CHANGES IN THE LIVER CELLS OF RATS DURING REPEATED EXERTION

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Rats were compelled to swim in a bath of water at 28-30°C for 3 h daily for 30 days. Groups of animals were sacrificed after swimming 2, 5, 12, 18, 24, and 30 times. The liver decreased in weight. Starting from the 12th day and until the end of the experiment this decrease was 7-15%. A decrease in the ploidy of the hepatocyte nuclei was determined by measuring the volume of the nuclei and by cytophotometric determination of the DNA content. The mitotic index of the hepatocytes fell but the number of binuclear cells remained at a high level throughout the experiment.

Only a few liver cells of adult animals reproduce by mitotic division, but by contrast the number of polyploid and binuclear cells is considerable [6]. The cell composition, however, can vary during regeneration [7], exposure of the liver to functional loads [5], deviations from the normal diet [2, 8], or during the action of stress factors [11, 12]. Further information on the character of the relations between mitotic activity, ploidy, and the occurrence of binuclear cells can be obtained by the cytological study of the changes which take place.

In the investigation described below the cellular composition of the liver was studied in rats compelled to swim daily for 1 month.

EXPERIMENTAL METHOD

Wistar rats weighing initially 95-100 g were compelled to swim in a bath of water at 28°C for 3 h daily for 1 month. Control and experimental animals in groups of five were sacrificed by decapitation at 7 A.M. after 2, 5, 12, 18, 24, and 30 days. Pieces of liver were fixed in Carnoy's fluid and paraffin sections were stained with hematoxylin-eosin. Under a magnification of 1200×, 100 nuclei were drawn in each section. The length (L) and breadth (B) of the nuclei were measured and their volume calculated in cubic microns by the equation

$$V = \frac{\pi}{6} L^2 B.$$

Logarithms were obtained of the nuclear volumes and subdivided into classes. The ploidy of the hepatocytes was determined from the volume of their nuclei: if the logarithm of their volume was 2.05 (geometric mean 112 μ^3) the nuclei were regarded as diploid, if 2.35 (224 μ^3) as tetraploid, and if 2.65 (448 μ^3) as octaploid. The volume of the liver cell nuclei is considered to correlate with their ploidy, calculated from the DNA concentration [9, 10]. However, for control purposes, cytophotometry of the nuclei was carried out on impressions obtained from rats sacrificed after swimming for 18 days.

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TABLE 1. Weight of Liver (in mg), Mitotic Index (per thousand cells), and Number of Binuclear Cells (in percent) in Liver of Control and Swimming Rats (M ± m)

Day of experiment	Animals	Weight of liver	Mitotic index	No. of binuclear cells
2	Control	5929±275	1,97±0,418	7,6±0,87
	Experimental	5624±158	0,10±0,006	10,6±1,07
	P	0,394	0,002	0,059
5	Control	5917±155	1,39±0,327	7,6±0,49
	Experimental	5741±243	0,34±0,152	8,5±0,71
	P	0,565	0,017	0,347
12	Control	6809±409	1,86±0,254	7,4±0,34
	Experimental	5822±150	0,37±0,007	7,5±0,89
	P	0,037	0,001	0,923
18	Control	6204±182	2,93±0,768	7,0±0,55
	Experimental	5768±152	0,89±0,123	7,1±0,55
	P	0,050	0,001	0,923
24	Control	7369±242	3,82±0,291	7,0±0,65
	Experimental	6543±269	2,75±0,273	5,8±0,35
	P	0,050	0,027	0,148
30	Control	8381±220	2,33±0,205	6,7±0,82
	Experimental	7346±373	0,59±0,282	6,9±0,41
	P	0,043	0,001	0,846

TABLE 2. Nuclear Composition of Hepatocytes (in percent) of Control and Swimming Rats

Day of experiment	Animals	Ploidy by nuclear volume				P χ^2
		n	$2n$	$4n$	$8n$	
5	Control	1	46	52	1	0,5221
	Experimental	0	54	45	1	
12	Control	0	32	53	15	0,1426
	Experimental	0	42	51	7	
18	Control	1	47	51	1	0,0039
	Experimental	1	67	32	0	
30	Control	3	34	59	4	0,0027
	Experimental	1	55	43	1	

