CHANGES IN THE LIVER OF RATS AT LATE STAGES OF REGENERATION

Z. A. Ryabinina

From the Laboratory of Growth and Development (Head: Prof. L. D. Liozner) of the Institute of Experimental Biology (Director: Prof. I. N. Maiskii) of the Akad. Med. Nauk SSSR, Moscow
(Presented by Active Member of the Akad. Med. Nauk SSSR N. N. Zhukov-Verezhnikov)
Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 51, No. 4, pp. 114-118, April, 1961
Original article submitted April 29, 1960

The regeneration process in the liver has been studied mainly in the early periods following infliction of traumas [1, 2, 4, 6, 7, 11]. Prolonged periods of observation have usually not exceeded 3 months. Investigations of the liver at later periods after partial resection has only been carried out in a few works, and on a small number of animals.

Investigators [3, 5, 12, 13] have not observed any peculiarities in the structure and function of the regenerating liver. Only Forti [8] writes that in rats, 1-2 yr after partial removal of the liver, the remaining portion of the organ has indeed increased in size, but almost always weighs somewhat less than the liver of the control animals. In addition, he observed a reduction in the functional activity of the organ operated upon, as judged by the quinine sulfate test.

Thus, investigation of the regenerating liver at prolonged intervals after the partial removal of the organ has been performed to an inadequate degree, and in the appraisal of the results obtained the opinions of the authors are divergent.

Meanwhile, it is of great theoretical and practical interest to elucidate how fully and stably the organs are regenerated after resection, and whether, with the passage of time, the regenerating organ begins to differ from the normal one.

Thus, it seemed timely to us to carry out a histological study of the regenerating liver at late intervals after partial removal of the organ.

METHOD

The experiments were performed on female rats with an initial body weight of 130-150 grams. The animals were operated upon according to the method of Higgins and Anderson [10]. We removed the left lateral and central lobes of the liver, which made up 2/3 of the organ.

The experimental and control animals received an abundant supply of white bread and water for the first 2 days following the operation. Then all the animals were transferred to the normal synthetic diet, and the rats were maintained under these conditions until the end of the experiment.

The experimental animals and their corresponding controls were sacrificed 2, 5, 11 and 17 months after the operation. The liver was quickly removed and weighed. Material was fixed in a 12% solution of formalin. The preparations were stained with hematoxylin-eosin. The reticular fibers were demonstrated with the use of Gomori's silver stain [9].

RESULTS

As the weighing showed, in the control rats the liver growth proceeds uninterruptedly, but the relative weight of the liver quickly attains a certain magnitude and, after this, remains more or less constant up to 17 months, equalling 3.4-3.8%.

Change in the Absolute and Relative Weight of the Control and Regenerating Liver in the Rat

Duration of experiment (in months)	Group of animals	Number of animals	Weight of animals at time of sacrifice, g	Weight of the liver	
				absolute (in g)	relative (in %)
2	Test	12	190	7.690	4.1
	Control	7	220	7.550	3.4
5	Test	13	237	7.960	3.4
	Control	6	230	8.600	3.8
17	Test	14	277	7.600	2.7
	Control	8	322	11.767	3.7

The absolute weight of the regenerating liver in the period from 2 to 17 months after the operation changes very little, as a result of which by the 17th month it weighs considerably less than the liver in the control animals of the same age. The relative weight of the regenerating liver also decreases, and by the 17th month comprises 2.7% of the body weight, i.e., it is 1.5 times less than the weight of the regenerating liver at earlier intervals of the investigations (2 months), when the relative weight is 4.1% (table).

