REGENERATION OF THE LIVER IN MICE IN EARLY PERIODS AFTER INJURY

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In a previously published work [1] we studied the mitotic activity in the liver after partial extirpation of that organ and its dependence on the time of the operation.

In the present work we investigated the changes in a number of morphological and cytochemical indices in the course of regeneration of the liver at different times of day after partial extirpation.

METHOD

From 60 to 70% of the liver (the central and left lateral lobes) was removed by the method of Higgins and Anderson from mice weighing 20-25 g. The weight of the part removed was 697-880 mg (3-4% of the body weight). The operations were carried out in February, in some of the mice in the morning (9 A.M. to 10 A.M.) and in others in the evening (6 P.M. to 7 P.M.). Altogether 90 mice were used, of which 65 were experimental and 25 controls.

The experimental and control animals were sacrificed 48, 54, 60, 66, and 72 hours after operation. The liver was weighed, the caudate lobe of the liver was fixed in Zenker's fluid with acetic acid, and the remaining part was fixed in 80° alcohol. The largest (D) and smallest (d) diameters of the liver cells were measured by means of an ocular micrometer in films stained by Gomori's method. Only mononuclear cells were measured, and only those cells were considered whose nuclei were in interkinesis. In each case we measured 100 liver cells. The diameter of the nuclei of the liver cells (d_n) was also determined. Measurements of the cell and nuclear diameters were made on the liver of 20 experimental and five control animals (in one animal for each period of fixation in mice undergoing operation in the morning and evening). Alkaline phosphatase was detected by Gomori's method and RNA was demonstrated by staining the films with methyl green pyronine.

RESULTS

On the second day after operation all the experimental mice lost from 2.5 to 5 g in weight. The absolute weight of the regenerating liver at this time was 780-1130 mg, or 4.2-5.7% of the body weight, and the weight of the liver in the control animals was 1190-1450 mg, or 5.5-6.1% of the body weight. These findings showed that the weight of the regenerating liver on the second to third day after operation reached 58-80% of the weight of the normal organ, irrespective of whether the operations were performed on the animals in the morning or evening.

Macroscopically, the lobes of the regenerating liver were considerably hypertrophied, although they had the normal dark red color.

The results of the measurements showed that the dimensions of the liver cells were increased on the second to third day after operation, and moreover that in the experimental mice undergoing operation in the morning, the smallest cell diameter d = 18.6μ and the largest D = 23.2 μ; in the control mice d = 17.7 μ and D = 20.7 μ; in the experimental mice undergoing operation in the evening, d = 20.5 μ and D = 24.1 μ, and in the controls d = 18.0 μ and D = 21.5 μ. An increase in the diameter of the nuclei was also observed. For instance, in the experimental mice undergoing operation in the morning, d_n = 8.7 μ, and in the controls d_n = 8.3 μ; in the experimental mice undergoing operation in the evening, d_n = 9.6 μ and in the controls d_n = 8.3 μ.

It can be seen from the figures in the table that hypertrophy of the nuclei and cells was more pronounced in the mice undergoing operation in the evening. The differences obtained were statistically significant. Hypertrophy of the liver cells in rats on the first day after operation was observed by several authors [2, 4, 6, 8]. In mice, however, observations in particular on the size of the liver cells at later periods after operation are not available. According to our findings, the hypertrophy of the liver cells was permanent in character.

Histological investigation showed that the regenerating liver was characterized by considerable polymorphism of the liver cells: side by side with very small cells, the diameter of which did not exceed 12.6 μ, were seen large cells, whose diameter was 36-43 μ; at the same

Results of Measurement of the Diameters of the Liver Cell and Nucleus After Partial Hepatectomy

