NATURE OF POSTTOXIC REPARATIVE REGENERATION OF THE LIVER IN RATS WITH INTACT ADRENALS AND WITH CORTICOSTEROID DEFICIENCY

G. S. Yakobson, O. A. Gromova,
S. G. Dobrovol'skaya, A. E. Kondakova,
L. V. Nosova, N. M. Seregina,
and G. N. Shorina

UDC 616.36-099-06 :616.45-008.64

The character of reparative regeneration of the liver was studied in experiments on rats. In adrenalectomized rats, by contrast with intact, administration of CCl_4 did not lead to changes indicative of activation of intracellular repair processes in the liver, as was confirmed indirectly by death of 67% of the animals after receiving CCl_4.

Administration of some hepatotropic poisons to animals induces necrotic and degenerative changes in the liver [9, 14]. The destruction is followed by repair aimed at restoring the function of the organ [4, 5]. Regenerative processes, induced by the metabolic situation in the liver [5], or by disturbance or loss of function of the organ [1], are controlled predominantly by hormones [3]. The influence of corticosteroids on both destructive and reparative processes in the liver in toxic hepatitis have been inadequately studied.

The authors have investigated a number of indices of reparative regeneration of the liver in animals after CCl_4 poisoning. The severity of the degeneration, the ploidy and dimensions of the nuclei and cytoplasm of the hepatocytes, their mitotic activity, and the DNA concentration in the liver were all determined. The state of protein synthesis in the liver was assessed from the RNA concentration, the activity of the liver acid RNase, and the size of the hepatocyte nucleoli. The state of the stroma was determined from the area of the connective tissue. To estimate the external function of the liver, the concentrations of the serum protein fractions were determined.

EXPERIMENTAL METHOD

Carbon tetrachloride was given as three separate doses by mouth to male Wistar rats weighing 120-180 g in the form of a 40% oily solution. Each dose was 0.1 ml/100 g body weight and the intervals between doses were 2 days. Corticosteroid deficiency was produced by bilateral adrenalectomy under ether anesthesia. The adrenalectomized rats received a special salt solution [2]. There were 2 series of experiments: I) on 11 intact rats receiving CCl_4, and II) on 30 adrenalectomized rats receiving CCl_4 under the same conditions, starting on the 4th day after adrenalectomy. The rats were decapitated 24 h after the last dose of CCl_4. The control consisted of 10 intact and 12 adrenalectomized rats respectively, and these animals received the solvent (olive oil) only (0.06 ml/100 g body weight).

The mitoses were counted in sections stained with hematoxylin and eosin, and the results expressed in promille. To estimate the area of the connective tissue formed in the liver quantitatively, a planimetric method was used.

Central Research Laboratory, Novosibirsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 72, No. 7, pp. 95-99, July, 1971. Original article submitted August 10, 1970.

TABLE 1. Reaction of Rats to CCl_4

Index studied	Intact rats				Adrenalectomized rats			
	control		receiving CCl_4		control		receiving CCl_4	
No. of mitoses in hepatocytes (in $^0/_{00}$)	0	(5)	3,6± 1,51	(8)	0	(5)	20,4± 5,84	(10)
DNA concn. in liver (in mg% P)	98,9± 3,1	(10)	127,4± 6,3 $P_{1-2}<0,001$	(11)	122,7± 6,4 $P_{1-3}<0,01$	(12)	141,6± 6,8	(10)
RNA concn. in liver (in mg% P)	226,4± 4,3	(10)	270,7± 8,4 $P_{1-2}<0,001$	(11)	253,0± 7,8 $P_{1-3}<0,01$	(12)	288,4± 7,3 $P_{3-4}<0,01$	(9)
RNA/DNA ratio	2,31± 0,08	(10)	2,15± 0,06	(11)	2,04± 0,05 $P_{1-3}<0,01$	(11)	1,99± 0,05 $P_{2-4}<0,1$	(10)
Activity of acid RNase in liver (in mg P RNA)	1315,7± 87,8	(7)	912,8± 121,2 $P_{1-2}<0,01$	(8)	1134,0± 103,5	(8)	1 207± 249,1	(5)
Area of nucleoli of hepatocytes (in μ^2)	2,85± 0,183	(5)	4,88± 0,617 $P_{1-2}<0,02$	(4)	2,66± 0,075 $P_{3-4}<0,05$	(3)	3,297± 0,238 $P_{2-4}<0,05$	(6)
Area of nucleus of hepatocytes (in μ^2)	21,66± 2,0	(8)	20,74± 1,26	(7)	24,41± 1,25	(6)	28,96± 3,08 $P_{2-4}<0,05$	(5)
Area of cytoplasm of hepatocytes (in μ^2)	139,4± 2,17	(8)	165,22± 1,72 $P_{1-2}<0,01$	(7)	128,47± 20,5	(6)	136,46± 2,37	(5)
Area of foci of infiltation of liver by lymphocytes and histiocytes (in relative units)	0,98± 0,05	(7)	4,0± 0,96	(15)	0,87± 0,08	(7)	2,71± 0,37 $P_{3-4}<0,001$	(16)

