THE STRUCTURE OF THE REGENERATING LIVER IN RATS

V. F. Sidorova

From the Laboratory of Growth and Development (Head — Dr. Biol. Sci. L. D. Liozner) of the Institute of Experimental Biology (Director — Prof. I. N. Maiskii) of the AMN SSSR, Moscow

(Received December 26, 1958. Presented by Active Member AMN SSSR V. N. Chernigovskii)

Large-scale investigations have recently been carried out into the study of the regenerative powers of mammalian organs, and especially the regeneration of the internal organs. The heightened interest of research workers in this problem has been due to an attempt to find an experimental basis for new theoretical concepts, and to the requirements of practical medicine.

The great regenerative powers of certain internal organs of mammals are not now in doubt. Relatively little attention has been paid, however, to the problem of the structural and functional qualities of the regenerating organ, which requires more detailed analysis.

For instance, it has been shown that removal of a large part of the liver, up to four fifths of its mass, in mammals, very quickly leads, in 7-14 days, to complete restoration of the initial size of the organ. The removed lobes are not reformed under these circumstances, but a regenerative hypertrophy of the residual lobes of the liver takes place [3, 8, 10, 11, 12].

This rapid restoration of the damaged liver is brought about by the intensive mitotic proliferation of the liver cells. Proliferation of the cells of the efferent ducts and the stroma takes place to a lesser degree [9].

After extirpation of two lobes of the liver in rats, on the 3rd day the number of liver cells in the regenerating liver is 65%, and on the 12th day 89%, of the number of liver cells in animals not undergoing operation [6]. The question whether such a rapid quantitative replacement of the cells is accompanied by structural changes in the organ has received only little study.

In earlier work [3, 11] it was asserted that the microsoopic structure of the regenerating liver (after the surgical procedure described above) was characterized by hypertrophy of the hepatic lobules. These authors did not describe the formation of new structural units of the organ. Later workers, however, concerned with the study of regeneration of the liver, began to hold that the formation of new lobules does take place in the liver, and that these are indistinguishable from normal in their structure [4, 8].

These divergent views may be explained by the absence of special research directed toward the solution of this problem. Little attention has been paid, in particular, to the study of the topography of the blood vessels in the regenerating liver, which could give much valuable evidence about its structure.

We know only one paper [5] in which it is pointed out that the blood pressure in the portal vein of a regenerating liver is lowered, and thus, according to the views of Elias [6], the arrangement of the liver lobules is inverted in character, i.e., the portal and not the hepactic vein is in the center of each lobule.

This state of affairs impelled us to carry out the present investigation, with the special object of ascertaining the dimensions of the lobules in the regenerating liver of rats, by demonstrating the topography of the arterial vessels of the liver.

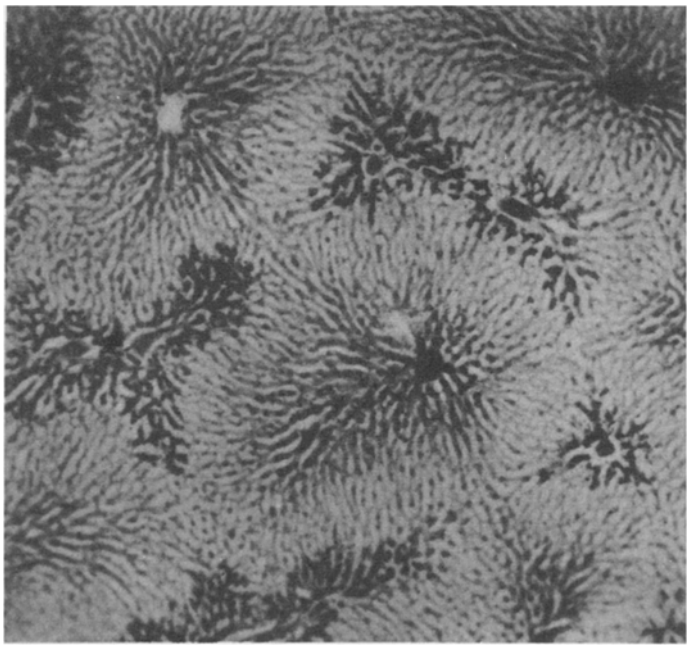

Fig. 1. Section of the normal liver of a rat after injection of the arterial vessels with India ink. The contours of the individual lobules are clearly seen. Magnification 75x .