Operation performed in morning, at 9-10 A.M.										Operation performed in evening, at 6-7 P.M.									
time of sacrifice after operation (hrs)	in hours	time of day at which fixation was carried out	Experiment			Control				time of sacrifice after operation (hrs)	in hours	time of day at which fixation was carried out	Experiment			Control			
			smallest cell, diameter, d (in μ)	largest cell, diameter, D (in μ)	diameter of nucleus, d_n (in μ)	smallest cell, diameter, d (in μ)	largest cell, diameter, D (in μ)	diameter of nucleus, d_n (in μ)					smallest cell, diameter, d (in μ)	largest cell, diameter, D (in μ)	diameter of nucleus, d_n (in μ)	smallest cell, diameter, d (in μ)	largest cell, diameter, D (in μ)	diameter of nucleus, d_n (in μ)	
48	48	9	18,4	22,9	7,4	17,6	21,2	8,4	8,4	48	48	21	21,2	23,8	9,3	18,3	20,5	8,0	8,0
54	54	15	18,6	23,1	8,7	18,0	21,8	8,1	8,1	54	54	3	19,4	23,2	9,0	17,7	21,9	8,4	8,4
60	60	21	18,6	22,6	8,7	18,3	20,5	8,0	8,0	60	60	9	19,3	24,1	8,4	17,6	21,2	8,4	8,4
66	66	3	18,9	24,1	9,4	17,7	21,9	8,4	8,4	66	66	15	19,3	23,2	9,0	18,0	21,8	8,1	8,1
72	72	9	18,7	23,1	9,0	16,8	20,7	8,3	8,3	72	72	21	21,2	26,1	10,4	18,3	21,8	8,3	8,3
		M	18,6	23,2	8,7	17,7	20,7	8,3				M	20,5	24,1	9,6	18,0	21,5	8,3	

time cells could be seen with very large and with very small nuclei. These observations are also confirmed by data in the literature [3-10]. On the second to third day after operation, an enormous number of binuclear cells was found in the liver, and liver cells with three, or even four nuclei were also present. Cells in different phases of mitotic and amitotic division were observed (Fig. 1).

During investigation of the RNA and alkaline phosphatase content in the normal and regenerating liver, an uneven distribution of these substances in the cytoplasm of the cells of the parenchyma was observed. The liver cells of the marginal zone, cells situated near the blood vessels and at the periphery of the liver lobules, were richer in RNA and alkaline phosphatase. In the liver of the control animals daily changes were observed in the content and localization of RNA. In the morning (9 A.M.) and afternoon (3 P.M.) more RNA was present in the cytoplasm of the liver cells than in the evening and at night (9 P.M. and 3 A.M.).

Our findings were not in agreement with the biochemical investigations of Jardetzky et al. [8], who showed that the RNA content of the liver in normal mice is increased from 4 to 8 P.M. and thereafter gradually falls. In the hours of day, globules of RNA filled the whole cytoplasm of the liver cells, but in the evening and night hours they were situated mainly along the periphery of the cells, with a small amount left also around the nucleus. This phenomenon was evidently connected with diurnal changes in the liver glycogen content. In the course of the 24 hours the staining of the nucleoli with pyronine remained unchanged. In the normal liver cells alkaline phosphatase was present in very small amounts.

The time of operation (morning or evening) had no effect on the cytochemical changes observed in the regenerating liver, so that the findings which we describe were obtained in the two groups of animals.

No diurnal periodicity in the RNA content was observed in the regenerating liver of the mice. In the process of regeneration other regular features were observed in relation to the content of this compound, namely in the liver in which regenerative processes had not yet appeared or were ill-developed, the RNA content remained unchanged or was slightly reduced, and in these cases the activity of the enzyme was slightly increased; conversely, in the liver in which intensive proliferation of liver cells was taking place by mitotic and amitotic division, an increase in the RNA content and the enzyme activity was observed. At the same time a change took place in the character of the distribution of these compounds in the cytoplasm of the liver cells, whether undergoing mitotic division or newly formed: RNA and alkaline phosphatase were distributed here in the form of fine granules, filling the whole cell (Fig. 2).

In the regenerating liver at all times of fixation the cells of the bile ducts, sinuses and connective tissue contained a large amount of RNA and showed a strong en-

