycogen is moderate (+ + +), although some cells show a relatively high response (+++).

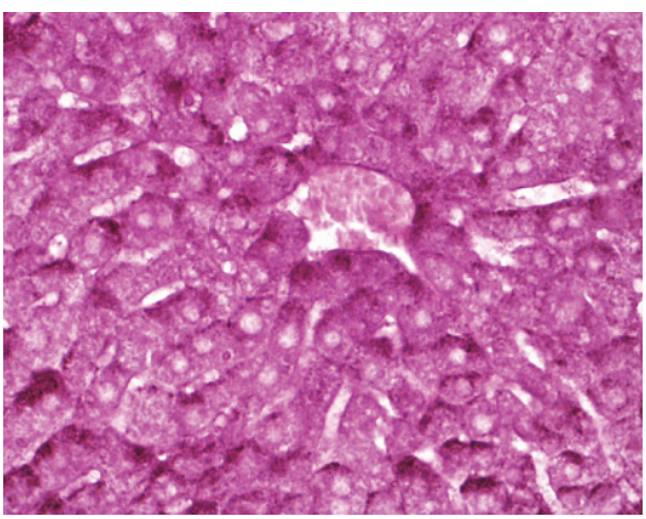

Fig. 21. Moderate histochemical reaction to glycogen in the liver of animals from the control group; Mac Manus method; micro-photo ocular 10, lens 40

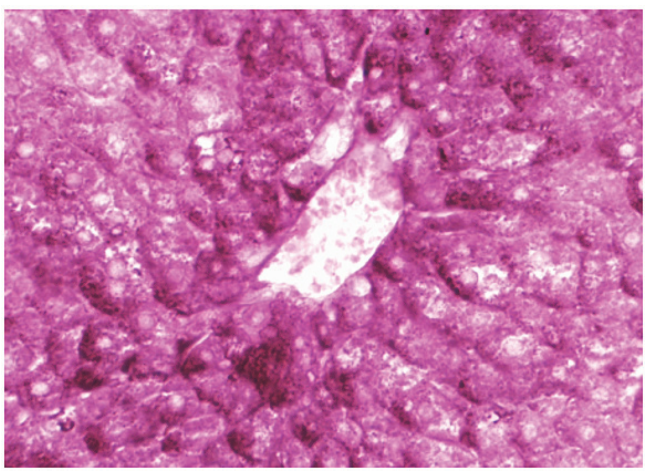

Fig. 22. High reaction to glycogen in hepatocytes close to the liver triad of an animal receiving the maral root tincture; Mac Manus method; micro-photo ocular 10, lens 40

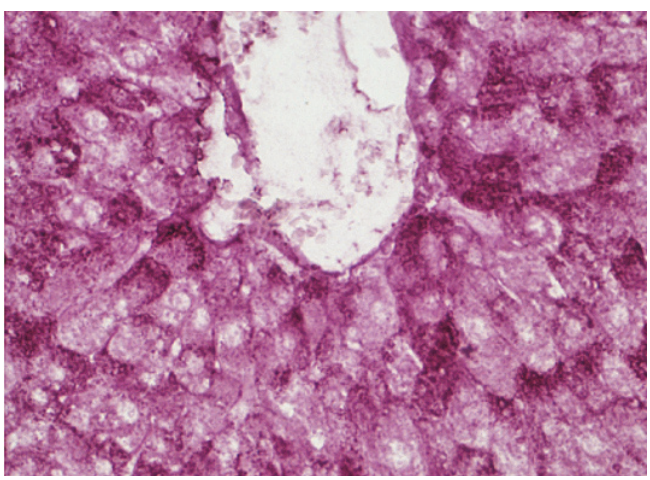

Fig. 23. Bulk of hepatocytes with a moderate reaction to glycogen, individual cells with a high reaction when using pantocrine; Mac Manus method; micro-photo ocular 10, lens 40

Table 2. Morphological parameters of blood of experimental mice after exercise and use of adaptogenes