Number of Central Veins in a Field of Vision and Area of the Lobule in the Liver of the Experimental and Control Rats

Group of animals	Indices used	Time of regeneration in days											
		1	2	3	7	12	14	20	30	50	75	90	180
Control	Number of central veins in a field of vision	8	—	8	—	8	9	—	—	8	—	9	9
	Magnified area of section of lobule, in mm²	147	—	127	—	153	141	—	—	164	—	169	178
Experiment	Number of central veins in a field of vision	7	6	5	4	4	4	5	5	4	5	5	4
	Magnified area of section of lobule, in mm²	193	231	386	373	397	340	293	343	416	358	371	383

EXPERIMENTAL METHOD

In male white rats, weighing 115-150 g, total extirpation of the left lateral and central lobe of the liver was performed , and ligatures applied to the bases of these lobes by the method of Higgins and Andersen. The portion removed accounted for 60-70 % of the weight of the organ. The regenerating liver was investigated 1, 2, 3, 7 , 12, 14, 20, 30, 50, 75, 90 and 180 days after operation. At each time 5 experimental and 3 control animals were sacrificed.

The main difficulty in demonstrating the strucgure of the regenerating liver was the absence of any clear division into lobules. The contours of the lobules could, however, be quite clearly distinguished by injection of a mixture of India ink and gelatin into the arterial system of the liver. A 4% solution of gelatin in ink, warmed to 37-40°, was injected into the anesthetized animal, through a thoracotomy directly into the aorta, in a volume of 1 cm^3. Before injection a first ligature was tied above the site of injection and after the injection a second ligature was tied below the site.

After injection of the ink the circulation must be stopped immediately, otherwise the ink escapes from the arterial capillaries into the venous, so that the contours of the lobule are obliterated. The sacrificed animal was placed without delay in the refrigerator for 30 minutes at a temperature of 0°, and was then kept for 24 hours at +2°. After 24 hours the liver was fixed in 10% formalin, and sections were cut to a thickness of 10 μ on a freezing microtome.

The upper part of the right lobe of the liver was removed, not far from its base, from both experimental and control animals; this was the most convenient place for the preparation of tangential sections. The structure of the lobule was seen best of all in sections passing just beneath the capsule. In these areas nearly all the lobules were cut transversely, which facilitated their counting and measurement.

For measuring the area of the lobules sections of equal size were used, passing at roughly the same distance from the capsule. By means of an Edinger drawing machine, with magnification 19 times, the contours of all the lobules in one section from each liver were traced on cleaned x-ray film. The section usually contained 12-26 lobules. Areas of film, corresponding to the individual lobules, were cut out and weighed on torsion scales. The values obtained were divided by the weight of 1 cm^3 of film. Having estimated in this way the area lobule, the mean area of the lobules was then calculated.

In order to analyze the structural relationships between the vessels of the regenerating liver and the parenchyma, and also to provide supplementary information on the dimensions of the lobules, the number of central veins in a field of vision of the microscope was counted, at a magnification of 20 times. Three fields of vision were used in each section.

EXPERIMENTAL RESULTS

In the liver sections, the arterial capillaries situated around the periphery of the lobule were filled with injected ink and clearly outlined its contours. The central veins were well marked, thanks to their central position in the transverse sections of the lobules, and to the almost complete absence of dye from them (Fig. 1).

In the first few days after operation, dilatation of the central and peripheral veins of all the lobules, and also of their venous and arterial capillaries, was observed.

The results of the counts of the number of central veins in a field of vision of the microscope, and of the measurement of the area of the lobules in the section in the experimental and control animals, are shown in the table. For each period of regeneration the mean values obtained in three animals are given.