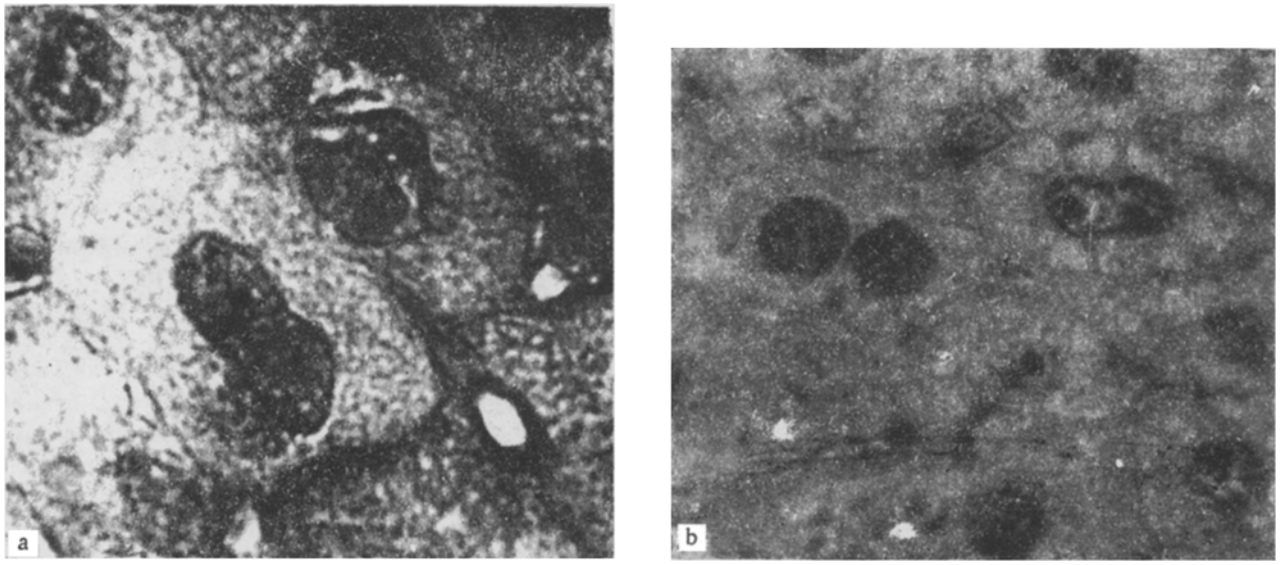

Fig. 1. Different stages of amitotic division in the regenerating liver of a mouse (on the second day). a) Constriction of the nucleus; b) dividing nucleus. Stained by Gomori's method for alkaline phosphatase. Magnification: ocular 6 ×, objective 90 ×.

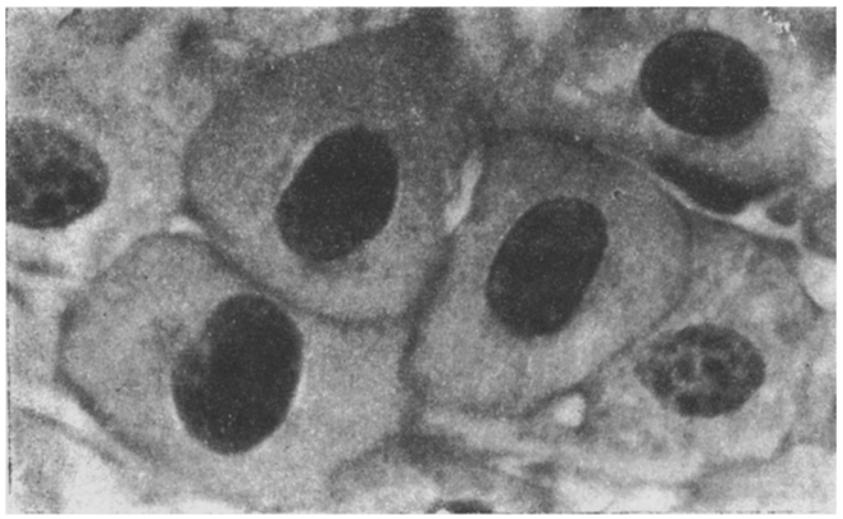

Fig. 2. A group of newly formed liver cells. Alkaline phosphatase in the form of tiny granules fills the whole cytoplasm of the liver cell. Stained by Gomori's method. Magnification: ocular 6 x, objective 90 x.

zymic reaction. The greatest increase in the RNA and alkaline phosphatase was observed 72 hours after operation (Fig. 3).

These findings were in agreement with the biochemical and histochemical investigations of Tsuboi and his coworkers [11-14].

In addition to newly formed cells, preserved liver cells with a normal content of RNA and alkaline phosphatase may be seen in the regenerating liver. The polymorphism of the liver cells during regeneration is thus increased still further by the considerable variations in the content of these compounds.