Indicators	Terms of the experiment					
	7 days			28 days		
	Experimental group 1	Experimental group 2	Control group	Experimental group 1	Experimental group 2	Control group
Erythrocytes × 10^{12}/l	7.18±0.360	6.87±1.8*	6.93±0.62	7.02±0.41*	7.41±2.1*	6.01±0.52
Haemoglobin, g/l	165±3.50	159±8.4	163±8.20	163±3.70*	168±8.8*	146±10.10
Leukocytes, × 10^9/l	7.29±0.16	6.89±1.2*	7.13±0.80	5.98±0.28*	5.5±0.9	8.81±0.90
Lymphocytes	66.05±3.60	66.19±9.70*	67.14±3.10	71.30±5.00*	71.98±9.10	65.40±2.90
Neutrophils	32.50±0.70	31.80±0.90*	33.01±0.70	23.01±0.63**	18.01±6.00	12.90±1.70
Stab neutrophils	4.96±1.20	5.96±0.90*	4.30±1.01	2.48±1.00**	2.60±1.10	3.68±0.33
Segmented neutrophils	29.91±1.10*	25.91±1.40	27.40±0.99	22.03±0.99*	15.08±2.10**	25.10±1.30
Eosinophils	1.04±0.01	1.00±0.06	1.08±0.002	2.86±0.04*	0.42±0.01	2.86±0.01
Basophils	0.91±0.004	0.55±0.001	0.85±0.001	1.46±0.002*	0.49±0.01	1.46±0.002
Monocytes	1.08±0.01	1.2±0.001*	1.10±0.002	1.57±0.03*	1.38±0.02	1.57±0.001

The study of the blood morphology (Table 2) of the experimental mice 7 days later after the start of the experiment showed that the content of red blood cells in the first group, receiving the maral root tincture before the physical stress, and mice of the control group was within the physiological norm. The ratio of the values of these indicators in the first experimental and control groups by the end of the experiment was 1:1.16, which is statistically by 14.3% significantly higher in the experimental group than in the control group.

By the end of the study there was a decrease by 18% in the number of leukocytes with respect to the initial values in the mice of the experimental group (from 7.29±0.16 to 5.98±0.28 x 10^9/l), ($p \leq 0.05$). In the control group, on the contrary, the increase was 1.23 times more or 19% (from 7.13±0.34 to 8.81±0.44×10^9/l) in comparison with their state at the beginning of the experiment ($p \leq 0.05$).

We also noticed an increase by 5.25 units in the number of lymphocytes, i.e. in 1.07 times (7.36%) in animals of the first group. Thus, at the beginning of the experiment the blood of animals of this group contained 66.05±3.60 lymphocytes, and at the end of the experiment they were – 71.30±5.00. The animals of this experimental group excelled by 5.9 units, or 8.27% at this indicator the mice of the control group. The relatively low content of lymphocytes in animals of the control group (65.40±3.80×10^9/l) under high physical activity, indicates a violation of physiological processes in the blood, probably due to insufficient stimulation of metabolic processes of the body.

At the beginning of the experiment in mice of both experimental groups, there was noticed a shift to the right of the neutrophil nucleus. That points at the predominance of segmental neutrophils in the blood, indicating the suppression of granulopoiesis when under physical stress. At the beginning of the experiment, the number of segmented neutrophils in mice of the control group was 27.40±1.20%, and in mice of the experimental group – 29.91±1.10%. By the end of the experiment in the control group the number of segmented neutrophils decreased by 8.4 units (25.10±1.01%), and in the experimental group – by 7.88 units (26.3%) and amounted to 22.03±0.99% ($p \leq 0.01$).

By the end of the experiment the number of monocytes in the experimental group increased by 1.45 times (by 31.2%) to the level of 1.57±0.03% ($p \leq 0.05$). During the experiment both in the experimental and in the control group of mice there was an increase by 2.73 times (by 63.6%) and by 1.48 times (by 32.5%) in the number of eosinophils and basophils respectively.