The results obtained show that the dimension of the hepatic lobules began to change quite early. Within 24 hours of operation, the dimensions of the hepatic lobules in the regenerating liver were increased. The number of central veins in a field of vision in the liver preparations from the control animals was 8, and from the experimental animals 7. The corresponding areas of the lobules were 147 and 193 mm^2. The differences between the experimental and control values were statistically significant. The increase in size of the liver lobules in the first 24 hours was evidently due both to an increase in the size of the hepatic cells, as pointed out by several authors [3, 10, 11], and to considerable dilatation of the blood vessels.

On the second day, when proliferation of hepatic cells began in the partially resected liver, the increase in the size of the liver lobules took place more intensively. The number of central veins in a field of vision was 6, the area of the lobule 231 mm^2. On the third day after operation, all the liver lobules were grossly hypertrophied. One field of vision included 5 central veins, whereas in the liver sections from the control animals there were 8 as before. At this time the area of the liver lobule in the experimental animals was 386, and in the controls 127 mm^2.

The difference in the size of the lobules on the second and third days after operation was statistically significant. Thus the dimensions of the liver lobules in the regenerating liver were roughly 2-3 times as great

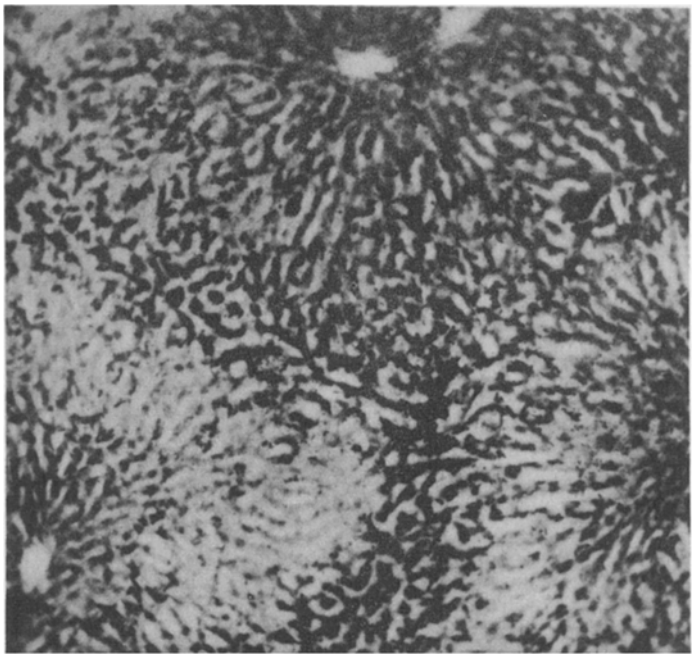

Fig. 2. Section through the regenerating liver 3 days after partial hepatectomy. Dilated arterial capillaries and veins are seen. The hepatic lobules are hypertrophied. Magnification 75 x.

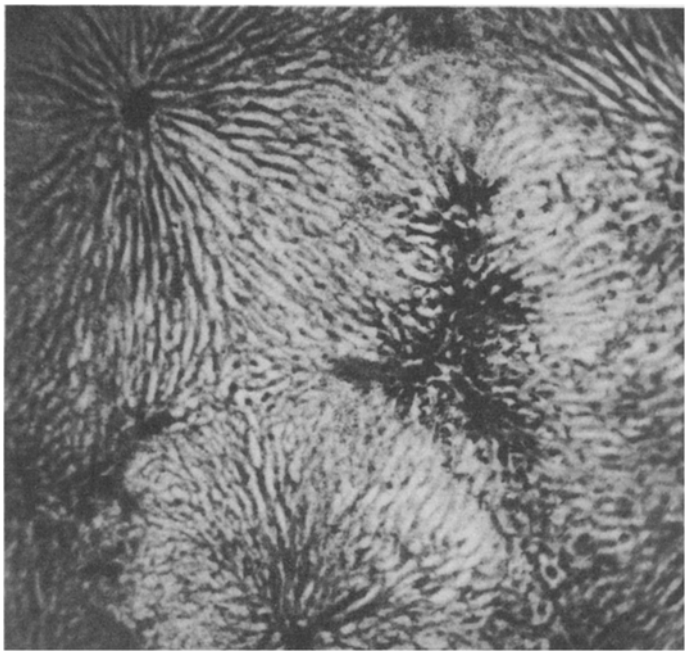

Fig. 3. Section through the regenerating liver 6 months after hepatectomy. Normalization of the filling of the blood vessels. Liver nodules hypertrophied. Magnification 75 x.

as those of the control animals on only the third day (Fig. 2). Under these circumstances all the lobules, both large and small, were enlarged and no individual lobules or groups of lobules showed any preferential enlargement.