Note. Number of animals shown in parentheses.

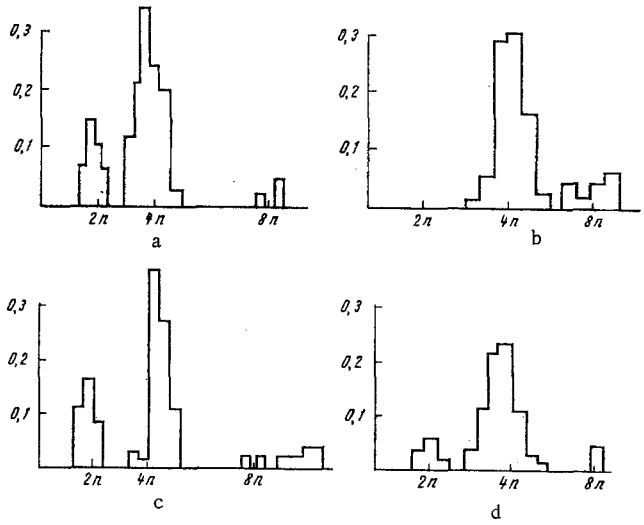

Fig. 1. Histograms of indices of rat liver studied after various procedures: a) intact rats; b) after 3 doses of CCl_4; c) 14 days after adrenalectomy; d) after 3 doses of CCl_4 given to adrenalectomized animals. Ordinate, frequency of occurrence of cells (after Wenzel); abscissa, DNA content in nuclei expressed in units of ploidy.

The relative DNA content in the nuclei of the hepatocytes was determined by a double-wave cytophotometric method [8] in impressions of liver sections fixed in Carnoy's fluid and stained by Feulgen's method.

The diameter of the nucleoli was measured in liver sections fixed in Carnoy's fluid and stained with a combination of gallocyanin and chrome alum, using a linear ocular micrometer giving a magnification of 1350. Control staining of the nucleoli was carried out with preliminary incubation of the sections with RNase or with treatment of the sections with 5% TCA solution. The area of the nucleoli was calculated by the formula for a circle.

The concentrations of RNA and DNA in the liver homogenates were determined spectrophotometrically by the method of Tsanev and Markov [7] after fractionation by the method of Schmidt and Thannhauser [12], and acid RNase activity was determined by the method of Schneider and Hogeboom [13]. The total protein concentration in the blood serum was determined by the method of Lowry et al. [10] and the protein fractions by electrophoresis on paper in veronal buffer (ionic strength 0.1) for 18 h under a voltage of 100 V.

EXPERIMENTAL RESULTS

Administration of CCl_4 to the adrenalectomized rats caused death of 67% of the animals; all the intact animals receiving the poison survived. Degenerative changes in the liver tissue of the adrenalectomized rats receiving CCl_4 were more widespread than in the intact rats receiving the poison. Infiltration of the organ by lymphocytes and histiocytes was about equal in degree in the adrenalectomized and intact rats. The number of mitoses in the liver of the adrenalectomized rats receiving CCl_4 was significantly higher (Table 1). No increase in the ploidy of the nuclei, such as was observed in the overwhelming majority of hepatocytes of the intact rats receiving CCl_4, was observed in the adrenalectomized rats receiving the poison (Fig. 1). In the adrenalectomized rats administration of CCl_4 was followed by a marked increase in the size of the nuclei (Table 1), presumably due to their disintegrative swelling [6], but there was no increase in the area of the cytoplasm of the hepatocytes (Table 1). This confirms the wider spread of the degenerative changes in the liver observed in adrenalectomized animals receiving CCl_4.

The area of the nucleoli in the hepatocytes of the adrenalectomized rats receiving CCl_4 was increased much less than in the intact rats (Table 1).

