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The Effect of Physical Load of Varying Intensity on the Activity of Liver Enzymes and Hepatocytes' Proliferation

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Abstract...

The paper establishes the relationship between enzymatic activity and proliferation of hepatocytes. Low-intensity physical activity is shown not to influence the activity of hepatocytes' enzymes and their proliferation. Hard work results in decreased activity of redox enzymes; at the same time, proliferation of hepatocytes is inhibited for a long time. The most favorable is moderate load, which leads to activation of oxidative-reducing enzymes and increases the number of binucleated hepatocytes, which persist for a long time after the end of the experiment.

Keywords: Liver; Enzymes; Hepatocytes; Proliferation; Physical activity; Mitoses.

Introduction

Currently, the structure of mortality and morbidity in developed countries has fundamentally changed. Infectious pathology, with the exception of a number of viral diseases, has faded into the background, and somatic diseases have taken the main place: coronary heart disease, hypertension, gastric ulcer, mental illness, diabetes, etc. With all the diversity of all these diseases in their etiology and pathogenesis often an overly intense and prolonged stress response is common. The study of the influence of repeated stressful effects (including excessive ones) on various body systems has been undertaken repeatedly [2-4], however, studies on the effect of physical activity on the morphological state of various organs and tissues have not been studied enough. In this regard, it can be considered appropriate to study the significance of physical activity of varying severity on the proliferative activity of some animal organs. Since the proliferation of functionally active elements is an indicator of structural and physiological well-being. Also little studied is the question of the influence of the intensity of physical activity on the enzymatic activity of the functionally active elements of the organ. Meanwhile, enzymatic activity is of exceptional importance for the implementation of various processes in the organ, including the proliferation of organ elements.

The purpose of this work was to study the influence of physical work on the activity of hepatocytes' enzymes and their proliferation.

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Material and methods

White sexually mature outbred male rats in the amount of 69 individuals weighing 250-265 grams were used as experimental animals. The animals were divided into two groups (series). The first series of rats were given light physical activity, for which they were placed in a bath with a water temperature of 29-32 degrees Celsius in which the animals were swimming for 15 minutes. The rats of the second series spent 30 minutes in the bath. We regarded this physical load on animals as a moderate load. Animals of the first and second series after their removal from the bath were active, mobile; signs of fatigue were not visually detected in them. To reproduce heavy physical activity (the third series), the animals swam in the bath until they began to lose strength and to drown. This usually occurred in 55-59 minutes after the animals' being in the water. After removing from the bath, the animals of this group were sluggish, stayed down for some time. Animals of all series underwent 10 sessions of water loading, after which they were taken out of the experiment immediately after the last session (8 animals per series) and in 30 days after the end of the experiment (8 animals per series) using zoletil anesthesia calculated as 5 mg per 100 g in accordance with International Rules for Working with Experimental Animals.

The experimental rats were kept in vivarium conditions in accordance with GOST 33216-2014 "Rules for Working with Laboratory Rodents and Rabbits".

After the liver was extracted, 1 x 1 cm pieces were cut out of it, which were fixed in 10% neutral formalin and embedded in paraffin; the resulting serial sections were stained with hematoxylin and eosin and using van Gieson's staining. The tetrazolium method was used to determine SDH, NADH and NADPH. Acid phosphatase was identified by simultaneous combination with naphthol phosphates AS and stable diazonium salts. Alkaline phosphatase was determined by the Burston method [9]. Binuclear hepatocytes were calculated per 7000 cells.

The quantitative assessment of hepatocytes' enzymatic activity in dynamics was carried out using photometry. Photometry was carried out in transmitted light on a "Micromed" microscope with a camera adapter FMEL-1 and FEU-79 and the amplifier output voltage of 1200 V. In order to obtain a monochromatic beam in the red region of the spectrum passing through the preparation, an interference light filter with a maximum light transmission at a wavelength of 620 nm was used. Light transmission was recorded using a digital voltmeter SCH 4300, after which, taking a negative decimal logarithm, the light transmission level was transformed into light absorption, which was expressed in conventional units (c.u.) of optical density. According to Beer-Lambert-Bouguer law, the optical density of the preparation is proportional to the amount of dye. The described method meets the requirements of proportionality of dye concentration and enzyme activity. The optical density was calculated using the formula OD = LgUi/100. Statistical processing of digital data was carried out in the program "Statstica" involving Microsoft Office software package (Word and Excel).