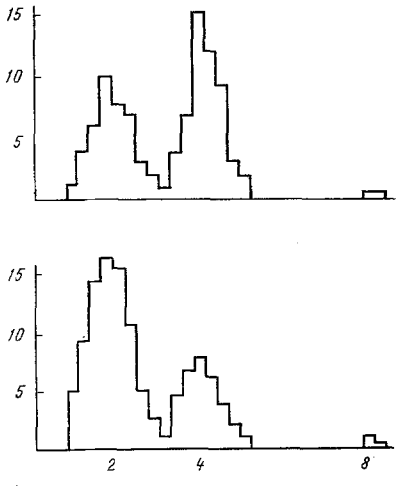

Fig. 1. Nuclear composition of hepatocytes in control (above) and experimental (below) rats on 18th day of swimming. Horizontal axis, ploidy of nuclei from DNA content; vertical axis, number of nuclei in percent.

The impressions were stained with Schiff's reagent and photometric measurements were made on 80-100 nuclei in the liver of each animal with the MUF-5 instrument in visible light at λ = 546 nm. For reference purposes, photometry was carried out on spermatozoa and lymphocytes. Besides determining the volume of the nuclei, the number of mitoses in the binuclear cells was counted among a total of 10,000-12,000 cells in sections of the liver.

EXPERIMENTAL RESULTS

The weight of the liver of the swimming rats was lower than that of the controls throughout the experiment, the difference becoming statistically significant on the 12th day (Table 1). Measurement of the volume of the nuclei showed a decrease in ploidy of the experimental rats: the number of tetraploid and octaploid nuclei fell while the number of diploid rose (Table 2). These results were confirmed in the 18-day experiment, in which the ploidy of the nuclei was determined from their DNA content (Fig. 1). In the control 42.0 ± 2.50% of diploid, 56.3 ± 2.08% of tetraploid, and 1.7 ± 0.37% of octaploid nuclei were found. Under the influence of swimming, the number of diploid nuclei rose to 70.8 ± 1.75% (P = 0.000), while the number of tetraploid and octaploid nuclei fell to 28.2 ± 1.62% (P = 0.000) and 1.0 ± 0.45% (P = 0.347) respectively.

Counting the mitoses showed that the number of mitotically dividing cells was lower in the swimming rats than in the control (Table 1). Meanwhile the number of binuclear cells remained at its initial level throughout the experiment.

Definite changes thus took place in the composition of the hepatocytes. In all probability these changes were directed toward ensuring maintenance of a high intensity of liver function throughout the period of exposure to exertion. Similar changes in the cell composition, i.e., a decrease in ploidy of the nuclei accompanied by low level of mitotic division and a constant number of binuclear cells, have been described in the literature in mice kept on a diet deficient in vitamin C [8].

Unfortunately at the present level of knowledge it is impossible to elucidate all the details of the mechanism of the cell transformations observed in the liver of the swimming rats. Nevertheless some factors can be discerned. For instance, it is unlikely that the decrease in ploidy of the nuclei was connected with death of some of the polyploid cells, for polyploid cells have been shown to be more resistant than diploid to death. In fasting rats, for example, the number of dying cells in the liver increased, but no polyploid cells were observed among them [2]. The inhibition of mitotic activity during functional loading increases the number of functioning cells. Maintenance of the number of binuclear cells evidently also has a definite purpose, for binuclear cells, as analogs of polyploid cells, at the same time differ from them in their more active metabolism as a result of an increase in the area of nucleo-cytoplasmic contact [1].

The claim has been made that if a functional load is placed on the liver the polyploid cells are partly transformed into binuclear [1, 3], which then divide by cytotomy into mononuclear diploid cells [2, 4, 8]. However, the material so far obtained is insufficient to allow any estimate to be made of the extent of such transformations in the present experiments.

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