We also observed morphological blood changes relative to the blood morphology of the control group in mice of the second group, receiving a tincture of pantocrine during exercise. The number of red blood cells at the beginning of experimental studies (on day 7) in mice of the experimental and control groups had an unreliable difference. By the end of the experiment in mice of the experimental group the number of red blood cells was at the level of 7.41±2, 1×10^{12}/l and exceeded the number of red blood cells in the control group by 1.40×10^{12}/l (i.e. in 1.23 times or by 20.2%) ($p \leq 0.05$). The number of white blood cells at the beginning of the experiment in the mice of the control group was slightly higher by 0.24×10^9/l than in the mice of the experimental group.

By the end of the experiment, there was a decrease by 20.2% in the number of leukocytes in the experimental group, and in the control group, on the contrary, this indicator increase by 19.06%. When calculating lymphocytes, at the beginning of the experiment, we found out that their number

in both groups was slightly different and is within the physiological norm. After the experiment, the indicator for this blood count in the second group was 71.98±9.10%, which is statistically much higher by 1.08 times (by 8.04%) than the initial value and by 6.58 units, by 1.1 times (by 7.7%) in relation to the control group. In the control group, this parameter remains unchangeable during the experiment with a slight oscillation from 67.14±7.89 to 65.40±8.80%. The shift of the leukocyte nucleus «to the right» in the leukocyte formula, that is, the predominance of segmented neutrophils shows the development of violations of physiological processes in animals in the control group, where experimental mice did not receive adaptogenes during exercise. In the second group, under physical stress and influenced by adaptogenes, the number of eosinophils and basophils are reduced by 28 or by 10.9%, respectively. In this case, the number of monocytes in the experimental group increases by 13% in relation to the initial value.

The use of pantocrine during exercise promotes an absolute increase in the live weight of mice by 15.7% respectively, while the maral root activates biological processes in the body and leads to its statistically significant reduction by 51.7% in relation to the control group. Physical activity leads to morphological disorders in the organs. In control mice, signs of blood supply disturbance are revealed, accompanied by hemorrhagic tissue impregnation, dystrophic changes in myocardial fibers, cytoplasm of cardiomyocytes and inflammatory cell infiltration in the pericardium, parenchymal cells of the organs: lungs, kidneys and liver to various degrees. The greatest dystrophic changes are observed in the tissue of skeletal muscles. Skeletal muscle tissue was characterized by a marked reaction of the vascular bed, dystrophic changes in muscle fibers with their partial fragmentation. Adaptogenes reduce the pathological effects of physical activity on the body. Morphological changes in the organs are observed to a lesser degree in animals receiving the maral root and pantocrine tinctures. Studies show that maximum physical activity leads to disorders in physiological and morphological functions of the heart muscle. The use of adaptogenes can significantly increase physical performance and reduce violations of physiological and morphological processes in mice and hamsters, which is confirmed by histological studies. However, complete recovery of skeletal muscle tissue does not occur. Adaptogenes contributed to the mobilization of complex organs of immunogenesis and of blood-forming organs, expressed by an increase in the number of lymph nodes with active germinative reproduction centers in the spleen and by the appearance of small islands of lymphoid tissue in the liver and lungs. Adaptogenes used after physical activity, contribute to the glycogen restoration in the liver cells. Intense histochemical glycogen reaction appears in the liver in animals that received the maral root tincture and pantocrine, which is indicative of the acceleration of hepatic metabolism and protective functions of the body under the influence of adaptogenes. The reaction is weak in hepatocytes of the control group. Receiving adaptogenes (maral root) under physical stress have a positive effect on the morphological parameters of the blood of mice : in peripheral blood they lead to the increase in the number of red blood cells, in groups by 1.16 (14.3%) – 1.41 (20%); in hemoglobin level – by 17 (10.42%) – 22 (13.1%); in the number of lymphocytes – by 5.9 (8.27%) – 6.58 (9.14%), in relation to the animals of the control group ($p \leq 0.05$). Leukocytes and neutrophilic granulocytes of peripheral blood underwent similar changes, but tended to decrease: the number of leukocytes decreased by 2.83 (16.72%) – 3.31 (37.6%); the number of neutrophils – by 2.9 (11.15%) – 32.9; the number of rod nuclear cells – by 1.13 (46.2%) – 1.08 (29.34%); the number of segmented neutrophils – by 2.8 (11.15%) – 10.02 (39.9%), the number of eosinophils – by 1.26 (44.05) – 1.59 (79.1%); the number of basophils – by 0.33 (29.45%) – 0.97 (66.4%), with ($p \leq 0.05$).

