REGENERATION OF THE MOUSE LIVER AFTER PROLONGED CCl_4 POISONING

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Mitotic activity and the intensity of DNA synthesis was investigated in the mouse liver regenerating after resection of the left lateral and central lobes. For several months beforehand the mice had been given CCl_4. The regenerating liver of mice not poisoned with CCl_4 was used as the control. During regeneration of the pathologically changed liver proliferation of the hepatocytes was found to take place at the same times and at the same intensity as during regeneration of the normal liver.

Even when cirrhotic changes in the liver have reached a considerable level of development the organ can still regenerate after partial hepatectomy [2-5]. However, it is not clear how regeneration of the pathologically changed liver differs from regeneration of an organ which was normal before surgical trauma. There is some evidence that the cirrhotic liver regenerates as the result of processes taking place throughout the residual part of the organ remaining after resection. Under these circumstances the process of regeneration of the liver under pathological conditions is similar to that of regeneration of the normal liver. There is also evidence of a slower course of regeneration of the pathologically changed than of the normal liver [6]. During regeneration of the liver in rats with cirrhotic changes following administration of thioacetamide, the times of development of proliferative processes in the residual organ are shifted slightly compared with the times of development of the same processes in the normal liver, but the intensity of proliferation is equally high as during regeneration of the normal liver [7].

This paper describes an investigation of the mitotic activity and the intensity of DNA synthesis during regeneration of the liver in mice poisoned by prolonged administration of CCl_4.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino mice. Pathological changes were produced in the liver by subcutaneous injections of CCl_4. The first experiment lasted from the end of June to the beginning of November. During this time 43 injections of CCl_4 were given. In the second experiment CCl_4 was injected from the end of August to the end of January (39 injections). The compound was given as injections of a 40% solution in oil in a dose of 0.2 ml per mouse.

On the fourth day after the end of administration of CCl_4 the left lateral and central lobes of the liver were removed from the mice. The operation was performed between 11 a.m. and noon. The mean weight of the experimental animals was 31 g. In the first experiment the mice were killed 37, 45, and 49 h after resection of part of the liver. These mice received a preliminary injection of thymidine-H^3, in a dose of 0.53 μCi/g, 1 h before sacrifice. Mice not poisoned with CCl_4 acted as the control. The operation was performed on these mice, and the animals were sacrificed simultaneously with the experimental group. The mean weight of the control mice was 35 g. Thymidine-H^3 was injected into the animals in a dose of 0.54 μCi/g. In the second experiment the animals were sacrificed 72 h after the operation. These mice received

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TABLE 1. Number of Labeled Hepatocytes in Regenerating Liver of Experimental and Control Animals (M±m)

Expt. No.	Time of sacrifice (in h)	Expt. (administr., CCl_4)		Control		P
		No. of mice	No. of labeled nuclei	No. of mice	No. of labeled nuclei	
1	37	10	3,33±0,81	7	7,29±2,3	0,25
	45	10	1,89±0,43	7	2,50±1,25	0,55
	49	3	1,31±0,34	—	—	
2	72	10	22,3±6,6	6	14,37±7,3	0,25

TABLE 2. Mitotic Activity in Regenerating Liver of Experimental and Control Animals (M±m)

Expt. No.	Time of sacrifice (in h)	Expt. (administr., CCl_4)		Control		P
		No. of mice	No. of mitoses (in $^0/_{00}$)	No. of mice	No. of mitoses (in $^0/_{00}$)	
1	37	10	0,58±0,17	7	1,30±0,55	0,24
	45	10	1,27±0,51	7	1,54±0,69	0,1
	49	3	0,65±0,07	—	—	
2	72	10	7,88±3,1	6	2,64±2,2	0,21

five injections of thymidine-H^3 (38, 42, 47, 52, and 58 h after the operation) in order to identify the proliferative pool. The dose of thymidine in the second experiment was 7 μCi per mouse per injection. The control for this experiment consisted of mice not poisoned with CCl_4.

Pieces of liver were fixed in Carnoy's fluid and embedded in paraffin wax. Type M nuclear emulsion was applied to the sections. Exposure lasted for 21 days. The sections were stained with hematoxylin and eosin. The number of mitoses and the number of labeled nuclei were counted in 6000 cells. Some sections were stained with azan by Heidenhain's method. The numerical data were subjected to statistical analysis by the Fisher–Student method.

EXPERIMENTAL RESULTS

As a rule the fibrotic changes in the liver were slight in degree and were manifested as a varied degree of development of collagenous connective tissue around the blood vessels and between the lobules. In some areas collagen fibers penetrated into the parenchyma of the lobules. Characteristically the liver of the experimental mice also contained areas of necrosis and round-cell infiltration of considerable size in the parenchyma. Marked polymorphism of the nuclei and fatty vacuolation of the hepatocytes were observed both in the liver of the animals poisoned with CCl_4 and in the liver of the control mice.

The mitotic activity and intensity of DNA synthesis in the regenerating liver both of the mice poisoned with CCl_4 and of the mice not so treated were low in both the first and the second experiments (Tables 1 and 2). This was evidently because partial hepatectomy was performed on old mice.

No significant differences in the number of mitoses or the number of labeled nuclei were found in the regenerating liver of the experimental and control animals (Tables 1 and 2). There were likewise no significant changes in these parameters at the different times after the operation.

In both the experimental and the control series considerable individual variations in mitotic activity and in the number of labeled nuclei were found in the regenerating liver of the different mice. For instance, 37 h after the operation the number of labeled hepatocytes in the regenerating liver of mice poisoned with CCl_4 varied from 0.06 to 6.65%. The number of labeled nuclei in the regenerating liver of the control mice varied from 0.10 to 27.30% and the number of mitoses from 0.14 to 3.42$^0/_{00}$. An equally marked degree of individual variation was found by investigation of the proliferation pool 72 h after the operation. In this experiment the number of labeled nuclei in the regenerating liver of the mice receiving CCl_4 varied

from 4.01 to 68.40%. In the regenerating liver of the control mice the number of labeled nuclei was 3.87-47.90%. The number of mitoses in the regenerating liver of the experimental mice varied between 1.13 and 30.1‰, compared with variation between 0.04 and 13.72‰ in the regenerating liver of the control mice.

The existence of individual variations in the course of proliferation in the regenerating liver of normal mice was noted previously [1]. The results given in this paper show that this is a characteristic feature also of regeneration of the pathologically changed liver.

The general conclusion can be drawn from these observations that after resection of part of the pathologically changed liver proliferation of the hepatocytes can take place at the same time and with the same intensity as during regeneration of the normal liver of animals of the same age.

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