The concentrations of RNA and DNA were increased in the liver of both the adrenalectomized and the intact rats receiving CCl_4. However, unlike in the intact animals, in the adrenalectomized rats this increase was not due to the toxic action of CCl_4, but to the corticosteroid deficiency. The increase in the

DNA content in the liver of the adrenalectomized rats compared with the intact animals also receiving CCl_4 was probably due to an increase in the number of mitoses and in the number of cells occurring in a weighed sample of tissue. This last result was due to a marked decrease in the glycogen content and in the volume of the hepatocytes of the adrenalectomized rats receiving CCl_4 compared with the intact animals also receiving the poison.

The activity of acid RNase in the liver of the adrenalectomized rats receiving CCl_4 was unchanged, whereas administration of the poison to the intact animals lowered the activity of the enzyme. The opposite directions of changes in the RNA concentration and in acid RNase activity will be noted.

It is interesting that the intensity of RNA metabolism increases with a decrease in the activity of the enzyme [11].

In the adrenalectomized rats, which differed from the intact in their lower concentrations of total protein, albumins, and β- and γ-globulins (9.43 ± 0.35; 3.62 ± 0.13; 1.5 ± 0.09; and 1.64 ± 0.07 g% compared with 7.17 ± 0.15; 2.59 ± 0.07; 1.17 ± 0.06; and 1.27 ± 0.09 g%, respectively), CCl_4 caused an increase in the concentration of α_1-globulins (1.44 ± 0.4 g% compared with 1.18 ± 0.09 g%). Under these circumstances, the characteristic increase in concentration of β-globulins observed in the intact rats (1.50 ± 0.09 and 2.17 ± 0.10 g%, respectively) was not observed (control 1.17 ± 0.06 g%; experiment 1.24 ± 0.07 g%).

The results thus indicate that administration of CCl_4 to adrenalectomized animals does not give rise to changes affording evidence of reparative intracellular regeneration of the liver. This is probably largely responsible for the death of 67% of the animals in this series. In adrenalectomized animals sacrificed at the same times after receiving the poison as the intact animals, the degenerative changes were less severe. In these same animals the effects of CCl_4 were reflected in a much smaller increase in the size of the hepatocyte nucleoli.

Characteristically, in these rats the action of CCl_4 did not alter the ploidy of the nuclei, and the RNA/DNA ratio showed a tendency to decrease.

At the same time, acid RNase activity determined in the liver homogenate remained unchanged.

The facts described above show that corticosteroids are highly important regulators of reparative processes in the liver. The results do not shed light on the relative importance of the individual adrenal hormones in maintenance of the optimal level of repair processes in the liver. It is thus necessary to study the isolated effects of glucocorticoids and mineralocorticoids on the processes of reparative regeneration after toxic action.

LITERATURE CITED

1. A. Glinos, in: Information Theory in Biology [in Russian], Moscow (1960), p. 148.
2. N. B. Medvedeva, Experimental Endocrinology [in Russian], Kiev (1946).
3. V. F. Sidorova, Postnatal Growth and Regeneration of the Internal Organs in Vertebrates [in Russian], Moscow (1969).
4. V. F. Sidorova, Z. A. Ryabinina, and E. M. Leikina, Regeneration of the Liver in Mammals [in Russian], Leningrad (1966).
5. N. Frunder, Vopr. Med. Khimii, No. 2, 130 (1968).
6. Ya. E. Khesin, Dimensions of the Nuclei and the Functional State of Cells [in Russian], Moscow (1967).
7. R. G. Tsanev and G. G. Markov, Biokhimiya, No. 1, 151 (1960).
8. A. I. Sherudilo, Cytophotometry (Physical and Biological Aspects of the Study of Cell Nuclei). Author's Abstract of Candidate's Dissertation, Novosibirsk (1965).
9. G. R. Cameron and W. A. E. Karunaratne, J. Path. Bact., 42, 1 (1936).
10. O. Lowry, N. Rosebrough, A. Farr, et al., J. Biol. Chem., 193, 265 (1951).
11. J. E. Rahman, Biochim. Biophys. Acta, 178, 68 (1969).
12. G. Schmidt and S. J. Thannhauser, J. Biol. Chem., 161, 83 (1945).
13. W. S. Schneider and G. H. Hogeboom, J. Biol. Chem., 198, 155 (1952).
14. R. E. Stowell and C. S. Lee, Arch. Path., 50, 519 (1950).