EFFECT OF PHYSICAL EXERTION ON THE DIURNAL RHYTHM OF THE NUCLEO-CYTOPLASMIC RATIOS AND HEPATOCYTE NUCLEAR VOLUMES IN SOME MAMMALS

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UDC 612.35.014.2"41"

The diurnal rhythm of the nucleo-cytoplasmic ratios and nuclear volumes were studied in mice and rats in a state of training or chronic fatigue brought about by swimming. Moderate measured physical exertion leads to an increase in the fluctuations of the diurnal rhythm of these cytological parameters of hepatocyte metabolism (volume and nucleo-cytoplasmic ratios), whereas maximal physical exertion disturbs the diurnal rhythm of these parameters and thus provides indirect evidence of serious disturbances of metabolism in the hepatocytes of the fatigued animal.

According to the results of recent investigations [1, 2, 5] regular changes take place in the nucleo-cytoplasmic ratios and the mean nuclear volumes of hepatocytes in mice and rats during the 24-h period.

Gubin [1] has shown that diurnal changes in the nucleo-cytoplasmic ratios and mean nuclear volumes, as well as the ratios between the numbers of diploid and polyploid hepatocyte nuclei in different species of vertebrates are closely connected with the level of their metabolism (with the concentrations of RNA, glycogen, total proteins, neutral lipids, etc.).

The object of this investigation was to study the character of the nucleo-cytoplasmic ratios and nuclear volumes for the hepatocytes of mice and rats under different physiological conditions: physical exertion within physiological limits (training) and prolonged, maximal physical exertion leading to chronic fatigue.

EXPERIMENTAL METHOD

The experiments were carried out in the spring on 24 sexually mature noninbred male mice, 24 male and 24 female BALB mice (weighing 18-20 g), and 24 noninbred male rats (weighing 180-200 g). The animals were subdivided into three groups: 1) control, 2) animals subjected to measured physical exertion by swimming in water, starting at 10 a.m. at 32-34°C for mice for 1 month (from 5 min at the beginning to 30 min at the end of the experiment), and at 34-36°C for rats for 2 months (from 15 min at the beginning to 90 min at the end of the experiment, with 2 days rest per week). The animals of group 3 were made to swim for 5-8 h daily until they were completely fatigued – for mice for 1 month and the rats for 2 months. During the experiment the animals gained in weight: the control mice by 20.8%, mice in training by 21%, mice swimming until fatigued by 1.5%; the control rats by 45%, the rats in training by 42.5%, and rats swimming until fatigued by 23.6%.

The animals were decapitated on the day after their last swim, at 3 and 9 a.m. and 3 and 9 p.m. Pieces of liver were fixed in Carnoy's fluid. Sections through the liver, 4 μ in thickness, from the various groups of animals were mounted on the same slide and stained with methyl green-pyronine. The nucleo-

Department of Biology with General Genetics, Tyumen' Medical Institute. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 77, No. 2, pp. 80-83, February, 1974.

TABLE 1. Mean Nuclear Volume at Different Times of Day or Night in Mice and Rats in Various Physiological States (M ± m)

Time of day	Group of animals	Mean nuclear volume (log μ^3)		
		control	training	fatigue
3 a.m.	Noninbred mice ♂	2,370±0,0240	—	2,084±0,0311
	BALB♂	2,422±0,0314	2,526±0,0224	2,222±0,0298
	BALB♀	2,416±0,0227	2,477±0,0204	2,300±0,0259
	Rats ♂	2,393±0,0118	2,400±0,0115	2,322±0,0138
9 a.m.	Noninbred mice ♂	2,244±0,0233	—	2,113±0,0238
	BALB♂	2,318±0,0307	2,427±0,0244	2,295±0,0310
	BALB♀	2,336±0,0252	2,376±0,0254	2,304±0,0314
	Rats ♂	2,369±0,0167	2,357±0,0128	2,000±0,0158
3 p.m.	Noninbred mice ♂	2,139±0,0242	—	2,026±0,0281
	BALB♂	2,180±0,0444	2,294±0,0403	2,172±0,0362
	BALB♀	2,261±0,0276	2,357±0,0305	2,322±0,0271
	Rats ♂	2,283±0,0151	2,296±0,0147	2,300±0,0181
9 p.m.	Noninbred mice ♂	2,151±0,0266	—	2,057±0,0101
	BALB♂	2,253±0,0407	2,186±0,0397	2,055±0,0301
	BALB♀	2,272±0,0252	2,270±0,0249	2,270±0,0207
	Rats ♂	2,293±0,0164	2,232±0,0130	2,281±0,0152
	Noninbred mice ♂	$P_{3,15}<0,001$	—	$P_{3,15}<0,5$
	BALB♂	$P_{3,15}<0,001$	$P_{3,15}<0,001$	$P_{3,15}<0,2$
	BALB♀	$P_{3,15}<0,001$	$P_{3,15}<0,001$	$P_{2,15}<0,5$
	Rats ♂	$P_{3,15}<0,001$	$P_{3,15}<0,001$	$P_{3,15}>0,5$

TABLE 2. Nucleo-Cytoplasmic Ratios at Various Times of Day or Night in Mice and Rats in Various Physiological States

