EFFECT OF PHYSICAL TRAINING ON REGENERATION OF THE RAT LIVER AFTER PARTIAL HEPATECTOMY

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Training rats by swimming was shown to cause changes in the functional state of the hepatocytes, accompanied by changes in the distribution of nuclei relative to their DNA ploidy classes. The blood sugar and liver glycogen concentrations of the trained animals 20 and 48 h after partial hepatectomy were significantly higher than in untrained rats. Reparative regeneration after partial hepatectomy in trained rats is characterized by a unique distribution of nuclei among DNA ploidy classes and by the entry of only diploid nuclei into the phase of synthesis 20 h after the operation.

KEY WORDS: swimming; partial hepatectomy; carbohydrates; DNA ploidy; mitoses.

Prolonged training can lead to changes in the functional state of the body [1, 5]. This, in turn, can affect the structural and functional characteristics and the course of reparative regeneration of the liver tissues [15].

The object of this investigation was to study the effect of preliminary physical training on changes in the level of liver function as reflected in several indices of carbohydrate metabolism and the corticosteroid status. The dynamics of reparative regeneration of the liver after partial hepatectomy was assessed by morphometric criteria.

EXPERIMENTAL METHOD

Experiments were carried out in May and June on male Wistar rats weighing 180-200 g. Training lasted 21 days. The animals were made to swim for 1.5 h daily in water at 28°C. The rats were deprived of food for 20 h before sacrifice and were decapitated at 10 a.m. 20 and 48 h after partial hepatectomy [11].

The blood plasma sugar concentration [3] and liver glycogen [14] were determined. Polysaccharides were detected histochemically by the PAS reaction and 11-hydroxycorticosteroids (11-HCS) in the blood plasma fluorometrically. The binding capacity of the blood plasma proteins [2] and rate of corticosteriod metabolism [8] also were determined. Activity of lactate dehydrogenase (LD) [6], glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD) [13], and tyrosine aminotransferase (TAT) [9] were determined in the soluble fraction of the liver after centrifugation of the homogenate at 105,000g for 1 h. Protein was determined by Lowry's method [12]. The ploidy of the hepatocyte nuclei in squash preparations of the liver was determined by cytophotometry of DNA-fuchsin [4]; the mitotic index was calculated after counting 10,000 hepatocytes.

EXPERIMENTAL RESULTS

Prolonged training led to changes in the state of the liver tissue of the rats: to a significant decrease in the glycogen content, the binding capacity of the plasma proteins (Table 1), and TAT activity (Table 2), and also an increase in the rate of corticosteroid metabolism (Table 1). Differences in the distribution of the nuclei by ploidy classes cor-

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TABLE 1. Indices of Carbohydrate Metabolism and Corticosteroid Status of Trained and Untrained Rats after Partial Hepatectomy (M ± m)

Experimental groups of animals	Blood sugar, mg%	Liver glycogen, mg/g tissue	Plasma 11-RCS, μg %	Binding capacity of plasma, μg %	Rate of corticosterone metabolism, μg/100 mg tissue/10 min
Intact:					
1) untrained	107±10 (5)	30±7 (4)	24±5 (5)	23±0,7 (5)	15,8±0,14 (6)
2) trained	80±9 (5)	12±2 (4)	30±3 (6)	15±1,6 (5)	16,4±0,13 (6)
Hepatectomized untrained					
20 h after operation	54±5 (4)	3±1 (4)	23±4 (6)	16,6±1,1 (5)	11,0±0,79 (5)
48 h after operation trained	88±12 (4)	8±3 (4)	16±1 (6)	21,0±1,1 (6)	13,9±0,59 (6)
20 h after operation	88±10 (8)	35±2 (4)	17±3 (8)	34,5±0,9 (8)	15,9±0,51 (6)
48 h after operation	120±11 (6)	44±8 (5)	8±2 (6)	20,0±0,4 (6)	12,1±0,58 (6)
P_{1-2}	—	<0,05	—	<0,01	<0,01
P_{1-3}	<0,05	<0,01	—	<0,01	<0,001
P_{1-4}	—	<0,05	—	—	<0,02
P_{2-5}	—	<0,001	<0,02	<0,001	—
P_{2-6}	<0,05	<0,02	<0,001	<0,001	<0,02
P_{3-4}	<0,05	—	—	<0,02	<0,02
P_{3-5}	<0,05	<0,05	—	<0,05	<0,05
P_{4-6}	—	<0,05	<0,05	—	—
P_{5-6}	—	—	<0,05	<0,001	<0,001