Results

Table 1 provides information on the activity of liver enzymes after physical load (in c.u.).

It follows from Table 1 that the activity of liver enzymes practically did not change in animals of the first series and insignificantly deviated from normal values in rats of the second series. In animals of the third series, there was a sharp increase in lysosomal enzymes and a statistically significant decrease in the activity of redox enzymes.

Table 1: Activity of rat hepatocyte enzymes immediately after the load (M ± m).

Series	Alkaline phosphatase	Acid phosphatase	SDH	NADH	NADPH
1	0,38 ± 0,03	0,28 ± 0,02	0,35 ± 0,02	0,24 ± 0,03	0,27 ± 0,01
2	0,40 ± 0,04	0,35 ± 0,04	0,41 ± 0,05	0,33 ± 0,03	0,36 ± 0,03
3	0,71 ± 0,006*	0,71 ± 0,05*	0,21 ± 0,04*	0,14 ± 0,08*	0,19 ± 0,06*
Control group	0,36 ± 0,002	0,39 ± 0,04	0,34 ± 0,02	0,27 ± 0,02	0,32 ± 0,01

*p<0,001

Table 2: Activity of hepatocyte enzymes in rats 30 days after the load (M ± m).

Series	Alkaline phosphatase	Acid phosphatase	SDH	NADH	NADPH
1	0,34 ± 0,03	0,32 ± 0,02	0,34 ± 0,02	0,28 ± 0,03	0,24 ± 0,03
2	0,32 ± 0,04	0,28 ± 0,05	0,36 ± 0,06	0,28 ± 0,04	0,26 ± 0,05
3	0,60 ± 0,004*	0,71 ± 0,07*	0,21 ± 0,03*	0,14 ± 0,07*	0,19 ± 0,08*
Control group	0,36 ± 0,002	0,39 ± 0,04	0,34 ± 0,02	0,27 ± 0,02	0,32 ± 0,01

*p<0,001

Table 2 shows the results of studying the enzymes' activity in 30 days after the end of the physical load test (in c.u.). It can be seen that in rats of series 1 and 2 that both lysosomal and redox enzymes approached the control values. At the same time, the control values of enzymes did not restore in group 3 rats.

Binucleate hepatocytes were found in the liver of animals of all series. Table 1 provides findings on the number of binucleate hepatocytes in rats immediately / and in 30 days after the end of the experiment.

Table 3: The number of bi nucleated hepatocytes immediately / in 30 days after the end of the experiment (M ± m).

Series	Experimental animals	Control animals
1	16,2 ± 2,5/16,3 ± 1,7	14,3 ± 3,5
2	41,8 ± 3,5/36,5, ± 1,4*	14,3 ± 3,5
3	5,4 ± 2,8/8,0 ± 0,9*	14,3 ± 3,5

*p<0,001

Discussion and conclusion

A comparative analysis of liver enzymes condition and the proliferative activity of hepatocytes shows that there is a direct relationship between them. Physical activity of low intensity (1 series) practically does not affect the enzyme activity and proliferation of hepatocytes. Physical activity of moderate intensity (series 2) slightly increases the activity of lysosomal enzymes and simultaneously promotes the activation of redox enzymes; at this, the proliferation of hepatocytes is stimulated. In 30 days, liver enzymes return to the original values, but the number of bi nucleated hepatocytes in the liver remains high. Heavy physical activity (series 3) negatively affects both indicators: the activity of lysosomal enzymes increases and the activity of redox enzymes decreases, which is accompanied by a decrease in the number of bi nucleated hepatocytes. Recovery of these indicators in 30 days in animals does not take place.

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