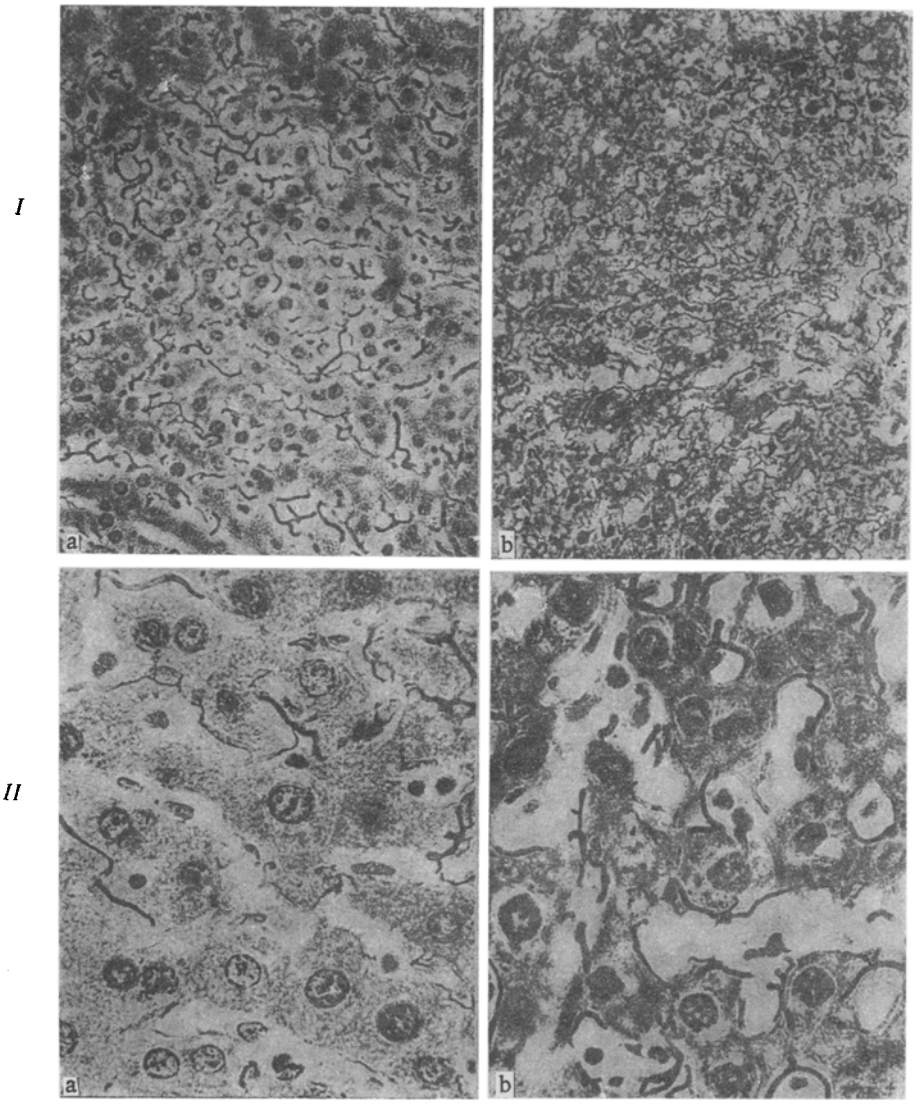
In the histological investigation of the regenerating liver 2 months after the operation we detected certain deviations from the normal. The nuclei and cytoplasm of the mononuclear hepatic cells still remain hypertrophied. Polymorphism of the liver cells, which stands out especially clearly in the liver at early periods of regeneration, decreases to an appreciable degree after 2 months; at this time we were unable to find mitotically dividing cells in the liver of the rats operated upon, nor did we encounter areas with destroyed cells; a significant number of binucleated hepatic cells was apparent in the preparations.

Staining the reticular fibers was also not observed to differ from the normal in any essential way. Fine branching threads of reticular fibers were arranged throughout the entire parenchyma of the liver; their distribution was observed to be more dense in the region of the large blood vessels. This type of reticular fiber distribution was observed in the liver of control animals of the same age.

Five months after the operation the general histological picture of the regenerating liver was the same as that which was observed at earlier intervals after the operation (2 months). However, study of the reticular fibers in the liver of the rats operated upon disclosed their heterogeneity. In the majority of the animals the usual distribution of reticular fibers was retained, being the same as that intrinsic to the liver in the control rats of that age. However, in two rats it was possible to observe proliferation and thickening of the reticular fibers. This proliferation was local in character; it appeared in the region of the large blood vessels, and spread out from there deep into the parenchyma of the liver. In the areas of proliferation and thickening of the reticular fibers we observed destructive changes in the parenchymal cells. The cytoplasm of the hepatic cells in this case stained weakly with the normal histologic stains, and several cells were missing their nuclei.

Eleven months after the operation a more clearly defined polymorphism was again observed in the hepatic cells; it was manifested by the appearance of a significant number of cells with small nuclei and a rather appreciable number of binucleated cells.

Well defined results were again not obtained for the reticular fibers. In certain cases we observed proliferation and thickening of the reticular fibers. They became more coarse and their network condensed, mainly in the region of the blood vessels. We observed this picture in 4 of the 8 rats, while in the remaining cases the reticular framework of the regenerating liver did not differ from that seen in the control animals.