Legend. Here and in Tables 2 and 3 number of animals shown in parentheses.

responded to differences in the functional state of the hepatocytes detected by biochemical tests in the trained and untrained rats. Diploid nuclei were predominant in the trained animals (Table 3). Prolonged training thus led to changes in certain structural and functional characteristics of the liver. Partial hepatectomy, performed against this background, was characterized by several differences from its course in untrained animals. The blood was unchanged (Table 1), whereas in the untrained animals it was reduced by half. Similar changes also affected the liver glycogens, demonstrated histochemically. Biochemical determination of glycogen revealed an increase in its content in the liver of the trained rats but, on the contrary, a marked decrease in the untrained animals. Similar results also were obtained 48 h after partial hepatectomy. The higher carbohydrate concentrations in the blood and liver of the trained animals than of the untrained 20 and 48 h after partial hepatectomy could be the result of a reduction in their catabolism or an increase in their synthesis. The lower activity of LD and enzyme of the oxidative branch of the hexose-monophosphate shunt (Table 2) could be evidence of a reduction in carbohydrate catabolism in the liver of the trained rats. The rate of carbohydrate synthesis depends on several factors, including glycocorticosteroids, which participate in the control of gluconeogenesis and which activate TAT [7]. The action of steroids depends on their concentration in the blood, the binding capacity of the plasma, and the rate of metabolism. As Table 1 shows, the concentration of total 11-HCS in the blood plasma fell after partial hepatectomy on the trained rats, the binding capacity of the plasma proteins increased, and the rate of corticosteroid metabolism was not below its level in the untrained animals. Changes in the last three indices could be evidence of a decrease in the efficiency of the action of glucocorticoids on the hepatocytes in the trained rats. Changes in TAT activity confirmed this hypothesis to some extent (Table 2). The rate of carbohydrate synthesis, controlled by glucocorticoids, was thus not increased. The high carbohydrate concentration in the trained animals after partial hepatectomy can probably be explained by an increase in gluconeogenesis in the earlier period under the influence of catecholamines and glucagon [10] or in the later period on account of fats. Another possible variant could be the appearance of a population of hepatocytes highly sensitive to the action of glucocorticoids.

Besides differences in the state of the liver function of the trained rats detectable after partial hepatectomy, distinctive changes also were found in the distribution of nuclei among ploidy classes. Whereas in the untrained animals both diploid and tetraploid nuclei

TABLE 2. Activity of TAT (in μmoles p-hydroxyphenylpyruvate/min/g protein at 37°C), LD (in μmoles NAD•H_2/min/mg protein at 25°C), G6PD, and 6PGD (in μmoles NADPH/min/g protein at 25°C) in the Liver of Trained and Untrained Rats after Partial Hepatectomy (M ± m)

Experimental groups of animals	Enzyme activity		
	TAT	LD	G6PD + 6PGD
Intact:			
1) untrained	31±9 (4)	3,2±0,30 (2)	188±15 (4)
2) trained	7,5±3,9 (4)	2,76±0,07 (4)	165±21 (4)
Hepatectomized untrained			
20 h after operation	85±34 (4)	1,45±0,04 (2)	240±56 (2)
48 h after operation	124±36 (3)	1,19±0,11 (3)	220±11 (3)
trained			
20 h after operation	40±7 (5)	0,92±0,15 (5)	170±11 (6)
48 h after operation	37± 16(3)	2,67±0,07 (6)	135±7 (6)
P_{1-2}	<0,05	—	—
P_{1-3}	—	<0,05	—
P_{1-4}	<0,05	<0,05	—
P_{2-5}	<0,01	<0,001	—
P_{3-5}	—	<0,05	—
P_{4-6}	—	—	<0,05
P_{5-6}	—	—	<0,05