Time of day	Group of animals	Control	Training	Fatigue
3 a.m.	Noninbred mice ♂	1:5,01	1:5,13	1:4,05
	BALB♂	1:4,23	1:4,68	1:3,62
	BALB♀	1:5,09	1:4,67	1:3,65
	Rats ♂	1:4,60	1:4,68	1:3,98
9 a.m.	Noninbred mice ♂	1:4,58	1:5,06	1:3,98
	BALB♂	1:4,03	1:4,57	1:3,84
	BALB♀	1:4,54	1:4,54	1:4,14
	Rats ♂	1:4,37	1:3,79	1:4,53
3 p.m.	Noninbred mice ♂	1:4,32	1:4,27	1:4,50
	BALB♂	1:3,60	1:3,70	1:3,19
	BALB♀	1:4,44	1:4,18	1:3,92
	Rats ♂	1:3,99	1:3,86	1:4,07
9 p.m.	Noninbred mice ♂	1:4,08	1:3,86	1:3,87
	BALB♂	1:3,42	1:3,78	1:3,21
	BALB♀	1:4,30	1:3,57	1:3,62
	Rats ♂	1:3,64	1:3,57	1:3,72

cytoplasmic ratios of the hepatocytes were determined [4]. The nuclear volume was calculated by the equation for an ellipsoid of rotation: $\Pi/6\ (L^2B)$ [3]. For each animal 100 nuclei distributed over the whole liver lobule were measured.

EXPERIMENTAL RESULTS

The results are given in Tables 1 and 2. As Table 1 shows, in the control and experimental animals (both mice and rats) the mean nuclear volumes of the hepatocytes reached a maximum at 3 a.m. As a rule the mean nuclear volumes of the hepatocytes in both intact and experimental animals were minimal at 3 or 9 p.m. However, the amplitude of the diurnal rhythm of fluctuations in the nuclear volumes of the hepatocytes differed significantly in the different groups of animals. For instance, it was 49.7% in the control mice, 25.5% in the control rats, 86.7 and 47.5%, respectively, in the trained mice and rats, but fell to 18.0

and 9.7%, respectively, in the mice and rats swimming until fatigued. Diminution and disappearance of the diurnal rhythm of fluctuations in hepatocyte nuclear volumes in the fatigued animals affected all the groups of mice and rats studied. The hepatocyte nuclear volume of the intact mice and rats differed significantly at 3 a.m. and 9 p.m. and also at 3 a.m. and 3 p.m. ($P<0.001$). Differences between the mean hepatocyte nuclear volumes of the intact rats also were significant at 9 a.m. and 3 p.m. and at 9 a.m. and 9 p.m. In some groups of intact mice (noninbred males and BALB males) the differences between the nuclear volumes were not statistically significant at these times ($P>0.05$; $P>0.2$). In all the groups of trained mice and rats studied, the differences between the hepatocyte nuclear volumes at 3 a.m. and 3 p.m., 3 a.m. and 9 p.m., 9 a.m. and 3 p.m., and 9 a.m. and 9 p.m. as a rule were statistically significant ($P<0.01$ or $P<0.001$).

A totally different picture was observed when the hepatocyte nuclear volumes were determined for mice and rats fatigued after swimming. The differences between the hepatocyte nuclear volumes of these animals at 3 a.m. and 3 p.m., at 3 a.m. and 9 p.m., at 9 a.m. and 3 p.m., and at 9 a.m. and 9 p.m. were not significant ($P>0.5$, $P>0.5$). A similar process of a decrease in amplitude of the diurnal rhythm of the cytological parameters of the hepatocytes is revealed by analysis of Table 2, which shows the dynamics of the nucleo-cytoplasmic ratios of the hepatocytes of intact and trained animals and animals fatigued after swimming. The minimal nucleo-cytoplasmic ratios of the hepatocytes were observed in all the animals tested at 3 a.m. and the maximal values at either 3 p.m. or 9 p.m. The amplitude of the diurnal rhythm of fluctuations in the nucleo-cytoplasmic ratios was 21% for the control mice, 26.3% for the control rats, 29 and 31%, respectively, for the trained mice and rats, falling to 12 and 21.7%, respectively, for mice and rats fatigued after swimming. Whereas the differences between the nucleo-cytoplasmic ratios for the control and trained animals at different times of the 24-h period were statistically significant ($P<0.001$; $P<0.01$ or $P<0.05$), the diurnal differences in nucleo-cytoplasmic ratios for the hepatocytes of the animals fatigued after swimming were not significant ($P>0.2$, $P>0.5$); i.e., a diurnal rhythm was absent.

Synchronously with these disturbances of the diurnal rhythm of changes in the dimensions of the hepatocyte nuclei and their nucleo-cytoplasmic ratios in the animals undergoing extreme physical exertion, the diurnal rhythm of certain parameters of intracellular metabolism (RNA, glycogen, total protein, neutral lipids) also was smoothed out or sharply reduced.

Disturbance of the rhythm of concentration of biologically active substances, reflecting catabolic and anabolic aspects of metabolism, is evidently the factor responsible for the disturbance of the diurnal rhythm of changes in the dimensions of the nuclei and the nucleo-cytoplasmic ratios.

It can therefore be concluded from these experimental results that training (moderate measured physical exertion leads to a more marked diurnal rhythm of the cytological parameters of metabolism studied. Meanwhile, prolonged physical exertion leading to chronic fatigue has a marked adverse effect on the course of the natural rhythm of changes in these properties of the hepatocytes.

These observations give indirect evidence of serious disturbances of metabolic processes in the hepatocytes of the fatigued animal.

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