Reticular fibers in the liver of a rat 17 months after the beginning of the experiment; a) control; b) regeneration. Stained according to Gomori. Magnification: oc. 5 ×, obj. 45 × (I); oc. 5 ×, obj. 100 × (II).

Subsequent to dissection of the test animals 17 months after the operation we noted a considerable development of the epiploön. In these animals the liver was soft, flaccid, and had many adhesions with surrounding organs and tissues, but its color remained normal.

Through histological investigation a number of morphological changes were detected. Along with unchanged liver cells there was a rather large number of hepatic cells with vacuolated cytoplasm. Cells were also encountered in the preparations whose nuclei were in the early stages of disintegration. The appearance of a significant number of binuclear cells was observed; the dimensions of their nuclei were most frequently smaller than the dimensions of the mononuclear hepatic cells, although the latter were also decreased.

Staining of the reticular fibers showed that they proliferated markedly in individual areas of the liver; the reticular fibers were notably thickened.

A series of age changes was also observed in the liver of the control animals by the 17th month, as well as a richer development of the reticular framework. However, the proliferation of the reticular fibers in the liver of the rats operated upon was always appreciably greater (figure).

The changes in the reticular fibers can probably be regarded as a result of reduction in the permeability of the walls of the blood vessels, which leads to deterioration of the nutritive supply and to the development of dystrophic changes in the liver tissue.

Actually, it was frequently possible to observe disintegrating liver cells in those areas where proliferation of the reticular fibers took place.

Thus, on the basis of the investigation carried out, it may be said that morphological changes occur in the regenerating liver of rats at late intervals after trauma which are dystrophic in nature. The development of adipose tissue observed 17 months after the operation is probably a result of disruption of the regenerating liver's normal functioning, which is closely related to the destructive changes arising in the organ at these intervals in the investigation.

Our investigations do not yet permit drawing wider conclusions, but it can be reasonably asserted that under conditions of our experiments clearly defined differences have been established between the growth changes of the normal and regenerated liver.

SUMMARY

The author carried out a histological study of the regenerating liver of rats at remote periods (2, 5, 11 and 17 months) after the operation.

Development of dystrophic processes was observed 17 months after the excision of $^2/_3$ of the organ by Higgins and Anderson's method. The weight of the liver decreased in experimental animals and was much lower than in controls. A large number of binuclear cells with small nuclei appeared; mononuclear hepatic cells decreased in size; proliferation and thickening of reticular fibers was detected by Gomori's staining.

LITERATURE CITED

1. Zhenevskaya, R. P., Works of the Severtsev Inst. of Animal Morphology [in Russian] (Moscow, 1954) 11, p. 40.
2. Liozner, L. D., Ryabinina, Z. A. and Sidorova, V. F., Byull. Éksper. Biol. i. Med. 47, 5, 96 (1959).
3. Meister, V. A., Regeneration of Liver Tissue Following Removal of Entire Lobes of the Liver up to 4/5 of the Total Mass of the Organ. Experimental Investigation [in Russian] (Kiev, 1894).
4. Ryabinina, Z. A., Byull. Éksper. Biol. i Med. No. 3, p. 105 (1960).
5. Sidorova, V. F., Byull. Éksper. Biol. i Med., No. 8, p. 99 (1959).
6. Brues, A. M., Drury, D. R. and Brues, M. C., Arch. Path. 22, 658 (1936).
7. Brues, A. M. and Marble, B. B., J. Exp. Med. 65, 15 (1937).
8. Forti, C., Boll. Soc. ital. Biol. sper. 29, 428 (1953).
9. Gomori, G., Cited by E. Pearse, Theoretical and Practical Histochemistry [Russian translation] (Moscow, 1956).
10. Higgins, G. M., Anderson, R. M., Arch. Path. 12, 186 (1931).
11. Jaffe, J. J., Anat. Rec. 120, 935 (1954).
12. Wilson, M. E., Stowell, R. E., Yakoyama, H. O. et al., Cancer Res. 13, 86 (1953).
13. Yakoyama, H. O., Wilson, M. E., Tsuboi, K. K. et al., Cancer Res. 13, 80 (1953).

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. ***Some or all of this periodical literature may well be available in English translation.*** **A complete list of the cover-to-cover English translations appears at the back of this issue.**