TABLE 3. Mitotic Index and Distribution of RNA among Ploidy Classes in Trained and Untrained Rats after Partial Hepatectomy (M ± m)

Experimental group of animals	Mitotic index, ‰	Ploidy of nuclei, %			P (χ^2)
		2 n	4 n	8n	
Intact:					
1) untrained	0,04 (6)	43,6 (9)	55,1 (9)	1,1 (9)	P_{1-2} <0,0005 (17)
2) trained	0,00 (6)	54,7 (6)	44,7 (6)	0,6 (6)	P_{1-3} <0,0005 (81)
Hepatectomized untrained					
20 h after operation	5,1±1,07 (4)	37,3 (3)	49,7 (3)	12,3 (3)	P_{2-5} <0,0005 (71)
48 h after operation	—	21,0 (4)	67,5 (4)	11,5 (4)	P_{3-4} <0,0005 (25)
trained					
20 h after operation	0,1±0,01 (8)	31,7 (7)	65,3 (7)	0,7 (7)	P_{3-5} <0,0005 (45)
48 h after operation		17,6 (5)	67,4 (5)	15,0 (5)	P_{4-6} <0,1 (3,36)
					P_{5-6} <0,0005 (75)

Legend. $P(\chi^2)$ denotes level of significance of differences in χ^2 (value of χ^2 shown in parentheses) during comparison of corresponding empirical distributions.

were found in the phase of DNA synthesis, in the trained rats after 20 h only the tetraploid class was increased. These changes were accompanied by some increase in the mitotic index after 20 h in the untrained and by no increase in the trained rats (Table 3). This corresponds to the later entry of the cells into the phase of DNA synthesis in the experimental animals (Table 3).

Training thus led to changes in the structural and functional state of the liver, including redistribution of the nuclei among the ploidy classes. The process of regeneration of the liver after partial hepatectomy in the trained animals also was marked by certain special features. The distribution of nuclei among the ploidy classes was significantly altered, possible evidence of the later entry of the cells into the phase of DNA synthesis, at least compared with the corresponding groups in the untrained rats. Consequently, the development of regenerative processes in rats subjected to prolonged training is probably determined by the preceding character of the functional state of the liver tissue and of the body as a whole, as is manifested in the course of regeneration.

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ACTION OF ALLOGENEIC AND SYNGENEIC SPLENIC EXTRACTS ON PRIMARY CELL CULTURES FROM INBRED MICE

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The effect of allogeneic and syngeneic extracts from the spleens of male and female inbred mice on primary cultures of fibroblasts obtained from the subcutaneous connective tissues of fetuses of CBA and C57BL/6J mice was studied. The cytotoxic and growth-inhibiting action on the cultures was successively enhanced by the use of extracts from syngeneic male and allogeneic female and male tissues. Consequently, an increase in the degree of antigenic difference between the target cells and extracts led to enhancement of the phenomenon of allogeneic inhibition. It was shown for the first time that in a syngeneic system extracts from male tissues (containing the weak H–Y antigen) have a cytotoxic action on cells from female inbred mice, i.e., that they induce a reaction of the allogeneic inhibition type.

KEY WORDS: target cell; extract; antigen; allogeneic inhibition.

Extracts that are foreign with respect to strong tissue compatibility antigens are known to have a cytotoxic action (allogeneic inhibition) on target cells in culture [2, 4, 5, 8, 9]. However, no data could be found in the accessible literature on the effect of extracts on target cells differing in the weak H–Y antigen. The problem of whether in this case the reaction is of the allogeneic inhibition type and whether this effect increases with an increase in the antigeneic differences between the target cells and extracts has not yet been studied.

The object of this investigation was to compare the cytotoxin effect of extracts differing with respect to strong (H–2) and weak (H–Y) transplantation antigens on primary cultures of cells from inbred mice.

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