All the above mentioned proves the high level of the oxidoreduction and of the body's resistance to physical stress. By the end of the experiment in the leucoformula of mice of the first group the number of monocytes increased with relevant difference by 0.49 (31.2%), and in relation to the control group it amounted to 0.77 units (49%), proving the activation of the immune reactions of the organism ($p \leq 0.05$). The control group of mice showed a decrease in the number of red blood cells by 0.92×10^{12}/l (13.2%), by 17 g/l (10.4%) in hemoglobin, and an increase in the number of white blood cells by 1.68×10^{9}/l (19%) with ($p \leq 0.05$). In leukocyte formula there was a shift of neutrophilic granulocytes "to the right", which proves the suppression of granulopoiesis against the backdrop of physical activity ($p \leq 0.05$). The blood biochemistry of the control group is characterized by a high level of lactic acid, alanine aminotransferase and aspartate aminotransferase. Thus, by the end of the experiment, the content of lactic acid increased by 0.52 mmol/l or 3.2 times (69.3%) with $p \leq 0.05$, aspartate aminotransferase by 4.54, 1.45 and 3.64% in accordance with the formed groups, but the difference was not significant. The content of alanine aminotransferase in the second group increased by 2.36% – with $p \geq 0.05$. Triglycerides as an indicator of fat metabolism during physical stress by the end of the experiment had a significant difference of 33.2, 33.3 and 50%, respectively, while cholesterol in the blood remained unchanged. Physical activity without

the use of adaptogenes contributed to an increase in triglycerides and glucose in the blood, followed by oxidation of this compound to lactic acid. Triglycerides, as the main component of the cell membrane, compensated and preserved the organism vitality during increased physical activity. The use of adaptogenes during physical stress shows positive physiological changes in mice, expressed by the normalization of biochemical processes. Thus, the glucose content increased in all experimental groups with a significant difference (by an average of 37.72%); lactic acid decreased, in the relation to the animals of the control group 2.68 times (by 62.6%), 2.6 (by 61.33%) and 1.7 (by 43.3%), respectively; urea in the first and second experimental groups 1.53 times less (by 34.85%), 1.8 (61.5%) with $p \leq 0.01$; the number of triglycerides in relation to the control group of animals increased 1.2 times on the average (by 16.3%), cholesterol in the first group by 10.3% and in the second by 17.6% ($p \leq 0.05$), which indicates the effectiveness of adaptogenes, increasing the physical endurance and providing metabolic processes of the body.

The mice of the experimental groups in comparison with the mice of the control group had an absolute increase in swimming activity. 14 days later after the beginning of the experiment, the duration of swimming in animals increased sharply in all groups, especially this figure was significant in the experimental group 2 and amounted to 180.0 s. Swimming activity in the group of animals receiving the maral root tincture increased by 128.40 s ($p \leq 0.01$). Maximal physical activity during 28 days in the experimental and control groups results in overtraining. This indicator in the experimental group 1 was higher by 171.0 s than that of the control group. The highest absolute increase in swimming activity we observed in the experimental group 2, where it was 371.40 s. Control group showed the lowest level of 106.30 s.

Conclusion

It is possible to use the maral root and pantocrine tinctures in recommended doses to enhance physiological functions and resistance of the animal organism to the maximal physical stress. To prevent the phenomenon of overtraining during maximal physical activity, after it the body needs rehabilitation even if adaptogenes were used. Therefore, to reduce the negative impact of physical activity on the body it is necessary to use not only adaptogenes, but also other drugs or substances that accelerate the recovery of the body. The research results should be used for the development of programs aimed to restore the physiological functions of the body after physical activity.

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