The mitotic activity of the liver cells reached its maximum intensity on the third day [6, 9, 10, 12, 13]. The weight of the residual lobes of the liver at this time was 70-80% of the weight of the whole organ [10].

The increase in the dimensions of the hepatic lobules is thus primarily the result of an increase in the number of liver cells.

The rapid compensatory reaction of the body in this case was thus not one of formation of new structural components of the organ, i.e., lobules, but of their hypertrophy. On the 7th day after partial hepatectomy, the anatomical structure of the liver was in no way indistinguishable from the picture that was observed on the 3rd day: the hepatic lobules remained hypertrophied.

The reorganization of the structure of the liver during regeneration of the organ evidently took place mainly during the first days, since on subsequent days it was impossible for us to detect any essential difference between the dimensions of the lobules at the early and late stages of regeneration. The differences in the areas of the lobules in the experimental animals (for example, on the 14th and 20th days of regeneration), given in the table, depended on individual variations and were not statistically significant. The hepatic lobules, which doubled or trebled their size in the initial stages of regeneration, maintained their large size later on, even for 6 months (the end of our period of observation). The filling of the blood vessels had returned to normal at this time (Fig. 3).

It must be pointed out that hypertrophy of the lobules took place not only at the periphery of the organ, but also in its depths, since in the deeper sections were observed enlarged complex and simple lobules, cut transversely and longitudinally. We were never once able to discover signs of formation of new lobules, which would inevitably have been accompanied by the appearance of new efferent ducts and blood vessels.

It can be concluded from our findings that after removal of the left lateral and central lobes of the liver, hypertrophy of the lobules of the remaining portion of the liver takes place.

Regarding the possibility of formation of new lobules, both in the experiments which we carried out and in any other type of damage to the liver, in perforating wounds [1, 2] or in death of part of the parenchyma as the result of pathological conditions, there is need for special investigation.

SUMMARY

60-70% of the liver was removed by Higgins and Anderson's method in white male rats, weighing 115 to 150 g. The changes in the size of hepatic lobules in regenerating liver were studied for a period of 1 to 180 days. India ink was injected into hepatic arteries which made possible clear delineation of individual lobules. As demonstrated, the size of the lobules increases 2-3 times during the 1st — 3rd days and remains so up to 6 months months. No newly formed lobules were revealed.

LITERATURE CITED

[1] R. P. Zhenevskaya, Transactions of the Severtsev Institute of Morphology of Animals, 11, pp. 40-91 1954 [In Russian].

[2] E. F. Kotovskii, The Influence of the Higher Divisions of the Nervous System on Inflammatory and Regenerative Processes, pp. 71-82. Moscow, 1957 [In Russian].

[3] B. P. Solopaev, Proceedings of a Conference on Regeneration and Proliferation of Cells, pp. 55-56. Moscow, 1958 [In Russian].

[4] P. Barone and D. Batalo, Biol. Catina, v. 11, N. 2 p. 252-266 (1958).

[5] A. M. Brues, D. R. Drury and M. C. Brues, Arch. Path. v. 22, p. 658-673 (1936).

[6] H. Elias, A. Sokol, Anat. Rec. v. 115, p. 71-80 (1953).

[7] F. C. Rishback, Arch. Path. v. 7, p. 955-977 (1929).

[8] R. D. Harkness, Brit. M. Bull. v. 13, p. 87-93 (1957).

[9] G. M. Higgins, R. M. Anderson, Arch. Path. v. 12, p. 186-202 (1931).

[10] J. J. Jaffe, Anat. Rec. v. 120, p. 935-954 (1954).

[11] E. Ponfrick, Arch. Path. Anat. Bd. 119, S. 193-240 (1890).

[12] H. O. Yokoyama, M. E. Wilson, K. K. Tsuboi, Cancer Res. v. 13, p. 80-92 (1953).