Side by side with the regenerative reaction in the liver of the mice on the second to third day after partial extirpation of the organ, destructive processes could be seen. These were primarily expressed in the presence of liver cells with a highly vacuolated cytoplasm. Cells were found with degenerative changes in their nuclei. In the process of destruction the picture of fragmentation and constriction of the nuclei could often be seen, and moreover this could not be confused with normal amitosis, for during the destructive changes the nuclear membrane acquired a ridged and uneven surface, the "amitosis" was not quite regular and the cytoplasm of such cells was highly vacuolated. The cells with degenerating nuclei also differed by their cytochemical characteristics from normal liver cells, the nuclei of which were undergoing amitotic division. Their cytoplasm contained little RNA and alkaline phosphatase.

In some cases it was difficult to differentiate the changes observed in the cells into regenerative or destructive processes. This concerned the distribution of

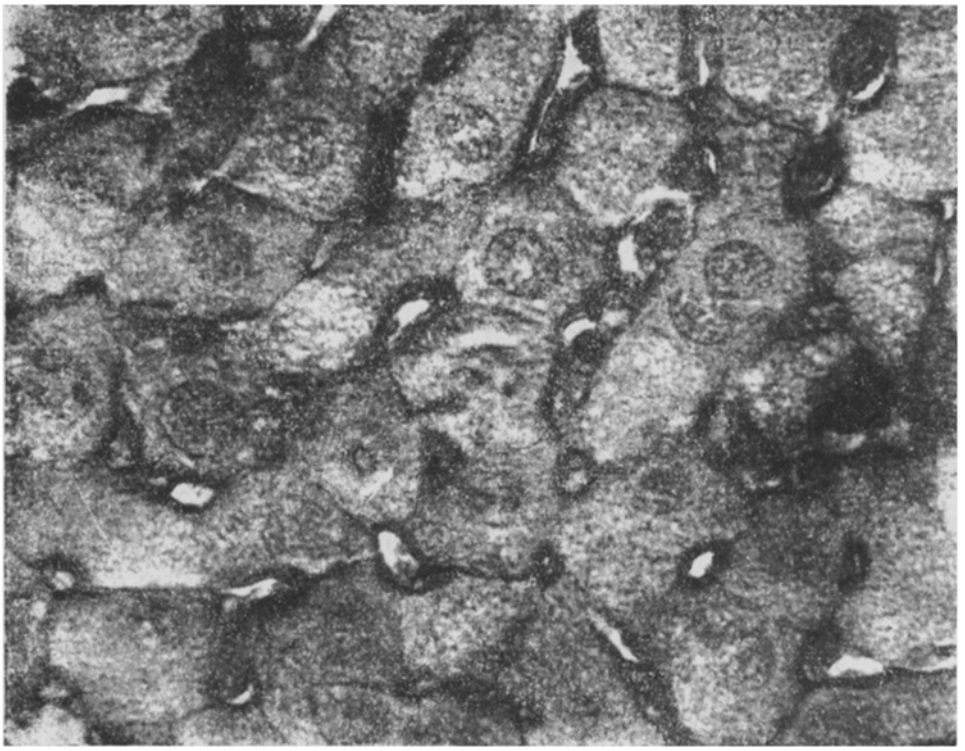

Fig. 3. Accumulation of alkaline phosphatase in the cells of the regenerating liver of a mouse (on the third day). Stained by Gomori's method. Magnification: ocular 5 x, objective 100 x.

the chromatin around the margins of the nuclei of the liver cells, and the vacuolation of the cytoplasm of certain liver cells, the nuclei of which were undergoing amitotic division, phenomena often observed in the regenerating liver.

In three cases large areas of necrosis of parenchymatous tissue were found in the regenerating liver, the liver cells being completely destroyed and staining badly with the ordinary histological stains, and considerable infiltration with blood cells being observed. Undamaged liver cells were preserved only around the blood vessels. No RNA nor alkaline phosphatase were found in the foci of necrosis.

SUMMARY

This work deals with the morphological and cytochemical changes in the regenerating liver of mice after removal of 65% of the liver tissue. Regenerative processes were found to occur shortly after the injury of the liver. Division of hepatic cells is both mitotic and amitotic. Hypertrophy of undividing hepatic cells continues for a period of 2-3 days. Cells adjacent to the blood vessels are more resistant to injury. The amount of RNA and alkaline phosphatase increases but their distribution in the cytoplasm of newly formed hepatic cells and in those undergoing mitosis is different. The time of the operation has no significant effect on the course of the regenerative process.

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